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Feeding by the heterotrophic nanoflagellate *Katablepharis remigera* on algal prey and its nationwide distribution in Korea

Jin Hee Ok[a], Hae Jin Jeong[a,b,*], An Suk Lim[a,c], Sung Yeon Lee[a], So Jin Kim[a]

[a] School of Earth and Environmental Sciences, College of Natural Sciences, Seoul National University, Seoul 08826, Republic of Korea
[b] Advanced Institutes of Convergence Technology, Suwon, Gyeonggi-do, 16229, Republic of Korea
[c] Brain Korea 21 Plus, School of Earth and Environmental Sciences, College of Natural Sciences, Seoul National University, Seoul 08826, Republic of Korea

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ABSTRACT

Heterotrophic nanoflagellates are ubiquitous in natural waters, and most heterotrophic nanoflagellates are known to grow on bacteria. Recently, the heterotrophic nanoflagellate *Katablepharis japonica* has been reported to be an effective predator of diverse toxic or harmful algal prey. To date, 7 *Katablepharis* species have been identified, and therefore important questions arise as to whether other *Katablepharis* species can feed on algal prey, and further whether the types of prey of other *Katablepharis* species differ from those of *K. japonica*. To answer these important questions, feeding by *Katablepharis remigera* on diverse algal prey was examined. Specific growth and ingestion rates of *K. remigera* feeding on the raphidophytes *Heterosigma akashiwo* and *Chattonella subsalsa* were determined. Furthermore, the abundance of *K. remigera* at 28 stations along the coastline of Korea from January 2015 to October 2017 was quantified using qPCR method and newly designed specific primer-probe sets. Among 25 potential algal prey tested, *K. remigera* fed on only *H. akashiwo* and *C. subsalsa*; however, it did not feed on a diatom, a prymnesiophyte, a prasinophyte, cryptophytes, dinoflagellates, *Mesodinium rubrum*, a mixotrophic ciliate, and another raphidophyte *Fibrocapsa japonica*. The number of prey types on which *K. remigera* could feed (2 species) was considerably smaller than that of *K. japonica* (14 species). With the increase in the mean prey concentration, the specific growth rates of *K. remigera* on *H. akashiwo* and *C. subsalsa* increased as well before becoming saturated. The maximum specific growth rates of *K. remigera* on *H. akashiwo* and *C. subsalsa* were 0.717 and 0.129 d^{-1}, respectively. In addition, the maximum ingestion rates of *K. remigera* on *H. akashiwo* and *C. subsalsa* were 0.333 and 0.661 ng C $predator^{-1}$ d^{-1} (3.33 and 0.23 cells $predator^{-1}$ d^{-1}), respectively. The results of this study clearly indicate that *K. remigera* is an effective predator of 2 red tide-causing raphidophyte species, and additionally, the feeding activity of *K. remigera* differs greatly from that of *K. japonica*. The abundance of *K. remigera* was $\geq$0.1 cells mL^{-1} at 24 stations located in the East, West, and South Sea of Korea. Thus, *K. remigera* has a nationwide distribution in Korea. The highest abundance of *K. remigera* in Korean waters was 24.9 cells mL^{-1} in March 2017, when there was no red tide caused by *H. akashiwo* or *Chattonella* spp. In most regions where red tides caused by *H. akashiwo* or *Chattonella* spp. occurred in 2000–2017, *K. remigera* was detected. Thus, the abundance of *K. remigera* may increase during red tides caused by *H. akashiwo* and *Chattonella* spp.

1. Introduction

Heterotrophic nanoflagellates (HNFs) are major components of marine ecosystems (Patterson and Larsen, 1991). Most HNFs are known to grow on bacteria; however, only a few HNFs feed on algae (Fenchel, 1982; Azam et al., 1983; Sherr, 1984; Sanders et al., 1992; Tanaka et al., 1997; Kamiyama et al., 2000; Seong et al., 2006). Recently, herbivory by HNFs has attracted considerable attention after the HNF *Katablepharis japonica* was shown to feed on diverse toxic and harmful algae (Kwon et al., 2017). Furthermore, this HNF has been shown to have considerable potential grazing impact on populations of the red tide-causing dinoflagellate *Akashiwo sanguinea*. Therefore, it has been suggested that feeding by *K. japonica* should be considered in studies on the dynamics of red tide-causing dinoflagellates.

To date, 7 *Katablepharis* species have been described (Skuja, 1939, 1948; Lee and Kugrens, 1991; Vørs, 1992a,b; Clay and

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

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Kugrens, 1999; Okamoto and Inouye, 2005), namely *Katablepharis hyalurus*, *K. japonica*, *K. notonectoides*, *K. oblonga*, *K. ovalis*, *K. phoenikoston*, and *K. remigera*. Hence, important questions arise as to whether *Katablepharis* species other than *K. japonica* can feed on algae, and whether the types of prey consumed by other *Katablepharis* species differ from those eaten by this species. Of the 7 *Katablepharis* species, ribosomal (r) DNA sequences of *K. japonica*, *K. remigera,* and an unidentified *Katablepharis* species were reported (Okamoto and Inouye, 2005; Kahn et al., 2014). Lack of clonal cultures may limit molecular analyses of the other *Katablepharis* species.

An HNF cell was isolated at Jinhae Bay, Korea in 2017. A clonal culture of this HNF was obtained by providing the raphidophyte *Heterosigma akashiwo* as prey. Based on molecular analyses, this HNF was identified to be *K. remigera*. This species (previously known as *Leucocryptos remigera*) was first described by Vørs (1992a) to have ovoid-oblong cells with two thick, unequal flagella. It has been reported to have a cytostome, or "cell mouth" (Clay and Kugrens, 1999). Using this clonal culture, feeding by *K. remigera* on various potential prey, including diatoms, cryptophytes, prymnesiophytes, prasinophytes, dinoflagellates, raphidophytes, and a mixotrophic ciliate, was examined. Furthermore, the type of algae that *K. remigera* can feed on was compared with that of *K. japonica*. Specific growth and ingestion rates of *K. remigera* feeding on *H. akashiwo* and another raphidophyte *Chattonella subsalsa,* which were revealed to be quite efficiently preyed upon by *K. remigera*, were further examined. Additionally, these growth and ingestion rates were compared with those of mixotrophic and heterotrophic dinoflagellates and ciliates preying on the same species, as reported in the literature. Moreover, the abundance of *K. remigera* at 28 stations located in the East, West, and South Sea of Korea from January 2015 to October 2017 were quantified using a quantitative real-time polymerase chain reaction (qPCR) method and newly designed specific primer-probe sets. The results of this study provide a basis for the better understanding of relationships between *K. remigera* and its potential prey species, as well as the dynamics of red tide-causing raphidophytes.

2. Materials and methods

2.1. Preparation of experimental organisms

Twenty-five potential prey species belonging to various taxa with diverse sizes and shapes were provided as potential prey (Table 1). All algal prey species, except *Skeletonema costatum*, *Cochlodinium polykrikoides*, and *Lingulodinium polyedra*, were grown at 20 °C in an enriched f/2-Si seawater medium (Guillard and Ryther, 1962) with illumination at 20 μE m^{-2} s^{-1} using cool white fluorescent light, under a 14/10-h light/dark cycle. The culture of *S. costatum* was done in an enriched f/2 seawater

Table 1
Taxa, size, and concentration of algal prey species offered to *Katablepharis remigera* and feeding ocuurence. Mean equivalent spherical diameter (ESD, μm) for algae is presented. The initial concentrations of *K. remigera* were approximately 2000–5000 cells mL^{-1}. T: Thecate, NT: Non-thecate, Y: Feeding by *K. remigera* or *K. japonica*, N: Not fed on by *K. remigera* or *K. japonica*.?: Questionable. NA: Not available.

Prey species	ESD (μm)	Initial prey concentration	By *K. remigera*		By *K. japonica*	
		(cell mL^{-1})	Attack	Feeding	Attack	Feeding
Bacillariophyte						
Skeletonema costatum	5.9	30,000	Y	N	Y	N
Prymnesiophyte						
Isochrysis galbana	4.8	150,000	N	N	Y	?
Prasinophyte						
Pyramimonas sp.	5.6	100,000	N	N	Y	Y
Cryptophytes						
Teleaulax sp.	5.6	50,000	N	N	Y	Y
Storeatula major	6.0	15,000	N	N	Y	NA
Rhodomonas salina	8.8	50,000	Y	N	Y	Y
Rhaphidophytes						
Heterosigma akashiwo (NT)	11.5	30,000	Y	Y	Y	Y
Fibrocapsa japonica (NT)	26.4	2000	Y	N	Y	NA
Chattonella subsalsa CCMP 217 (NT)	36.5	2000	Y	Y	Y	Y
Phototrophic dinoflagellates						
Heterocapsa rotundata (T)	5.8	100,000	N	N	Y	Y
Amphidinium carterae (NT)	9.7	30,000	Y	N	Y	Y
Prorocentrum cordatum (T)	12.1	5000	N	N	Y	N
Prorocentrum donghaiensis (T)	13.3	5000	N	N	Y	Y
Heterocapsa triquetra (T)	15.0	1000	N	N	Y	N
Alexandrium minutum CCMP 1888(T)	20.4	2000	N	N	Y	Y
Scrippsiella acuminata (T)	22.8	5000	N	N	Y	N
Cochlodinium polykrikoides (NT)	25.9	2000	N	N	Y	Y
Prorocentrum micans (T)	26.6	2000	N	N	Y	N
Akashiwo sanguinea (NT)	30.8	2000	N	N	Y	Y
Coolia malayensis (T)	30.4	2000	N	N	Y	Y
Alexandrium tamarense CCMP1493 (T)	32.6	1500	N	N	Y	N
Gymnodinium catenatum (NT)	33.9	100	N	N	Y	Y
Lingulodinium polyedra (T)	38.2	1000	N	N	Y	N
Gambierdiscus caribaeus (T)	67.4	100	N	N	Y	N
Naked ciliate						
Mesodinium rubrum	22.0	500	Y	N	Y	Y
Reference			This study	This study	Kwon et al. (2017)	Kwon et al. (2017)