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Evaluation of the effects of light intensity on growth of the benthic dinoflagellate *Ostreopsis* sp. 1 using a newly developed photoirradiation-culture system and a novel regression analytical method

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ABSTRACT

Benthic dinoflagellates of the genus *Ostreopsis* are found all over the world in temperate, subtropical, and tropical coastal regions. Our recent studies revealed that a putative "cryptic" species of *Ostreopsis ovata* is present widely along Japanese coasts. This organism, *Ostreopsis* sp. 1, possesses palytoxin analogs and thus its toxic blooms may be responsible for potential toxification of marine organisms. To evaluate the bloom dynamics of *Ostreopsis* sp. 1, the present study examined the growth responses of *Ostreopsis* sp. 1 strain s0716 to various light intensities (photon flux densities: μmol photons m^{-2} s^{-1}) using a newly devised photoirradiation-culture system. This novel system has white light-emitting diodes (LEDs) capable of more closely simulating the wavelength spectrum of light entering the oceanic water column than do fluorescent tubes and halogen lamps. In this system, the light intensity of the white LEDs was reduced through two polarizing filters by varying the rotation angles of the filters. Thereby, the new system was capable of culturing microalgae under well-controlled light intensity conditions. *Ostreopsis* sp. 1 grew proportionally when light intensity was increased from 49.5 to 199 μmol photons m^{-2} s^{-1}, but its growth appeared to be inhibited slightly at $\geq$263 μmol photons m^{-2} s^{-1}. The relationship between observed growth rates and light intensity was calculated at $R > 0.99$ ($P < 0.01$) using a regression analysis with a modified equation of the photosynthesis-light intensity (P-L) model. The equation determined the critical light intensities for growth of *Ostreopsis* sp. 1 and the organism's growth potential as follows: (1) the threshold light intensity for growth: 29.8 μmol photons m^{-2} s^{-1}; (2) the optimum light intensity (L_m) giving the maximum growth rate (μ_{max} = 0.659 divisions day^{-1}): 196 μmol photons m^{-2} s^{-1}; (3) the optimum light intensity range (L_{opt}) giving $\geq$95% μ_{max}: 130–330 μmol photons m^{-2} s^{-1}; (4) the semi-optimum range (L_{sopt}) giving $\geq$80% μ_{max}: 90 to over 460 μmol photons m^{-2} s^{-1}. The L_{sopt} represents 4.5–23% ambient light intensity present in surface waters off of a temperate region of the Japanese coast, Tosa Bay; putatively, this semi-optimum range of light intensity appears at depth of 12.9–27.8 m. Considering these issues, our data indicate that *Ostreopsis* sp. 1 in coastal environments may form blooms at ca. ~28 m depth in regions along Japanese coasts.

1. Introduction

Blooms of marine dinoflagellates of the genus *Ostreopsis* may be associated with toxification of marine organisms (Yasumoto et al., 1986; Taniyama et al., 2003; Aligizaki and Nikolaidis, 2006; Shears and Ross, 2009; Aligizaki et al., 2011) and outbreaks of aerosol particles (Ciminiello et al., 2006, 2008). The toxic dinoflagellate *Ostreopsis* sp. 1 is present in Japanese (Sato et al., 2011; Hariganeya et al., 2013) and Korean (Kang et al., 2013) coastal waters. This organism has similar morphological features to those of *Ostreopsis ovata*, but shows sequences in the 28S ribosomal DNA D8–D10 and the ITS (internal transcribed spacer) regions that differ from those

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of the other *Ostreopsis* spp., including *O. ovata*/*O.* cf. *ovata* (Sato et al., 2011). In consideration of these issues, Sato et al. (2011) reported that *Ostreopsis* sp. 1 belongs to a novel phylo-type and is a putative "cryptic" species of *O. ovata*/*O.* cf. *ovata*.

Ostreopsis sp. 1 is distributed widely along temperate Japanese coastal regions, such as Tosa Bay (Sato et al., 2011). Suzuki et al. (2012) demonstrated that Japanese *Ostreopsis* sp. 1 cultures possess not only palytoxin but also its analogs such as ovatoxin-a AC. Strong toxicities of *Ostreopsis* sp. 1 cultures for mouse have also been reported by Sato et al. (2011). These reports support supposition that *Ostreopsis* sp. 1 is a ubiquitous and toxic species of the genus *Ostreopsis* in Japan. Thus, blooms of *Ostreopsis* sp. 1 may be one of the causative agents for potential toxification of marine organisms in Japan.

To understand the bloom dynamics of *Ostreopsis* sp. 1, it is important to clarify the critical environmental factors affecting algal growth. Our recent studies established a suitable culture method for Japanese *Ostreopsis* species (Yamaguchi et al., 2012a) and thereby clarified significant effects of temperature-salinity interactions on the growth of *Ostreopsis* sp. 1 (Tanimoto et al., 2013), Japanese *O.* cf. *ovata* (Yamaguchi et al., 2012b) and a novel clade *Ostreopsis* sp. 6 (Tanimoto et al., 2013). Tanimoto et al. (2013) suggested that *Ostreopsis* sp. 1 is well-adapted to cold waters of 15 °C and is consequently present widely around Japanese temperate coasts. However, the effects of light intensity (photon flux density: μmol photons m^{-2} s^{-1}) on growth of *Ostreopsis* sp. 1 have not yet been clarified.

In order to examine the light-responsive growth of *Ostreopsis* spp., it is necessary to use an appropriate light source which effectively simulates the natural photosynthesis available radiation (PAR) in oceanic water. Furthermore, to elucidate the effects of light on the growth of *Ostreopsis* spp. under various light intensities, we needed a system with a photoirradiation device capable of adjusting light intensities accurately. Several light sources and photoirradiation devices have previously been used for research on the light-responsive growth of harmful algae, including *Ostreopsis* species (Faust et al., 1982; Anderson et al., 1984; Bomber et al., 1988; Morton et al., 1992; Yamaguchi et al., 2010). Cool-white and/or Vita fluorescent tubes and halogen lamps have been used as general light sources for algal cultures; however, the illumination from these sources, produced by AC (alternating current), is incapable of simulating closely the continuous irradiation and the light spectrum of natural PAR in oceanic water, due to the intermittent (pulsed) light and the lack of blue light (Guillard and Morton, 2003; Carvalho et al., 2011).

To estimate and predict algal growth under various levels of light intensity, it is also essential to formularize the relationship between growth and light intensity. In several harmful algae, such as *Chattonella marina*/*C. ovata* (Yamaguchi M. et al., 1991; Yamaguchi H. et al., 2010), *Karenia mikimotoi* (Yamaguchi and Honjo, 1989), and *Gambierdiscus* species (Kibler et al., 2012), the growth-light intensity relationships have been estimated quantitatively by formularizing with one of the model equations described below: Michaelis–Menten (MM) (Yamaguchi and Honjo, 1989), modified MM (mMM) (Yamaguchi M. et al., 1991; Yamaguchi H. et al., 2010) and Gaussian/Lorentzian model (probability density function of the normal distribution) equations (Kibler et al., 2012). Among them, the MM and mMM model equations are incapable of displaying algal growth inhibition at intense levels of light intensity, due to the appearance of a saturated growth rate at infinity light intensity. In contrast, the Gaussian/Lorentzian model equation is capable of displaying algal growth inhibition as well as promotion with increases in light intensity when the growth-light intensity curve resembles a normal distribution curve; however, such curve-forms are infrequently observed in dinoflagellates (Morton et al., 1992; Kibler et al., 2012). Importantly, none of these model equations are capable of displaying algal growth inhibition and promotion with varying light intensity and of also determining the threshold of light intensity required for algal growth.

An empirical equation, the photosynthesis-light intensity (P-L) model equation, describes the photo-promotion and -inhibition of algal photosynthesis (Platt et al., 1980) and has been used for *Thalassiosira pseudonana* and *Dunaliera tertiolecta* monocultures (Bates and Platt, 1984) and phytoplankton assemblages (Glibert et al., 1985). This equation has not previously been used to describe the threshold light intensity required for photosynthesis; however, by configuring the intercept on the *x*-axis of the P-L model equation, it may potentially be used to estimate the threshold light intensity for algal growth. The 'modified' P-L model equation which would estimate the threshold light intensity required for algal photosynthesis may be used to formularize the relationship between growth and light intensity in harmful algae.

In this study, we used a newly developed photoirradiation-culture system to closely simulate natural PAR in oceanic water at varying levels of light intensity. By using this system and a novel regression analytical method with the modified P-L (mP-L) model equation, we precisely estimated the growth responses of the toxic Japanese strain of *Ostreopsis* sp. 1 to various light intensities in order to evaluate the organism's bloom dynamics in coastal waters.

2. Materials and methods

2.1. Development of the photoirradiation-culture system

2.1.1. System components

The photoirradiation-culture system is shown in Fig. 1. This system was composed of a light source, a water bath, a water circulator, and photoirradiation-adjustable device. The source of white light was composed of 98-pcs flux light-emitting diodes (LEDs) (3.3 V/30 mA, No. 405, audio-Q, Japan) and 49-pcs Φ5 mm LEDs (3.2 V/20 mA, No. 077, audio-Q, Japan) coupled with an electric circuit board. A 24-V DC (direct current) adapter (Go Forward Enterprise Corp., Taiwan) was used as a power supply for the LEDs. A clear acrylic tank (250 mm × 200 mm × 300 mm) was used as the water bath in which ca. 2 L water was contained. A water circulator (Cool Line-100, Yamato Co. Ltd., Japan) was used for maintaining the temperature of the water bath (Fig. 1). A unit of the photoirradiation-adjustable device was fabricated as follows:

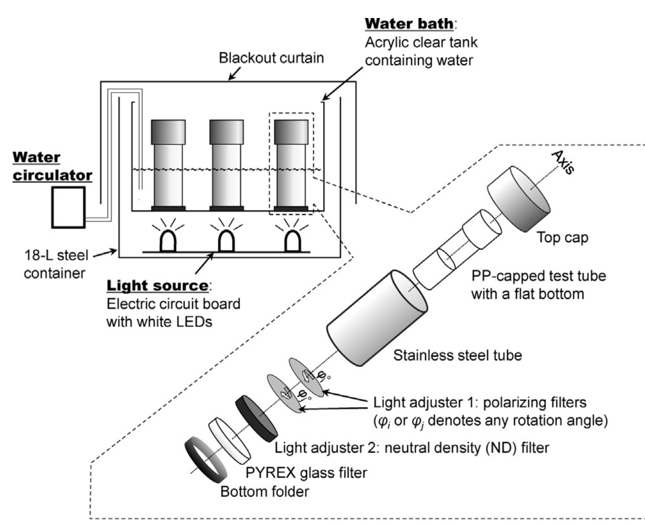

Fig. 1. Simplified schematic of the culture system with photoirradiation-adjustable devices developed in the present study.