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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

in Masan Bay were investigated. The aim of this study was to investigate the interactions between *T. amphioxeia* and co-occurring bacterioplankton to better understand the population dynamics of *T. amphioxeia* during the development stages of cryptophyte-caused red tides, as well as the evolutionary processes in marine photosynthetic organisms.

The results of the present study may provide a basis on better understanding the interactions between *T. amphioxeia* and co-occurring bacterioplankton and the population dynamics of *T. amphioxeia* during the development stages of its red tides.

2. Materials and methods

2.1. Preparation of experimental organisms

The cyanobacterium *Synechococcus* sp. strain CC9311 (clade I) and phytoplankton species were grown at 20 °C in enriched f/2-Si seawater medium (Guillard and Ryther, 1962) under continuous illumination of 20 μE m^{-2} s^{-1} of cool white fluorescent light in the walk-in incubator system of the MarineBio Research and Education Center, Kunsan National University (Table 1). The *Synechococcus* strain has two phycoerythrin proteins (i.e., PE I and PE II) (Ong and Glazer, 1991). The mean equivalent spherical diameter (ESD ± SD) and cell volume of the phytoplankton were measured using a particle counter (Coulter counter Z2, Beckman Coulter, Fullerton, CA, USA). The carbon content for each phytoplankton species was estimated from its cell volume according to Menden-Deuer and Lessard (2000). The cell volume and carbon content for *Synechococcus* sp. strain CC9311 was obtained from Apple et al. (2011).

For isolation and cultivation of the cryptophyte *T. amphioxeia* CR-MAL01, water samples were collected from Gomso Bay, Korea, during February 2002 when the water temperature and salinity were 7.8 °C and 30.1, respectively. A culture of *T. amphioxeia* was established by serial single-cell isolation (Yih et al., 2004). *T. amphioxeia* was maintained at 20 °C in enriched f/2-Si seawater medium (Guillard and Ryther, 1962) under continuous illumination of 20 μE m^{-2} s^{-1}. The ESD and carbon content per cell of *T. amphioxeia* were 5.6 μm and 0.017 ng C, respectively.

2.2. Feeding occurrence

Experiment 1 was designed to investigate whether *T. amphioxeia* was able to feed on prey cells (i.e. heterotrophic bacteria, *Synechococcus* sp., and unialgal species) when these were provided (Table 1). The initial abundance provided of each prey species was similar in terms of carbon biomass. To confirm that a prey species was not ingested by *T. amphioxeia*, higher concentrations of the prey species were additionally offered.

A dense culture of phototrophically growing *T. amphioxeia* was transferred to one 2-l polycarbonate bottle containing f/2-Si medium and maintained for 2 days. Three 1-ml aliquots were then removed from the bottle and *T. amphioxeia* densities were determined with Sedgwick-Rafter chambers under a light microscope.

To explore the feeding of *T. amphioxeia* on heterotrophic bacterial prey, the bacterial cells (that originated from a non-axenic culture of *T. amphioxeia*) were fluorescently labeled one day prior to the feeding experiment, as per Sherr et al. (1987). To remove any aggregate of FLB, the FLBs were dispersed throughout the medium using a sonicator for 10–30 s and then filtered through a 3-μm filter.

The FLBs and heterotrophic bacteria were added to each of the six 80-ml polycarbonate bottles (final concentration = ~5.5×10^6 bacterial cells ml^{-1} and ~50,000 *T. amphioxeia* cells ml^{-1}). Triplicate 80-ml polycarbonate experimental bottles (containing mixtures of *T. amphioxeia* and added heterotrophic bacteria plus FLB) and triplicate predator control bottles (containing *T. amphioxeia* only) were established. The bottles were filled to capacity with freshly filtered seawater, capped, placed on shelves and incubated at 20 °C under illumination of 20 μE m^{-2} s^{-1} of cool white fluorescent light. After 5, 30, and 60 min and 6 h, a 3-ml aliquot was removed from each bottle and fixed with formalin (final concentration = 3%). The fixed samples were stained with 4′, 6-diamidino-2-phenylindole (DAPI, final concentration = 1 μM) and filtered with 3-μm polycarbonate white membrane filters. Ingested FLB inside *T. amphioxeia* cells were observed under an epifluorescence microscope (Olympus BX50, Olympus Co., Tokyo, Japan) with UV, blue, and green-light excitation at a magnification of 1000× (Porter and Feig, 1980). *T. amphioxeia* cells containing ingested FLB cells were photographed using a digital camera.

When determining if *Synechococcus* sp. was ingested as prey, the initial concentrations of *T. amphioxeia* (ca. 50,000 cells ml^{-1}) and *Synechococcus* sp. (ca. 5.5×10^6 cells ml^{-1}) were established using an autopipette to deliver a predetermined volume of culture with a known cell density to the experimental bottles. Triplicate 80-ml polycarbonate experimental bottles (containing mixtures of *T. amphioxeia* and *Synechococcus* sp.) and triplicate predator control bottles (containing *T. amphioxeia* only) were set up at a single prey concentration for the predator. During incubation, a 3-ml aliquot was subsampled from each bottle and fixed with formalin at 5, 30, and 60 min and 6 h. The fixed aliquots were filtered onto 3-μm polycarbonate white membrane filters, and the concentrated cells on the membranes were then examined under the epifluorescence microscope with UV, blue, and green-light excitation at a magnification of 1000× to determine whether or not the predator was able to feed on *Synechococcus* sp. *T. amphioxeia* cells containing ingested *Synechococcus* sp. cells were photographed using the digital camera on the epifluorescence microscope with UV, blue and green-light excitation at a magnification of 1000×.

To best observe the ingestion of target algal prey under a light microscope and/or an epifluorescence microscope, the initial concentrations of *T. amphioxeia* (~50,000 cells ml^{-1}) and each target algal species (~10,000–100,000 cells ml^{-1}) were established as described above (Table 1). Triplicate 80-ml polycarbonate experimental bottles and duplicate predator control bottles were set up for each target algal species following the methods described above. After 6, 24, and 48 h incubation, a 3-ml aliquot was removed from each bottle and transferred into scintillation

Table 2
Water temperature (T) and salinity (S) of the collected waters, the size of cryptophytes, volume of fluorescent labeled bacteria (FLB), and abundance of the natural population of heterotrophic bacteria (HB) in Masan Bay.

Date	T (°C)	S	Cryptophytes			Volume of FLBs (μm^3)	HB (cells ml^{-1})	FLB (cells ml^{-1})	Abundance ratio (%)
			Length (μm)	Width (μm)	Volume (μm^3)				
8-Apr-06	13.6	29.5	11.1 ± 0.69	5.4 ± 0.29	2620 ± 432	0.32 ± 0.03	1.7×10^7	6.5×10^5	3.9
23-Apr-06	14.2	27.5	7.2 ± 0.25	5.4 ± 0.21	1094 ± 94	0.32 ± 0.03	8.7×10^6	3.1×10^5	3.5
24-Apr-06	14.4	25	7.2 ± 0.26	5.3 ± 0.19	1062 ± 93	0.32 ± 0.03	1.1×10^7	3.4×10^5	3.2

Abundance ratio, actual initial abundance of FLBs to the abundance of HB (%).
Values are presented as mean ± standard error.

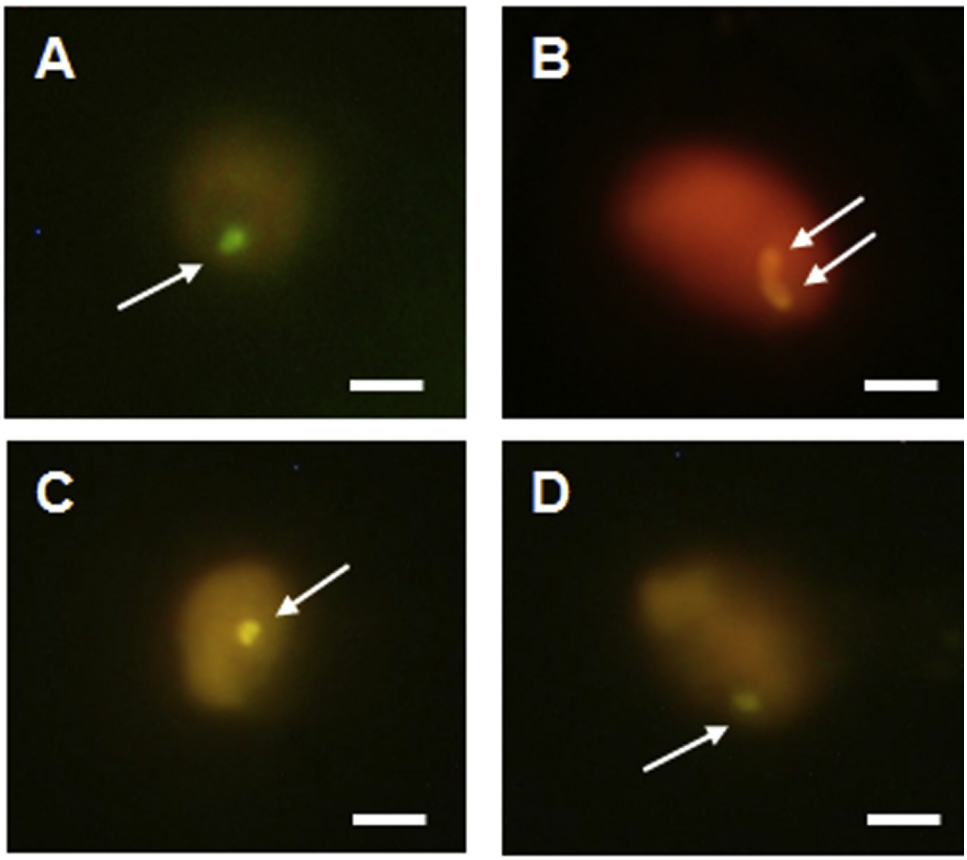

Fig. 1. Epifluorescence micrographs of the photosynthetic cryptophyte *Teleaulax amphioxeia* on fluorescently-labeled bacteria (FLB) and *Synechococcus* sp. (A) *T. amphioxeia* cell with a single ingested FLB with blue light excitation. (B) A *T. amphioxeia* cell with two ingested FLBs with green light excitation. (C) *T. amphioxeia* cell with a single ingested *Synechococcus* sp. with green light excitation. (D) *T. amphioxeia* cell with a single ingested *Synechococcus* sp. with blue light excitation. Arrows indicate ingested prey cells. Scale bars = 5μm.

vials. Aliquots (0.2 ml) were placed on slides with cover-glasses. Under these conditions, the *T. amphioxeia* cells were alive, but almost motionless. The protoplasms of >100 *T. amphioxeia* cells were carefully examined using a light microscope or an epifluorescence microscope at a magnification of 100–1000× to determine whether *T. amphioxeia* was able to feed on the target algal prey species.

For transmission electron microscopy (TEM) observation of *T. amphioxeia* feeding on *Synechococcus* sp., the initial concentrations of *T. amphioxeia* (~50,000 cells ml^{-1}) and *Synechococcus* sp. (~2.0×10^6 cells ml^{-1}) were established using an autopipette. One 250-ml experimental flask (mixtures *T. amphioxeia* and *Synechococcus* sp.), one predator control flask (containing only *T. amphioxeia*), and one prey control flask (containing only *Synechococcus*) were set up. The flasks were placed on shelves and incubated at 20 °C under continuous illumination of 20 $\mu E\, m^{-2}\, s^{-1}$. At the beginning and after 2-day incubation periods, the contents of one experimental bottle were distributed into five 50-ml centrifuge tubes and then concentrated at 1610 g for 10 min using a centrifuge (Vision Centrifuge VS-5500, Vision Scientific Co., Bucheon, Korea). Five pellets from the five centrifuge tubes were then transferred into 1.5-ml tubes and fixed for 1 h in 4% (w/v) glutaraldehyde in a culture medium. Afterward, the fixative was removed and the pellets were rinsed using a 0.2 M cacodylic acid/sodium salt solution (pH 7.4). The pellet was then embedded in 1% nutrient agar (NA) (w/v). After several rinses with the medium, the cells were post-fixed in 1% (v/v) osmium tetroxide in deionized water. Dehydration was accomplished using a graded ethanol series (50%, 60%, 70%, 80%, 90%, and 100% ethanol, followed by two 100% ethanol steps). The material was embedded in Spurr's low-viscosity resin (Spurr, 1969). Sections were obtained with a RMC MT-XL ultramicrotome (Boeckeler Instruments Inc., Tucson, AZ, USA) and post-stained with 3% (w/v) aqueous uranyl acetate followed by lead citrate. The stained sections were viewed with a JEOL-1010 electron microscope (JEOL Ltd., Tokyo, Japan).

2.3. Ingestion rate of T. amphioxeia on heterotrophic bacteria as a function of the prey concentration measured in the laboratory

Experiment 2 was designed to measure the ingestion rate of *T. amphioxeia* as a function of the prey concentration when fed on heterotrophic bacteria in the laboratory.

Two days prior to Experiment 2, the heterotrophic bacterial cells from non-axenic culture of *T. amphioxeia* were filtered onto 1.2-μm pore size filter paper and incubated on plate medium for 5 days in the NA broth (Andersen et al., 1974). FLBs were prepared with DTAF as per Sherr et al. (1987) as described above. The length and width of over 30 FLB cells under an epifluorescence microscope were also measured. Carbon contents of heterotrophic bacteria were estimated from cell volumes by using the following formula: pg C $cell^{-1} = 0.12 \times V^{0.7}$ (Simon and Azam, 1989; Norland, 1993), where V is the cell volume (μm^3) of the heterotrophic bacteria.

For these experiments, dense cultures of *T. amphioxeia* (300,000 cells ml^{-1}) and FLBs (9.9×10^8 cells ml^{-1}) were used. Three 1 ml aliquots were subsampled from each *T. amphioxeia* culture for the cell counting under a light microscope (Olympus BX50, Olympus Co., Tokyo, Japan). For the FLB cell counting, 5-ml aliquots from

each FLB culture were removed and then fixed with formalin (final concentration = 4%). The fixed sample was stained using DAPI (final concentration = 1 μM) and then filtered onto 25-mm polycarbonate black membrane filters of 0.2 μm-pore-size. The FLB cells on the membranes were observed under an epifluorescence microscope as per the methodology described above.

The initial concentrations of *T. amphioxeia* (10,000 cells ml^{-1}) and heterotrophic bacteria plus FLB (5.4×10^3–1.5×10^7 cells ml^{-1}) were established using a pipette to deliver predetermined volumes of known cell concentrations to the bottles. For each prey concentration triplicate 80-ml polycarbonate experimental bottles (containing mixtures of *T. amphioxeia* and added heterotrophic bacteria plus FLB), triplicate prey control bottles (containing added heterotrophic bacteria plus FLB only), and triplicate predator control bottles (containing *T. amphioxeia* only) were established. All the culture bottles were placed on shelves and incubated under the conditions described above.

After 1, 10, 20, and 30 min incubation, 5-ml aliquots were removed from each bottle, transferred to 20-ml scintillation vials, and then fixed with formalin (final concentration = 5%). The fixed samples were stained using DAPI and then filtered onto 3 μm polycarbonate white membrane filters. Then, *T. amphioxeia* cells with FLBs and FLBs inside a *T. amphioxeia* were enumerated under an epifluorescence microscope with UV, blue, and green-light excitation at a magnification of 1000× by scanning the *T. amphioxeia* body at consecutive intervals of 1–2 μm focal depth along the z-axis. Furthermore, at the beginning of the experiment, a 1-ml fixed aliquot was stained with DAPI and then filtered onto 0.2-μm polycarbonate black membrane filters. Heterotrophic bacteria (both FLBs and non-FLBs) outside the *T. amphioxeia* cells were also enumerated under an epifluorescence microscope with UV light excitation for non-FLBs, and blue light excitation for FLBs.

The ingestion rate of a predator on FLBs (cells $predator^{-1}\,h^{-1}$) was calculated by linear regression of the number of FLBs per predator cell as a function of incubation time as described by Sherr et al. (1987). Furthermore, the ingestion rate of the grazer on heterotrophic bacteria including FLBs was calculated by multiplying the ingestion rate of the predator on FLBs by the ratio of total abundance of FLBs plus non-FLB bacteria relative to that of FLBs.

The clearance rates were calculated as follows:

$$CR = IR/PC, \quad (1)$$

where IR (cells $predator^{-1}\,h^{-1}$) is the ingestion rate of cryptophyte on heterotrophic bacteria and PC (cells ml^{-1}) is the heterotrophic bacteria prey concentration.

All ingestion data (IR, cells $predator^{-1}\,h^{-1}$) were fitted to a Michaelis-Menten equation:

$$IR = \frac{MIR\,(x)}{K_{IR} + (x)}, \quad (2)$$

where MIR = the maximum ingestion rate (cells $predator^{-1}\,h^{-1}$); x = PC (cells ml^{-1}), and K_{IR} = the prey concentration sustaining 1/2 I_{max}.

2.4. Ingestion rate of T. amphioxeia on Synechococcus sp. as a function of the prey concentration measured in the laboratory

Experiment 3 was designed to measure the ingestion rate of *T. amphioxeia* as a function of the prey concentration when fed on *Synechococcus* sp.

For these experiments, dense cultures of *T. amphioxeia* (300,000 cells ml^{-1}) and *Synechococcus* sp. (5.5×10^7 cells ml^{-1}) were used. Three 1 ml aliquots were subsampled from each *T. amphioxeia* culture for the cell counting under a light microscope. For *Synechococcus* sp. cell counting, 5-ml aliquots from each *Synechococcus* sp. culture were removed and then fixed with formalin (final concentration = 4%). The fixed sample was stained using DAPI (final concentration = 1 μM) and then filtered onto 25-mm polycarbonate black membrane filters of 0.2 μm-pore-size. The *Synechococcus* sp. cells on the membranes were observed under an epifluorescence microscope as per the methodology described above.

The initial concentrations of *T. amphioxeia* (10,000 cells ml^{-1}) and *Synechococcus* sp. (5.4×10^3–1.5×10^7 cells ml^{-1}) were established using a pipette to deliver predetermined volumes of known cell concentration to the bottles. For each prey concentration triplicate 80-ml polycarbonate experimental bottles (containing mixtures of *T. amphioxeia* and *Synechococcus* sp.), triplicate prey control bottles (*Synechococcus* sp. only), and triplicate predator control bottles (containing *T. amphioxeia* only) were established. All the culture bottles were placed on shelves and incubated as per the methodology described above.

After 1, 10, 20, and 30 min incubation, 5-ml aliquots were removed from each bottle, and then fixed with formalin. The fixed samples were stained using DAPI and then filtered onto 3 μm-pore-sized polycarbonate white membrane filters. Then, *T. amphioxeia* cells with *Synechococcus* cells and *Synechococcus* sp. inside a *T. amphioxeia* were enumerated under an epifluorescence microscope with UV, blue, and green-light excitation at a magnification of 1000× by scanning the *T. amphioxeia* body at consecutive intervals of 1–2 μm focal depth along the z-axis.

Each value of the ingestion and clearance rates was calculated, as described above.

2.5. Ingestion rates of cryptophytes on the natural populations of heterotrophic bacteria in Masan Bay

Experiment 4 was designed to measure the ingestion rates and grazing impacts of cryptophytes co-occurring natural populations of marine heterotrophic bacteria in Masan Bay, Korea. Water samples were taken from the surface in April 2006 by using water samplers. Water temperature and salinity of the surface waters were measured using YSI 30 (YSI, Inc. Baton Rouge, LA, USA) (Table 2).

To determine the abundance of cryptophytes, aliquots of the water samples were poured into 500-ml polyethylene (PE) bottles and preserved with acidic Lugol's solution. The preserved samples were concentrated by 1/5–1/10 of the original volumes following the settling and siphoning method (Welch, 1948). After through mixing, all or >200 cells for cryptophytes in 1-ml Sedgwick-Rafter counting chamber were counted under a light microscope (Olympus BX50, Olympus Co., Japan).

In order to determine the abundance of marine heterotrophic bacteria, aliquots of the water samples were poured into 100-ml PE bottle and preserved with glutaraldehyde (final concentration = 1% v/v). Three to twelve 1-ml aliquots of the fixed samples were stained with DAPI (final concentration = 1 μM) and then filtered through 0.2-μm nucleopore polycarbonate black membrane filters (Whatman, GE Co., Pittsburgh, PA, USA). Marine heterotrophic bacteria were enumerated under an epifluorescence microscope with UV light excitation.

The samples for feeding experiments were screened gently through a 90-μm Nitex mesh and placed in 250-ml polycarbonate bottles. Two to three days before experiment 1, the marine heterotrophic bacteria cells that were collected from the same site were fluorescently labeled (FLBs) using the method as per Sherr et al. (1987). To remove any aggregated FLBs, the FLBs were dispersed throughout the medium using a sonicator (Cleaner 5510E-DTH; Bransoic Ultrasonics, Danbury, CT, USA) for 30 s and then filtered through a 3-μm filter (Whatman, GE Co., Pittsburgh, PA, USA). These experiments revealed that the FLBs were mostly

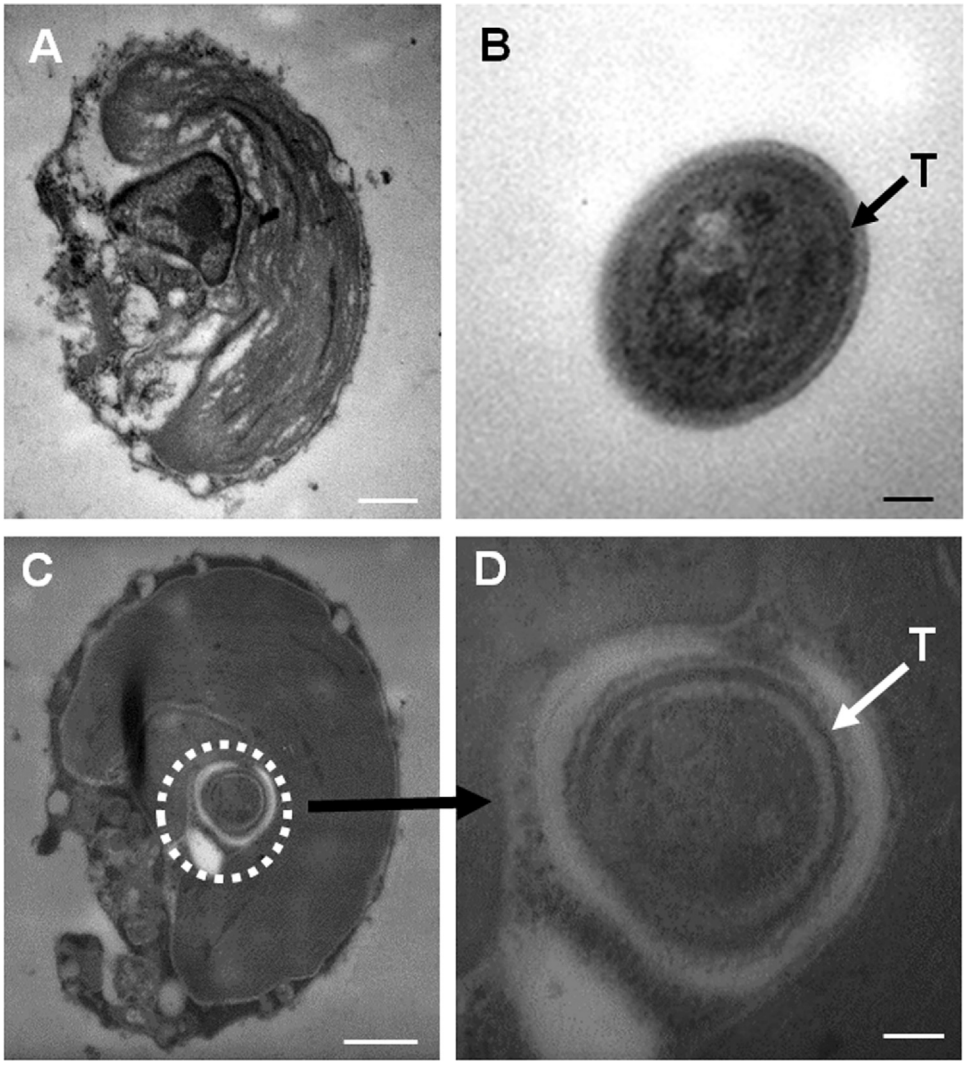

Fig. 2. Transmission electron micrographs (TEM) of *Teleaulax amphioxeia* on *Synechococcus* sp. (A) Unfed *T. amphioxeia* cell. (B) Un-ingested *Synechococcus* sp. cell with thylakoid layer (T). (C) *T. amphioxeia* cell with a single ingested *Synechococcus* cell (white arrow inside the circle). (D) Enlarged from (C). Scale bars = 0.5 μm for (A, C) and 0.1 μm for (B, D).

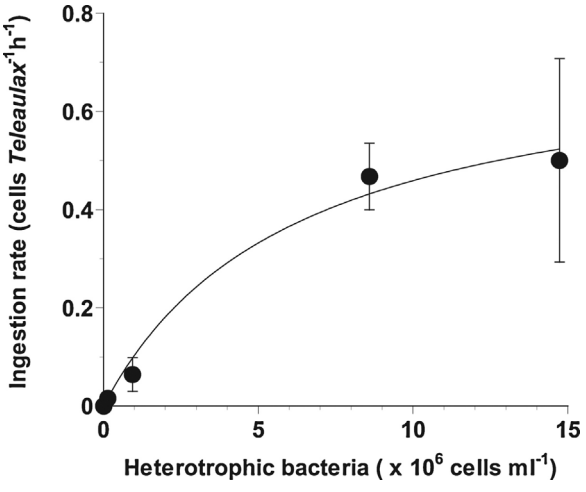

Fig. 3. Ingestion rates (IR) of *Teleaulax amphioxeia* on fluorescently labeled bacteria as a function of mean prey concentration (x). Symbols represent treatment means ± 1 standard error. The curves are fitted to a Michaelis-Menten equation [Eq. (2)] using all treatments in the experiment. IR (cells predator^{-1} h^{-1}) = 0.74 [x/(6.2 × 10^6 + x)], r^2 = 0.741.

rods (a cylinder) and rarely spherical. The length and the width of more than 35 FLB cells for each experiment were measured under an epifluorescence microscope, as per Lee and Fuhrman (1987). And, the cell volume was calculated according to the following equation: volume = [π (3*L* − *W*)/3 × (*W*/2)^2] for a rod (cylinder) and 4/3 × (π *R*^{3}) for a sphere, where *L* is length, *W* is width, and *R* is radius, as described by Lee (1993). The size of fluorescent beads (0.47 μm, size data supplied by the manufacturer; Polyscience Inc., Warrington, PA, USA) was also measured to calibrate our results. The ranges of the length and width of FLB (±standard error [SE]) used in this field study were 1.04 (±0.06) μm, and 0.64 (±0.02) μm, respectively. The mean (±SE) volume of FLB was 0.32 (±0.03) μm^3. The ratios of the actual initial abundance of FLBs relative to that of natural populations of heterotrophic bacteria were 3–4% (Table 2). FLBs were added to triplicate bottles with whole water samples collected from Masan Bay. One control bottle (without FLB) was also set up for each experiment. The bottles were placed on shelves and incubated at a temperature equivalent to that of the water temperature at the sampling site under continuous illumination of 20 μE m^{-2} s^{-1} of cool white fluorescent light. After 1, 10, 20, and 30 min incubation, 10-ml aliquots were removed from each bottle, transferred into 20-ml scintillation vials, and then fixed with borated-buffered formalin (final

Table 3
Maximum ingestion and clearance rates of *Teleaulax amphioxeia* on heterotrophic bacteria and *Synechococus* sp. CC9311.

Prey species	PV	MIR	K_{IR}	MCR
Heterotrophic bacteria	0.3	0.74	6.2×10^6	0.11
Synechococcus sp. CC9311	0.6	0.26	3.9×10^6	0.03

PV, prey volume (μm^3); MIR, maximum ingestion rate (cells predator^{-1} h^{-1}); K_{IR}, the prey concentration sustaining 1/2 MIR (cell ml^{-1}); MCR, maximum clearance rate (nl predator^{-1} h^{-1}).

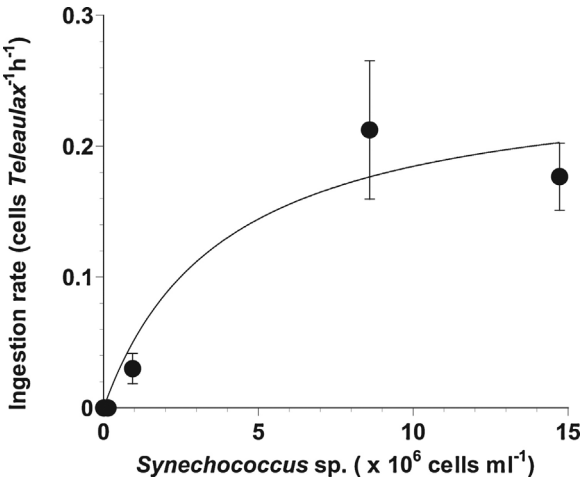

Fig. 4. Ingestion rates (IR) of *Teleaulax amphioxeia* on *Synechococcus* sp. as a function of mean prey concentration (x). Symbols represent treatment means ± 1 standard error. The curves are fitted to a Michaelis-Menten equation [Eq. (2)] using all treatments in the experiment. IR (cells predator^{-1} h^{-1}) = 0.26 [x/(3.9 × 10^6 + x)], $r^2 = 0.827$.

concentration = 5%). The fixed samples were filtered onto polycarbonate black membrane filters of 0.2-μm pore size. Green inclusions (FLB) inside approximately 35 cryptophytes cells on the polycarbonate black membrane filters were enumerated under an epifluorescence microscope with blue light excitation. The ingestion rate of a predator on FLBs (cells predator^{-1} h^{-1}) was calculated as described above. Furthermore, the ingestion rate of the predator on heterotrophic bacteria was also calculated by multiplying the ingestion rate of the predator on FLBs by the ratio of the abundance of natural populations of heterotrophic bacteria relative to that of FLBs.

The grazing coefficient (GC, d^{-1}) was calculated as follows:

$$GC = CR \times CC \times 24, \quad (3)$$

where CR (ml predator^{-1} h^{-1}) is the clearance rate of cryptophyte cell on heterotrophic bacterial prey at a given bacterial concentration and CC is the cryptophyte concentration (cells ml^{-1}). The clearance rates were calculated as described above.

3. Results

3.1. Feeding occurrence

Among the diverse prey items provided, *T. amphioxeia* ingested only heterotrophic bacteria and *Synechococcus* sp. (Figs. 1 and 2). Under epifluorescence microscopy, a single FLB or *Synechococcus* cell was frequently observed inside the protoplasms of *T. amphioxeia* cells (Fig. 1). Thus, these results suggest that *T. amphioxeia* is able to ingest bacteria one by one. Under TEM, ingested *Synechococcus* cells were clearly observed inside the protoplasms of *T. amphioxeia* cells (Fig. 2C and D). However, *T. amphioxeia* did not feed on any algal species such as *Isochrysis galbana*, *Tetraselmis* sp., *Rhodomonas salina*, *Heterosigma akashiwo*, *Heterocapsa rotundata*, *Amphidinium operculatum*, *Symbiodinium voratum*, or *Prorocentrum concavum* (Table 1).

3.2. Ingestion rate of T. amphioxeia on heterotrophic bacteria as a function of the prey concentration measured in the laboratory

When the initial prey concentrations of heterotrophic bacteria were 5.4×10^3–1.5×10^7 cells ml^{-1}, the ingestion rates of *T. amphioxeia* on heterotrophic bacteria increased rapidly with increasing prey concentrations up to 8.6×10^6 cells ml^{-1}, but slowly at higher prey concentrations. (Fig. 3). When the data were fitted to Eqs. (1) and (2), the maximum ingestion and clearance rates of *T. amphioxeia* on heterotrophic bacteria were 0.74 cells predator^{-1} h^{-1} (18 cells predator^{-1} d^{-1}) and 0.11 nl predator^{-1} h^{-1}, respectively (Table 3).

Table 4
The abundances of marine cryptophytes and co-occurring heterotrophic bacteria (NPHB), ingestion rate (IR), clearance rate (CR), and calculated grazing coefficient (GC) by cryptophytes on the populations of heterotrophic bacteria in Masan Bay in April 2006.

Date	Cryptophytes (cells ml^{-1})	NPHB (× 10^6 cells ml^{-1})	IR (cells predator^{-1} h^{-1})	CR (nl predator^{-1} h^{-1})	GC (d^{-1})
8-Apr-06	2633 ± 129	16.8 ± 1.78	8.29 ± 1.17	0.52 ± 0.112	0.033 ± 0.008
23-Apr-06	27,825 ± 1273	8.7 ± 0.53	0.38 ± 0.29	0.04 ± 0.029	0.026 ± 0.018
24-Apr-06	19,305 ± 866	10.7 ± 0.33	0.32 ± 0.32	0.03 ± 0.028	0.012 ± 0.012

Values are presented as mean ± standard error.

Table 5
Maximum ingestion rate of marine and freshwater cryptophyte species on heterotrophic bacteria.

Species name	Habitat	PDV	Prey	MIR	Reference
Teleaulax amphioxeia	Marine	76	FLB.	0.74	This study
Cryptomoans sp.	Marine	NA	FLB	20.8	Epstein and Shiaris (1992)
Cryptomonas erosa	Freshwater	1478	FLB	3.22	Sinistro et al. (2006)
Cryptomonas marsonii	Freshwater	620	FLB	15.38	Sinistro et al. (2006)
Cryptomonas marsonii	Freshwater	620	FLB	15.5	Izaguirre et al. (2012)
Cryptomonas ovata	Freshwater	1983	FLB	NA	Sinistro et al. (2006)

PDV, predator volume (μm^3); FLB, fluorescently labeled bacteria; MIR, maximum ingestion rate (cells predator^{-1} h^{-1}); NA, not available.

Table 6
Maximum ingestion rate of marine protistan grazers on heterotrophic bacteria measured in the laboratory and their carbon acquisition from the heterotrophic bacteria.

Predator species	PDV	CPD	MIR	CA	BC	Reference
Cryptophyte						
Teleaulax amphioxeia	76	17	0.05	1.1	6.5	This study
Dinophyte						
Heterocapsa rotundata	102	20	0.63	15.1	75.5	Seong et al. (2006)
Pfiesteria piscicida	240	39	1.19	28.6	73.3	Jeong et al. (2008)
Oxyrrhis marina	470	72	4.71	113.0	156.9	Jeong et al. (2008)
Gyrodinium cf. *guttula*	660	98	1.53	36.7	37.5	Jeong et al. (2008)
Prorocentrum minimum	927	130	1.47	35.3	27.2	Seong et al. (2006)
Heterocapsa triquetra	1766	220	0.33	7.9	3.6	Seong et al. (2006)
Cochlodinium polykrikoides	9092	930	0.61	14.6	1.6	Seong et al. (2006)
Raphidophyte						
Heterosigma akashiwo	697	110	0.57	13.7	12.5	Seong et al. (2006)
Chattonella ovata	33,493	2870	1.72	41.3	1.4	Seong et al. (2006)
Ciliate						
Mesodinium rubrum	5316	1064	2.76	66.1	6.2	Myung et al. (2006)

PDV, predator volume (μm^3); CPD, carbon content of a predator cell (pg C $cell^{-1}$); MIR, maximum ingestion rate (pg C $predator^{-1}$ h^{-1}); CA, carbon acquired from prey by predator per day (pg C $predator^{-1}$ d^{-1}); BC, acquired carbon as percentage of predator's carbon (%).

3.3. Ingestion rate of T. amphioxeia on Synechococcus sp. as a function of the prey concentration measured in the laboratory

When the initial prey concentration of *Synechococcus* sp. were 5.4×10^3–1.5×10^7 cells ml^{-1}, the ingestion rates of *T. amphioxeia* on *Synechococcus* sp. increased rapidly with increasing prey concentrations up to 8.6×10^6 cells ml^{-1}, but slowly at higher prey concentrations. (Fig. 4). When the data were fitted to Eqs. (1) and (2), the maximum ingestion and clearance rates of *T. amphioxeia* on heterotrophic bacteria were 0.26 cells $predator^{-1}$ h^{-1} (6.2 cells $predator^{-1}$ d^{-1}) and 0.032 nl $predator^{-1}$ h^{-1}, respectively (Table 3).

3.4. Ingestion rates of cryptophytes on the natural populations of heterotrophic bacteria in Masan Bay

The ingestion rates of natural populations of cryptophytes on natural populations of marine heterotrophic bacteria in Masan Bay, Korea in 2006 were measured (Table 4). During all field experiments, the water temperature ranged from 13.6 to 14.4 °C, whereas the salinity ranged from 25.0 to 29.5, respectively (Table 2).

During field experiments, cryptophytes were observed to ingest FLBs. No green inclusion was observed inside the protoplasms of cryptophytes in the control bottle (without FLBs). The mean abundance of cryptophytes and heterotrophic bacteria were 2633–27,825 cells ml^{-1} and 8.7×10^6–1.7×10^7 cells ml^{-1}, respectively (Table 4). The mean ingestion rates of cryptophytes on natural populations of heterotrophic bacteria were 0.3 to 8.3 cells $predator^{-1}$ h^{-1}, whereas their mean clearance rates were 0.03 to 0.52 nl $predator^{-1}$ h^{-1} (Table 4). The calculated grazing coefficients on natural populations of heterotrophic bacteria attributable to cryptophytes were 0.012–0.033 d^{-1} (Table 4).

4. Discussion

4.1. Mixotrophic ability and the kind of prey

The present study clearly shows that the common marine cryptophyte *Teleaulax amphioxeia* is a mixotrophic organism. Previously, a few studies had reported that marine cryptophyte *Cryptomonas* sp., *Geminigera cryophila*, and unidentified marine cryptophytes were able to feed on heterotrophic bacteria in natural environments (Epstein and Shiaris, 1992; Gast et al., 2014; Unrein et al., 2014). The mixotrophic ability of *T. amphioxeia* should be taken into consideration in investigations on population dynamics of this species. In addition to heterotrophic bacteria, *T. amphioxeia* was also able to feed on *Synechococcus* prey. *T. amphioxeia* usually co-occurs with heterotrophic bacteria and cyanobacteria in most marine environments (Cloern and Dufford, 2005; Jeong et al., 2013; Cañvate et al., 2015). Thus, the results of this study suggest that *T. amphioxeia* may play a role as a grazer of heterotrophic bacteria and cyanobacteria in the marine planktonic food webs. Therefore, feeding by *T. amphioxeia* on heterotrophic bacteria and cyanobacteria should be incorporated in the investigations on population dynamics of bacterioplankton. Several cryptophyte species form red tides (e.g., Andreoli et al., 1986; Laza-Martínez, 2012) which can have severe effects on the surrounding environment. It is, therefore, worth exploring the mixotrophic ability of the other species in the genus *Teleaulax* and the species in the other cryptophyte genera.

4.2. Ingestion rates of Teleaulax amphioxeia on bacterial prey

The maximum ingestion rates (MIR) of *T. amphioxeia* on heterotrophic bacteria obtained from this study is considerably lower than that of the freshwater cryptophytes *Cryptomonas erosa, C. marsonii,* and *C. ovata*, and the marine cryptophyte *Cryptomonas* sp. (Table 5). Furthermore, the MIR of *T. amphioxeia* on heterotrophic bacteria is much lower than that of the other marine protistan grazers (Table 6). The size of *T. amphioxeia* is smaller than that of the freshwater cryptophytes and the other marine protistan grazers. Thus, the smallest size of *T. amphioxeia* may be partially responsible for the lowest MIR on heterotrophic bacteria. However, the MIR of the cryptophytes or all marine protistan grazers on heterotrophic bacteria was not significantly correlated with the cell volume of the predators ($p > 0.1$, ANOVA; Fig. 5A and B). Thus, other factors such as taxonomical characterizations and trophic modes may affect the MIR. The ratio of the daily carbon acquisition of *T amphioxeia* from heterotrophic bacteria relative to its body carbon is 6.5% (Table 6). Theoretically, *T. amphioxeia* may divide once when it feeds on heterotrophic bacteria for 31 days even when its growth efficiency is assumed to be 50%. Thus, heterotrophic bacteria may not be a critical growth factor, but a supplementary factor.

The MIR of *T. amphioxeia* on *Synechococcus* sp. is lower than that of any other mixotrophic organism reported so far (Table 7). The

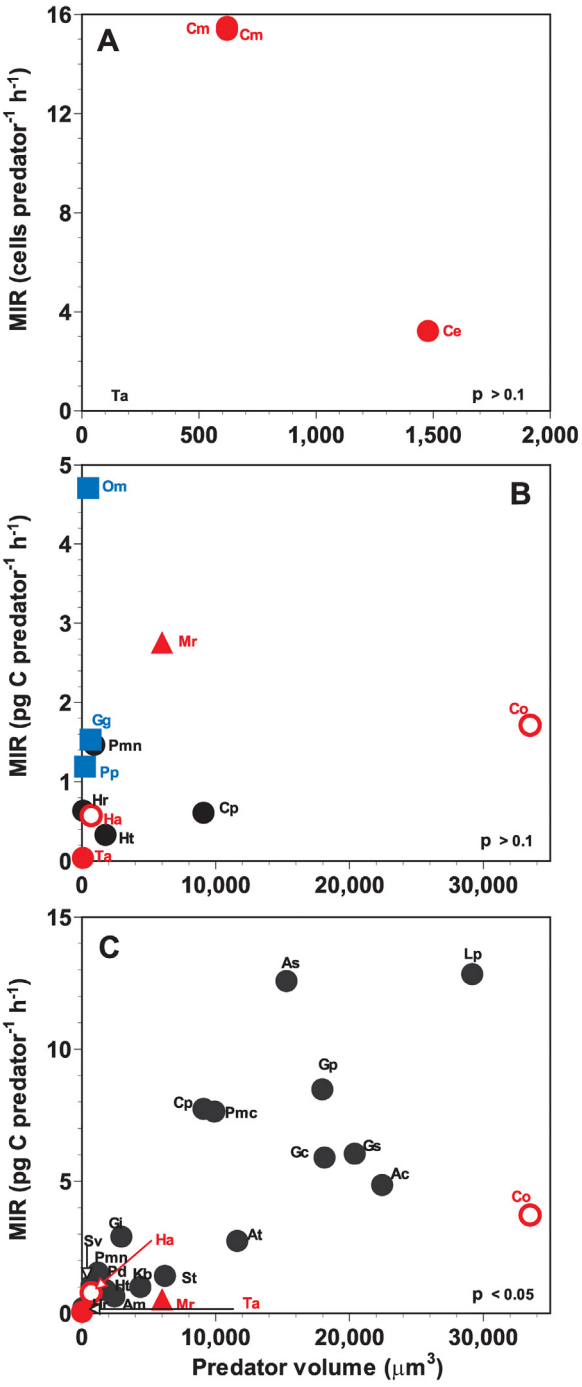

Fig. 5. Maximum ingestion rate (MIR) of protistan grazers on heterotrophic bacteria (A, B) and *Synechococcus* (C) as a function of predator volume (μm^3). (A) MIR of marine and freshwater cryptophytes on heterotrophic bacteria. (B) MIR of mixotrophic and heterotrophic protistan grazers on heterotrophic bacteria. (C) MIR of mixotrophic protistan grazers on *Synechococcus* sp. The p-value in (A) and (B) were $p > 0.1$, but the p-value in (C) was $p < 0.05$ (linear regression ANOVA). As, *Akashiwo sanguinea*; Ac, *Alexandrium catenella*; Am, *A. minutum*; At, *A. tamarense*; Ce, *Cryptomonas erosa*; Cm, *Cryptomons marson*; Co, *Chattonella ovata*; Cp, *Cochlodinium polykrikoides*; Gc, *Gymnodinium catenatum*; Gg, *Gyrodinium* cf. *guttula*; Gi, *G. impudicum*; Gp, *Gonyaulax polygramma*; Gs, *G. spinifera*; Ha, *Heterosigma akashiwo*; Hr, *Heterocapsa rotundata*; Ht, *H. triquetra*; Kb, *Karenia brevis*; Lp, *Lingulodinium polyedrum*; Mr, *Mesodinium rubrum*; Om, *Oxyrrhis marina*; Pd, *Prorocentrum donghaiense*; Pmc, *P. micans*; Pmn, *P. minimum*; Pp, *Pfiesteria piscicida*; St: *Scrippsiella trochoidea*,; Sv, *Symbiodinium voratum*; Ta, *Teleaulax amphioxeia*. Mixotrophic dinoflagellates (black closed circles); heterotrophic dinoflagellates (blue closed squares); raphidophytes (red open circles); cryptophyte (red closed circles); mixotrophic ciliate (red closed triangle).

MIR of mixotrophic organisms feeding on *Synechococcus* sp. is significantly positively correlated with the predator cell volume ($p < 0.05$, ANOVA, Fig. 5C). This relationship suggests that the sizes of the mixotrophic organisms may be an important factor affecting their ingestion rates on *Synechococcus* species. The smallest size of *T. amphioxeia* may be partially responsible for the lowest MIR on *Synechococcus*. The ratio of the daily carbon acquisition of *T. amphioxeia* from *Synechococcus* sp. relative to its body carbon is 7.6% (Table 7). Theoretically *T. amphioxeia* may divide once when it feeds on *Synechococcus* sp. for 26 days even when its growth efficiency is assumed to be 50%. *Synechococcus* sp. may, therefore, also not be a critical growth factor, but a supplementary factor.

4.3. Ingestion rates and grazing impacts of cryptophytes on the natural populations of heterotrophic bacteria

Prior to this study, there have been many field studies providing the ingestion rates and grazing impacts of mixotrophic protists on heterotrophic bacteria (Seong et al., 2006, 2017; Unrein et al., 2014; Millette et al., 2017) and also those of freshwater cryptophytes (Sinistro et al., 2006; Izaguirre et al., 2012). However, there have been few field studies reporting the ingestion rates and/or grazing impact of marine cryptophytes on heterotrophic bacteria (Unrein et al., 2014). To understand the dynamics of marine cryptophytes and bacteria, the ingestion rates of cyptophytes on natural populations of marine heterotrophic bacteria should be measured in other marine environments. The present study addressed this lack of information and has provided valuable data on the ingestion rates of cryptophytes on natural populations of marine heterotrophic bacteria and the calculated grazing impacts.

The mean of the ingestion rates of marine cryptophytes on natural populations of heterotrophic bacteria in Masan Bay on April 8, 2006 (8.3 cells predator^{-1} h^{-1}) was much greater than the ingestion rate of *T. amphioxeia* on heterotrophic bacteria (0.3 cells predator^{-1} h^{-1}), calculated at the same abundance of heterotrophic bacteria using the equation in Fig. 3 with a correction of ingestion rates using $Q_{10} = 2.8$ (Hansen et al., 1997). However, the means of the ingestion rates of marine cryptophytes on natural populations of heterotrophic bacteria April 23 and 24, 2006 (0.3–0.4 cells predator^{-1} h^{-1}) were comparable to the ingestion rate of *T. amphioxeia* on heterotrophic bacteria (0.2–0.3 cells predator^{-1} h^{-1}), calculated at the same abundance of heterotrophic bacteria using the equation in Fig. 3 with a correction of ingestion rates using $Q_{10} = 2.8$ (Hansen et al., 1997). The cell volume of the cryptophytes on April 8, 2006 ($n = 12$) was considerably greater than that of *T. amphioxeia*, whereas the cell volume of the cryptophytes on April 23 and 24, 2006 ($n = 15$ for each day) was similar to that of *T. amphioxeia* (Table 2). Thus, larger sized cryptophyte cells on April 8 may be partially responsible for the ingestion rates being much higher than those of *T. amphioxeia*, and the similar sized cryptophyte cells on April 23 and 24 resulting in similar ingestion rates to those of *T. amphioxeia*. The MIR of marine cryptophytes on natural populations of heterotrophic bacteria in Blanes Bay, western Mediterranean Sea (2.3 cells predator^{-1} h^{-1}; Unrein et al., 2014) falls in the range of the ingestion rates of marine cryptophytes on natural populations of heterotrophic bacteria in Masan Bay in 2006 (Table 8). However, the MIR of *Cryptomons* sp. (20.8 cells predator^{-1} h^{-1}; Epstein and Shiaris, 1992) in Boston Harbor, USA was greater than that in Masan Bay in 2006. This evidence suggests that the range of the ingestion rates of marine cryptophytes is likely to be wide and MIR may be species specific. It is worthwhile to investigate ingestion rates of the other marine cryptophyte species on heterotrophic bacteria.

The MIR of cryptophytes (8.3 cells predator^{-1} h^{-1}) on natural populations of heterotrophic bacteria in Masan Bay in April 2006 measured in this study were comparable to or higher than those of

Table 7
Maximum ingestion rate of mixotrophic organisms on the cyanobacteria *Synechococcus* species measured in the laboratory and their carbon acquisition from the *Synechococcus* species.

Predator species	PDV	CPD	MIR	CA	BC	Reference
Cryptophyte						
Teleaulax amphioxeia	76	17	0.06	1.3	7.6	This study
Dinophyte						
Heterocapsa rotundata	102	45	0.20	5.9	10.7	Jeong et al. (2005a)
Symbiodinium voratum	716	188	1.06	25.4	13.5	Jeong et al. (2012)
Prorocentrum minimum	927	227	1.18	28.3	12.5	Jeong et al. (2005a)
Prorocentrum donghaiense	1200	274	1.54	37.0	13.5	Jeong et al. (2005a)
Heterocapsa triquetra	1766	364	0.88	21.1	5.8	Jeong et al. (2005a)
Alexandrium minutum	2437	462	0.64	15.4	3.3	Jeong et al. (2005a)
Gymnodinium impudicum	2951	531	2.90	69.6	13.1	Jeong et al. (2005a)
Karenia brevis	4378	710	1.00	24.0	3.4	Jeong et al. (2005a)
Scrippsiella trochoidea	6203	917	1.42	34.1	3.7	Jeong et al. (2005a)
Cochlodinium polykrikoides	9092	1215	7.74	185.8	15.3	Jeong et al. (2005a)
Prorocentrum micans	9900	1294	7.64	183.4	14.2	Jeong et al. (2005a)
Alexandrium tamarense	11,612	1455	2.74	65.8	4.2	Jeong et al. (2005a)
Akashiwo sanguinea	15,291	1781	12.58	301.9	17.0	Jeong et al. (2005a)
Gonyaulax polygramma	17,965	2005	8.48	203.3	10.1	Jeong et al. (2005a)
Alexandrium catenella	18,131	2018	5.90	141.6	7.0	Jeong et al. (2005a)
Gymnodinium catenatum	20,388	2200	6.04	145.0	6.6	Jeong et al. (2005a)
Gonyaulax spinifera	22,438	2360	4.86	116.6	4.9	Jeong et al. (2005a)
Lingulodinium polyedrum	29,172	2863	12.84	308.2	10.8	Jeong et al. (2005a)
Raphidophyte						
Heterosigma akashiwo[a]	697	101	0.78	18.7	18.5	Jeong et al. (2010a)
Chattonella ovata[a]	33,493	3837	3.72	89.3	2.3	Jeong et al. (2010a)
Ciliate						
Mesodinium rubrum	5245	1050	0.53	12.6	1.2	Yoo et al. (2015)

PDV, predator volume (μm^3); CPD, carbon content of a predator cell (pg C $cell^{-1}$); MIR, maximum ingestion rate (pg C $predator^{-1}$ h^{-1}); CA, carbon acquired from prey by predator per day (pg C $predator^{-1}$ d^{-1}); BC, acquired carbon as a percentage of the predator's carbon (%).
[a] The maximum value among the mean ingestion rates measured at given prey concentrations.

Table 8
Ingestion rates (IRs) and grazing coefficients (GCs) of natural populations of mixotrophic protists on natural populations of heterotrophic bacteria.

Predator	Location	PDA	HB($\times 10^6$)	IR	CR	GC (d^{-1})	Ref.[a]
Cryptophytes	Masan Bay, Korea	2633–27,825	8.7–1.7	0.3–8.3	0.03–0.50	0.012–0.034	(1)
Cryptophytes	Masan Bay, Korea	2–392,000	0.5–14.3			0.000–2.317[b]	(2)
Cryptophytes	Blances Bay, Spain	5–2173	0.56–1.4	0.0–2.3	0.00–2.13	0.000–0.188	(3)
Cryptomonas sp.	Boston Harbor, USA	NA	2.2	7.3–20.8	3.3–9.5	NA	(4)
Cochlodinium polykrikoides	Yeosu waters, Korea	1300–2330	2.5–8.5	2.9–9.3	1.1–1.2	0.043–0.067	(5)
Heterocapsa rotundata	Keum Estuary, Korea	37,640	3.5	2.2	0.6	0.569	(5)
Heterocapsa rotundata	Choptank River, USA	297–11,475	0.9–2.9	1.2–12.3	1.0–5.6	0.049–0.753	(6)
Heterocapsa triquetra	Keum Estuary, Masan Bay, Korea	200–8410	0.8–3.5	2.6–3.5	0.7–4.6	0.019–0.151	(5)
Heterosigma akashiwo	Shiwha, Masan, Jinhae Bay, Korea	550–59,670	6.5–13.0	2.7–9.0	0.4–1.4	0.020–0.857	(5)
Prorocentrum minimum	Masan, Jinhae Bay, Korea	3760–30,120	0.8–13.0	2.0–15.3	0.6–2.7	0.113–0.850	(5)
Prorocentrum triestinum	Shiwha Bay, Korea	20,650	6.5	7	1.1	0.498	(5)
Mesodinium rubrum	Keum Estuary, Masan Bay, Saemankeum Area, Korea	100–16,879	1.6–14.7	2.3–16.8	0.2–10.9	0.003–0.245	(7)

PDA, predator's abundance (cells ml^{-1}); HB, heterotrophic bacteria concentration (cells ml^{-1}); IR, ingestion rate (cells $predator^{-1}$ h^{-1}); CR, clearance rate (nl $predator^{-1}$ h^{-1}); NA, not available.
[a] 1, This study; 2, Jeong et al. (2013); 3, Unrein et al. (2007, 2014); 4, Epstein and Shiaris (1992); 5, Seong et al. (2006); 6, Millette et al. (2017); 7, Seong et al. (2017).
[b] Grazing impacts attributable to cryptophytes on heterotrophic bacteria were calculated using Eq. (3), field data on the abundances of cryptophytes and co-occurring heterotrophic bacteria, and the ingestion rates of the *T. amphioxeia* on heterotrophic bacteria obtained in the present study (see text for details).

the red tide mixotrophic dinoflagellates *Heterocapsa triquetra* (3.5 cells $predator^{-1}$ h^{-1}), *Prorocentrum triestinum* (7.0 cells $predator^{-1}$ h^{-1}), and *Cochlodinium polykrikoides* (9.3 cells $predator^{-1}$ h^{-1}) and the raphidophyte *Heterosigma akashiwo* (9.0 cells $predator^{-1}$ h^{-1}), but lower than those of *Heterocapsa rotundata* (12.3 cells $predator^{-1}$ h^{-1}), *Prorocentrum minimum* (15.3 cells $predator^{-1}$ h^{-1}), and the ciliate *M. rubum* (16.8 cells $predator^{-1}$ h^{-1}) in Korean waters (Table 8). Thus, the dominant cryptophytes in Masan Bay may be able to compete with some red tide dinoflagellates and raphidophytes for heterotrophic bacteria prey at sea.

The maximum grazing coefficient (MGC) attributable to cryptophytes on co-occurring heterotrophic bacteria in Masan Bay in April 2006 (0.04 d^{-1}) is much lower than those attributable to cryptophytes in Blanes Bay, western Mediterranean Sea (0.19 d^{-1}; Unrein et al., 2007, 2014). The grazing coefficient is proportional to the clearance which, in turn, is proportional to ingestion rate, but inversely proportional to prey concentration (Eqs. (1) and (3)). The maximum clearance rate (MCR) of marine cryptophyte on co-occurring heterotrophic bacteria in Masan Bay in April 2006 was much lower than that in Blanes Bay. In contrast,

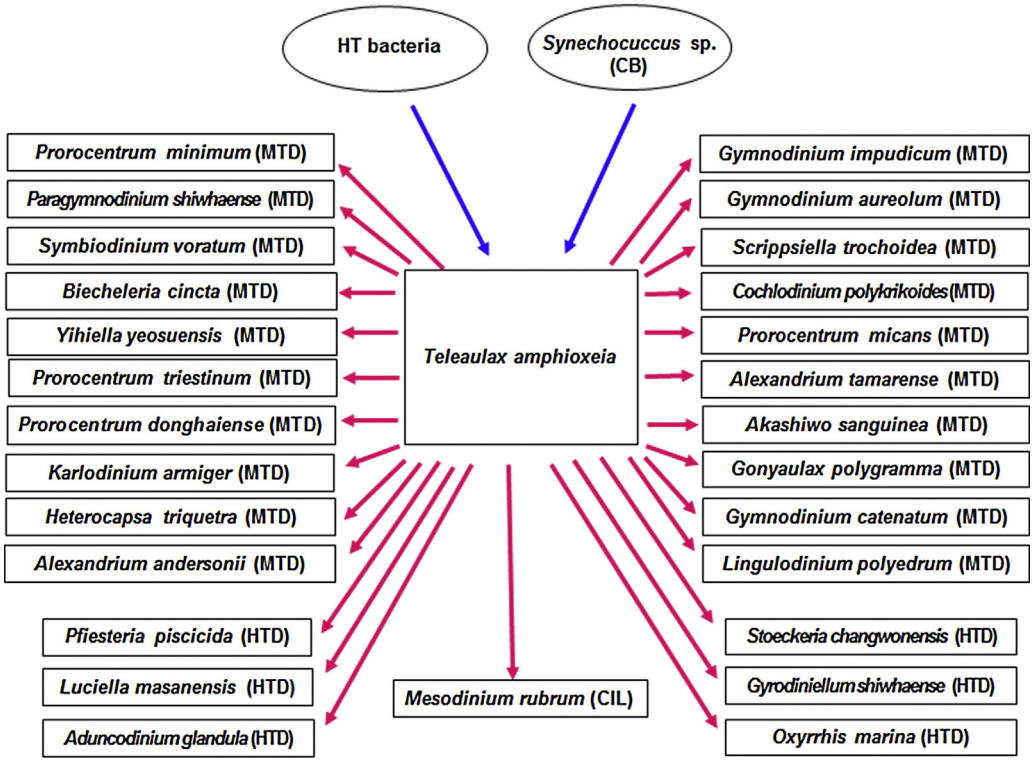

Fig. 6. The "*T. amphioxeia* hub" presenting the confirmed prey and predator species of *Teleaulax amphioxeia*. The blue-colored arrows emerge from prey species and point to cryptophyte species. The red-colored arrows emerge from cryptophyte species and point to the predator species. CB: cyanobacterium, MTD: mixotrophic dinoflagellate, HTD: heterotrophic dinoflagellate, CIL: ciliate.

the abundance of heterotrophic bacteria in Masan Bay in April 2006 was much greater than that in the western Mediterranean Sea. Thus, lower MCR and higher bacteria abundance in Masan Bay in 2006 may be partially responsible for this MGC being lower than that in Blanes Bay. The abundances of marine cryptophytes and co-occurring heterotrophic bacteria in Masan Bay from June 2004 to May 2005 (n = 115) were 2–392,000 cells ml^{-1} and 0.5–14.3 $\times 10^6$ cells ml^{-1} (Jeong et al., 2013). The mean (±standard error) and maximum grazing coefficients attributable to cryptophytes on co-occurring heterotrophic bacteria calculated using Eq. (3) and the abundance data with an assumption that the ingestion rates of the other cryptophytes on heterotrophic bacteria were identical to that of *T. amphioxeia* were 0.09 d^{-1} (±0.03, n = 115) and 2.32 d^{-1}, respectively. Thus, up to 90% of the population of heterotrophic bacteria could be consumed by marine cryptophytes in 1 day. The MGC of marine cryptophytes on heterotrophic bacteria in Masan Bay is greater than that of the mixotrophic dinoflagellates and *M. rubrum* (Table 8). Therefore, marine cryptophytes sometimes have considerable potential grazing impact on populations of heterotrophic bacteria in Masan Bay and sometimes their contribution to grazing on heterotrophic bacteria may be greater than the mixotrophic dinoflagellates and the ciliate.

The abundances of *T. amphioxeia* and *Synechococcus* spp. in Masan Bay, Korea in 2004–2005 wereas 2–392,000 cells ml^{-1} and 70–16,490 cells ml^{-1}, respectively (Jeong et al., 2013). The mean (± standard error) and maximum grazing coefficients attributable to cryptophytes on co-occurring *Synechococcus* spp., calculated using Eq. (3) and the abundance data with an assumption that the ingestion rates of the other cryptophytes on *Synechococcus* spp. is identical to that of *T. amphioxeia*, were 0.017 h^{-1} (±0.058, n = 115) and 0.518 h^{-1}, respectively. Thus, up to 40% of the population of *Synechococcus* could be consumed by marine cryptophytes in 1 h. Therefore, marine cryptophytes sometimes have considerable potential grazing impact on populations of *Synechococcus* spp. in Masan Bay.

4.4. Ecological implication

The results of the present study show that *T. amphioxeia* can be an effective predator on both heterotrophic bacteria and cyanobacteria. Furthermore, this species was revealed to be an excellent prey species for a diverse array of mixotrophic and heterotrophic dinoflagellates and the ciliate *M. rubrum* (Jeong et al., 2004, 2005b, 2006, 2007, 2010b, 2011; Yih et al., 2004; Yoo et al., 2010; Kang et al., 2011; Johnson, 2015; Jang et al., 2016; Lee et al., 2016). Thus, the interactions of this species with bacteria and numerous protist species indicate that it may play diverse roles in marine plankton food webs. Based on the present study and previous findings reported in the literature, a diagram depicting predator-prey relationships of *T. amphioxeia*, i.e., the "*T. amphioxeia* hub", can be established (Fig. 6). This hub can be used to establish models for predicting red tide dynamics of *T. amphioxeia* (e.g., Jeong et al., 2015). Furthermore, in the cycling of materials, bacterial components can be transferred to the mixotrophic and heterotrophic dinoflagellates and ciliates via marine cryptophytes. Previous studies concurred with the findings of the present study in that *M. rubrum* is a predator of *T. amphioxeia* and also showed that *M. rubrum* in turn was fed on by the dinoflagellate *Dinophysis*spp. (Yih et al., 2004; Park et al., 2006; Kim et al., 2015).

The results of the present study are ecologically important for a clearer understanding of marine ecosystems because (1) the marine cryptophyte *T. amphioxeia* was found to be mixotrophic, (2) this species is able to feed on both heterotrophic bacteria and a cyanobacterium, and (3) *T. amphioxeia* may sometimes have a

considerable potential grazing impact on populations of co-occurring heterotrophic bacteria and *Synechococcus* sp. Further studies on the mixotrophic ability of other marine cryptophyte species should be explored to better understand the marine food webs and cycling of materials that occur within them.

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