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Mixotrophy in the marine red-tide cryptophyte *Teleaulax amphioxeia* and ingestion and grazing impact of cryptophytes on natural populations of bacteria in Korean coastal waters

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ABSTRACT

Cryptophytes are ubiquitous and one of the major phototrophic components in marine plankton communities. They often cause red tides in the waters of many countries. Understanding the bloom dynamics of cryptophytes is, therefore, of great importance. A critical step in this understanding is unveiling their trophic modes. Prior to this study, several freshwater cryptophyte species and marine *Cryptomonas* sp. and *Geminifera cryophila* were revealed to be mixotrophic. The trophic mode of the common marine cryptophyte species, *Teleaulax amphioxeia* has not been investigated yet. Thus, to explore the mixotrophic ability of *T. amphioxeia* by assessing the types of prey species that this species is able to feed on, the protoplasms of *T. amphioxeia* cells were carefully examined under an epifluorescence microscope and a transmission electron microscope after adding each of the diverse prey species. Furthermore, *T. amphioxeia* ingestion rates heterotrophic bacteria and the cyanobacterium *Synechococcus* sp. were measured as a function of prey concentration. Moreover, the feeding of natural populations of cryptophytes on natural populations of heterotrophic bacteria was assessed in Masan Bay in April 2006. This study reported for the first time, to our knowledge, that *T. amphioxeia* is a mixotrophic species. Among the prey organisms offered, *T. amphioxeia* fed only on heterotrophic bacteria and *Synechococcus* sp. The ingestion rates of *T. amphioxeia* on heterotrophic bacteria or *Synechococcus* sp. rapidly increased with increasing prey concentrations up to 8.6×10^6 cells ml^{-1}, but slowly at higher prey concentrations. The maximum ingestion rates of *T. amphioxeia* on heterotrophic bacteria and *Synechococcus* sp. reached 0.7 and 0.3 cells $predator^{-1} h^{-1}$, respectively. During the field experiments, the ingestion rates and grazing coefficients of cryptophytes on natural populations of heterotrophic bacteria were 0.3–8.3 cells $predator^{-1} h^{-1}$ and 0.012–0.033 d^{-1}, respectively. Marine cryptophytes, including *T. amphioxeia*, are known to be favorite prey species for many mixotrophic and heterotrophic dinoflagellates and ciliates. Cryptophytes, therefore, likely play important roles in marine food webs and may exert a considerable potential grazing impact on the populations of marine bacteria.

1. Introduction

Cryptophytes are one of the major phototrophic components in marine planktonic communities with wide distributions from coastal to oceanic waters in the tropical, temperate, and polar regions (Buma et al., 1992; Moline et al., 2004; Jeong et al., 2013; Johnson et al., 2013; Piwosz et al., 2013; Šupraha et al., 2014; Unrein et al., 2014). Cryptophytes have often caused red tides in the waters of many countries (Andreoli et al., 1986; Dame et al., 2000; Jeong et al., 2013; Kang et al., 2013; Bazin et al., 2014; Šupraha et al., 2014). For example, a year-long, daily monitoring of the Masan Bay, Korea (June 2004–May 2005), revealed that cryptophytes caused red tides 19 times with a maximum abundance (biomass) of approximately 392,000 cells ml^{-1} (equivalent to ~6680 ng C ml^{-1}) (Jeong et al., 2013). This maximum biomass of cryptophytes was greater than any other phytoplankton groups, except raphidophytes (Jeong et al., 2013), confirming cryptophytes as one of the

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Table 1
Taxon, size, and concentration of prey species offered as food to *Teleaulax amphioxeia* in Experiment 1. To confirm no ingestion by the predators on some prey species, additional higher prey concentrations were additionally provided.

Species	ESD (±SD)	Initial concentration (cells ml^{-1})	Feeding by *T. amphioxeia*
Bacteria			
Heterotrophic bacteria	0.9 (0.3)	5,500,000	Y
Synechococcus sp.CC9311	1.0 (0.2)	5,500,000	Y
Prasinophyceae			
Tetraselmis sp.	5.9 (1.9)	100,000	N
Prymnesiophyceae			
Isochrysis galbana	6.2 (1.3)	100,000	N
Cryptophyceae			
Rhodomonas salina	8.1 (2.1)	30,000	N
Raphidophyceae			
Heterosigma akashiwo	12.6 (2.3)	15,000	N
Dinophyceae			
Heterocapsa rotundata	7.4 (1.0)	30,000	N
Amphidinium operaculatum	7.6 (2.6)	30,000	N
Symbiodinium voratum	10.1 (1.7)	15,000	N
Prorocentrum concavum	19.5 (7.6)	10,000	N

Y – *T. amphioxeia* was observed to feed on prey cell; N – *T. amphioxeia* was observed not to feed on prey cell. Mean equivalent spherical diameter (ESD ± SD, μm) for algae and heterotrophic bacteria were measured by a coulter particle counter (Coulter counter Z2; Beckman Coulter, Fullerton, CA, USA) and under an epifluorescence microscope ($n > 1000$ for each algal species and $n > 30$ for bacteria). The ESD for *Synechococcus* was obtained from Apple et al. (2011). The initial abundance of *T. amphioxeia* for each target prey was 50,000 cells ml^{-1}.

major red-tide causative groups in Masan Bay (2004–2005). The high abundance of red tide organisms has often caused large-scale mortality of shellfish by hypoxia (Paerl et al., 2001; Paerl and Justic, 2011). Red tides that are dominated by cryptophytes are, therefore, a serious concern to scientists and those involved in aquaculture industries. A critical step in predicting aspects of cryptophyte-dominated red tides is understanding the mechanisms of the outbreak, persistence, and decline of red tides (e.g., Jeong et al., 2015). The unveiling of the trophic modes of cryptophytes is of primary importance because these modes determine how their growth materials are acquired. Exclusively autotrophic organisms acquire growth materials via photosynthesis, whereas mixotrophic organisms acquire growth materials through both photosynthesis and feeding (Jeong et al., 2010c; Stoecker et al., 2017). It is, therefore, vital to understand if red tide cryptophytes are mixotrophic in nature to understand cryptophyte eco-physiology and red tide dynamics.

Several freshwater cryptophyte species such as *Cryptomonas erosa, C. marsonii*, and *C. ovata* were revealed to be mixotrophic organisms (Sinistro et al., 2006; Izaguirre et al., 2012). In case of marine cryptophytes, however, two species has been revealed to be mixotrophic (Epstein and Shiaris, 1992; Gast et al., 2014); the marine cryptophytes *Cryptomonas* sp. which fed on fluorescently labeled bacteria (FLBs) and *Geminigera cryophila* was able to ingest microspheres. Recently, Unrein et al. (2014) showed that marine cryptophyte populations fed on FLBs, however, taxonomic identification of the bacterivorous cryptophytes or the consumed bacterial species was not documented. Moreover, whether these cryptophytes are able to feed on any other prey items such as cyanobacteria or micro-algae were not investigated. Determination of the presence or absence of a mixotrophic ability and in turn, the kind of prey that marine cryptophyte species consume are important in understanding certain evolutionary processes among photosynthetic organisms, i.e., their formation by secondary endosymbiosis and the link between red algae and dinoflagellates (Douglas and Penny, 1999; Petersen et al., 2006).

The cryptophyte *Teleaulax amphioxeia* is one of the most well-known marine species and has been observed in the coastal waters of many countries (Seppauml;lä and Balode, 1999; Yih et al., 2004; Cloern and Dufford, 2005; Novarino, 2005; Peter and Sommer, 2012; Johnson et al., 2016). This cryptophyte sometimes causes red tides or dense blooms (e.g., Johnson et al., 2013). Moreover, *T. amphioxeia* (indicated as a unidentified cryptophyte with an ESD of 5.6 μm in some papers) is empirically known to be a preferred prey species that supports positive growth of many protistan grazers including many mixotrophic dinoflagellates such as *Alexandrium andersoni* (Lee et al., 2016), *Cochlodinium polykrikoides* (Jeong et al., 2004), *Gonyaulax polygramma* (Jeong et al., 2005b), *Gymnodinium aureolum* (Jeong et al., 2010b), *Karlodinium armiger* (Berge et al., 2008), *Paragymnodinium shiwhaense* (Yoo et al., 2010), *Prorocentrum minimum* (Johnson, 2015), and *Woloszynskia cincta* (Kang et al., 2011), the heterotrophic dinoflagellates such as *Aduncodinium glandula* (Jang et al., 2016), *Gyrodiniellum shiwhaensis* (Jeong et al., 2011), *Luciella masanensis* (Jeong et al., 2007), and *Pfiesteria piscicida* (Jeong et al., 2006), and the mixotrophic ciliate *Mesodinium rubrum* (Yih et al., 2004). In particular, *T. amphioxeia* has drawn much attention because this species is a donor of plastids to *M. rubrum* and in turn to the dinoflagellate *Dinophysis* spp. (e.g., Kim et al., 2015). Moreover, the occurrence of *T. amphioxeia* has often increased the abundance of its grazers in natural environments (Hansen and Fenchel, 2006; Jeong et al., 2013; Yih et al., 2013). *T. amphioxeia* may, therefore, play important roles in marine food webs. Most previous studies on *T. amphioxeia*, however, assumed that this species was exclusively autotrophic (Berge et al., 2010; Kim et al., 2015; Peter and Sommer, 2015).

In the present study, using the cryptophyte strain of *T. amphioxeia* which has previously been used in many feeding experiments (e.g., Yih et al., 2004; Park et al., 2007; Myung et al., 2011), its mixotrophic ability and the kind of prey that it is able to feed on were investigated. In addition, the ingestion rates of *T. amphioxeia* on heterotrophic bacteria and cyanobacteria *Synechococcus* sp. as a function of prey concentration were measured in the laboratory. The maximum ingestion rates of *T. amphioxeia* on heterotrophic bacteria and/or *Synechococcus* sp. were compared to those of freshwater cryptophytes and other mixotrophic red tide organisms as reported in the literature. Furthermore, the ingestion rates and grazing coefficients of the natural populations of cryptophytes on the natural populations of heterotrophic bacteria