Journal of Experimental Marine Biology and Ecology 459 (2014) 114–125

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

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

Feeding by the newly described mixotrophic dinoflagellate *Gymnodinium smaydae*: Feeding mechanism, prey species, and effect of prey concentration

Kyung Ha Lee [a], Hae Jin Jeong [a,b,*], Tae Young Jang [a], An Suk Lim [a], Nam Seon Kang [a], Ju-Hyoung Kim [c], Kwang Young Kim [c], Ki-Tae Park [d], Kitack Lee [d]

[a] School of Earth and Environmental Sciences, College of Natural Sciences, Seoul National University, Seoul 151-747, Republic of Korea
[b] Advanced Institutes of Convergence Technology, Suwon, Gyeonggi-do 443-270, Republic of Korea
[c] Department of Oceanography, Chonnam National University, Gwangju 500-757, Republic of Korea
[d] School of Environmental Science and Engineering, Pohang University of Science and Technology, Pohang 790-784, Republic of Korea

ARTICLE INFO

Article history:
Received 13 May 2014
Accepted 15 May 2014
Available online 7 June 2014

Keywords:
Growth
Ingestion
Mixotrophy
Peduncle
Protist
Red tide

ABSTRACT

To investigate feeding by the newly described mixotrophic dinoflagellate *Gymnodinium smaydae*, we explored the feeding mechanism and the kinds of prey species that *G. smaydae* is able to feed on. In addition, we measured the growth and ingestion rates of *G. smaydae* on optimal and suboptimal algal prey *Heterocapsa rotundata* and *Heterocapsa triquetra* as a function of prey concentration. Among the 19 algal prey species offered, *G. smaydae* ingested only thecate dinoflagellates *H. rotundata*, *H. triquetra*, *Heterocapsa* sp., and *Scrippsiella trochoidea*. Among the peduncle-feeding dinoflagellates so far reported, *G. smaydae* is the only grazer that is able to feed on *S. trochoidea* and one of the two species that are able to feed on *H. triquetra*. However, *G. smaydae* did not feed on the raphidophyte *Heterosigma akashiwo*, the cryptophytes *Teleaulax* sp. and *Rhodomonas salina*, and the small dinoflagellate *Amphidinium carterae* which all the other peduncle-feeding dinoflagellates except *Stoeckeria algicida* are able to feed on. *G. smaydae* fed on algal prey using a peduncle after anchoring the prey by a tow filament. All *Heterocapsa* species supported high positive growth of *G. smaydae*, *S. trochoidea* only helped in merely maintaining the predator population. With increasing mean prey concentration, the growth and ingestion rates for *G. smaydae* on *H. rotundata* increased rapidly, but became saturated at concentrations of 455 ng C ml^{-1} (3500 cells ml^{-1}), while that on *H. triquetra* increased rapidly, but slowly at concentrations of 293 ng C ml^{-1} (945 cells ml^{-1}). The maximum specific growth rates (i.e., mixotrophic growth) of *G. smaydae* on *H. rotundata* and *H. triquetra* were 2.226 d^{-1} and 1.053 d^{-1}, respectively, at 20 °C under a 14:10 h light–dark cycle of 20 μE m^{-2} s^{-1}, while the growth rates (i.e., phototrophic growth) under the same light conditions without added prey were 0.005 to −0.051 d^{-1}. The maximum ingestion rates of *G. smaydae* on *H. rotundata* and *H. triquetra* were 1.59 ng C $grazer^{-1}$ d^{-1} (12.3 cells $grazer^{-1}$ d^{-1}) and 0.24 ng C $grazer^{-1}$ d^{-1} (0.8 cells $grazer^{-1}$ d^{-1}), respectively. The calculated grazing coefficients for *G. smaydae* on co-occurring *H. rotundata* or *H. triquetra* were up to 0.23 h^{-1} or 0.02 h^{-1}, respectively (i.e., 21% or 2% of the population of *H. rotundata* or *H. triquetra* was removed by *G. smaydae* populations in 1 h). The results of the present study suggest that *G. smaydae* can sometimes have a considerable grazing impact on the population of *H. rotundata*.

1. Introduction

Phototrophic dinoflagellates are one of the major components in marine planktonic communities (Jeong et al., 2013; J.Y. Park et al., 2013; Smayda, 1997). They live in diverse habitats, such as in the water column, on the surface of macroalgae, and inside sediments and organisms (Jeong et al., 2012, 2013; Kang et al., 2013; Lee et al., 2013; Lim et al., 2013). In the last 2 decades, several phototrophic dinoflagellates have been revealed to be mixotrophic (Burkholder et al., 2008; Jeong et al., 2005b, 2010b, 2012; Kang et al., 2011; Stoecker, 1999; Turner, 2006). These mixotrophic dinoflagellates are known to feed on diverse prey, such as heterotrophic bacteria, cyanobacteria, small flagellates, other mixotrophic dinoflagellates, and ciliates (Berge et al., 2008; Glibert et al., 2009; Jeong et al., 1999, 2005b, 2005c, 2005d, 2010a, 2012; Kang et al., 2011; Li et al., 2000; Park et al., 2006; Seong et al., 2006; Stoecker et al., 1997; Yoo et al., 2009). However, the number of phototrophic dinoflagellates of known mixotrophy is only ca. 50 species among ca. 1200 reported phototrophic dinoflagellates (i.e., 4–5%)

* Corresponding author at: School of Earth and Environmental Sciences, College of Natural Sciences, Seoul National University, Seoul 151-747, Republic of Korea. Tel.: +82 2 880 6746; fax: +82 2 874 9695.
E-mail address: hjjeong@snu.ac.kr (H.J. Jeong).

http://dx.doi.org/10.1016/j.jembe.2014.05.011
0022-0981/

(Gómez, 2012; Jeong et al., 2010b). Furthermore, the phototrophic dinoflagellates whose growth and ingestion rates have been quantified are less than 30 species. Therefore, to understand the eco-physiology of phototrophic dinoflagellates and their roles in planktonic food webs of the ecosystem, the kind of prey that a certain phototrophic dinoflagellate is able to feed on, optimal prey species, growth and ingestion rates, and grazing impact should be fully understood.

Recently, we found a new mixotrophic dinoflagellate *Gymnodinium smaydae* in Shiwha Bay, Korea (Kang et al., 2014). This dinoflagellate possesses nuclear chambers, a nuclear fibrous connective, an apical groove running in a counterclockwise direction around the apex, and peridinin as major accessory pigment, which are 4 key features of the genus *Gymnodinium*. This dinoflagellate (6–11 μm long and 5–10 μm wide) is smaller than any other *Gymnodinium* species so far reported, with the exception of *Gymnodinium nanum*. Cells are covered with polygonal amphiesmal vesicles arranged in 11 horizontal rows (Kang et al., 2014). This dinoflagellate has a sharp and elongated ventral ridge reaching halfway down the hypocone, which is unlike other *Gymnodinium* species. The new species possesses a peduncle, permanent chloroplasts, pyrenoids, and trichocysts, which suggest that it is a mixotrophic dinoflagellate.

We established a clonal culture of *G. smaydae* and observed its feeding behavior under high-resolution video-microscopy in order to explore the feeding mechanisms and determine the prey species when diverse algal species were provided. We also conducted experiments to determine the effects of prey concentration on the growth and ingestion rates of *G. smaydae* on the optimal and suboptimal algal prey species *Heterocapsa rotundata* and *Heterocapsa triquetra*, respectively, as a function of prey concentration. In addition, we estimated the grazing coefficients attributable to *G. smaydae* on *H. rotundata* and *H. triquetra* using the ingestion rate data obtained from the laboratory experiments and the abundances of predators and prey in the field. The abundance of *G. smaydae* was quantified using real-time polymerase chain reaction (PCR). The results of the present study provide a basis for understanding the feeding mechanisms and ecological roles of *G. smaydae* in marine planktonic food webs.

2. Materials and methods

2.1. Preparation of experimental organisms

The mixotrophic dinoflagellates *Cochlodinium polykrikoides* and *Lingulodinium polyedrum* were grown at 20 °C in enriched f/2 seawater media (Guillard and Ryther, 1962) under a continuous illumination of 50 μE m^{-2} s^{-1} of cool white fluorescent light, while the other phytoplankton species were grown under an illumination of 20 μE m^{-2} s^{-1} of cool white fluorescent light on a 14:10 h light–dark cycle. *C. polykrikoides* and *L. polyedrum* did not grow well under an illumination on a light–dark cycle. The mean equivalent spherical diameter (ESD) ± standard deviation was measured by an electronic particle counter (Coulter Multisizer II, Coulter Corporation, Miami, Florida, USA). The size of the phototrophic dinoflagellate *H. rotundata* strain used in the present study (HRSH1201; ESD = 9.5 μm) was bigger than that of the *H. rotundata* strain previously used in our papers (ESD = 5.8 μm; Jeong et al., 2006, 2007, 2010a, 2011b, 2012; Kang et al., 2011; Yoo et al., 2010).

Plankton samples were collected with a water sampler from Shiwha Bay, Korea (37° 18′ N, 126° 36′ E), during May 2010, when the water temperature and salinity were 19 °C and 27.7, respectively (Kang et al., 2014). The samples were filtered gently through a 154-μm Nitex mesh and placed in 6-well tissue culture plates. A clonal culture of *G. smaydae* was established following two serial single-cell isolations. *H. rotundata* was provided as prey for *G. smaydae* at concentrations of ca. 60,000–100,000 cells ml^{-1}. When the concentration of *G. smaydae* increased, cells were transferred to 32-, 270-, and 500-ml polycarbonate (PC) bottles containing fresh cultures of *H. rotundata*. The bottles were filled to capacity with filtered seawater, capped, and placed on a rotating wheel at 0.9 rpm at 20 °C under an illumination of 20 μE m^{-2} s^{-1} cool white fluorescent light in a 14:10 h light–dark cycle. Once dense cultures of *G. smaydae* were obtained, they were transferred daily to 500-ml PC bottles of fresh cultures of *H. rotundata* containing ca. 100,000 cells ml^{-1}.

The carbon contents of *H. rotundata* (0.13 ng C per cell), *Heterocapsa* sp. CCMP 3244 (0.10), *H. triquetra* (0.31), and *Scrippsiella trochoidea* (0.7) were measured using a CHN Analyzer (vario MICRO, Elementar, Germany) and those of the other phytoplankton species were obtained from our previous studies (Jeong et al., 2010a, 2011b, 2012; Kang et al., 2011; Yoo et al., 2010).

2.2. Prey species

Experiment 1 was designed to investigate whether or not *G. smaydae* was able to feed on each target algal species when unialgal diets of diverse algal species were provided (Table 1). The initial concentrations of each algal species offered were similar, in terms of carbon biomass. To confirm no ingestion by *G. smaydae* on some algal species, additional higher prey concentrations were provided.

A dense culture (ca. 10,000 cells ml^{-1}) of *G. smaydae* growing mixotrophically on *H. rotundata* in f/2 media and under 14:10 h light–dark cycle of cool white fluorescent light at 20 μE m^{-2} s^{-1} was transferred to one 1-l PC bottle containing f/2 medium when *H. rotundata* was undetectable. This culture was maintained in f/2 media for 2 d under 14:10 h light–dark cycle at 20 μE m^{-2} s^{-1}. Three 1-ml aliquots were then removed from the bottle and examined using a compound microscope to determine the *G. smaydae* concentration.

In this experiment, the initial concentrations of *G. smaydae* and each target algal species were established by using an autopipette to deliver a predetermined volume of culture with a known cell density to the experimental bottles. Triplicate 80-ml PC bottles with mixtures of *G. smaydae* and the target prey and duplicate predator control bottles containing *G. smaydae* only were set up for each target algal species. The bottles were filled to capacity with freshly filtered seawater, capped, and then placed on a vertically rotating plate rotating at 0.9 rpm and incubated at 20 °C under a 14:10 h light–dark cycle of cool white fluorescent light at 20 μE m^{-2} s^{-1}. After 6, 12, 24, and 48 h, a 5-ml aliquot was removed from each bottle and transferred into a 20-ml bottle. Two 0.1 ml aliquots were placed on slides and then cover-glasses were added. Under these conditions, the *G. smaydae* cells were alive, but almost motionless. The protoplasms of >200 *G. smaydae* cells were carefully examined with a compound microscope and/or an epifluorescence microscope (Zeiss-Axiovert 200M, Carl Zeiss Ltd., Göttingen, Germany) at a magnification of 100–630× to determine whether or not *G. smaydae* was able to feed on the target prey species. Pictures showing the ingested cells of each target algal species inside a *G. smaydae* cell were taken using digital cameras on these microscopes at a magnification of 630–1000×.

2.3. Feeding mechanisms

Experiment 2 was designed to investigate the feeding behaviors of *G. smaydae* when a unialgal diet of *H. rotundata*, *H. triquetra*, and *S. trochoidea* was provided as prey. Feeding by *G. smaydae* on these prey species had been observed in Experiment 1. The initial concentrations of predators and prey were the same as above.

The initial concentrations of *G. smaydae* and the target algal species were established using an autopipette to deliver a predetermined volume of culture with a known cell density to the experimental bottles. One 80-ml PC bottle with a mixture of *G. smaydae* and the algal prey was set up for each target algal species. The bottle was filled to capacity with freshly filtered seawater, capped, and then well mixed. After 1-min incubation, a 1-ml aliquot was removed from the bottle and transferred