Harmful Algae 73 (2018) 98–109

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

# Feeding and grazing impact by the bloom-forming euglenophyte *Eutreptiella eupharyngea* on marine eubacteria and cyanobacteria

Yeong Du Yoo[a,**], Kyeong Ah Seong[a], Hyung Seop Kim[a], Hae Jin Jeong[b,c,*], Eun Young Yoon[c], Jaeyeon Park[c], Jong Im Kim[d], Woongghi Shin[d], Brian Palenik[e]

[a] Department of Marine Biotechnology, College of Ocean Science and Technology, Kunsan National University, Kunsan, 54150, Republic of Korea
[b] School of Earth and Environmental Sciences, College of Natural Sciences, Seoul National University, Seoul 08826, Republic of Korea
[c] Environment and Resource Convergence Center, Advanced Institutes of Convergence Technology, Suwon 16229, Republic of Korea
[d] Department of Biology, Chungnam National University, Daejeon, 34134, Republic of Korea
[e] Marine Biology Research Division, Scripps Institution of Oceanography, University of California at San Diego, La Jolla, CA 92093-0202, USA

ARTICLE INFO

*Article history:*
Received 7 October 2017
Received in revised form 19 December 2017
Accepted 12 February 2018
Available online xxx

*Keywords:*
Ecology
Food web
Algal bloom
Mixotrophy
Red tide
*Synechococcus*

ABSTRACT

The phototrophic euglenophyte *Eutreptiella eupharyngea* often causes blooms in the coastal waters of many countries, but its mode of nutrition has not been assessed. This species has previously been considered as exclusively auxotrophic. To explore whether *E. eupharyngea* is a mixotrophic species, the protoplasm of *E. eupharyngea* cells were examined using light, epifluorescence, and transmission electron microscopy after eubacteria, the cyanobacterium *Synechococcus* sp., and diverse algal species were provided as potential prey. Furthermore, the ingestion rates of *E. eupharyngea* KR on eubacteria or *Synechococcus* sp. as a function of prey concentration were measured. In addition, grazing by natural populations of euglenophytes on natural populations of eubacteria in Masan Bay was investigated. This study is the first to report that *E. eupharyngea* is a mixotrophic species. Among the potential prey organisms offered, *E. eupharyngea* fed only on eubacteria and *Synechococcus* sp., and the maximum ingestion rates of these two organisms measured in the laboratory were 5.7 and 0.7 cells predator$^{-1}$ h$^{-1}$, respectively. During the field experiments, the maximum ingestion rates and grazing impacts of euglenophytes, including *E. eupharyngea*, on natural populations of eubacteria were 11.8 cells predator$^{-1}$ h$^{-1}$ and 1.228 d$^{-1}$, respectively. Therefore, euglenophytes could potentially have a considerable grazing impact on marine bacterial populations.



## 1. Introduction

Euglenophytes are unicellular organisms that live in diverse environments including freshwaters, estuaries, intertidal zones, tidal pools, coastal embayments, and pelagic zone (Olli et al., 1996; Stonik and Selina, 2001; Brown et al., 2002; Kingston, 2002; Ansotegui et al., 2003; Rat'kova et al., 2004; Zimba et al., 2004; Stone, 2006; Gameiro et al., 2007; Jeong et al., 2011; Yamaguchi et al., 2012; Kang et al., 2013). They have diverse morphologies and wide genetic variations that are used for their classification (Marin et al., 2003; Linton, 2010; Karnkowska et al., 2015). Furthermore, euglenophytes have diverse modes of nutrition such as phototrophy, phagotrophy, osmotrophy, and mixotrophy (Leander, 2004; Yamaguchi et al., 2012; Lakey and Triemer, 2017).

Phototrophic euglenophytes have diverse roles in marine planktonic food webs: they are primary producers (Kingston, 1999); predators that feed on prey species such as eubacteria and *Tetraselmis* sp. (Seong et al., 2006; Yamaguchi et al., 2012); and prey for diverse grazers such as *Gyrodinium dominans, Oxyrrhis marina, Pfiesteria piscicida, Polykrikos kofoidii, Protoperidinium bipes, Stoeckeria algicida, Strobilidium* sp., *Strombidinopsis* sp., *Acartia omorii,* and *Pseudodiaptomus marinus* (Uye and Takamatsu, 1990; Jeong et al., 2011). In addition, some euglenophytes sometimes cause dense blooms in diverse environments and produce the alkaloid toxin (Kingston, 2002; Zimba et al., 2017). Therefore, the outbreak, persistence, and decline of blooms by an euglenophyte species can be affected by interactions with predators, prey, and competitors (Stonik, 2007; Jeong et al., 2013).

The euglenophyte *Eutreptiella eupharyngea* was first described by Walne in 1986 (Walne et al., 1986); it has fusiform morphology

* Corresponding author at: School of Earth and Environmental Sciences, College of Natural Sciences, Seoul National University, Seoul 08826, Republic of Korea.
** Corresponding author.
*E-mail addresses:* ydyoo77@kunsan.ac.kr (Y.D. Yoo), hjjeong@snu.ac.kr (H.J. Jeong).

https://doi.org/10.1016/j.hal.2018.02.003
1568-9883/

with 18–28 fine striations per 10 μm cell length. The cells are 26–70 μm × 6–12 μm in size and have two stellate chloroplasts at the anterior and posterior positions adjacent to the nucleus. The mean biovolume of this species was 398 $\mu m^3$ (Trottet et al., 2007). Furthermore, two clusters of pyrenoids are surrounded by large paramylon granules. This species lives in neritic environments and is widely distributed along the coasts of many countries such as Canada, Denmark, Japan, Russia, Spain, and the USA (Walne et al., 1986; Roy et al., 2006; Seoane et al., 2006; Stonik, 2007). Furthermore, this species is the one of the most common causative species of blooms in Peter the Great Bay, East/Japan Sea (Stonik, 2007). Several studies have been conducted on the taxonomy and physiology of *E. eupharyngea* (Walne et al., 1986; Stonik, 2007); however, in most of these studies, this species has been assumed to be as phototrophic (Walne et al., 1986; Stonik and Selina, 2001; Stonik, 2007). To better understand the population dynamics of *E. eupharyngea* in marine environments, its mode of nutrition should be determined.

Previously, *E. eupharyngea* has been considered exclusively auxotrophic; however, recently, food vacuoles inside *E. eupharyngea* have been observed by our team. This observation implies that *E. eupharyngea* may be a mixotrophic species. In this study, two strains of *E. eupharyngea* were separately given several eukaryotic algal species, eubacteria, and the cyanobacterium *Synechococcus* sp. as potential prey. Cells of *E. eupharyngea* were examined using epifluorescence and transmission electron microscopy. Furthermore, the ingestion rates of *E. eupharyngea* on eubacteria and *Synechococcus* sp. as a function of prey concentration were measured in the laboratory. In addition, the ingestion rates of the natural populations of euglenophytes on the natural populations of eubacteria were measured in Masan Bay. Using the data on the ingestion rates and abundance of predators and prey, grazing coefficients by the euglenophytes on co-occurring eubacteria were calculated. The results of the present study provide a basis for understanding the interactions between *E. eupharyngea* and co-occurring bacterioplankton, as well as the dynamics of red tides dominated by *E. eupharyngea* in marine environments.

## 2. Materials and methods

### 2.1. Preparation of experimental organisms

Two strains of *Eutreptiella eupharyngea* were tested, including one isolated from Masan Bay, Korea (designated KR) and the other from the Scripps Institution of Oceanography pier, La Jolla, California, USA (designated SIO) (Table 1). A clonal culture of each *E. eupharyngea* strain was established by serial single-cell isolations. As the concentration of *E. eupharyngea* increased, it was subsequently transferred to 50-, 125-, and 500-ml polycarbonate bottles containing f/2 medium without silicate (f/2-Si) seawater medium (Guillard and Ryther, 1962). The bottles were placed on a shelf at 20 °C in f/2-Si medium under continuous illumination with 20 $\mu E\ m^{-2}\ s^{-1}$ cool white fluorescent light in the walk-in incubator system of the Marine Biology Education and Research Center, Kunsan National University. These conditions were also used for the experiments below.

The equivalent spherical diameter (ESD) and cell volume of protists were measured using an electron particle counter (Coulter counter Z2; Beckman Coulter, Fullerton, CA, USA). The carbon content of the protists was estimated from cell volume according to Menden-Deuer and Lessard (2000). The cell volume and carbon content of *Synechococcus* sp. was adopted from Apple et al. (2011). All observations under light or epifluorescence microscopy were made at 100–1000× magnification with an Olympus BX50 microscope using UV-, blue-, and green-light excitation (Olympus Corporation, Tokyo, Japan) and Nikon digital camera (Nikon Corporation, Tokyo, Japan).

### 2.2. DNA extraction, amplification, sequencing, and alignment

The DNA from the two strains of *Eutreptiella eupharyngea* was extracted and the cytoplasmic 18S small subunit (SSU) rDNA was amplified and sequenced as previously described (Kim et al., 2010). Two new sequences were visually aligned using the Genetic Data Environment (GDE 2.4) program (Smith et al., 1994); the secondary structure of the rRNA gene sequences of *Euglena gracilis* Klebs (Wuyts et al., 2001) was used as a guide. The conserved regions of the cytoplasmic encoded SSU rDNA genes were similarly aligned across related taxa and used for phylogenetic analyses. Unalignable nucleotides were excluded from the phylogenetic analysis.

### 2.3. Phylogenetic analysis

A dataset of 1578 characters was generated for the phylogenetic analyses (Table 2). Twenty species were used as outgroup taxa to root the tree because they have been shown to be taxa of photosynthetic euglenoids and the genus *Eutreptiella* in previous molecular studies (Marin et al., 2003; Kim et al., 2015). Maximum likelihood (ML) phylogenetic analyses were performed using RAxML version 8.0.0 (Stamatakis, 2014) with the general time-reversible plus gamma (GTR + GAMMA) model. The data were analyzed by 1000 independent tree inferences using the –# option to identify the best tree. The model parameters with gamma correction values and proportion of invariable sites in the combined dataset were obtained automatically by the program. Bootstrap support values (MLBS) were calculated using 1000 replicates with the same substitution model. Bayesian analyses were run using MrBayes 3.2.6 (Ronquist et al., 2012) with a random starting tree, two simultaneous runs (nruns = 2) and four Metropolis-coupled Markov chain Monte Carlo ($MC^3$) algorithms for $2 \times 10^6$ generations, with one tree retained every 1000 generations. The burn-in point was identified graphically by tracking the likelihoods (Tracer v.1.6; http://tree.bio.ed.ac.uk/software/tracer/). The first 500 trees were discarded, and the remaining 1501 trees were used to calculate the posterior probabilities (PP) for each clade. Trees were visualized using FigTree v.1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

### 2.4. Prey types consumed by E. eupharyngea

Experiment 1 was designed to investigate whether each *Eutreptiella eupharyngea* strain was able to feed on eubacteria, coccoid cyanobacteria, and eukaryotic microalgal species (Table 3).

**Table 1**
Location and time for sampling, including temperature (T, °C) and salinity (S), of seawater for the isolation of the experimental strains. Mean equivalent spherical diameter (ESD, data given as means ± 1 standard deviation, SD, μm) was measured using an electronic particle counter (Coulter counter Z2; Beckman Coulter, Fullerton, CA, USA).

| Species name | ESD | Location | Time | T | S | Reference |
|---|---|---|---|---|---|---|
| *Eutreptiella eupharyngea* KR | 13.6 ± 1.6 | Masan Bay, Korea | May 2016 | 17.3 | 30.0 | This study |
| *Eutreptiella eupharyngea* SIO | 14.1 ± 1.7 | SIO pier, USA | Jan. 2013 | 12.8 | – | This study |