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Effects of light intensity, temperature, and salinity on the growth and ingestion rates of the red-tide mixotrophic dinoflagellate *Paragymnodinium shiwhaense*

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ABSTRACT

Among mixotrophic dinoflagellates, the maximum mixotrophic growth rate of the red-tide dinoflagellate *Paragymnodinium shiwhaense* is relatively high, whereas mortality due to predation is low. To investigate the effects of major environmental parameters on *P. shiwhaense*, growth and ingestion rates of one strain of *P. shiwhaense* on the algal prey species *Amphidinium carterae* (also a dinoflagellate) were determined under various light intensities (0–500 µE $m^{-2}s^{-1}$), water temperatures (5–30 °C), and salinities (5–40). Cells of *P. shiwhaense* did not grow well in darkness but grew well at light intensities ≥ 10 µE $m^{-2}s^{-1}$. There were no significant differences in either growth or ingestion rates of *P. shiwhaense* fed *A. carterae* at light intensities between 10 and 500 µE $m^{-2}s^{-1}$. Furthermore, *P. shiwhaense* did not grow at 5 °C or ≥ 28 °C. Its growth rates between 7 and 26 °C were significantly affected by temperature, and the optimal temperature for maximal growth was 25 °C. With increasing salinity from 5 to 20, the growth rate of *P. shiwhaense* fed *A. carterae* increased and became saturated at salinities between 20 and 40, while the ingestion rate at salinities between 10 and 40 did not significantly change. Thus, overall, the growth and ingestion rates of *P. shiwhaense* fed *A. carterae* were affected by temperature and salinity, but not by light intensity other than darkness. These findings provide a beginning basis for understanding the ecology of this potentially harmful algal species in marine coastal ecosystems.

1. Introduction

Phototrophic dinoflagellates are ubiquitous and a major component of marine ecosystems (Juhl, 2005; Anderson et al., 2012; Glibert et al., 2012; Sellers et al., 2014; Sala-Pérez et al., 2016). In the last three decades, many phototrophic dinoflagellates have been shown to be mixotrophic (i.e., they obtain energy through both photosynthesis and phagotrophy or osmotrophy) (Bockstahler and Coats, 1993; Stoecker, 1999; Burkholder et al., 2008). They play diverse roles as primary producers, predators of bacteria and microalgae, and prey for protists and metazoans in marine food webs (Tillmann, 2004; Jeong et al., 2010; Hansen, 2011; Johnson, 2015; Lee et al., 2016; Stoecker et al., 2017). Furthermore, many mixotrophic dinoflagellates cause “red tides” or “harmful algal blooms”, which can result in significant losses for diverse industries (Place et al., 2012; Park et al., 2013; Adolf et al., 2015; Menden-Deuer and Montalbano, 2015). Consequently, there is a great focus from federal and local governments, scientists, aquaculture farmers, and the public on processes of dinoflagellate red tides. Understanding and predicting the processes responsible for dinoflagellate red tides, thus providing early warning to aquaculture farmers, is one of the critical steps needed to minimize potential losses. To understand the bloom dynamics of a dinoflagellate species, its growth and mortality due to predation should be explored (Jeong et al., 2015).

The mixotrophic dinoflagellate *Paragymnodinium shiwhaense* (Dinophyceae) was described as a new species in a new genus in 2010 (Kang et al., 2010). This species (ca 14 µm in cell length x ca. 11 µm in cell width) has rows of amphisiemal plates, and it has both chloroplasts and nematocysts, which is unusual among phototrophic dinoflagellates (Kang et al., 2010). Its cells possess peridinin as the major accessory pigment. This species has caused red tides in Korean waters (Kang et al.,

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2013), but mortality of finfish or shellfish has not been reported yet. Cells of *P. shiwhaense* are known to feed on diverse small algal prey and grow well on the dinoflagellate *Amphidinium carterae*, the raphidophycean *Heterosigma akashiwo* (Raphidophyceae), and the cryptophytes *Teleaulax* sp. and *Rhodomonas salina* (Cryptophyceae) (Yoo et al., 2010). Furthermore, it can divide almost twice per day when fed on the optimal prey *A. carterae*, and its maximum rate is relatively high among the mixotrophic dinoflagellates (Yoo et al., 2010; Jeong et al., 2015). In contrast, effective predators of *P. shiwhaense* are lacking (Jeong et al., 2017a); the growth rates of the common heterotrophic protists *Oxyrrhis marina* (Dinophyceae), *Gyrodinium dominans* (Dinophyceae), and *Strombidinopsis* sp. (Oligotrichea, ciliate) feeding on *P. shiwhaense* were very low or negative, although they were able to feed on *P. shiwhaense*. In addition, the maximum ingestion rate of *Strombidinopsis* sp. on *P. shiwhaense* was much lower than ingestion rates reported for other mixotrophic dinoflagellate prey species (Jeong et al., 2017a), and it was suggested that *P. shiwhaense* has defense mechanisms against potential protistan predators.

For mixotrophic dinoflagellates, biological properties such as suitable prey availability, and physical/chemical properties such as nutrients, light, temperature, and/or salinity have been shown to affect growth and ingestion rates (Li et al., 2000; Skovgaard et al., 2000; Montagnes et al., 2003; Guerrini et al., 2007; Salgado et al., 2015). In coastal environments, these properties readily change spatially and temporally over broad gradients, and mixotrophic dinoflagellates often experience wide ranges of these properties (Cullen and Eppley, 1981; Aoki et al., 2017; Jeong et al., 2017b). Cells of *P. shiwhaense* have not grown without suitable prey, even under nutrient-replete conditions (Yoo et al., 2010). Therefore, other physical and chemical properties such as light, temperature, and/or salinity may affect its growth and ingestion rates, and these effects must be investigated to understand its bloom dynamics.

Light intensity varies widely in a day and in a year at sea (e.g., Côté and Platt, 1983). Furthermore, many red-tide dinoflagellates are known to conduct diurnal vertical migration (Eppley et al., 1968; Blasco, 1978; Anderson and Stolzenbach, 1985; Olsson and Granéli, 1991; Schofield et al., 2006; Hu et al., 2016), ascending to the well-lit surface waters during daytime and descending to the nutrient-rich deep waters at night. Thus, they experience a wide range of light intensity. In population dynamics, whether mixotrophic dinoflagellates can feed and/or grow in darkness is important because they experience darkness for several hours a day in most regions. In addition, whether the growth or ingestion rate of a mixotrophic dinoflagellate is affected by light intensity is also important. Feeding and/or growth of mixotrophic dinoflagellates in darkness, as well as light intensity effects, are known to be species-specific (Skovgaard, 1996; Hansen and Nielsen, 1997; Stoecker et al., 1997; Jeong et al., 1999; Li et al., 2000; Skovgaard et al., 2000) and thus these effects should be explored for *P. shiwhaense*.

Water temperatures and salinities in estuaries and coastal marine waters substantially vary. Temperature is an important factor affecting the survival and growth rates of mixotrophic dinoflagellates (Watras et al., 1982; Morton et al., 1992; Navarro et al., 2006; Matsubara et al., 2007). The lower and/or upper limits of temperature and optimal temperature for the growth of mixotrophic dinoflagellates vary (Aelion and Chisholm, 1985; Montagnes et al., 2003; Scalco et al., 2012; Aquino-Cruz et al., 2018). The salinity of coastal and estuarine waters fluctuates widely, which can also substantially affect the growth rates of mixotrophic dinoflagellates (Grzebyk et al., 2003; Magana and Villareal, 2006; Maier Brown et al., 2006; Guerrini et al., 2007). Thus, the effects of both temperature and salinity on growth and ingestion rates of *P. shiwhaense* should be investigated.

Recently, *P. shiwhaense* was found to have high contents of eicosapentaenoic acid (EHA) and docosahexaenoic acid (DHA), two major components of omega-3 (Jang et al., 2017). For commercial uses, finding the optimal light intensity, temperature, and salinity supporting its maximum growth rate is important for large-scale cultivation of *P. shiwhaense*.

In the present study, the growth and ingestion rates of *P. shiwhaense* on the optimal prey *A. carterae* were measured under various light intensities (0–500 µE m^{-2}s^{-1}), water temperatures (5–30 °C), and salinities (5–40). Specifically, it was explored whether *P. shiwhaense* can feed and grow in darkness with added prey; the lower and upper limits of temperature and salinity for survival; and whether the growth and/or ingestion rates of *P. shiwhaense* are affected by light intensity, temperature, or salinity when the lower and upper limits are excluded. The results provide a basis for understanding the ecophysiology and population dynamics of the newly discovered mixotrophic dinoflagellate *P. shiwhaense* in marine ecosystems.

2. Materials and methods

2.1. Collection and culturing of *Paragymnodinium shiwhaense*

Plankton samples were taken from the surface waters of Shiwha Bay, Korea, during May 2006 when the water temperature and salinity were 18.8 °C and 30.4, respectively (Kang et al., 2010). The samples were gently screened through a 154-µm Nitex mesh and placed in 6-well tissue culture plates. A clonal culture of *P. shiwhaense* PSSW0605 (carbon content per cell = 0.13 ng C) was established by two serial single cell isolations. The clones were unialgal but not axenic. The mixotrophic dinoflagellate *Amphidinium carterae* (5,000–8,000 cells mL^{-1}) was provided as prey. As the concentration of *P. shiwhaense* increased, the predators were subsequently transferred to 32-, 270-, and 500-mL polycarbonate (PC) bottles containing fresh *A. carterae*. The bottles were again filled to capacity with freshly filtered seawater, capped, and placed on a rotating wheel at 0.9 rpm at 20 °C under a light intensity of 20 µE m^{-2} s^{-1} from cool white fluorescent lights on a 14:10 h light–dark cycle. Once dense cultures of *P. shiwhaense* (ca 5000 cells mL^{-1}) were obtained, they were transferred to 500-mL PC bottles with fresh prey (ca 20,000 cells mL^{-1}) daily.

2.2. Light effects

Expt 1 was conducted to investigate the effects of light intensity on the growth and ingestion rates of *P. shiwhaense* on *A. carterae* (carbon content per cell = 0.07 ng C), at a single prey concentration at which the growth and ingestion rates of *P. shiwhaense* on *A. carterae* were saturated, according to the results of Yoo et al. (2010) (Table 1). This

Table 1
Design of experiments. The numbers in prey (*Amphidinium carterae*) and predator (*Paragymnodinium shiwhaense*) columns are the actual initial concentrations (cells mL^{-1}) of prey and predator. LI: Light intensity (µE m^{-2}s^{-1}). T: temperature (°C). S: Salinity.

Expt No.	Variable	Prey Concentration	Predator Concentration
1 (light)	0, 10, 20, 50, 100, 200, 500	31800, 33280, 23670, 30890, 30750, 29480, 29680	690,770, 760, 760, 790, 840, 950
2 (temperature)	5, 10, 15, 20, 25, 30	27310, 23530, 28390, 39940, 39400, 28,340	1010, 910, 820, 790, 1080, 890
3 (temperature)	5, 6, 8, 10	29730, 25580, 22210, 26,320	1020, 790, 650, 900
4 (temperature)	25, 26, 27, 28, 29	27280, 29920, 27990, 27310, 26110	620, 1260,1360, 1510, 1300
5 (salinity)	5, 10, 15, 20, 25, 30, 35, 40	22800, 25810, 24370, 20890, 21000, 22240, 24190, 28660	510, 660, 690, 620, 640, 610, 670, 730