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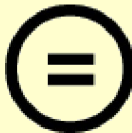
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이학석사학위논문

Differential responses by common heterotrophic protistan predators to four different dinoflagellates *Scrippsiella* species and their interactions with the heterotrophic nanoflagellate *Katablepharis japonica*

와편모류인 스크립시엘라 4종에 대한 종속영양성 포식자의 서로 다른 반응 및 종속영양성 미세편모류 카타블레페리스 자포니카와의 상호작용 연구

2019년 02월

서울대학교 대학원

지구환경과학부 해양학전공

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지도 교수 정 해 진

이 논문을 이학석사 학위논문으로 제출함

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김소진의 이학석사 학위논문을 인준함

2018년 12월

위 원 장 ____________________ (인)

부위원장 ____________________ (인)

위 원 ____________________ (인)

Abstract

Differential responses by common heterotrophic protistan predators to four different dinoflagellates *Scrippsiella* species and their interactions with the heterotrophic nanoflagellate *Katablepharis japonica*

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The dinoflagellate genus *Scrippsiella* is widely distributed and known to cause red tides. However, the nationwide distributions of *Scrippsiella* species in Korea have not been fully understood yet. Recently, four *Scrippsiella* species (*S. acuminata*, *S. donghaiensis*, *S. lachrymosa*, and *S. masanensis*) were isolated from the Korean coastal waters. To investigate nationwide distributions of these *Scrippsiella* species in Korean coastal waters, the species specific primers of each species were developed and then the abundance of

each species in the 28 stations was measured using the quantitative real-time PCR method. These four *Scrippsiella* species had different temporal and spatial distributions; *S. donghaiensis* was usually observed at 18 stations in July 2016-2017 and *S. lachrymosa* was often found at 9 stations in March 2016-2017. *S. masanensis* was detected at 11 stations in January, March, July, October, and December in 2016 and March and July in 2017, while *S. acuminata* was rarely observed in the study period.

To understand population dynamics of *Scrippsiella* species, mortality due to predation as well as growth should be determined. To investigate predation by heterotrophic protists on *Scrippsiella* species, interactions between four *Scrippsiella* species and common heterotrophic dinoflagellates and a ciliate were explored. All heterotrophic protists tested were able to feed on all four *Scrippsiella* species, however, the growth and ingestion rates of the heterotrophic dinoflagellates *Oxyrrhis marina*, *Gyrodinium dominans*, and *Polykrikos kofoidii* on one of four *Scrippsiella* species were clearly different from those on the other *Scrippsiella* species. Therefore, it may cause a selection of the bloom causative species among these 4 *Scrippsiella* species. Furthermore, differential responses by each predator to four *Scrippsiella* species may cause different ecological niches of both the predators and prey species.

Recently, the heterotrophic nanoflagellate *Katablepharis japonica* has been revealed to feed on diverse red tide species and thus could be an effective grazer of red-tide organisms. However, if there are effective predators feeding on *K. japonica*, its grazing

impact may be reduced. To investigate potential effective predators on *K. japonica*, feeding by diverse heterotrophic dinoflagellates and the naked ciliates on *K. japonica* was explored. None of heterotrophic protists fed on actively swimming *K. japonica* cells. To the contrary, *K. japonica* was able to feed on six heterotrophic protists. The results of this study suggest that predation impact by heterotrophic protists on *K. japonica* be negligible and thus grazing impact by *K. japonica* on populations of red tide species may not be reduced by mortality due to predation. Furthermore, to understand their population dynamics, I investigated the distribution and abundance of *K. japonica* in 2016–2017 using quantitative real-time PCR. Cells of *K. japonica* were widely distributed around Korean coastal waters and detected four seasons in the study period.

This study provides a basis on understanding the interactions between common heterotrophic protists and 4 *Scrippsiella* species and also between common heterotrophic protists and the heterotrophic nanoflagellate *K. japonica,* which are able to feed on red-tide species.

Keywords: Dinoflagellates, Distribution, Feeding, Harmful algal bloom, Heterotrophic nanoflagellates, Heterotrophic protists

Student Number: 2016-26602

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Chapter 1. Overall introduction

In marine ecosystems, a large number of organisms from primary producers to top carnivores live together and interact. In the conventional grazing food chain, phytoplankton grown up by uptaking nutrient was fed on by zooplankton that is also eaten by fish (Pomeroy 1974, Fuhrman et al. 1980). In this food chain, most bacteria are attached bacteria that decompose the dead organisms. In 1983, “the microbial loop” was first established by Azam (1983). According to the microbial loop, most bacteria are free−living forms and fed on by diverse heterotrophic protists (HTPs). Thus, a loop was added to conventional grazing food chain (Fig 1.1). Therefore, heterotrophic protists such as heterotrophic dinoflagellates, heterotrophic nanoflagellates, and ciliates become important more than previously known.

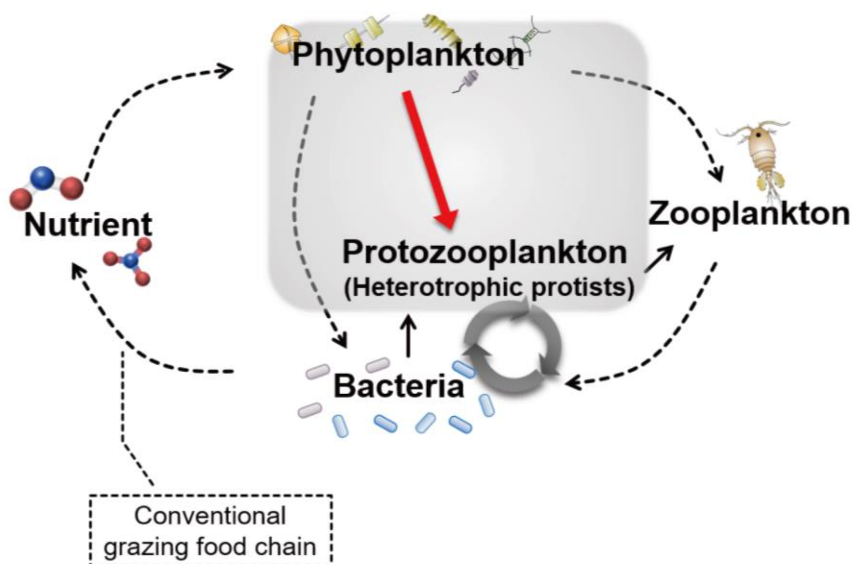


Fig. 1. 1. A diagram of the microbial loop.

There are diverse species in phytoplankton that is the major primary producer and important prey for diverse zooplankton and metazoans in marine planktonic food webs. Among the phytoplankton, dinoflagellates are a major component of marine ecosystems throughout the world (Smayda 1997, Shears and Ross 2009, Jeong et al. 2013, Lee et al. 2016, Lim et al. 2017b). Each of them has one of the 3 major trophic modes (autotrophic, heterotrophic, mixotrophic) and plays diverse roles as prey, predator, symbiotic partner, and parasite (Stentiford et al. 2002, Jeong et al. 2010b, LaJeunesse et al. 2015, Lim et al. 2017a). In addition, some dinoflagellate species lead to red tides or harmful algal blooms which sometimes disturb marine ecosystems and often cause large scale mortalities of marine organisms such as fin-fish, shellfish, and shrimps (Widdows et al. 1979, Guzmán et al. 1990, Robineau et al. 1991, Silke and Cronin 2005, Leverone et al. 2006, Park et al. 2013). To minimize great loss due to red tides or harmful algal blooms, we must understand and predict their outbreak using models. In the models of predicting the population dynamics of a red-tide dinoflagellate, data on its growth rate and mortality rate due to predation are needed.

In this thesis, I investigate interactions between common heterotrophic protists (HTPs) and the autotrophic dinoflagellates (ATD) *Scrippsiella* species and also interactions between common HTPs and the heterotrophic nanoflagellate (HNFs) *K. japonica* that are important members of the microbial loop (Fig 1.2). In addition, the genera *Scrippsiella* and *Katablepharis* are globally distributed

and have continuously appeared in Korean coastal waters. However, the distributions of 4 *Scrippsiella* species (*S. acuminata*, *S. donghaiensis*, *S. lachrymosa*, and *S. masanensis*) and *Katablepharis* are poorly understood. Thus, in chapter 2, I explored four *Scrippsiella* species nationawide distributions in the Korean coastal waters by determing the abundance of each species in the waters of 28 stations in 2016–2017 using the quantitative real–time PCR method after developing the specific primers and probes.

In chapter 3, differential interactions between common heterotrophic protists and four *Scrippsiella* species tested in chapter 2 were investigated. In the genus *Scrippsiella*, *S. acuminata* is a common species that often causes red tides or harmful algal blooms (HABs) and its growth and mortality rates due to predation have been relatively well known. However, the growth and mortality of the other *Scrippsiella* species are poorluy known. To explore the mortality of four *Scrippsiella* species isolated from the Korean coastal waters, the growth and ingestion rates of each of common heterotrophic protist predators on each of *Scrippsiella* species were determined. Furthermore, to understand eco–physiological characteristics of four *Scrippsiella* species, I investigated relationships between feeding rates of heterotrophic protists and swimming speeds or toxicity of four *Scrippsiella* species.

In chapter 4, the interactions between heterotrophic protists including the predators used in chapter 3 and the heterotrophic nanoflagellates *Katablepharis japonica* were examined. Recently, *K. japonica* has been known to feed on various phytoplankton including

red-tide species, and it may give significant grazing impact on the population dynamics of red-tide species (Kwon et al. 2017). However, if mortality of *K. japonica* due to predation is high, the grazing impact of *K. japonica* may be reduced. Therefore, to understand the mortality rate of *K. japonica* and ecological roles each of heterotrophic protists and *K. japonica*, the interactions between *K. japonica* and heterotrophic protists which are able to feed on red-tide organisms were investigated. In addition, the nationwide distribution of *K. japonica* in Korean coastal waters were explored using the same method used in chapter 2.

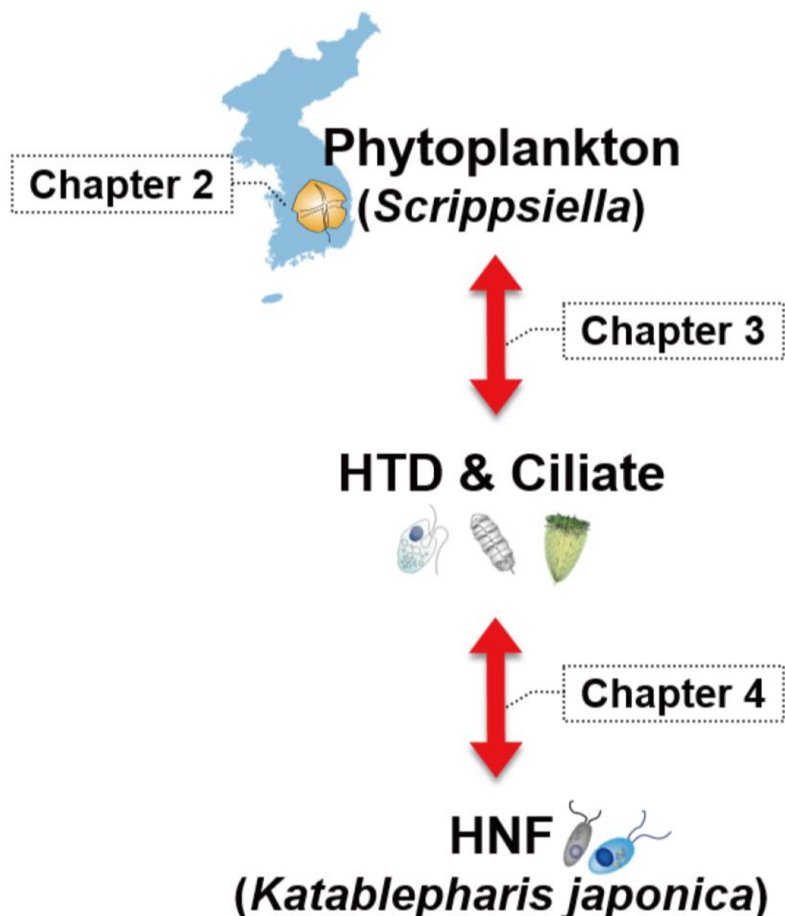


Fig. 1. 2. Thesis outline

Chapter 2. Distributions of four *Scrippsiella* species in Korean coastal waters during 2016–2017 using quantitative real-time PCR

2.1. Introduction

The genus *Scrippsiella* is one of the major genera of the dinoflagellates. Some species are known to cause red tides (Hallegraeff 1992, Wang et al. 2007). They can survive in unfavorable environments by forming calcareous cysts. Therefore, they have been distributed globally as motile forms in the water columns or as resting cysts in the sediments (Ishikawa and Taniguchi 1993, Nehring 1994, Blanco 1995, Olli and Trunov 2010, Soehner et al. 2012, Luo et al. 2016, Rahmadyani et al. 2017). Especially, *Scrippsiella acuminata* is widely distributed in the waters of many countries such as Japan, Italy, USA, Mexico, UK, France, China, Australia, Iceland, Norway and Turkey (Lewis 1991, Ishikawa and Taniguchi 1993, Montresor et al. 2003, Gu et al. 2008, Gárate-Lizárraga et al. 2009, Soehner et al. 2012, Tang and Gobler 2012, Guilloux et al. 2013, McCarthy 2013, Scholz and Einarsson 2015, Balkis et al. 2016). However, the distributions of many other *Scrippsiella* species such as *S. lachrymosa*, *S. donghaiensis*, and *S. masanensis* have not been well documented.

Since the genus *Scrippsiella* was first established by Blaech (1959), many species have been established as new species, but

currently approximately 30 species are taxonomically accepted (Attaran-Fariman and Bolch 2007, Gu et al. 2008, Kretschmann et al. 2015, Luo et al. 2016). The vegetative cells of *Scrippsiella* spp. have a stable plate formula as Po, x, 4', 3a, 7'', 6c(5c+t), 5s, 5''', and 2'''' with little difference among the species (Balech 1959, Zinssmeister et al. 2011). Therefore, it is difficult to distinguish one *Scrippsiella* species from others using light microscope.

Scrippsiella acuminata, one of the most commonly found species in the genus has formed red tides or harmful algal blooms which often cause mortality of fin-fish, shellfish, and shrimp (Gárate-Lizárraga et al. 2009, Tang and Gobler 2012). This species caused a red tide in Ulsan, Korea in 1983 and also in the waters of the southern coasts of Korea in August 2009, May 2011, and April 2017 (National Institute of Fisheries Science, 2018). *Scrippsiella* species can survive by forming cysts under the conditions unfavorable for growth, but become free-living cells which can cause red tides when the conditions is favorable for growth. Therefore, since *Scrippsiella* species have a potential to cause red tides, temporal and spatial distributions of *Scrippsiella* should be monitored.

Recently, cells of *S. acuminata*, *S. donghaiensis*, *S. lachrymosa*, and *S. masanensis* were isolated in the Korean coastal waters. Although there have been reports on red tides by *Scrippsiella* species, but nationwide distributions of these species in Korea have not been explored yet. Thus, to investigate temporal and spatial distributions of each *Scrippsiella* species in Korean

coastal waters, species−specific primers and probes for each species were developed and then the abundances of each species in the sampling waters collected from 28 stations in 2016−2017 using the quantitative real−time PCR (qPCR) method.

2.2. Material and Method

Samples

Water samples were collected from the surface of 28 stations in East, West, and South Sea of Korea, in January, March, July, October, and December in 2016 and March, July, and October in 2017. Samples for qPCR (50−300 mL) were filtered through 25−mm GF/C filters (Whatman Inc., Floreham Park, NJ, USA). The filters were put into a 1.5−mL tube and preserved under −20℃. To extract the DNA from cells on the filters of each sample, the AccuPrep® Genomic DNA Extraction Kit (Bioneer Cooperation, Daejeon, Korea) was used with following manufacturer's instructions.

Culture of four *Scrippsiella* species

Four *Scrippsiella* species tested in the present study were collected by using water samplers from the coastal waters off Jeju, Gijang, Busan, and Masan, Korea during 2009−2017 (Table 2.1).

Nucleic acid extraction, polymerase chain reaction (PCR) amplification, and sequencing

For PCR amplification, nucleic acids from approximately 10-mL of a dense culture of each of four *Scrippsiella* species were extracted as described above. To amplify small subunit (SSU), internal transcribed spacer (ITS), and large subunit (LSU) of the ribosomal DNA (rDNA), the following primers were used: EukA, EukB (Stoeck et al. 2005), Euk1209F (Giovannoni et al. 1988), ITSF2, LSU500R, ITSR2 (Litaker et al. 2003), D1RF (Scholin et al. 1994), LSUB (Litaker et al. 2003), and 1483R (Daugbjerg et al. 2000) (Table 2.2.). The DNA was amplified in a Mastercycler ep gradient (Eppendorf, Hamburg, Germany) using the following cycling conditions: 3 min at 94℃ pre-denaturation, followed by 35 cycles of 1 min at 94℃, 1 min at the annealing temperature, and 2 min at 72℃, with a final extension of 5 min at 72℃. PCR products were purified using an AccuPrep® DNA Purification Kit (Bioneer Cooperation, Daejeon, Korea), following manufacturer's instructions. The obtained sequences were edited and aligned using Contig Express (Invitrogen, Carlsbad, CA, USA).

Design of species specific probe and primer set

The program MEGA v.4 was used to identify the species-specific region of each target *Scrippsiella* species. To align and sort ITS sequences of the target *Scrippsiella* species used in this study, ITS rDNA sequences of the other strains of the target *Scrippsiella* species and related dinoflagellates, available from GenBank, were obtained from PCR amplification (Tamura et al. 2007). Manual searches of the alignments were conducted to determine unique

sequences and develop each target *Scrippsiella* species-specific qPCR assay. The sequences for the primer-probe set were selected from the regions that were conserved within the strains of each target *Scrippsiella* species, but allowed discrimination with other dinoflagellates (Table 2.2). The primer and probe sequences of each target species were analyzed with Primer 3 (Whitehead Institute, Cambridge, MA, USA; Howard Hughes Medical Institute, Chevi Chase, MD, USA) and Oligo Calc: Oligonucleotide Properties Calculator (Kibbe 2007) software for optimal melting temperature and secondary structure. NCBI blast was used to confirm whether there are matching specific primer and probe sequences with other eukaryotes or not. The primers and probe were synthesized by Bioneer Cooperation (Daejeon, Korea). The probe was dual-labeled with the fluorescent dyes, FAM and BHQplus (Bioneer Cooperation, Daejeon, Korea), at the 5' and 3' ends, respectively.

Table 2. 1. Four *Scrippsiella* species used in the present study. Sampling location and time; water temperature (T, ℃); salinity (S) for isolation; mean equivalent spherical diameter (ESD, μm); cell volume (CV, x 10^3 $μm^3$).

Organism	Location	Time	T	S	ESD	CV
Scrippsiella acuminata (STJJ1005)	Jeju, Korea	May. 2010	-	-	22.8	6.20
Scrippsiella donghaiensis (SDGJ1703)	Gijang, Korea	Mar. 2017	13.2	33.9	19.4	4.01
Scrippsiella lachrymosa (SLBS1703)	Busan, Korea	Mar. 2017	10.9	33.5	17.7	3.01
Scrippsiella masanensis (SMMS0908)	Masan Bay, Korea	Aug. 2009	27	31.5	22.0	5.68

Table. 2. 2. Oligonucleotide primers used to amplify the SSU, ITS, and LSU regions of ribosomal DNA and the species−specific primers and TaqMan probes of each *Scrippsiella* species used in this study.

Name	Type	Primer region	5′ −3′	Reference
EukA	Forward	SSU	AAC CTG GTT GAT CCT GCC AGT	Stoeck et al. (2005)
EukB	Reverse	SSU	TGA TCC TTC TGC AGG TTC ACC TAC	Stoeck et al. (2005)
Euk1209F	Forward	SSU−ITS	GGG CAT CAC AGA CCT G	Giovannoni et al. (1988)
ITSF2	Forward	SSU−ITS	TAC GTC CCT GCC CTT TGT AC	Litaker et al. (2003)
ITSR2	Reverse	ITS	TCC CTG TTC ATT CGC CAT TAC	Litaker et al. (2003)
LSU500R	Reverse	ITS−LSU	CCC TCA TGG TAC TTG TTT GC	Litaker et al. (2003)
D1RF	Forward	LSU	ACC CGG TGA ATT TAA GCA TA	Scholin et al. (1994)
LSUB	Reverse	LSU	ACG AAC GAT TTG CAC GTC AG	Litaker et al. (2003)
1483R	Reverse	LSU	GCT ACT ACC ACC AAG ATC TGC	Daugbjerg et al. (2000)
Sacuminata_F	Forward	ITS	CCT GCT TCA GTG TCG ACT TCC	This study
Sacuminata_R	Reverse	ITS	CCA TGC ATT GAA CGC GTC AG	This study
Sacuminata_P	Probe	ITS	CAC TTT TGT GGT ACC GTT GCT TGG GTG	This study
Sdonghaiensis_F	Forward	ITS	GCA GGG GCT GGA TTC AGG	This study
Sdonghaiensis_R	Reverse	ITS	CAA CCA CTT CGG AGT ATA TCG	This study
Sdonghaiensis_P	Probe	ITS	TCG TCA CCC TCC TTT GCT TCA TTT TG	This study
Slachrymosa_F	Forward	ITS	TGC TTG CCA CCT CCT TTC AG	Lee et al. (2018)

Slachrymosa_R	Reverse	ITS	TCG ATC ACT GAA AGT TGT AAG AAG GAA A	Lee et al. (2018)
Slachrymosa_P	Probe	ITS	TCA CCC ACC TTT TGC CTT GAT CTT GCC	Lee et al. (2018)
Smasanensis_F	Forward	ITS	ATG GAG GTG CTT GCA TCG ATG	This study
Smasanensis_R	Reverse	ITS	CAG AAG CAA CAA TGG AGC AA	This study
Smasanensis_P	Probe	ITS	CCT TTC AGT TCT TGT CGT CAC CTT CCC	This study

Specificity test and establishing standard curve

To confirm whether the developed probe and primer set is species specific, other dinoflagellates which are associated with each target *Scrippsiella* species were conducted using the following steps modified from Lee et al. (2017): 1 μL of DNA template, 0.2 μM of primers (forward and reverse) and 0.15 μM of probe (final concentrations), and 5 μL of HiFast Probe Hi-Rox (Genepole, Gwangmyung, Korea) were combined and DDW was added to each sample, resulting in the total final volume of 10 μL. The qPCR analyses were performed using Rotor-Gene Q (Qiagen, Hilden, Germany) under following thermal cycling conditions: 3 min at 95℃, followed by 40 cycles of 10 s at 95℃, and 40 s at 58℃.

To obtain the standard curve, DNA was extracted from a dense clonal culture of the target *Scrippsiella* species, targeting 100,000 cells in the final elution volume of 100 μL, using the identical method mentioned above. The extracted DNA of the target *Scrippsiella* species was then diluted to 1, 10, 100, 1,000, 10,000, 100,000 cells by adding deionized sterile water (DDW) (Bioneer) to the 1.5-mL tubes. The samples were stored under -20℃ in the freezer.

The qPCR assays were performed as Lee et al. (2017) to establish the standard curve of each *Scrippsiella* species and extracted DNA of each samples were analyzed in quadruplicate to improve the accuracy of results. Samples using DDW as the template were used as the negative control, whereas the DNA used to construct standard curve was used as positive and standard

control.

2.3. Result

Morphological differences of four *Scrippsiella* species

The cell length and width (length–width) of *S. acuminata*, *S. masanensis*, *S. donghaiensis*, and *S. lachrymosa* were 32–22, 23–21, 22–17, and 20–16 μm, respectively (Table 2.3). *S. acuminata*, *S. donghaiensis*, and *S. lachrymosa* were observed to be conical in epitheca, and rounded in hypotheca and also the apical horn was upright collar with smooth circular–ridged shape. However, *S. masanensis* were rounded in epitheca and hypotheca and no upright collar. *S. acuminata* and *S. lachrymosa* form calcareous cysts, while cyst ornament of *S. donghaiensis* and *S. masanensis* were noncalcareous. All four *Scrippsiella* species has apical pore plate(Po) and canal plate(x). *S. donghaiensis* and *S. lachrymosa* have long and narrow canal plate(x). In addition, the canal plate of *S. acuminata* was short and wide, but that of *S. masanensis* were short and narrow. Four *Scrippsiella* species has almost same plate formulae (Po, x, 4', 3a, 7'', 6c, 5s, 5''', 2''''), but *S. donghaiensis* has one different number of the sulcal plates (Po, x, 4', 3a, 7'', 6c, 6s, 5''', 2'''').

Molecular differences of four *Scrippsiella* species

When the sequences of the SSU rDNA of four *Scrippsiella* species were compared, *S. acuminata* was different by 17 bp

(1.0%) from *S. donghaiensis*, 24 bp (1.5%) from *S. lachrymosa*, and 9 bp (0.5%) from *S. masanensis*. In addition, *S. donghaiensis* was different by 25 bp (1.5%) from *S. lachrymosa*, and 15bp (0.9%) from *S. masanensis* and also *S. lachrymosa* was different by 20 bp (1.2%) from *S. masanensis* (Table 2.4A). Furthermore, the sequence of the LSU rDNA of *S. acuminata* was 26 bp (3.1%), 35 bp (4.2%), and 34 bp (4.0%) different from *S. donghaiensis*, *S. lachrymosa*, and *S. masanensis*, respectively. *S. donghaiensis* was 41 bp (4.6%) and 39 bp (4.2%) different from *S. lachrymosa* and *S. masanensis*. In addition, 50 bp (4.0%) difference between *S. lachrymosa* and *S. masanensis* (Table 2.4B).

Table 2. 3. Morphological differences among four *Scrippsiella* species observed by light microscopy (LM) and scanning electron microscopy (SEM).

	S. acuminata (STJJ1005)	*S. donghaiensis* (SDGJ1703)	*S. lachrymosa* (SLBS1703)	*S. masanensis* (SMMS0908)
Cell length (μm)	25−37 (32) [a]	18.5−28.4 (22.2) [b]	16.3−23.2 (19.5)	19.3−27.1 (23.3)
Cell width (μm)	17−27 (22) [a]	13.5−20.8 (16.7) [b]	13.0−21.5 (15.9)	17.9−24.6 (20.6)
Shape of Epitheca/ hypotheca	Conical / Rounded	Conical / Rounded	Conical / Rounded	**Rounded** / Rounded
Shape of Apical horn	Upright collar, pore plate with smooth circular ridge	Upright collar, pore plate with smooth circular ridge	Upright collar, pore plate with smooth circular ridge	**No upright collar**, pore plate with smooth circular ridge
Cyst ornament	**calcareous**	**noncalcareous**	**calcareous**	**noncalcareous**
Apical pore plate (Po)	Yes	Yes	Yes	Yes
canal plate (x)	**short, wide** [a]	**long, narrow**	**long, narrow**	**short, narrow**
Number of Apical plate	4	4	4	4
Number of intercalary plate	3	3	3	3
Number of precingular plate	7	7	7	7
Number of cingular plate	6	6	6	6
Number of sulcal plate	**5**	**6**	**5**	**5**
Number of postcingular plate	5	5	5	5
Number of Antapical plate	2	2	2	2

Plate Formulae	Po, x, 4', 3a, 7'', 6c, 5s, 5''', 2''''	Po, x, 4', 3a, 7'', 6c(5c+t), 6s, 5''', 2''''	Po, x, 4', 3a, 7'', 6c, 5s, 5''', 2''''	Po, x, 4', 3a, 7'', 6c(5c+t), 5s, 5''', 2''''
Reference	Lewis (1991), Kretschmann et al. (2015) [a]	Gu et al. (2008), This study [b]	Lee et al. (2018)	Lee et al. in revision

Table 2. 4. Comparison of the sequences of the small subunit (SSU) and large subunit (LSU) ribosomal DNA of the *Scrippsiella* species. The numbers are base pairs different from each other. The value in parenthesis indicate dissimilarity (%).

A. SSU

	S. acuminata (STJJ1005)	*S. donghaiensis* (SDGJ1703)	*S. lachrymosa* (SLBS1703)	*S. masanensis* (SMMS0908)
S. acuminata (STJJ1005)	–	17(1.0%)	24(1.5%)	9(0.5%)
S. donghaiensis (SDGJ1703)	–	–	25(1.5%)	15(0.9%)
S. lachrymosa (SLBS1703)	–	–	–	20(1.2%)

B. LSU

	S. acuminata (STJJ1005)	*S. donghaiensis* (SDGJ1703)	*S. lachrymosa* (SLBS1703)	*S. masanensis* (SMMS0908)
S. acuminata (STJJ1005)	–	26(3.1%)	35(4.2%)	34(4.0%)
S. donghaiensis (SDGJ1703)	–	–	41(4.6%)	38(4.2%)
S. lachrymosa (SLBS1703)	–	–	–	50(4.0%)

Nationwide distributions of four *Scrippsiella* species

In this study, all four *Scrippsiella* species (*Scrippsiella acuminata*, *S. donghaiensis*, *S. lachrymosa*, and *S. masanensis*) were detected in Korean coastal waters in January 2016. The ranges of temperature and salinity of the Korean coastal waters when the four *Scrippsiella* were found were 3.5−15.1℃ and 28.7−34.4. In contrast, theses *Scrippsiella* species rarely appeared in October and December 2016 and October 2017 when water temperature and salinity were 5.9−24.5℃ and 11.5−35.6, respectively.

Moreover, any of four *Scrippsiella* species was not detected in Gosan, Seogwipo, Wimi, Seongsan, and Gimnyeong in the coastal waters of Jeju Island, Donghae on the east coasts, Kwangyang on the south coasts, and Seocheon on the west coasts of Korea among the 28 stations in the study period (Fig 2.1). On the other hand, the *Scrippsiella* species except *S. acuminata* appeared most frequently in Mokpo in January and July 2016, March, July and October 2017 when the temperature and salinity ranged from 7.6 to 25.8℃ and from 27.7 to 31.4, respectively (Table 2.5−8).

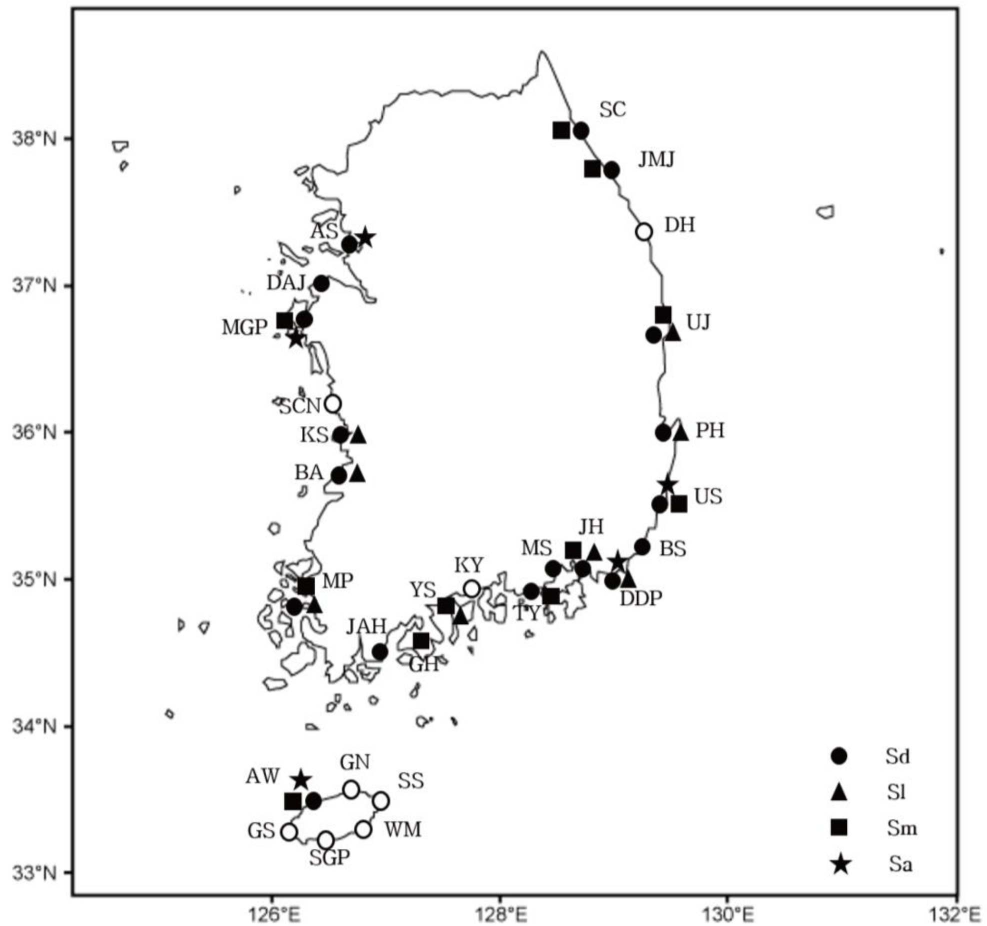


Fig 2. 1. Map of the sampling stations of the study area in Korea. The closed circles (●), triangles (▲), square(■), and stars(★) indicate the stations where *Scrippsiella donghaiensis*, *S. lachrymosa*, *S. masanensis* and *S. acuminata* were detected. The stations where four *Scrippsiella* species were not detected are indicated by opened circles (○). SC, Sokcho; JMJ, Jumunjin; DH, Donghae; UJ, Uljin; PH, Pohang; US, Ulsan; BS, Busan; DDP, Dadaepo; MS, Masan; JH, Jinhae; TY, Tongyoung; YS, Yeosu; KY, Kwangyang; GH, Goheung; JAH, Jangheung; AS, Ansan; DAJ, Dangjin; MGP, Mageompo; SCN, Seocheon; KS, Kunsan; BA, Buan; MP, Mokpo; AW, Aewol; GS, Gosan; SGP, Seogwipo; WM, Wimi; SS, Seongsan; GN, Gimnyeong.

Scrippsiella acuminata

Cells of *S. acuminata* were detected in 5 stations (Ulsan, Busan, Ansan, Mageompo, Aewol) in January, July, and October 2016, and October 2017 (Table 2.5), furthermore, the ranges of temperature and salinity were 9.6−22.5℃ (avr 17.9℃) and 23.6−33.6 (avr 29.7), respectively (Table 2.9, Fig 2.2). The highest density of *S. acuminata* (1,825 cells L^{-1}) was observed in Dadaepo in January 2016 when the temperature and salinity of this region was 9.6℃ and 33.6.

Scrippsiella donghaiensis

S. donghaiensis was found in 18 stations (Sokcho, Jumunjin, Uljin, Pohang, Ulsan, Busan, Dadaepo, Jinhae, Masan, Tongyoung, Jinhae, Ansan, Dangjin, Mageompo, Kunsan, Buan, Mokpo, Aewol) in January, March, and July 2016 and March, July, and October 2017, which means the most variety distributed among the four *Scrippsiella* speices (Table 2.6) and the ranges of temperature and salinity at this time was 9.3−28.0℃ (avr 20.4℃) and 9.9−34.3. (avr 29.6) (Table 2.9, Fig 2.2). They were usually observed between 1 and 538 cells L^{-1}. In July 2017, the highest abundance (1,175 cells L^{-1}) was detected in Busan where the water temperature and salinity were 20.9℃ and 29.8, respectively. In addition, cells of *S. donghaiensis* were found in 10 stations (Sokcho, Jumunjin, Uljin, Dadaepo, Masan, Jangheung, Kunsan, Buan, Mokpo, Aewol) in July 2016, and 12 stations (Sokcho, Jumunjin, Pohang, Ulsan, Busan, Jinhae, Masan, Ansan, Dangjin, Mageompo, Buan,

Mokpo) in July 2017.

Scrippsiella lachrymosa

Cells of *S. lachrymosa* appeared at 9 stations (Uljin, Pohang, Busan, Dadaepo, Jinhae, Yeosu, Kunsan, Buan, Mokpo) in January, and March 2016, and March 2017 (Table 2.7) and the ranges of water temperature and salinity at the stations were 5.4−14.9℃ (avr 11.4℃) and 30.5−34.3 (avr 32.4) (Lee et al. 2018) (Table 2.9, Fig 2.2). The highest abundance (26,325 cells L^{-1}) was found in Jinhae in March 2016, and the temperature and salinity were 13.2℃ and 32.4. Except at that time, *S. lachrymosa* were normally found between 21 and 6,188 cells L^{-1} in the surface waters of Korea and mainly in March 2016, 2017, except for January 2016 (Buan, 1,100 cells L^{-1}). However, there was no detection in the coastal water off Jeju Island.

Scrippsiella masanensis

Cells of *S. masanensis* appeared in 11 stations (Sokcho, Jumunjin, Uljin, Ulsan, Jinhae, Tongyoung, Yeosu, Goheung, Mageompo, Mokpo, Aewol) among 28 stations and 7 months (January, March, July, October, and December 2016, and March, and July 2017) of total 8 months (Table 2.8). The ranges of the temperature and salinity at the stations where *S. masanensis* was detected were 5.7−25.4℃ (avr 14.9℃), 19.4−34.1 (avr 31.6) (Table 2.9, Fig 2.2). In addition, they usually appeared between 1 and 21 cells L^{-1}. In 2016, the highest abundance was detected in

Goheung (25 cells L^{-1}) in October and Ulsan (25 cells L^{-1}) in December when the water temperature and salinity at each stations were 21.8℃, 19.4 and 13.7℃, 33.4, respectively.

Table 2. 5. The abundance of *Scrippsiella acuminata* (cells L^{-1}) in the surface water of Korean coasts.

	2016 01	2016 03	2016 07	2016 10	2016 12	2017 03	2017 07	2017 10	Max
SC	–	–	–	–	NA	–	–	–	–
JMJ	–	–	–	–	–	–	–	–	–
DH	–	–	–	–	–	–	–	–	–
UJ	–	–	–	–	–	–	–	–	–
PH	–	–	–	–	–	–	–	–	–
US	–	–	–	50	–	–	–	–	50
BS	–	–	–	–	–	–	–	–	–
DDP	1,825	–	–	–	–	–	–	–	1,825
JH	–	–	–	–	–	–	–	–	–
MS	–	–	–	–	–	–	–	–	–
TY	–	–	–	–	–	–	–	–	–
YS	–	–	–	–	–	–	–	–	–
KY	–	–	–	–	–	–	–	–	–
GH	–	–	–	–	–	–	–	–	–
JAH	–	–	–	–	–	–	–	–	–
AS	–	–	–	–	–	–	–	338	338
DAJ	–	–	–	–	–	–	–	–	–
MGP	–	–	–	–	–	–	–	550	550
SCN	–	–	–	–	–	–	–	–	–
KS	–	–	–	–	–	–	–	–	–
BA	–	–	–	–	–	–	–	–	–
MP	–	–	–	–	–	–	–	–	–
AW	–	–	775	–	–	–	–	1	775
GS	–	NA	NA	–	–	–	–	–	–
SGP	–	–	–	–	–	–	–	–	–
WM	–	–	–	–	–	–	–	–	–
SS	–	–	–	–	–	–	–	–	–
GN	–	NA	NA	–	–	–	–	–	–

Max, maximum abundance (cells L^{-1}); NA, not available; –, no detection of *S. acuminata.*

Table 2. 6. The abundance of *Scrippsiella donghaiensis* (cells L^{-1}) in the surface water of Korean coasts.

	2016 01	2016 03	2016 07	2016 10	2016 12	2017 03	2017 07	2017 10	Max
SC	–	–	38	–	NA	13	375	–	375
JMJ	–	–	1	–	–	–	50	–	50
DH	–	–	–	–	–	–	–	–	–
UJ	–	–	38	–	–	–	–	–	38
PH	–	–	–	–	–	–	1	–	1
US	–	–	–	–	–	–	175	–	175
BS	50	–	–	–	–	363	1,175	–	1,175
DDP	–	–	50	–	–	–	–	–	50
JH	–	–	–	–	–	–	54	–	54
MS	–	–	50	–	–	–	113	–	113
TY	–	–	–	–	–	1	–	–	1
YS	–	–	–	–	–	–	–	–	–
KY	–	–	–	–	–	–	–	–	–
GH	–	–	–	–	–	–	–	–	–
JAH	–	–	38	–	–	–	–	–	38
AS	–	–	–	–	–	1	538	–	538
DAJ	–	–	–	–	–	–	75	–	75
MGP	–	–	–	–	–	–	250	–	250
SCN	–	–	–	–	–	–	–	–	–
KS	–	–	88	–	–	–	–	–	88
BA	–	–	50	–	–	–	375	–	375
MP	–	–	50	–	–	–	25	1	50
AW	–	50	38	–	–	–	–	–	50
GS	–	NA	NA	–	–	–	–	–	–
SGP	–	–	–	–	–	–	–	–	–
WM	–	–	–	–	–	–	–	–	–
SS	–	–	–	–	–	–	–	–	–
GN	–	NA	NA	–	–	–	–	–	–

Max, maximum abundance (cells L^{-1}); NA, not available; –, no detection of *S. donghaiensis*.

Table 2. 7. The abundance of *Scrippsiella lachrymosa* (cells L^{-1}) in the surface water of Korean coasts.

	2016 01	2016 03	2016 07	2016 10	2016 12	2017 03	2017 07	2017 10	Max
SC	–	–	–	–	NA	–	–	–	–
JMJ	–	–	–	–	–	–	–	–	–
DH	–	–	–	–	–	–	–	–	–
UJ	–	–	–	–	–	275	–	–	275
PH	–	–	–	–	–	513	–	–	513
US	–	–	–	–	–	–	–	–	–
BS	–	–	–	–	–	213	–	–	213
DDP	–	1,128	–	–	–	–	–	–	1,128
JH	–	26,325	–	–	–	–	–	–	26,325
MS	–	–	–	–	–	–	–	–	–
TY	–	–	–	–	–	–	–	–	–
YS	–	238	–	–	–	–	–	–	238
KY	–	–	–	–	–	–	–	–	–
GH	–	–	–	–	–	–	–	–	–
JAH	–	–	–	–	–	–	–	–	–
AS	–	–	–	–	–	–	–	–	–
DAJ	–	–	–	–	–	–	–	–	–
MGP	–	–	–	–	–	–	–	–	–
SCN	–	–	–	–	–	–	–	–	–
KS	–	–	–	–	–	1,075	–	–	1,075
BA	1,100	1,475	–	–	–	–	–	–	1,475
MP	–	–	–	–	–	6,188	–	–	6,188
AW	–	–	–	–	–	–	–	–	–
GS	–	NA	NA	–	–	–	–	–	–
SGP	–	–	–	–	–	–	–	–	–
WM	–	–	–	–	–	–	–	–	–
SS	–	–	–	–	–	–	–	–	–
GN	–	NA	NA	–	–	–	–	–	–

Max, maximum abundance (cells L^{-1}); NA, not available; –, no detection of *S. lachrymosa.*

Table 2. 8. The abundance of *Scrippsiella masanensis* (cells L^{-1}) in the surface water of Korean coasts.

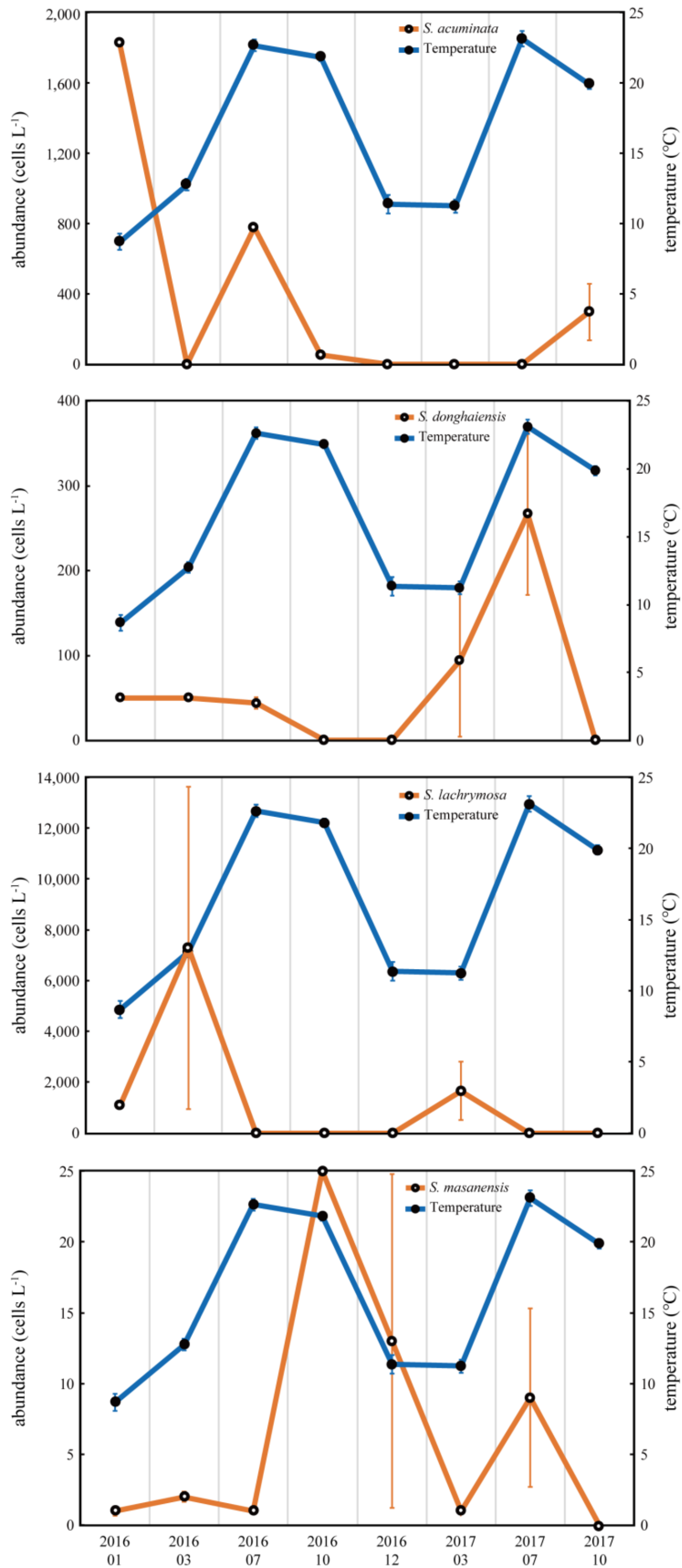
	2016 01	2016 03	2016 07	2016 10	2016 12	2017 03	2017 07	2017 10	Max
SC	–	1	1	–	NA	–	–	–	1
JMJ	–	3	1	–	–	1	–	–	3
DH	–	–	–	–	–	–	–	–	–
UJ	–	3	1	–	–	–	–	–	3
PH	–	–	–	–	–	–	–	–	–
US	–	–	–	–	25	–	–	–	25
BS	–	–	–	–	–	–	–	–	–
DDP	–	–	–	–	–	–	–	–	–
JH	–	–	–	–	–	–	4	–	4
MS	–	–	–	–	–	–	–	–	–
TY	3	–	–	–	–	–	21	–	21
YS	–	–	–	–	1	–	–	–	1
KY	–	–	–	–	–	–	–	–	–
GH	–	–	–	25	–	–	–	–	25
JAH	–	–	–	–	–	–	–	–	–
AS	–	–	–	–	–	–	–	–	–
DAJ	–	–	–	–	–	–	–	–	–
MGP	1	1	1	–	–	–	–	–	1
SCN	–	–	–	–	–	–	–	–	–
KS	–	–	–	–	–	–	–	–	–
BA	–	–	–	–	–	–	–	–	–
MP	1	–	–	–	–	–	1	–	1
AW	1	–	–	–	–	–	–	–	1
GS	–	NA	NA	–	–	–	–	–	–
SGP	–	–	–	–	–	–	–	–	–
WM	–	–	–	–	–	–	–	–	–
SS	–	–	–	–	–	–	–	–	–
GN	–	NA	NA	–	–	–	–	–	–

Max, maximum abundance (cells L^{-1}); NA, not available; –, no detection of *S. masanensis*.

Table 2. 9. Temperature, salinity, number of appeared months, and maximum density of four *Scrippsiella* species.

	Scrippsiella acuminata	*Scrippsiella donghaiensis*	*Scrippsiella lachrymosa*	*Scrippsiella masanensis*
T_{max}	22.5	28.0	14.9	25.4
T_{min}	9.6	9.3	5.4	5.7
T_{avr}	17.9	20.4	11.4	14.9
S_{max}	33.6	34.3	34.3	34.1
S_{min}	23.6	9.9	30.5	19.4
S_{avr}	29.7	29.6	32.4	31.6
no. of appeared months /total months	4/8	6/8	3/8	7/8
D_{max}	1,825	1,175	26,325	25

T_{max}, T_{min}, and T_{avr}, maximum, minimum, and average of temperature of the waters collected at the stations where each *Scrippsiella* species was detected; S_{max}, S_{min}, and S_{avr}, maximum, minimum, and average of salinity of the waters collected at the stations where each *Scrippsiella* species was detected; D_{max}, maximum density of each *Scrippsiella* species



S. acuminata
Temperature
abundance (cells L-1)
temperature (°C)
S. donghaiensis
Temperature
abundance (cells L-1)
temperature (°C)
S. lachrymosa
Temperature
abundance (cells L-1)
temperature (°C)
S. masanensis
Temperature
abundance (cells L-1)
temperature (°C)
2016 01
2016 03
2016 07
2016 10
2016 12
2017 03
2017 07
2017 10

Fig 2. 2. The average abundances (cells L^{-1}) of four *Scrippsiella* species in the Korean coastal waters where each species were detected and the average coastal water temperature (℃) from Jan 2016 to Oct 2017. (a) Average abundances of *S. acuminata* (b) *S. donghaiensis* (c) *S. lachrymosa* (d) *S. masanensis*. Open circles and closed circles represent average abundance of each *Scrippsiella* species and water temperature ± standard error.

2.4. Discussion

In this study, four *Scrippsiella* species are frequently distributed in the Korean coastal waters. These four *Scrippsiella* species have similar morphological characteristics (Table 2.3). In addition, dissimilarity of SSU and LSU rDNA are 0.5−1.5% and 3.1−4.6%, respectively (Table 2.4). However, they had different temporal and spatial distributions.

The ranges of water temperature and salinity at the stations where cells of *S. lachrymosa* appeared were 5.4−14.9℃ and 30.5−34.3, respectively, which were the narrowest among the four *Scrippsiella* species (Table 2.9). In addition, *S. lachrymosa* cells were usually detected in March 2016−2017. This results suggest that they may have seasonality (Fig 2.2 and 3), and the average of water temperature and salinity at 28 station in March were 12℃ and 32.6, respectively, indicating a relatively low water temperature. *S. lachrymosa* is located at the latitudes of 35° 07′ − 36° 54′ N and the other strains are 27° 12′ − 63° 42′ N (Zinssmeister et al. 2011, Soehner et al. 2012).

On the other hand, unlike *S. lachrymosa*, *S. donghaiensis* have a tendency to appear relatively high abundance in July, when

water temperature is high (Fig 2.2 and 3). Therefore, this species may also have seasonality and appear to have different ecological niche by reacting differently to the environment with *S. lachrymosa.* Furthermore, *S. donghaiensis* were most distributed around Korea peninsula among the four *Scrippsiella* species. Although cells of *S. donghaiensis* were detected relatively low density, they were distributed not only in the southern coast (Busan) in January 2016, when the water temperature is low, but also at 4 stations in March 2017. In addition, *S. donghaiensis* has the widest salinity range (9.9–34.3) among the four *Scrippsiella* species (Table 2.9). *S. donghaiensis* has been found in Australia, Sweden, Chile, Uruguay, and China (Gu et al. 2008, Zinssmeister et al. 2011). The Korean strain of *S. donghaiensis* is located at the latitudes of 33° 46′ – 38° 17′ N and the other strains are 29° 00′ – 58° 54′ N. However, the temperature and salinity data of the other strains were not indicated. Therefore, to better understand the ecophysiological characteristics of *S. donghaiensis*, this information would be a basis data.

Cells of *S. masanensis* were found in seven months of the total sampling eight months. Thus, *S. masanensis* has the broadest ranges of water temperature and salinity among four *Scrippsiella* species, but they were detected quite low density (1–25 cells L^{-1}) (Table 2.8). In addition, *S. masanensis* has a wide range of latitude. It has recently been established as a new species that is only found in China, Norway, and Korea. Korean strains are located at the latitudes of 33° 46′ – 38° 17′ N and the other strains are 27°

21′ – 63° 41′ N (Gu et al. 2008, Zinssmeister et al. 2011, Lee et al. 2018).

Furthermore, since the highest density (1,825 cells L^{-1}) of *S. acuminata* was observed during the study period in Dadaepo in January 2016, they tend to rarely observed in the surface waters of Korea (Table 2.5). However, this species is one of the most common species globally in the genus *Scrippsiella* and found in cyst or motile form in the sediments or surface waters of various marine environments even near the equator or polar regions. It has the widest latitude range among the other strains of the four *Scrippsiella* species (11° 54′ – 58° 45′ N), and Korean strains are located at the latitudes 33° 46′ – 37° 29′ N.

The results of this study did not show correlation between water temperature and salinity. But, since their distribution may also affect the abundance of predators, further studies should be needed. Consequently, I analyzed the distribution of *Scrippsiella* species in motile forms in surface waters of the Korean coastal. However, *Scrippsiella* species also exist in cyst form in the sediment and thus the distribution of *Scrippsiella* species may become wider if cycts are analyzed in Korean coastal waters. Therefore, the results of the present study provide a basis on understanding the distributions of these four *Scrippsiella* species and predicting of their red tides.

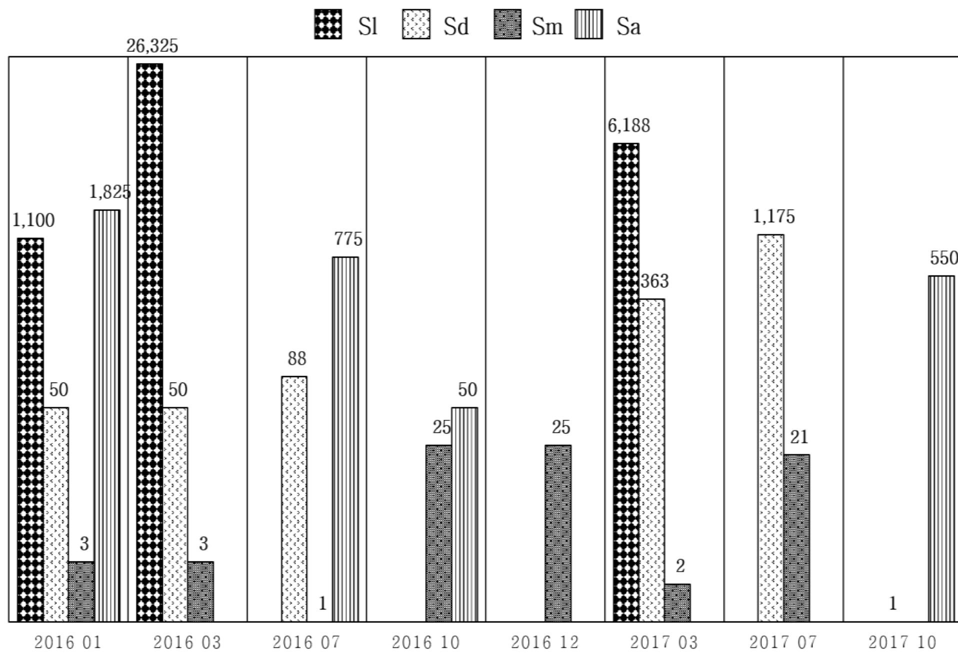


Fig 2. 3. The highest abundance of four *Scrippsiella* species from January 2016 to October 2017. The numbers on the bar indicate density (cells L^{-1}) of each *Scrippsiella* species. Sl, *Scrippsiella lachrymosa*; Sd, *Scrippsiella donghaiensis*; Sm, *Scrippsiella masanensis*; Sa, *Scrippsiella acuminata*.

Chapter 3. Differential interactions between four *Scrippsiella* species and common heterotrophic protists

3.1. Introduction

Since the genus *Scrippsiella* was first established by Balech (1959), approximately 30 species in the genus have been taxonomically accepted (Guiry and Guiry 2018). The *Scrippsiella* species are common phototrophic dinoflagellates in Korean waters and distributed worldwide (Estrada 1984, Ishikawa and Taniguchi 1993, Nehring 1994, Eker et al. 2000, Janofske 2000, Kim and Han 2000, Orlova and Morozova 2003, Giannakourou et al. 2005, Gottschling et al. 2005, Gu et al. 2008, Lee et al. 2018). The most common species in this genus is *Scrippsiella acuminata,* which is a non-toxic and cause red tides or harmful algal blooms globally (Licea et al. 2002, Yan et al. 2002, Lu and Hodgkiss 2004, Wong and Wong 2004). Its red tides can cause hypoxia near sea bottoms, eventually bringing the fish, larvae (Tang and Gobler 2012), shrimp (Gárate-Lizárraga et al. 2009) to death. Therefore, to understand their population dynamics, growth rate and mortality due to predation should be studied. However, there have been studies on mortality of only *S. acuminata* due to predation (Yoo et al. 2013b, Lee et al. 2014a, Löder et al. 2014, Saiz et al. 2014), although there have been studies on growth rates of several *Scrippsiella* species

(Montresor and Marino 1989, Taira et al. 2004, Zhuo−Ping et al. 2009, Luo et al. 2016). The maximum ingestion rate of *P. kofoidii* on *S. acuminata* was 16.6 ng C pseudocolony^{-1} d^{-1} (19.5 cells pseudocolony^{-1} d^{-1}) (Jeong et al. 2001b). Unfortunately, predation on other *Scrippsiella* species such as *S. donghaiensis* and *S. lachrymosa* have not been explored, although they have global distributions (Lewis 1991, Kuylenstierna and Karlson 2000, Olli and Anderson 2002, Hoppenrath 2004, Gu et al. 2008, Zinssmeister et al. 2011, Satta et al. 2014, Lee et al. 2018). Meanwhile, *Scrippsiella masanensis* has recently been established as a new species (Lee et al. in revision), however, mortality rate of *S. masanensis* due to predation has not been explored yet. Some morphological and biochemical characters of prey cells, such as swimming speed and toxicity, may affect feeding by predators (Hansen 1989, Jeong et al. 2018a, Tillmann and John 2002, Waggett et al. 2008). Swimming speed of *S. acuminata* has been reported (Jeong et al. 1999b), but there have been no studies on swimming speed of *S. donghaiensis*, *S. lachrymosa*, and *S. masanensis*. Furthermore, *S. acuminata* has been reported to be non−toxic (Bardouil et al. 1993, Hold et al. 2001), but there have been no studies on toxicity of *S. donghaiensis*, *S. lachrymosa*, and *S. masanensis*. Thus, it is worthwhile to investigate relationships between feeding rates and swimming speed or toxicity of prey species, which have not been reported yet.

Heterotrophic dinoflagellates and ciliates, major components of heterotrophic protists, are known to feed on diverse red−tide dinoflagellates (Hansen 1992, Matsuyama et al. 1999, Jeong et al.

2010b, Anderson and Menden-Deuer 2017). Grazing impact by these predators on populations of red tide dinoflagellates is high enough to control prey populations (e.g., Yoo et al. 2013a). In general, grazing impact by heterotrophic dinoflagellates and ciliates on a red tide dinoflagellate is greater than that by metazoans because the abundance of the former predators is much higher than that of the latter predators (Lee et al. 2017, Lim et al. 2017b). Thus, to assess grazing impact by predators on populations of a red tide dinoflagellate, the kind of heterotrophic protist predators that are able to feed on the red tide dinoflagellate and growth and ingestion rates of the protist predator on the red tide dinoflagellate should be determined. The heterotrophic dinoflagellates *Oxyrrhis marina*, *Gyrodinium dominans*, *P. kofoidii*, *Oblea rotunda*, and *Pfiesteria piscicida* and the ciliates *Strombidinopsis* sp. have been commonly found in diverse marine environments (Edward and Burkill 1995, Nakamura et al. 1995a, Burkholder and Glasgow 1997, Matsuoka et al. 2000, Jeong et al. 2004a, 2007b, Watts et al. 2010, Lee et al. 2014b). Thus, it is worthwhile to explore interactions between these protistan predators and *Scrippsiella* species whose predators have not been reported yet.

In the present study, feeding occurrence by common heterotrophic protistan predators (*O. marina*, *G. dominans*, *P. kofoidii*, *O. rotunda*, *P. piscicida* and ciliate *Strombidinopsis* sp.) on *S. acuminata*, *S. donghaiensis*, *S. lachrymosa*, and *S. masanensis* were investigated. Furthermore, growth and ingestion rates of *P. kofoidii* on *S. donghaiensis*, *S. lachrymosa*, and *S. masanensis* as a

function of prey concentration were determined. Moreover, the growth and ingestion rates of *G. dominans* and *O. marina* on 4 *Scrippsiella* species (*S. acuminata*, *S. donghaiensis*, *S. lachrymosa*, and *S. masanensis*) were explored at prey concentrations at which growth and ingestion rates of *P. kofoidii* on *S. acuminata* became saturated. In addition, although swimming speed of *S. acuminata* has been reported previous study (Jeong et al. 1999b), *S. acuminata* (STJJ1005), *S. donghaiensis*, *S. lachrymosa*, and *S. masanensis* were measured at identical conditions. Also, toxicity of these *Scrippsiella* species was investigated using nauplii of the brine shrimp *Artemia*. The results of the present study provide a basis on understanding predator-prey relationships between four *Scrippsiella* species and common heterotrophic protistan predators, bloom dynamics of these four species, differential feeding by predators on different species in the same genus.

3.2. Material and Method

Preparation on experimental organisms

The collection and culture of four *Scrippsiella* species (*S. acuminata*, *S. donghaiensis*, *S. lachrymosa*, and *S. masanensis*) were described in Table 2.1. All *Scrippsiella* species were grown at 20℃ in enriched F/2-Si seawater media (Guillard and Ryther, 1962) under 20 μE m^{-2} s^{-1} illumination on a 14:10 h light:dark cycle. The mean equivalent spherical diameter (ESD) ± standard deviation was measured using an electronic particle counter (Coulter

Multisizer II; Coulter Corporation, Miami, Florida, USA). The ESD of *S. acuminata* and *S. lacrymosa* were obtained from the previous studies (Jeong et al. 2005b, Lee et al. 2018), but that of *S. donghaiensis* and *S. masanensis* was measured in this study.

For the isolation and culture of the heterotrophic dinoflagellate (HTD) predators *Gyrodinium dominans*, *Oblea rotunda*, *Oxyrrhis marina* and *Polykrikos kofoidii* plankton samples were collected by using water samplers, from the coastal waters off Masan, Jinhae, Kunsan, and Jangheung, Korea during 2001–2016 (Table 3.1). A clonal culture of each species was established by using two serial single-cell isolations (Table 3.1). The culture of *Pfiesteria piscicida* (CCMP2091) was obtained from National Center for Marine Algae and Microbiota, USA. For the isolation and culture of the ciliate *Strombidinopsis* sp. (approximate cell length = 100 μm), plankton samples were collected by using a 10-μm mesh, in coastal waters off Yeosu, Korea in July 2018 when the water temperature and salinity were 27.5℃ and 32.4, respectively (Table 3.1).

The carbon contents of the HTDs were estimated from the cell volume (Menden-Deuer and Lessard 2000). The cell volumes of the predators were estimated using the methods of Kim and Jeong (2004) for *G. dominans*, that of Jeong et al. (2008b) for *O. marina*, Jeong et al. (2001a) for *P. kofoidii*, Jeong et al. (2007b) for *P. piscicida*, and Ok et al. (2017) for *O. rotunda*. The cell volume of *Strombidinopsis* sp. was measured in this study (Table 3.1).

Table 3. 1. Isolation and maintenance conditions for the experimental organisms.

Organism	Location	Time	T	S	FM	CV	Prey species	PC
Heterotrophic dinoflagellate								
Gyrodinium dominans (GDMS0907)	Masan Bay, Korea	Apr. 2007	15.1	33.4	EG	4.2	*Amphidinium carterae*	30,000–40,000
Oblea rotunda (ORJH1504)	Jinhae Bay, Korea	Apr. 2015	12.6	31.2	PA	5.3	*Amphidinium carterae*	10,000–20,000
Oxyrrhis marina (OMKS0105)	Kunsan Bay, Korea	May. 2001	16.1	27.7	EG	2.0	*Amphidinium carterae*	~8,000
Polykrikos kofoidii (PKJH201607)	Jangheung Bay, Korea	Jul. 2016	23.6	26.4	EG	43.0	*Alexandrium lusitanicum*	~7,000
Pfiesteria piscicida (CCMP2091)	Neuse River, USA	Jan. 1998	–	–	PE	1.3	*Amphidinium carterae*	~5,000
Ciliate								
Strombidinopsis sp. (SSYS1807)	Yeosu, Korea	Jul. 2018	27.5	32.4	EG	383.0	*Heterocapsa triquetra*	~7,000

Sampling location and time; water temperature (T, ℃); salinity (S) for isolation; feeding mechanisms (FM); cell volume (CV, $\times 10^3$ μm^3); prey species and concentrations (PC, cells · mL^{-1}) for maintenance. EG, engulfment feeder; PA, pallium feeder; PE, peduncle feeder.

Interactions between four *Scrippsiella* species and heterotrophic protists

Experiment (Expt) 1 was designed to examine interactions between each of four *Scrippsiella* species and the target heterotrophic protists by mixing two components (Table 3.2). In this experiment, it was tested whether the target heterotrophic protist is able to feed on each of the *Scrippsiella* species and also whether any of four *Scrippsiella* species is able to lyse or immobilize the target heterotrophic protist.

The initial concentrations of four *Scrippsiella* species and each target heterotrophic protists were settled as described in Table 3.2. It was performed using an autopipette to deliver a predetermined volume of culture with a known cell density into the wells of 6-well plate chambers. For each heterotrophic protists species, duplicate wells containing mixtures of each of four *Scrippsiella* species and heterotrophic protists (a total of 5 mL in each well), duplicate predator and prey control wells containing a culture of heterotrophic protists and four *Scrippsiella* species only (a total of 5 mL in each well) were established. The plate chambers were placed on a shelf and incubated at 20℃ under a 14:10 h light:dark cycle of 20 μE m^{-2} s^{-1} provided with cool white fluorescent light.

After 2- and 24-h incubation, to determine whether or not the target predator species were able to feed on the target *Scrippsiella* species, more than 30 target predator cells in the well were tracked using a dissecting microscope at a magnification of

×10–63 (SZX10, Olympus, Tokyo, Japan). Predators containing ingested *Scrippsiella* cells were photographed on slides with cover-glasses at a magnification of ×100–400 with a digital camera (Zeiss AxioCam HRc5; Carl Zeiss Ltd., Göttingen, Germany) attached to the microscope. The feeding and lysis process of the target heterotrophic protist on the target *Scrippsiella* species was recorded using a video analyzing system (Sony DXC-C33, Sony Co., Tokyo, Japan) and also captured using the digital camera.

Cells of *P. kofoidii* were lysed at a high *S. donghaiensis* concentration (concentration = ca. 8,000 cells mL^{-1}) in Expt 1. To investigate whether cell or filtrate of *S. donghaiensis* lyses the *P. kofoidii* cells, an additional experiment was designed. Dense cultures of *S. donghaiensis* (concentration = ca. 10,000 cells mL^{-1}) were filtered through 0.2-μm disposable syringe filter (DISMIC-25CS type, 25mm; Advantec, Toyo Roshi Kaisha, Ltd, Chiba, Japan) to get *S. donghaiensis* culture filtrates. Cells of *P. kofoidii* and a predetermined volume of *S. donghaiensis* culture filtrate were transferred to 6-well plate chambers and filled with freshly-filtered seawater to the target volume as described in Table 3.2 (a total of 5 mL in each well). Cells of *P. kofoidii* were monitored after 0, 24 and 48 h incubation.

Table 3. 2. Experimental design. The numbers in the prey and predator columns are the actual initial densities (cells mL^{-1}) of prey and predator. The values within parentheses in the predator column are the predator densities in the control bottles.

Expt No.	Prey		Predator	
	Species	Density	Species	Density
1	*Scrippsiella* species	5,000−8,000	*Gyrodinium dominans*	1,000
			Oxyrrhis marina	1,000
			Polykrikos kofoidii	50
			Oblea rotunda	1,000
			Pfiesteria piscicida	1,500
			Strombidinopsis sp.	5
2	*Scrippsiella donghaiensis*	91, 167, 359, 1,457, 2,978, 5,002, 8,746, 9,725, 10,733	*Polykrikos kofoidii*	16, 28, 33, 63, 68, 83, 78, 89, 97 (60)

3	*Scrippsiella lachrymosa*	87, 154, 348, 701, 1,461, 3,006, 5,188, 7,782, 11,276	*Polykrikos kofoidii*	16, 23, 28, 38, 41, 53, 60, 78, 93 (40)
4	*Scrippsiella masanensis*	93, 189, 373, 875, 1,459, 3,063, 5,472, 8,281, 10,645	*Polykrikos kofoidii*	16, 28, 39, 56, 64, 69, 80, 91, 110 (66)
5	*Scrippsiella acuminata*	9,549	*Gyrodinium dominans*	485 (524)
6	*Scrippsiella donghaiensis*	4,946	*Gyrodinium dominans*	183 (260)
7	*Scrippsiella lachrymosa*	9,700	*Gyrodinium dominans*	524 (569)
8	*Scrippsiella masanensis*	5,232	*Gyrodinium dominans*	233 (265)
9	*Scrippsiella acuminata*	3,148	*Oxyrrhis marina*	172 (127)
10	*Scrippsiella donghaiensis*	2,753	*Oxyrrhis marina*	131 (127)
11	*Scrippsiella lachrymosa*	2,735	*Oxyrrhis marina*	131 (127)
12	*Scrippsiella masanensis*	2,552	*Oxyrrhis marina*	201 (127)

Growth and ingestion rates of *Polykrikos kofoidii* on *Scrippsiella* species as a function of prey concentration

In Expt 2-4, the growth and ingestion rates of *P. kofoidii* on three *Scrippsiella* species (*S. donghaiensis*, *S. lachrymosa*, and *S. masanensis*) as a function of prey concentration were determined (Table 3.2). For comparison, data on the growth and ingestion rates of *P. kofoidii* on *S. acuminata* as a function of prey concentration were obtained from Jeong et al. (2001b).

In these experiments, a dense cultures of *P. kofoidii* growing on *Alexandrium minutum* CCMP 1888 (= *A. lusitanicum*) was transferred to a 250-mL culture flask when the prey cells were no longer detectable 1 d before these experiments. The bottle was filled to capacity with freshly-filtered seawater, capped, and placed on plankton wheels rotating at 0.9 rpm, and incubated at 20℃ under illumination of 20 μE m^{-2} s^{-1} on a 14:10 h light:dark cycle. This was done to minimize possible residual growth resulting from the ingestion of prey during batch culture. One day later, the cells in three 1-mL aliquots from the bottle was counted using a compound microscope to determine the concentrations of predator cells, and the cultures were then used to conduct experiments.

For each experiment, the initial concentrations of *P. kofoidii* and the target *Scrippsiella* species were established using an autopipette (Table 3.2). Triplicate 42-mL PC experimental bottles (mixtures of predator and prey), triplicate prey control bottles (*Scrippsiella* prey only), and triplicate predator control bottles (*P. kofoidii* predator only) at a single predator concentration were

settled for each predator–prey combination. The same amount the prey filtrate as the volume of the prey culture added into the experimental bottles were added to the predator control bottles to make the water conditions similar. The bottles were filled to capacity with freshly-filtered seawater and capped. To determine the actual initial predator and prey densities at the beginning of the experiment, a 5-mL aliquot was removed from each bottle, fixed with 5% Lugol's solution, and examined with a light microscope to determine predator and prey abundances by enumerating the cells in three 1-mL Sedgwick–Rafter chambers (SRCs). The bottles were refilled to capacity with F/2-Si medium, capped, and placed on rotating wheels under the conditions as described above. Dilution of the cultures associated with refilling the bottles was considered ingestion rates were calculated. After 48h, 10-mL aliquot was taken from each bottle and fixed with 5% Lugol's solution, and then the abundances of predators and prey were determined by counting all or more than 200 cells in three 1-mL SRCs. The conditions of predators and prey were assessed using a dissecting microscope as described above prior to subsampling.

The specific growth rates of heterotrophic protist predators, $\mu (d^{-1})$ were calculated as follows:

$$\mu (d^{-1}) = [\mathrm{Ln}\ (P_t/P_0)]/t \qquad (1)$$

where P_0 and P_t = the concentrations of predators at 0 d and 2 d, respectively.

Data for growth rates of heterotrophic protist predators were fitted to a modified Michaelis-Menten equation:

$$\mu(d^{-1}) = \mu_{max}(x-x')/[K_{GR}+(x-x')] \qquad (2)$$

where μ_{max} = the maximum growth rate (d^{-1}); x=prey concentration (cells mL^{-1} or ng C mL^{-1}), x' =threshold prey concentration (the prey concentration where μ=0), K_{GR}=the prey concentration sustaining ½ μ_{max}. One pseudocolony of *P. kofoidii* was treated as one predator as in Jeong et al. (2001b). Data were iteratively fitted to the model using DeltaGraph® (Red Rock Software Inc., Salt Lake, Ut).

Ingestion rates and mean prey concentration were calculated using the equations of Frost (1972) and Heinbokel (1978). The incubation time for calculating ingestion rates was the same as that for estimating growth rate. Data for heterotrophic protist predators ingestion rates (IR, cells $predator^{-1}$ d^{-1} or ng C $predator^{-1}$ d^{-1}) were fitted to a modified Michaelis-Menten equation:

$$IR = I_{max}(x)/[K_{IR}+(x)] \qquad (3)$$

Where I_{max}=the maximum ingestion rate (cells $predator^{-1}$ d^{-1} or ng C $predator^{-1}$ d^{-1}); x=prey concentration (cells mL^{-1} or ng C mL^{-1}), and K_{IR}=the prey concentration sustaining ½ I_{max}.

Growth and ingestion rates of heterotrophic protists on four *Scrippsiella* species at single prey concentrations

Expt 5-8 (or Expt 9-12) were designed to measure the growth and ingestion rates of *Gyrodinium dominans* (or *Oxyrrhis marina*) on each of four *Scrippsiella* species at single high prey concentrations (Table 3.2). Dense cultures of potential predators were transferred into 500-mL PC bottles for *G. dominans* (or *O.*

marina) when the target prey cells were no longer detectable. The bottles were filled to capacity with freshly-filtered seawater, capped, and placed on plankton wheels rotating at 0.9 rpm. These bottles were incubated at the temperature and light conditions described above. One day later, three 1-mL aliquots from each bottle were counted using a compound microscope to determine the cell concentrations of predators, and the cultures were then used to conduct experiments.

For each experiment, the initial concentrations of *G. dominans* (or *O. marina*; predator) and each of four *Scrippsiella* species (prey) were established using an autopipette (Table 3.2). For *G. dominans* (or *O. marina*), triplicate 42-mL PC experimental bottles (mixtures of predator and prey), triplicate control bottles (prey only), and triplicate control bottles (predator only) were set up. To obtain similar water conditions, the water of the prey culture was filtered and then added to the predator control bottles as described above. All the bottles were then filled to capacity with freshly-filtered seawater and capped. The actual predator and prey densities at the beginning of the experiment were determined as stated above. The bottles were refilled to capacity with F/2-Si medium, capped, and placed on rotating wheels under the conditions described above. Dilution of the cultures associated with refilling the bottles for *G. dominans* (or *O. marina*) was considered as described above. The incubation time was 48 h. The abundances of predators and prey at the end of the experiments were determined as described above.

Swimming speed of *Scrippsiella* species

A culture of four *Scrippsiella* species (approximately 8,000–10,000 cells mL^{-1}) growing autotrophically in a 38-mL cell culture flask (BD Biosciences, MA, USA) was examined under a dissecting microscope. The video camera focused on an individual field, viewed as a single circle, in the flask under a dissecting microscope (SZX10, Olympus, Tokyo, Japan) at 20℃. Swimming of *Scrippsiella* cells was recorded at a magnification of ×25 using a video analyzing system (Samsung Techwin SRD-1673DN, Korea). The mean and maximum swimming speeds of all swimming cells viewed during 30 min were analyzed. The average swimming speed (n = 20) was calculated based on the linear displacement of cells in 1 s during a single-frame playback.

Cells of *S. donghaiensis* usually moved forward with spin, but sometimes suddenly jumped in a straight line. Thus, the swimming speed was measured when the cells jumped in a straight line.

Bioassay of toxicity of the four *Scrippsiella* species

The potential toxicity of four *Scrippsiella* species (*S. acuminata*, *S. donghaiensis*, *S. lachrymosa*, *S. masanensis*) was tested using the brine shrimp (*Artemia salina*) lethality test (Solis et al., 1993). The eggs of *A. salina* were hatched after being incubated for 2 days in freshly filtered seawater at 20℃ under cool white fluorescent light. Ten A. salina were added to each triplicate experimental wells containing *Scriippsiella* cells and filtrate,

respectively. Ten *A. salina* nauplii were added to each of triplicate experimental wells containing *Scriippsiella* cells (target initial abundances = 10, 100, 500, 1,000, 5,000, 8,000, and 10,000 cells mL^{-1}) and filtrate from the equivalent abundances, respectively. Triplicate wells of containing *A. salina* nauplii only were also set up for control. The filtrate was obtained through 0.2-μm disposable syringe filter (DISMIC-25CS type, 25mm). The plate chambers were incubated on a shelf in a culture room at 20℃ under an illumination of 20 μE m^{-2} s^{-1} on a 14:10 h light:dark cycle. At the beginning of the incubation and 6, 12, 24, and 48h later, live and dead nauplii were counted using a dissecting microscope.

3.3. Result

Interactions between four *Scrippsiella* species and common heterotrophic protists

All heterotrophic protist predators tested in the present study were able to feed on all four *Scrippsiella* species (Table 3.3). Cells of *Oxyrrhis marina*, *Gyrodinium dominans*, *Polykrikos kofoidii* and ciliate *Strombidinopsis* sp. engulfed whole bodies of actively swimming cells of each species (Fig 3.1-2). Moreover, the pallium feeder *Oblea rotunda* cells anchored actively swimming prey cells by deploying a filament to the surface of the prey cells and then enclosing whole body of the prey cells using the pallium. In contrast, the peduncle feeder *P. piscicida* did not feed on actively swimming prey, but it rarely fed on motionless prey cells sitting on the bottom

of a multi-well chamber using the peduncle.

Among the four *Scrippsiella* species tested, cells and filtrates of only *S. donghaiensis* lysed the cell bodies of *P. kofoidii* (Fig 3.2e). This evidence suggests that *S. donghaiensis* lyses the cells of *P. kofoidii* by using excreted chemicals.

Table 3. 3. Taxa and size of potential heterotrophic dinoflagellate (HTD) and naked ciliate predators on four *Scrippsiella* species in Expt 1. The values within parentheses in the predator column are the predator's equivalent spherical diameter (ESD, μm). O: feeding, O[a]: rarely feeding on motionless cells.

prey species	ESD (μm)	Potential predators						Growth		
		P. piscicida (13.5)	*O. marina* (15.6)	*G. dominans* (20.0)	*O. rotunda* (21.6)	*P. kofoidii* (43.5)	*Strombidinopsis* sp. (113.3)	P. k	O. m	G. d
S. acuminata	22.8	O[a]	O	O[b]	O	O[c]	O	+++	++	++
S. donghaiensis	19.4	O[a]	O	O	O	O	O	−−	++	+
S. lachrymosa	17.7	O[a]	O	O	O	O	O	+	+++	+++
S. masanensis	22.0	O[a]	O	O	O	O	O	−	+	+

Growth rate: +++ : > $0.7d^{-1}$; ++: 0.7 ~ > $0.4d^{-1}$; +: 0.4 ~ > $0d^{-1}$; −: 0 ~ > $-0.2d^{-1}$; −−: < $-0.2d^{-1}$

O[b] : Nakamura et al. (1995b), O[c] : Jeong et al. (2001b)

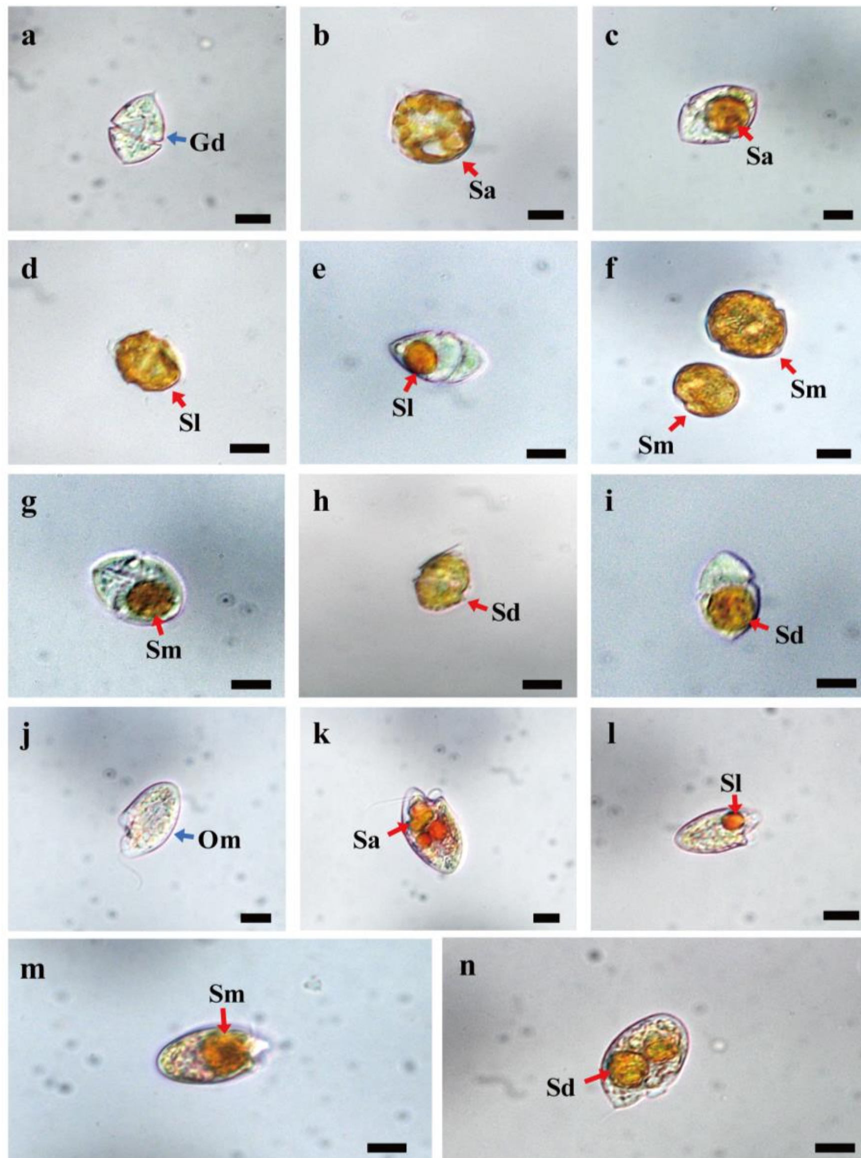


Fig 3. 1. Feeding by the heterotrophic protists (blue arrows) on *Scrippsiella* spp. (red arrows) using a light microscope. (a) An unfed *Gyrodinium dominans* (Gd) cell. (b) *Scrippsiella acuminata* (Sa) cell. (c) *G. dominans* cell with an ingested *S. acuminata* cell. (d) *Scrippsiella lachrymosa* (Sl) cell. (e) *G. dominans* cell with an ingested *S. lachrymosa* cell. (f) Two *Scrippsiella masanensis* cells. (g) *G. dominans* cell with an ingested *S. masanensis* cell. (h) *Scrippsiella donghaiensis* cell. (i) *G. dominans* cell with an ingested *S. donghaiensis* cell. (j) An unfed *Oxyrrhis marina* (Om) cell. (k) *O. marina* cell with several ingested *S. acuminata* cells. (l) *O. marina* cell with an ingested *S. lachrymosa* cell. (m) *O. marina* cell with an ingested *S. masanensis* cell. (n) *O. marina* cell with two ingested *S. donghaiensis* cells. Scale bars = 10 μm.

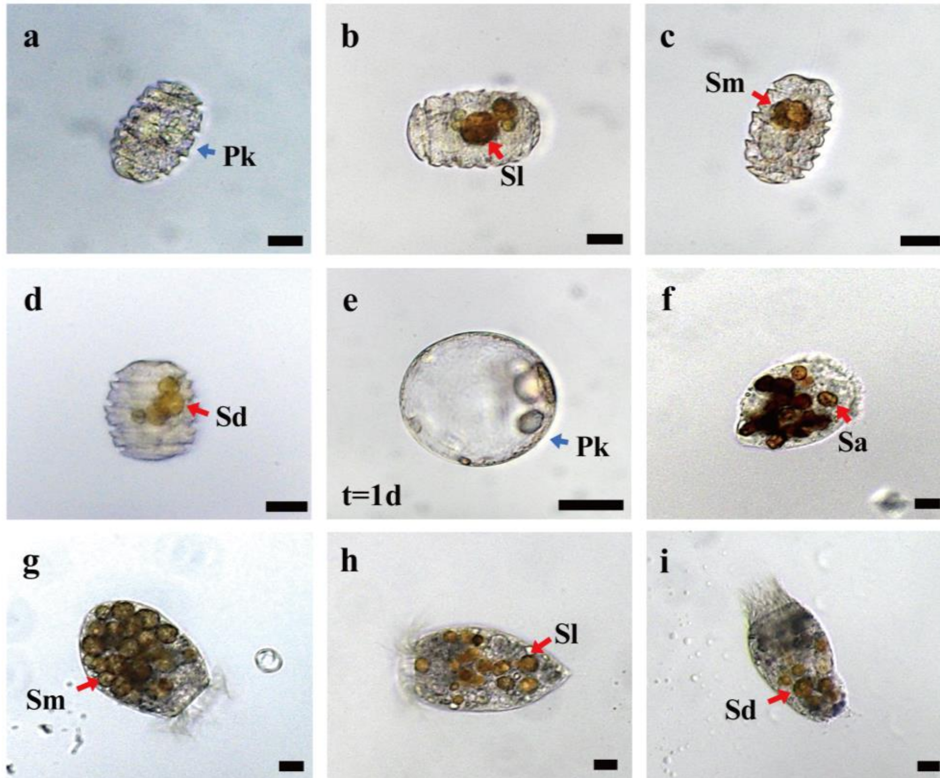


Fig 3. 2. Heterotrophic protists (blue arrows) feeding on *Scrippsiella* species (red arrows) and lysis of a *Polykrikos kofoidii* (Pk) cell incubated with *Scrippsiella donghaiensis* (Sd) observed under a light microscope. (a) An unfed *P. kofoidii* cell. (b) *P. kofoidii* cell with two ingested *Scrippsiella lachrymosa* (Sl) cells. (c) *P. kofoidii* cell with an ingested *Scrippsiella masanensis* (Sm) cell. (d) *P. kofoidii* cell with several *S. donghaiensis* cells. (e) Lysed *P. kofoidii* cell one day after incubation with *S. donghaiensis* cells. (f) The naked ciliate *Strombidinopsis* sp. cell with several ingested *Scrippsiella acuminata* (Sa) cells. (g) *Strombidinopsis* sp. cell with several ingested *S. masanensis* cells. (h) *Strombidinopsis* sp. cell with several ingested *S. lachrymosa* cells. (i) *Strombidinopsis* sp. cell with several ingested *S. donghaiensis* cells. Scale bars = 20 μm.

Effect of *Scrippsiella* concentration on growth and ingestion rates of *Polykrikos kofoidii*

The numerical (growth) and functional (ingestion) responses by *P. kofoidii* to the concentrations of *S. lachrymosa*, *S. donghaiensis*, and *S. masanensis* were clearly different from one another prey species (Fig 3.3–4).

With increasing mean prey concentration, the specific growth rate of *P. kofoidii* on *S. lachrymosa* increased rapidly at the prey concentrations < 672 ng C mL^{-1} (equivalent to 1,919 cells mL^{-1}), but slowly at higher prey concentrations (Fig 3.3a). When the data were fitted to Eq. (2), the maximum specific growth rate of *P. kofoidii* on *S. lachrymosa* was 0.519 d^{-1}.

There was no clear pattern in the specific growth rates of *P. kofoidii* on *S. masanensis* as a function of mean prey concentration (Fig 3.3b). The range of the specific growth rates of *P. kofoidii* on *S. masanensis* were from −0.05 d^{-1} to −0.442 d^{-1}, but the rates were not significantly affected by mean prey concentration ($p>0.1$, ANOVA).

The range of the specific growth rates *P. kofoidii* on *S. donghaiensis* were from −0.034 d^{-1} to −0.671 d^{-1} (Fig 3.3c). The specific growth rates of *P. kofoidii* on *S. donghaiensis* at the prey concentrations < 2,469 ng C mL^{-1} (5,610 cells mL^{-1}) were not significantly different from one another ($p>0.1$, ANOVA), but they decreased at higher prey concentrations with increasing mean prey concentration.

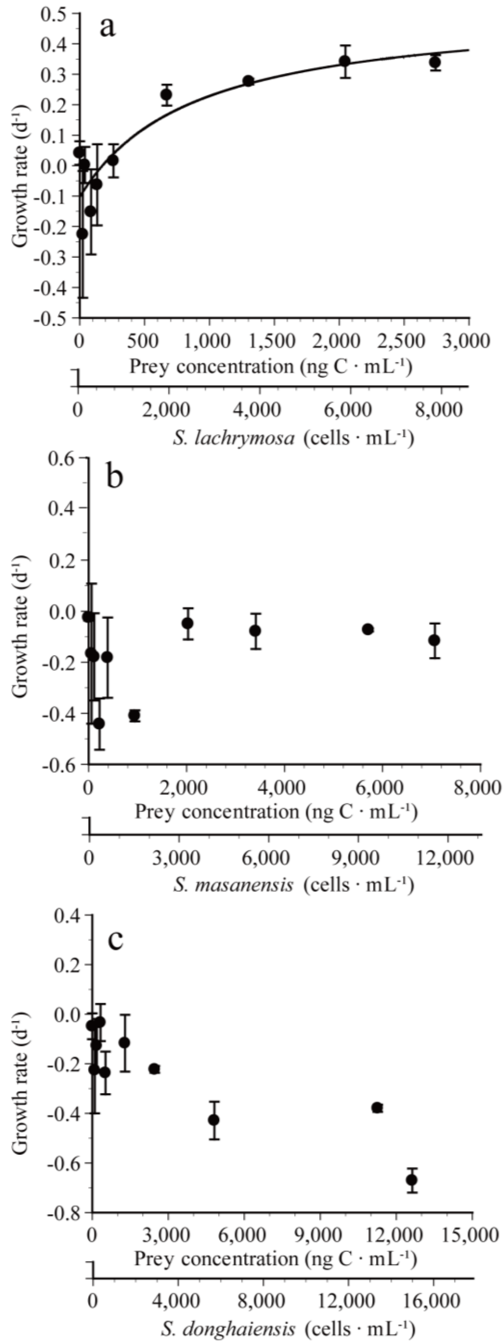


Fig 3. 3. Specific growth rates of *Polykrikos kofoidii* on (a) *Scrippsiella lachrymosa*, (b) *Scrippsiella masanensis*, and (c) *Scrippsiella donghaiensis* as a function of mean prey concentration (x, ng C mL^{-1}). Symbols represent treatment means ± standard error. (a) The curve is fitted to the Michaelis–Menten equation (Eq. 2) using all treatments in the experiment. Growth rate (GR, d^{-1}) = 0.519 {[x + 183.5]/[1058 + (x – 183.5)]}, r^2 = 0.550.

With increasing mean prey concentrations, the ingestion rates of *P. kofoidii* on *S. lachrymosa* increased rapidly at the prey concentrations < 672 ng C mL−1 (1,919 cells mL^{-1}), but slowly at higher prey concentrations (Fig 3.4a). When the data were fitted to Eq. (3), the maximum ingestion rate of *P. kofoidii* on *S. lachrymosa* was 9.4 ng C $predator^{-1}$ d^{-1} (26.9 cells $predator^{-1}$ d^{-1}).

With increasing mean prey concentrations, the ingestion rates of *P. kofoidii* on *S. masanensis* increased up to 10.4 ng C $predator^{-1}$ d^{-1} (17.1 cells $predator^{-1}$ d^{-1}) at the prey concentrations < 3,413 ng C mL^{-1} (5,596 cells mL^{-1}), but decreased down to 3.7 ng C $predator^{-1}$ d^{-1} (6.0 cells $predator^{-1}$ d^{-1}) (Fig 3.4b). The ingestion rates of *P. kofoidii* on *S. masanensis* were significantly affected by mean prey concentration ($p<0.01$, ANOVA).

The range of the ingestion rates of *P. kofoidii* on *S. donghaiensis* were 0.9 – 4.5 ng C $predator^{-1}$ d^{-1} (1.1−5.3 cells $predator^{-1}$ d^{-1}) (Fig 3.4c). The ingestion rates of *P. kofoidii* on *S. donghaiensis*, however, were not significantly affected by mean prey concentration ($p>0.1$, ANOVA).

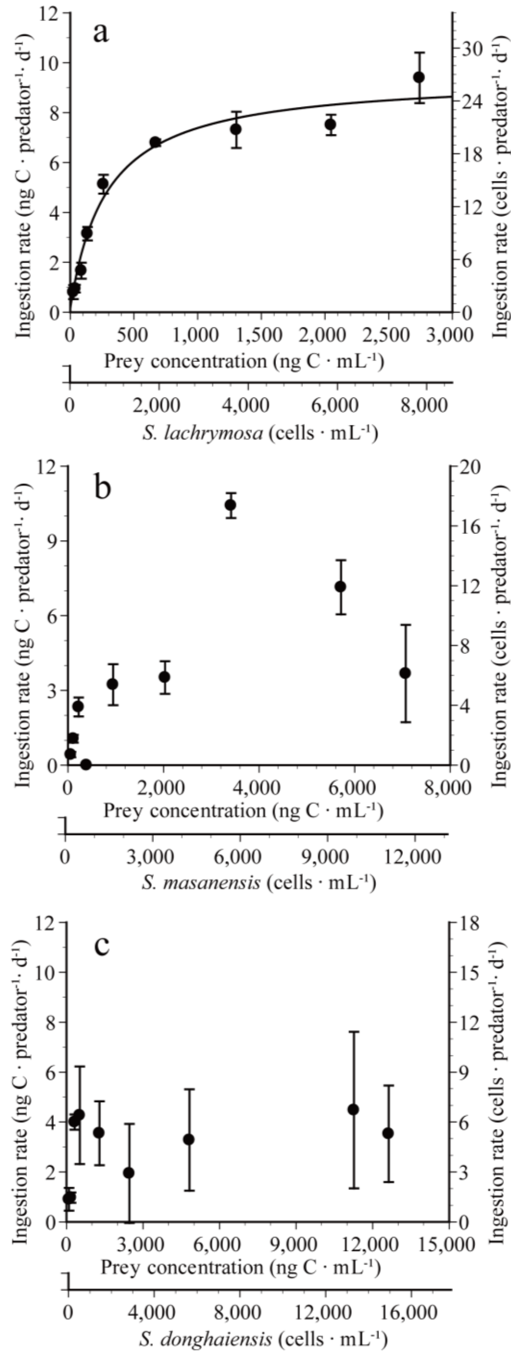


Fig 3. 4. Ingestion rates of *Polykrikos kofoidii* on (a) *Scrippsiella lachrymosa*, (b) *Scrippsiella masanensis*, and (c) *Scrippsiella donghaiensis* as a function of mean prey concentration (x, ng C mL^{-1}). Symbols represent treatment means ± standard error. (a) The curve is fitted to the Michaelis–Menten equation (Eq. 3) using all treatments in the experiment. Ingestion rate (IR, ng C $predator^{-1}$ day^{-1}) = 9.423 [(x)/(281.6 + x)], r^2 = 0.925.

Comparison of growth and ingestion rates of three heterotrophic protists on different *Scrippsiella* species at single high mean prey concentration

At mean prey concentrations of 2,742−5,715 ng C mL^{-1}, the specific growth rates of *P. kofoidii* on *S. acuminata*, *S. lachrymosa*, *S. masanensis*, and *S. donghaiensis* (0.989, 0.338, −0.073, and −0.429 d^{-1}, respectively) were significantly different from one another species ($p<0.001$, ANOVA; Fig 3.5a). Furthermore, the ingestion rates of *P. kofoidii* on *S. acuminata*, *S. lachrymosa*, *S. masanensis*, and *S. donghaiensis* (10.5, 9.4, 7.1, and 3.3 ng C $predator^{-1}$ d^{-1}, respectively) were also significantly different from one another species ($p<0.01$, ANOVA; Fig 3.5b).

At the mean prey concentrations of 4,078−5,265 ng C mL^{-1}, the specific growth rates of *Gyrodinium dominans* on *S. lachrymosa*, *S. acuminata*, *S. donghaiensis*, and *S. masanensis* (0.723, 0.419, 0.124, and 0.011 d^{-1}, respectively) were significantly different from one another species ($p<0.01$, ANOVA; Fig 3.6a). The ingestion rates of *G. dominans* on *S. lachrymosa*, *S. acuminata*, *S. donghaiensis*, and *S. masanensis* (2.0, 0.7, 1.4, and 2.1 ng C $predator^{-1}$ d^{-1}, respectively), however, were not significantly different from one another species ($p>0.1$, ANOVA; Fig 3.6b).

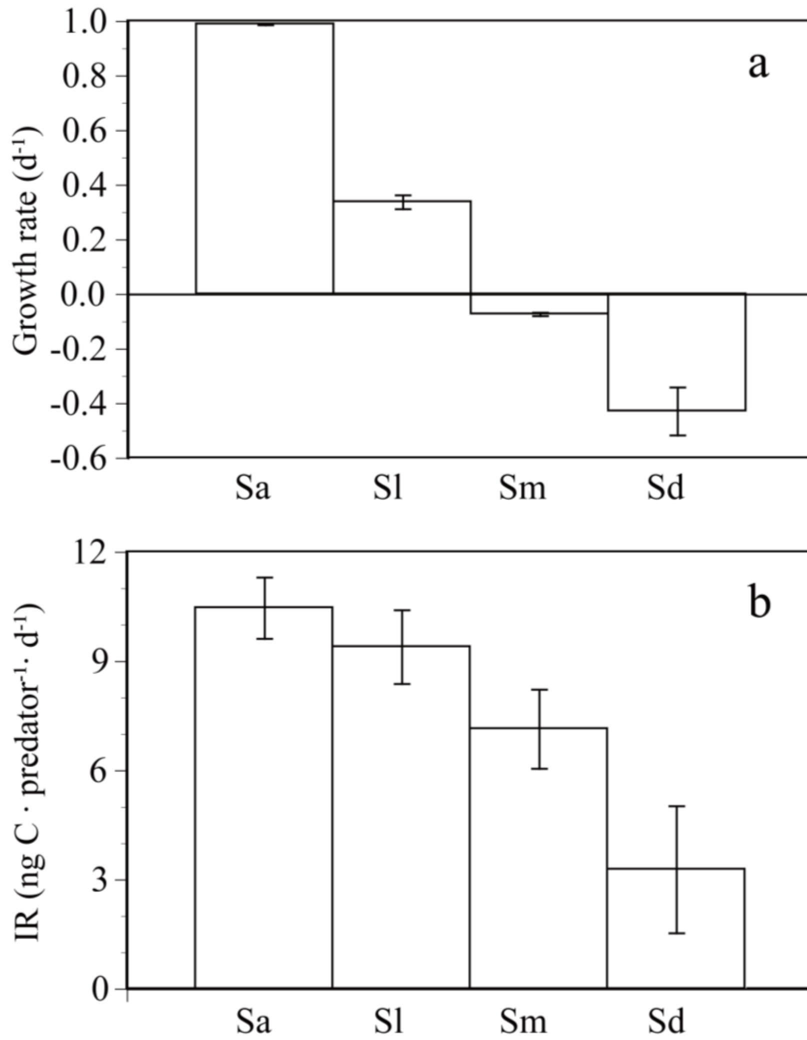


Fig 3. 5. (a) Growth and (b) ingestion rates of *Polykrikos kofoidii* on four *Scrippsiella* species at single high prey concentrations (mean prey concentrations of 2,742–5,715 ng C mL^{-1}). Symbols represent treatment means ± standard error.

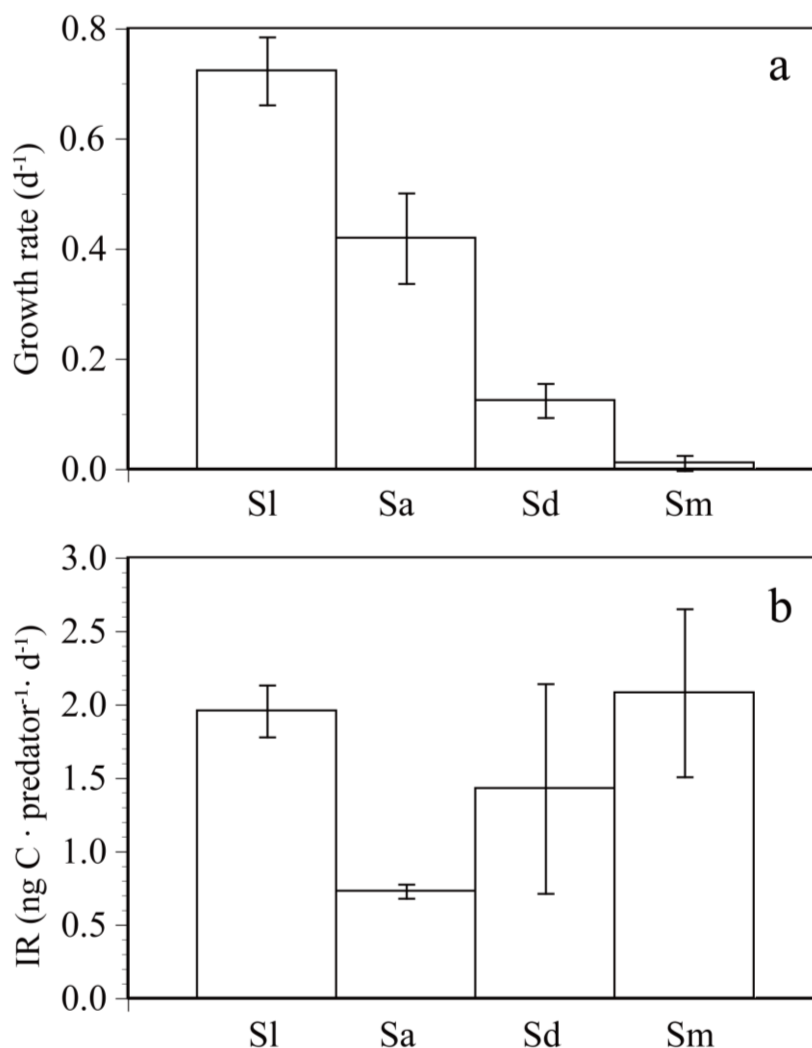


Fig 3. 6. (a) Growth and (b) ingestion rates of *Gyrodinium dominans* on four *Scrippsiella* species at single high prey concentrations (mean prey concentrations of 4,078–5,265 ng C mL^{-1}). Symbols represent treatment means ± standard error.

At mean prey concentrations of 1,336–2,092 ng C mL^{-1}, the specific growth rates of *Oxyrrhis marina* on *S. lachrymosa*, *S. acuminata*, *S. donghaiensis*, and *S. masanensis* (0.793, 0.656, 0.415, and 0.079 d^{-1}, respectively) were significantly different from one another species ($p<0.01$, ANOVA; Fig 3.7a). The ingestion rates of

O. marina on *S. lachrymosa*, *S. acuminata*, *S. donghaiensis*, and *S. masanensis* (0.8, 0.1, 0.4, and 0.1 ng C predator^{-1} d^{-1}, respectively), however, were not significantly different from one another species ($p>0.1$, ANOVA; Fig 3.7b).

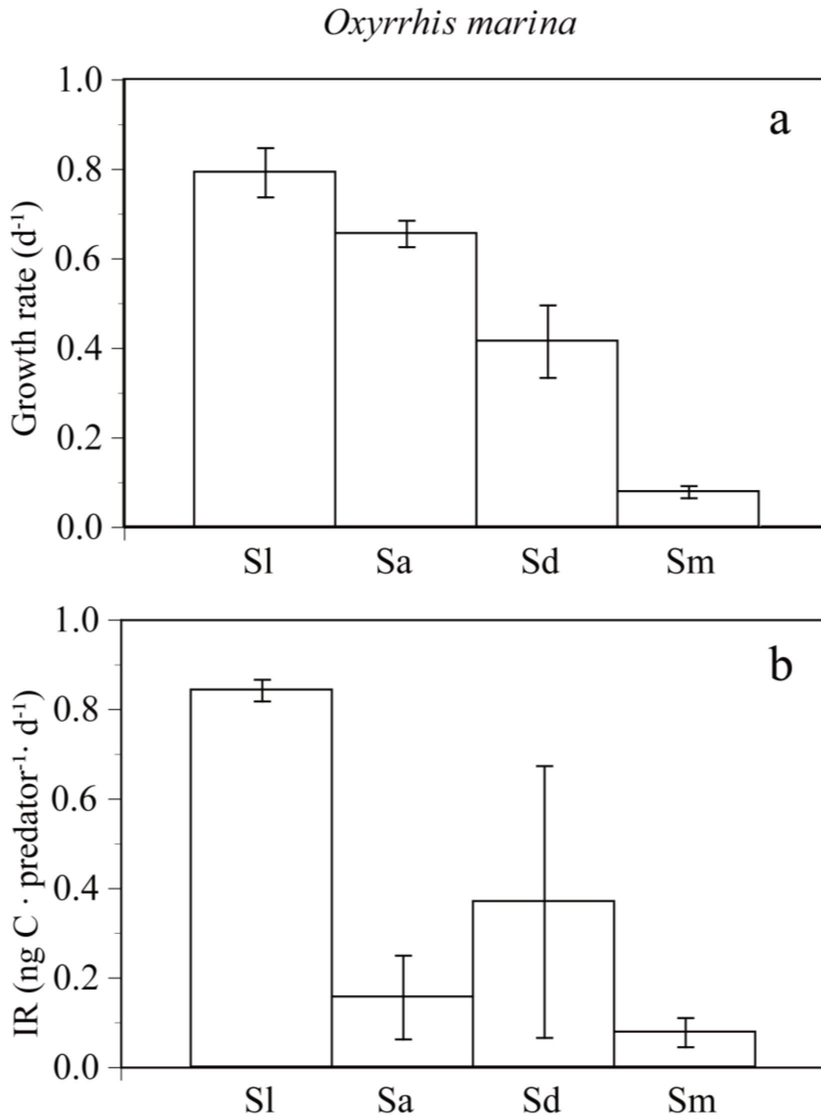


Fig 3. 7. (a) Growth and (b) ingestion rates of *Oxyrrhis marina* on four *Scrippsiella* species at single high prey concentrations (mean prey concentrations of 1,336−2,092 ng C mL^{-1}). Symbols represent treatment means ± standard error.

Swimming speed of *Scrippsiella* species

The average (± standard error) and maximum swimming speeds of *Scrippsiella* species (n=20 for each species) were 640 (± 28) and 786 μm s^{-1} for *S. lachrymosa*, 473 (± 23) and 788 μm s^{-1} for *S. donghaiensis*, 455 (± 19) and 642 μm s^{-1} for *S. acuminata*, and 382 (± 19) and 531 μm s^{-1} for *S. masanensis* (Table 3.4).

Bioassay of toxicity of the four *Scrippsiella* species

In comparison with triplicate wells of containing *A. salina* nauplii only, mortality rate of *A. salina* incubated with cells and filtrate of four *Scrippsiella* species were not significantly different ($p>0.05$, two-tailed *t*-test; Table 3.4).

Table 3. 4. Comparison of cell size, swimming speeds and toxicity of four *Scrippsiella* species.

Organism	Cell size		SS (MSS)	Toxicity
	Length (μm)	Width (μm)		
S. acuminata (STJJ1005)	25.0–37.0 (32.0)	17.0–27.0 (22.0)	454(642)	X
S. donghaiensis (SDGJ1703)	18.5–28.4 (22.2)	13.5–20.8 (16.7)	473(788)	X
S. lachrymosa (SLBS1703)	16.3–23.2 (19.5)	13.3–21.5 (16.7)	640(786)	X
S. masanensis (SMMS0908)	19.3–27.1 (23.3)	17.9–24.6 (20.6)	382(531)	X

SS, averaging swimming speed (μm s^{-1}); MSS, maximum swimming speed (μm s^{-1});

3.4. Discussion

This study shows that the common heterotrophic dinoflagellates *Oxyrrhis marina*, *Gyrodinium dominans*, *Polykrikos kofoidii*, *Oblea rotunda*, *Pfiesteria piscicida* and the common ciliate *Strombidinopsis* sp. were able to feed on *Scrippsiella acuminata*, *S. donghaiensis*, *S. lachrymosa*, and *S. masanensis*. However, numerical and functional responses by *P. kofoidii* to the concentration of one of *S. donghaiensis*, *S. lachrymosa*, and *S. masanensis* were different from those of another *Scrippsiella* species. Although the pattern in numerical and functional responses by *P. kofoidii* to the concentration of *S. acuminata* is similar to those to *S. lachrymosa* (Jeong et al., 2001b), the maximum growth and ingestion rates of this predator on the former prey species is different from those on the latter prey species. Furthermore, growth and ingestion rates of *G. dominans* and *O. marina* on *S. acuminata*, *S. donghaiensis*, *S. lachrymosa*, and *S. masanensis* were different from one another prey species. Therefore, the ecological niche of one *Scrippsiella* species is different from that of the other three species. All these four *Scrippsiella* species have been found in Korean coastal waters and the East China Sea (Gu et al. 2008, Zinssmeister et al. 2011, Jeong et al. 2017b, Lee et al. 2018, this study). Furthermore, the cells and/or cysts of *P. kofoidii*, *G. dominans*, and *O. marina* have also been found in these waters (Yu-Zao et al. 1996, Jeong et al. 2001a, 2001b, Wang et al. 2004, Liu 2008, Zhang et al. 2010, Ke et al. 2012, Yoo et al. 2013a). Based on

differential feeding by the common heterotrophic dinoflagellates on these four *Scrippsiella* species may affect causative species of a red tide or harmful algal bloom. In addition, the abundance of these four *Scrippsiella* species are likely to differentially affect the population dynamics of *P. kofoidii*, *G. dominans*, and *O. marina*.

The size range of four *Scrippsiella* species tested (17.7 – 22.8 μm) is somewhat narrow. The upper prey size limit that each of *G. dominans*, *P. kofoidii*, *O. rotunda*, *P. piscicida*, and *Strombidinopsis* sp. has previously reported to be able to feed on (35.3 μm, 38.2 μm, 120.0 μm, 40.0 μm, and 38.2 μm, respectively) is greater than the size of four *Scrippsiella* species, and the upper prey size limit for *O. marina* (20.4 μm) is slightly smaller than *S. acuminata* and *S. masanensis* (Jacobson and Anderson 1986, Nakamura et al. 1992, Jeong et al. 1999c, 2001b, 2006, Tillmann and Reckermann 2002). Thus, the size of all four *Scrippsiella* species are likely not to affect feeding occurrence by these heterotrophic protistan predators except for *O. marina*. However, there was a big difference in both growth and ingestion rates of *P. kofoidii*, *G. dominans*, or *O. marina* on four *Scrippsiella* species. At mean prey concentrations at which growth and ingestion rates of *P. kofoidii* on *S. acuminata* became saturated, the growth and ingestion rates of *P. kofoidii* on *S. acuminata* and *S. lachrymosa* are greater than those on *S. donghaiensis* and *S. masanensis*. The growth rates of *P. kofoidii* on four *Scrippsiella* species are significantly positively correlated with the ingestion rates (Fig 3.8a). Therefore, the efficiency of conversion of ingested prey carbon to body carbon of

the predator is similar to one another *Scrippsiella* prey species and the ingestion rates of *P. kofoidii* on *Scrippsiella* prey species affect its growth rates. *P. kofoidii* is likely to detect, capture, ingest, and digest *S. acuminata* and *S. lachrymosa* cells more readily than *S. donghaiensis* and *S. masanensis* cells. The maximum swimming speeds of *S. acuminata* and *S. lachrymosa* (642 and 786 μm s^{-1}) are not clearly different from *S. donghaiensis* and *S. masanensis* (788 and 531 μm s^{-1}). The maximum swimming speed of *P. kofoidii* (911 μm s^{-1}) is greater than that of any of these four prey species (Jeong et al. 2002). Therefore, prey swimming speed is likely not to affect this differential ingestion rates. Other factors rather than size and swimming speed of prey species are likely to affect ingestion and growth rates of *P. kofoidii*. Lysis of *P. kofoidii* cells at high *S. donghaiensis* cell concentration were observed. Thus, excreted materials from *S. donghaiensis* may be partially responsible for low ingestion and growth rates. However, the excreted materials did not affect *Artemia* nauplius and thus these materials may not be toxic enough to kill the nauplii.

At similar high mean prey concentrations, the growth rates of *G. dominans* on *S. acuminata* and *S. lachrymosa* are also greater than those on *S. donghaiensis* and *S. masanensis*, although the ingestion rates of *G. dominans* on *S. donghaiensis* and *S. masanensis* are greater than or comparable to those on *S. acuminata* and *S. lachrymosa*. Furthermore, the growth rates of *G. dominans* on four *Scrippsiella* species are not significantly positively correlated with the ingestion rates (Fig 3.8b). Thus, the efficiency

of conversion of ingested prey carbon to body carbon of *G. dominans* is different from one another *Scrippsiella* prey species and the ingestion rates of *G. dominans* on *Scrippsiella* prey species affect its growth rates; *S. acuminata* gives the highest conversion efficiency for *G. dominans*, whereas *S. masanensis* the lowest one. For *O. marina* predator, the growth rates on *S. acuminata* and *S. lachrymosa* are also greater than those on *S. donghaiensis* and *S. masanensis*, although the ingestion rate on *S. donghaiensis* is greater than or comparable to those on *S. lachrymosa* (Fig 3.8c). In the plot of the growth and ingestion rates, *S. lachrymosa*, *S. donghaiensis* and *S. masanensis* are likely to have similar conversion efficiencies, but *S. acuminata* has a conversion efficiency higher than the other *Scrippsiella* species. Overall, *P. kofoidii*, *G. dominans* and *O. marina* show a different pattern on conversion efficiency on four different *Scrippsiella* prey species. *P. kofoidii* converts ingested prey carbon to its own carbon proportionally because it may not spend much energy in capturing and ingesting prey, whereas *G. dominans* and *O. marina* may not convert ingested prey carbon to its own carbon proportionally because they may spend much energy in capturing and ingesting prey.

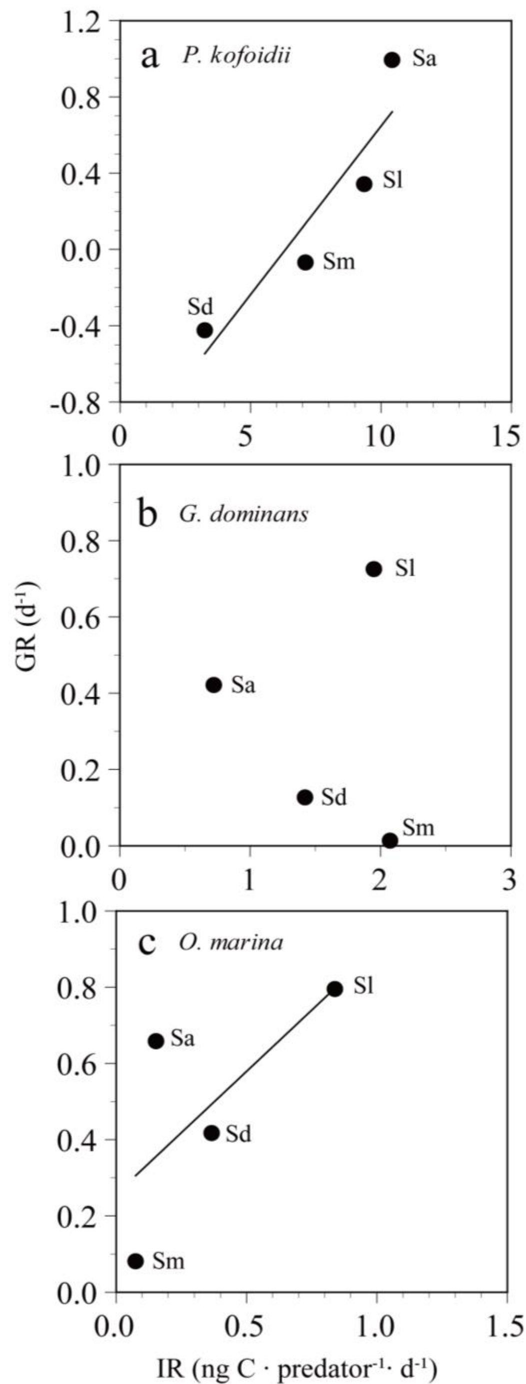


Fig 3. 8. Growth rates (GR) of (a) *Polykrikos kofoidii*, (b) *Gyrodinium dominans*, and (c) *Oxyrrhis marina* on four *Scrippsiella* species as a function of ingestion rates (IR). *Scrippsiella acuminata* (Sa), *Scrippsiella lachrymosa* (Sl), *Scrippsiella masanensis* (Sm) and *Scrippsiella donghaiensis* (Sd). The equation of the linear regression is (a) Growth rate (GR, d^{-1}) = $0.177x$(IR)-1.13, r^2 = 0.850.

The maximum ingestion rate of *P. kofoidii* on *S. acuminata* is comparable to that on *Gymnodinium catenatum*, but lower than on *Ostreopsis* cf. *ovata*, *Alexandrium tamarense*, and *Lingulodinium polyedrum* (Table 3.5). However, the maximum growth rate of *P. kofoidii* on *S. acuminata* is lower than that on *G. catenatum*, but greater than on *Ostreopsis* cf. *ovata*, and *L. polyedrum*. Thus, conversion efficiencies of *P. kofoidii* on *S. acuminata* is lower than that on *G. catenatum*, but greater than on *Ostreopsis* cf. *ovata*, and *L. polyedrum*. Although *S. acuminata* is non−toxic, *G. catenatum* and *A. tamarense* (PSP toxins), *O.* cf. *ovata* (Osterol A, palytoxin), and *L. polyedrum* (Yessotoxin) are known to be toxic (Oshima et al. 1987, Asakawa et al. 1995, Paz et al. 2004, Ciminiello et al. 2013, Hwang et al. 2013). *Polykrikos kofoidii* is known to detoxify phytotoxins of *G. catenatum* within its body (Jeong et al. 2003). Therefore, the presence or absence of toxins might not affect feeding by *P. kofoidii* on these dinoflagellates. Furthermore, the maximum ingestion rate of *P. kofoidii* on *S. lachrymosa* is comparable to that on *Alexandrium minutum* CCMP 1888 (= *A. lusitanicum*), *Effrenium* (*Symbiodinium*) *voratum*, and *Tripos furca* (Table 3.5). However, the maximum growth rate of *P. kofoidii* on *S. lachrymosa* is lower than that on *A. minutum* CCMP 1888, but greater than on *E. voratum*, and *T. furca*. Thus, conversion efficiencies of *P. kofoidii* on *S. lachrymosa* is lower than that on *A. minutum* CCMP 1888, but greater than on *E. voratum*, and *T. furca*.

Table 3. 5. Maximum growth and ingestion rates for the heterotrophic dinoflagellate *Polykrikos kofoidii* on algal prey species.

Prey species	Type	ESD	MGR	MIR	RMGI
Effrenium voratum [a]	MTD	11.1	0.03	10.0	0.02
Scrippsiella lachrymosa [b]	ATD	17.7	0.52	9.4	0.00
Scrippsiella donghaiensis [b]	ATD	19.4	−0.03	4.5	0.01
Gymnodinium aureolum [c]	MTD	19.5	0.11	2.3	0.04
Alexandrium minutum [d] (CCMP 1888)	ATD	20.4	0.77	11.1	0.07
Scrippsiella masanensis [b]	ATD	22.0	−0.05	10.4	0.05
Scrippsiella acuminata [e]	MTD	22.8	0.97	16.6	0.01
Gymnodinium impudicum [e]	MTD	23.2	0.06	5.4	0.03
Prorocentrum micans [e]	MTD	26.0	0.06	4.6	0.00
Ostreopsis cf. *ovata* [f]	ATD	26.4	0.73	33.3	−0.01
Tripos furca [e]	MTD	29.0	0.35	9.8	0.06
Alexandrium tamarense [d]	ATD	31.2	1.01	26.2	0.07

Species	Type	ESD	MGR	MIR	RMGI
Gymnodinium catenatum [e]	ATD	34.0	1.12	17.1	0.07
Lingulodinium polyedrum [e]	MTD	37.9	0.83	24.4	0.06

ESD, equivalent spherical diameter (μm); MGR, maximum growth rate (d^{-1}); MIR, maximum ingestion rate (ng C $predator^{-1}$ day^{-1}). RMGI, ratio of MGR relative to MIR. ATD, autotrophic dinoflagellate; MTD, mixotrophic dinoflagellate. [a] Jeong et al. (2014), [b] This study, [c] Yoo et al. (2010), [d] Kang et al. (2018), [e] Jeong et al. (2001b), [f] Yoo et al. (2015).

When the growth rates of *G. dominans* on four *Scrippsiella* species measured at the mean prey concentrations of 4,070–5,270 ng C mL^{-1} in the present study and growth rates of *G. dominans* on the other microalgal prey species and the mixotrophic ciliate *Mesodinium rubrum* at the prey concentration range calculated from the equations in Lee et al. (2014b) were compared, the growth rate of *G. dominans* on *S. lachrymosa* is lower than that on *Eutreptiella gymnastica*, *Gymnodinium aureolum*, but greater than that on *E. voratum* and *M. rubrum* (Table 3.6). However, the growth rates of *G. dominans* on *S. donghaiensis* and *S. masanensis* are much lower than that on the other prey species (Table 3.6). Therefore, the abundance of *G. dominans* is likely to be high when *S. lachrymosa* is abundant, but low when *S. donghaiensis* or *S. masanensis* is abundant. In addition, the difference in the growth rates of *G. dominans* on the four *Scrippsiella* prey species (i.e., 0.011–0.723 d^{-1}) was higher than that between *S. lachrymosa* and *G. aureolum* (0.723 d^{-1} vs. 0.880 d^{-1}) and *M. rubrum* (0.723 d^{-1} vs. 0.450 d^{-1}). Therefore, the difference in the growth rates of *G. dominans* on different species of the same genus is sometimes higher than that on different species of different genus. Thus, differential feeding of common heterotrophic protist predators on different species of the same genus might partially cause evolution in the same genus.

Table 3. 6. Growth and ingestion rates for the heterotrophic dinoflagellate *Gyrodinium dominans* on algal prey species and the ciliate *Mesodinium rubrum*.

Prey species	Type	ESD	MGR	MIR	x	at 4,078−5,265 ng C mL^{-1}		
						GR(μ)	IR	RGI
Thalassiosira sp. [a]	DIA	5.4	0.73		4,694*			
Rhodomonas salina [b]	CR	6.5	0.21	0.8	4,694*		0.79#	
Dunaliella teriolecta [b]	CH	6.5	0.28	1.9	4,694*		1.89#	
Effrenium voratum [c]	MTD	11.1	0.61	1.9	4,694*	0.60#	1.72#	0.35
Prorocentrum minimum [d]	MTD	12.1	1.13	1.2	4,694*		1.19#	
Biecheleria cincta [e]	MTD	12.2	0.07	0.1	4,694*			
Eutreptiella gymnastica [f]	EUG	12.6	1.13	2.7	4,694*	1.02#	2.54#	0.40
Heterocapsa triquetra [a]	MTD	15.3	0.54	2.9	4,694*		2.87#	
Karenia mikimotoi [a]	MTD	16.8	0.48		4,694*			
Scrippsiella lachrymosa [g]	ATD	17.7			5,107	0.72	2.00	0.36
Scrippsiella donghaiensis [g]	ATD	19.4			4,325	0.12	1.40	0.09
Gymnodinium aureolum [h]	MTD	19.5	0.92	2.0	4,694*	0.88#	1.73#	0.51
Mesodinium rubrum [i]	MNC	22.0	0.48	0.6	4,694*	0.45#	0.59#	0.76

Scrippsiella masanensis [g]	ATD	22.0			4,078	0.01	2.10	0.01
Scrippsiella acuminata [g]	MTD	22.8			5,265	0.42	0.70	0.60
Chattonella antiqua [j]	RA	35.3	0.5	2.3	4,694*			

ESD, equivalent spherical diameter (μm); MGR, maximum growth rate (d^{-1}); MIR, maximum ingestion rate (ng C predator^{-1} day^{-1}); x, prey concentration (ng C mL^{-1}); GR(μ), growth rate (d^{-1}); IR, ingestion rate (ng C predator^{-1} day^{-1}); RGI, ratio of GR relative to IR. Rates are corrected to 20℃ using Q_{10}=2.8 (Hansen et al. 1997); DIA; diatom; CR, cyptophyte; CH, chlorophyte; MTD, mixotrophic dinoflagellate; EUG, euglenophyte; MNC, mixotrophic naked ciliate; RA, raphidophyte; ATD, autotrophic dinoflagellate. . *Value is the mean of all prey concentrations for the four *Scrippsiella* spp. #Rates are calculated by interpolation using x values and the equations in the reference. [a] Nakamura et al. (1995b), [b] Calbet et al. (2013), [c] Jeong et al. (2014), [d] Kim and Jeong (2004), [e] Yoo et al. (2013c), [f] Jeong et al. (2011), [g] This study, [h] Yoo et al. (2010), [i] Lee et al. (2014b), [j] Nakamura et al. (1992).

Similarly, when the growth rates of *O. marina* on four *Scrippsiella* species measured at the mean prey concentrations of 1,336−2,092 ng C mL^{-1} in the present study and growth rates of *O. marina* on the other microalgal prey species at the prey concentration range calculated from the equations in Jeong et al. (2018b) were compared, the growth rate of *O. marina* on *S. lachrymosa* is lower than that on the raphidophyte *Heterosigma akashiwo*, comparable to that on *E. gymnastica* and *E. voratum*, but greater than that on the other microalgal prey species (Table 3.7). The growth rate of *O. marina* on *S. masanensis* is lower than that on the other prey species (Table 3.7). Therefore, the abundance of *O. marina* may also be high when *S. lachrymosa* is abundant, but low when *S. masanensis* is abundant.

Table 3. 7. Growth and ingestion rates for the heterotrophic dinoflagellate *Oxyrrhis marina* on diverse prey species.

						at 1,336−2,092 ng C mL^{-1}		
Prey species	Type	ESD	MGR	MIR	x	GR(μ)	IR	RGI
Azadinium cf. *poporum* [a]	ATD	10.0	0.5	4.99	1,806*	0.48#	4.31#	0.11
Brachiomonas submarina [b, c]	CH	10.5	0.73	1.29	1,806*	0.71#	1.19#	0.60
Gymnodinium smaydae [d]	MTD	10.5	0.41	0.27	1,806*	0.41#	0.26#	1.58
Effrenium voratum [e, f]	MTD	11.1	0.87	2.10	1,806*	0.84#	1.75#	0.48
Heterosigma akashiwo [g]	RA	11.5	1.43	1.25	1,806*	1.35#	0.90#	1.50
Biecheleria cincta [f, h]	MTD	12.2	0.49	0.35	1,806*	0.49#	0.35#	1.40
Eutreptiella gymnastica [i, j]	EUG	12.6	0.81	2.70	1,806*	0.81#	2.48#	0.33
Luciella masanensis [k, l]	HTD	13.5	0.04	0.07	1,806*		0.07#	
Pfiesteria piscicida [k, m]	HTD	13.5	0.66	0.33	1,806*	0.65#	0.32#	2.07
Stoeckeria algicida [k, n]	HTD	13.9	0.22	0.14	1,806*	0.21#	0.13#	1.59
Scrippsiella lachrymosa [o]	ATD	17.7			1,336	0.79	0.80	0.99
Scrippsiella donghaiensis [o]	ATD	19.4			1,812	0.42	0.40	1.04
Gymnodinium aureolum [p]	MTD	19.5	0.71	0.51	1,806*	0.69#	0.49#	1.40
Fibrocapsa japonica [q]	RA	20.4	0.72	1.18	1,806*			

Scrippsiella masanensis [o]	ATD	22.0	1,983	0.08	0.10	0.79
Scrippsiella acuminata [o]	MTD	22.8	2,092	0.66	0.10	6.56

ESD, equivalent spherical diameter (μm); MGR, maximum growth rate (d^{-1}); MIR, maximum ingestion rate (ng C $predator^{-1}$ day^{-1}); x, prey concentration (ng C mL^{-1}); GR(μ), growth rate (d^{-1}); IR, ingestion rate (ng C $predator^{-1}$ day^{-1}); RGI, ratio of GR relative to IR. Rates are corrected to 20℃ using Q_{10}=2.8 (Hansen et al. 1997); CH, chlorophyte; EUG, euglenophyte; RA, raphidophyte; ATD, autotrophic dinoflagellate; MTD, mixotrophic dinoflagellate; HTD, heterotrophic dinoflagellate. *Value is the mean of all prey concentrations for the four *Scrippsiella* spp. #Rates are calculated by interpolation using x values and the equations in the reference. [a] Potvin et al. (2013), [b] Fuller (1990), [c] Bauerfeind et al. (1986), [d] Jeong et al. (2018b), Lee et al. (2014a), [e] Jeong at al. (2014), [f] Yoo et al. (2015), [g] Jeong et al. (2003), Yamochi and Abe (1984), [h] Yoo et al. (2013c), [i] Jeong et al. (2011), [j] Throndsen (1973), Sommer (1988), [k] Jeong et al. (2007b), [l] Jeong et al. (2007a), Jang et al. (2016), [m] Jeong et al. (2006), Burkholder and Glasgow (1997), [n] Jeong et al. (2005a), Lim et al. (2014), [o] This study, [p] Yoo et al. (2010), Jeong et al. (2010a), [q] Tillmann and Reckermann (2002).

Most morphological characters of four *Scrippsiella* species tested in this study are not largely different (Table 2.3). Furthermore, the difference in small subunit (SSU) and large subunit (LSU) among the four *Scrippsiella* species are 0.5–1.5% and 3.1–4.6%, respectively (Table 2.4). However, the difference in maximum growth and ingestion rates of *P. kofoidii* on the four *Scrippsiella* species based on the highest value of growth and ingestion rates are 46– more than 100% and 10–73%. In addition, the differences in growth and ingestion rates are 42–99% and 5–67% for *G. dominans* and 17–90% and 0–88% for *O. marina* (Table 3.8). Thus, differential feeding by these common heterotrophic protist predators can be used for a proxy for different genomes among these four *Scrippsiella* species. It is worthwhile to explore relationships between differences in predators and genomes in this genus. Conclusively, difference in the growth and/or ingestion rates of *O. marina*, *G. dominans*, and *P. kofoidii* on four *Scrippsiella* species may cause different ecological niches of both the predators and prey species. In addition, *S. acuminata* and *S. lachrymosa* are nutritious for predators. Although *Scrippsiella* species have similar morphological characteristics, their eco-physiological characteristics are considerably different such as the result of this study.

Table 3. 8. Comparison of growth and ingestion rates of predators on *Scrippsiella* species. The numbers within parentheses in *Scrippsiella* species column are growth and ingestion rates of each predator feeding on four *Scrippsiella* species. The values are dissimilarity (%) between the values on two *Scrippsiella* species.

A. *P. kofoidii*

	S. acuminata (0.97, 16.6)	*S. donghaiensis* (−0.03, 4.5)		*S. lachrymosa* (0.52, 9.4)		*S. masanensis* (−0.05, 10.4)	
		MGR	MIR	MGR	MIR	MGR	MIR
S. acuminata (0.97, 16.6)	−	>100	72.9	46.3	43.4	>100	37.3
S. donghaiensis (−0.03, 4.5)	−	−		>100	52.1	−	56.7
S. lachrymosa (0.52, 9.4)	−	−		−		>100	9.6

B. *G. dominans*

	S. acuminata (0.42, 0.7)	*S. donghaiensis* (0.12, 1.4)		*S. lachrymosa* (0.72, 2)		*S. masanensis* (0.01, 2.1)	
		GR	IR	GR	IR	GR	IR
S. acuminata (0.42, 0.7)	–	71.4	50.0	41.7	65.0	97.6	66.7
S. donghaiensis (0.12, 1.4)	–	–		83.3	30.0	91.7	33.3
S. lachrymosa (0.72, 2.0)	–	–		–		98.6	4.8

C. *O. marina*

	S. acuminata (0.66, 0.1)	*S. donghaiensis* (0.42, 0.4)		*S. lachrymosa* (0.79, 0.8)		*S. masanensis* (0.08, 0.1)	
		GR	IR	GR	IR	GR	IR
S. acuminata (0.66, 0.1)	–	36.4	75.0	16.5	87.5	87.9	0
S. donghaiensis (0.42, 0.4)	–	–		46.8	50.0	81.0	75.0
S. lachrymosa (0.79, 0.8)	–	–		–		89.9	87.5

Chapter 4. Interactions between the voracious heterotrophic nanoflagellate *Katablepharis japonica* and common heterotrophic protists and distribution of *K. japonica*

4.1. Introduction

Marine heterotrophic nanoflagellates (HNFs) are major components of marine planktonic food webs (e.g., Patterson and Larsen 1991, Jeong et al. 2013). They are known to be major predators of marine bacteria and occasionally control their populations (Fenchel 1982, Azam et al. 1983, Sieburth 1984). Recently, the free-living HNF *Katablepharis japonica* has been reported to feed on red-tide dinoflagellates and raphidophytes (Kwon et al. 2017). In turn, some HNFs such as *Cafeteria* spp., *Spumella* spp., and *Bodo* spp. are known to be prey for some heterotrophic dinoflagellates (HTDs) and ciliates (Jürgens et al. 1996, Jeong et al. 2007c). Therefore, they are known to play an important role in the transfer of bacteria and microalgae to HTDs and ciliates in marine microbial loops. However, still feeding by common heterotrophic protists on several important HNF species that can affect the dynamics of red-tide species or bacteria is poorly understood until date.

Katablepharis japonica is a common HNF in Korean waters and was found in the waters of Japan and the United States (Okamoto and Inouye 2005, Kahn et al. 2014, Kwon et al. 2017). Recently, this species has been reported to feed on diverse phytoplankton species (Kwon et al. 2017). Interestingly, it can grow fast by feeding on suitable phytoplankton species, but it does not grow on bacteria, unlike most HNFs (Kwon et al. 2017). Furthermore, it was suggested that *K. japonica* may have a considerable potential grazing impact on populations of a red-tide species. However, if its mortality due to predation is high, the effect of *K. japonica* on populations of the red-tide species could be reduced. To the best of our knowledge, no studies have been reported on the protist predators of *K. japonica*. To understand the roles of *K. japonica* in red tide dynamics, mortality due to predation should be determined.

In addition, HTDs and ciliates are major components in marine ecosystems (Stoecker et al. 1984, Hansen 1991, Jeong 1999a, 1999c, Levinsen and Nielsen 2002, Sherr and Sherr 2007, Jeong et al. 2010b, 2015, Yoo et al. 2013a, Lim et al. 2017b). They feed on diverse types of prey, such as bacteria, phytoplankton including red-tide species, mixotrophic, or heterotrophic protists, eggs and early naupliar stages of metazooplankton, and sometimes control populations of their prey (Hansen 1992, Jeong 1994, Monta gnes et al. 1996, Jeong et al. 2004b, 2008a, 2008b, Kamiyama and Matsuyama 2005, Turner 2006, Yoo et al. 2013b, Lee et al. 2014a, Jang et al. 2016, Lim et al. 2017b). In general, due to much higher

abundances of HTDs or ciliates than those of metazooplankton, effect of grazing on prey populations by the former grazers is usually greater than that by the latter grazers (Kim et al. 2013, Yoo et al. 2013a). Thus, to understand the roles of potential prey species in food webs, feeding by HTDs or ciliates on the prey should be explored.

In general, HTDs and ciliates have two major feeding mechanisms, raptorial feeding and filter feeding (e.g., Fenchel 1980, Hansen and Calado 1999). In raptorial feeding, there are three major feeding mechanisms—engulfment feeding, peduncle feeding, and pallium feeding (Jacobson 1987, Jeong and Latz 1994, Kiφrboe and Titelman 1998, Berge et al. 2008, Jeong et al. 2010b, Lim et al. 2014, Lee et al. 2015). Using these diverse feeding mechanisms, they are able to feed on prey species having diverse size, shape, behavior, biochemistry etc. (Jeong et al. 2010b). Therefore, in the present study, we aimed to investigate the potential effects of protist predators on *K. japonica*, feeding by the engulfment-feeding HTDs *Oxyrrhis marina*, *Gyrodinium dominans*, *Gyrodinium moestrupii*, *Polykrikos kofoidii*, and *Noctiluca scintillans*, the peduncle-feeding HTDs *Luciella masanensis* and *Pfiesteria piscicida*, the pallium-feeding HTD *Oblea rotunda*, and the naked ciliates *Strombidium* sp. (approximately 20 μm in cell length), *Pelagostrobilidium* sp., and *Miamiensis* sp. on *K. japonica* was explored. The strain of *K. japonica* used in the present study was originally isolated from Masan Bay, where most of these heterotrophic protists were isolated or reported to be abundant at

certain times (Jeong et al. 2007a, 2007b, Yoo et al. 2013b, Kwon et al. 2017). Furthermore, these heterotrophic protists have often been found in the waters of many other countries as well (Strom and Buskey 1993, Watts et al. 2010, Moestrup et al. 2014, Grzebyk et al. 2017). Some dinoflagellates are known to feed on, kill, lyse, or immobilize some common heterotrophic protists (Kim et al. 2017, Ok et al. 2017). Therefore, we investigated whether *K. japonica* attacks common heterotrophic protists or not. The results of the present study provide a basis for understanding the interactions between *K. japonica* and common heterotrophic protist species, and their ecological roles in the marine planktonic community.

In addition, to understand the population dynamics of *K. japonica*, their distribution and abundance in the field should be studied. Prior to this study, the abundance of *K. japonica* at Masan Bay in January, May, September, and October 2016 has been reported (Kwon et al. 2017). However, species-specific primer and probe set was designed small subunit ribosomal DNA (SSU rDNA) region which may not be conserved region of only *K. japonica*. Thus, the distribution and abundance data of *K. japonica* may not be accurate. Therefore, since only the abundance data in Masan in 2016 were described, distribution and abundance at 28 stations of the East, West, and South Sea of Korea from January 2016 to October 2017 were analyzed by using improved species-specific primer and probe set and qPCR.

4.2. Material and Method

Preparation of experimental organisms

A clonal culture of *K. japonica* (KJMS1610), which was isolated from plankton samples collected from Masan Bay, Korea in October 2016, was used in the present study (Kwon et al. 2017). The mixotrophic dinoflagellate *Akashiwo sanguinea* was provided as prey every day. As the concentration of *K. japonica* increased, the culture was sequentially transferred to 50-, 250-, and 800-mL flasks containing fresh prey (approximately 10,000 cells mL^{-1}). The flasks were filled to capacity with freshly filtered seawater, capped, and placed on a shelf at 20℃ under an illumination of 20 μE m^{-2} s^{-1} provided by cool white fluorescent lights, under a 14:10 h light:dark cycle. The mean equivalent spherical diameter (ESD) and carbon content of *K. japonica* were 8.1 μm and 0.043 ng C per cell, respectively (Kwon et al. 2017).

For the isolation and culture of the HTD predators *G. dominans*, *G. moestrupii*, *L. masanensis*, *N. scintillans*, *O. rotunda*, *O. marina*, and *P. kofoidii*, plankton samples were collected by using water samplers, from the coastal waters off Saemankeum, Jangheung, Jinhae, or Shiwha, Korea during 2008-2016 (Table 4.1). A clonal culture of each species was established by using two serial single-cell isolations (Table 4.1). A clonal culture of *P. piscicida* was obtained from National Center for Marine Algae and Microbiota, USA.

For the isolation and culture of the ciliate *Strombidium* sp. (approximate cell length 20 μm), plankton samples were collected by using a 10-μm mesh from Jinhae Bay, Korea in July 2017, when the water temperature and salinity were 23.8℃ and 28.3, respectively (Table 4.1). A clonal culture of *Strombidium* sp. was established by using two serial single-cell isolations. For the isolation and culture of the ciliate *Pelagostrobilidium* sp. (approximate cell length 50 μm), plankton samples were collected by using a 10-μm mesh, in coastal waters off Tongyoung, Korea in August 2017 when the water temperature and salinity were 27.2℃ and 31.5, respectively (Table 4.1). Furthermore, the scuticociliate *Miamiensis* sp. B1 was isolated from an infected larva of *Clithon retropictus* in the waters off Bieung Island, western Korea in May 2016, when water temperature and salinity were 20.8℃ and 31.3, respectively (Kim et al. 2017).

The carbon contents of the HTDs and the ciliate were estimated from the cell volume, according to the procedure of Menden-Deuer and Lessard (2000). The cell volumes of the predators were estimated using the methods of Kim and Jeong (2004) and Yoon et al. (2012) for *G. dominans* and *G. moestrupii*, that of Jeong et al. (2008b) for *O. marina*, Jeong et al. (2001b) for *P. kofoidii*, Jeong et al. (2007a, 2007b) for *L. masanensis* and *P. piscicida*, and the cell volumes of *N. scintillans*, *O. rotunda*, *Strombidium* sp., *Pelagostrobilidium* sp., and *Miamiensis* sp. were measured in this study (Table 4.1).

Table 4. 1. Isolation and maintenance conditions for the experimental organisms.

Organism	Location	Time	T	S	CV	FM	Prey species	PC
Heterotrophic dinoflagellate								
Gyrodinium dominans	Shiwha Bay, Korea	Nov 2011	19.7	31.0	4.2	EG	*Amphidinium carterae*	30,000–40,000
Gyrodinium moestrupii	Off Saemankeum, Korea	Oct 2009	21.2	31.0	3.3	EG	*Alexandrium minutum*	3,000–5,000
Luciella masanensis	Jinhae Bay, Korea	Jul 2016	22.6	30.7	1.3	PE	*Apistonema* sp. (CCMP 3085)	~20,000
Noctiluca scintillans	Jangheung Bay, Korea	Jul 2016	23.6	26.4	39,385	EG	*Lingulodinium polyedra*	1,500–2,000
Oblea rotunda	Jinhae Bay, Korea	Apr 2015	12.6	31.2	5.3	PA	*Amphidinium carterae*	10,000–20,000
Oxyrrhis marina	Shiwha Bay, Korea	Dec 2008	16.8	27.0	2.0	EG	*Amphidinium carterae*	8,000
Polykrikos kofoidii	Jangheung Bay, Korea	Jul 2016	23.6	26.4	43.1	EG	*Scrippsiella acuminata*	~5,000
Pfiesteria piscicida (CCMP2091)	Neuse River, USA	Jan 1998	–	–	1.3	PE	*Amphidinium carterae*	~5,000
Ciliate								
Pelagostrobilidium sp.	Tongyoung, Korea	Aug 2017	27.2	31.5	40.6	EG	*Prorocentrum minimum*	~3,000

Species	Sampling location	Sampling time	T	S	CV	FM	Prey species	PC
Miamiensis sp.	Bieung Island, Korea	May 2016	20.8	31.3	276.5	EG	Yeast	0.2 g 100 mL^{-1}
Strombidium sp.	Jinhae Bay, Korea	Jul 2017	23.8	28.3	5.4	EG	*Heterosigma akashiwo*	2,000–3,000

Sampling location and time, water temperature (T, ℃), salinity (S) for isolation, cell volume (CV, $\times 10^3$ μm^3), and feeding mechanisms (FM), prey species, and prey concentrations (PC, cells mL^{-1}) for maintenance.

EG, engulfment feeder; PE, peduncle feeder; PA, pallium feeder.

Effect of the *Katablepharis japonica* concentration on the growth rate of *Oxyrrhis marina*

In Exp. 1, there was no heterotrophic protist that capable of feeding on actively swimming *K. japonica*. To the contrary, *K. japonica* fed on *O. marina*. Thus, Exp. 3 was designed to measure the growth rate of *O. marina* as a function of *K. japonica* concentration (Table 4.2).

For the experiment on *A. sanguinea* prey, cultures of approximately 11,000 cells mL^{-1} of *K. japonica* growing on *A. sanguinea* were transferred to a single 250-mL culture flask containing freshly filtered seawater after *A. sanguinea* became undetectable. This culture was maintained for 1 day. Three 1-mL aliquots were then collected from the flask and examined using a light microscope to determine the concentration of *K. japonica*.

The initial concentrations of *K. japonica* and *A. sanguinea* were established using an autopipette to deliver predetermined volumes of known cell concentrations to the flasks. For each predator-prey combination, triplicate experimental 50-mL culture flasks (containing a mixture of *K. japonica* and *O. marina*) and triplicate control flasks (containing a culture of *K. japonica* only) were established. Moreover, triplicate control flasks containing only *O. marina* were established at a single concentration. The flasks were filled to 20 mL with freshly filtered seawater, capped, and then placed on a shelf at 20℃ under a 14:10 h light:dark cycle of 20 μE m^{-2} s^{-1} of cool white fluorescent light. To determine the actual initial predator and prey densities (cells mL^{-1}) at the beginning of

the experiment (Table 4.2) and after a 2-day incubation period, 10-mL aliquots were removed from each flask and fixed with 5% Lugol's solution. Then, all *K. japonica* cells and all or >300 prey cells in three 1-mL Sedgwick-Rafter chambers were enumerated. Only 10 mL water in each 50-mL flask after subsampling at the beginning of the experiment was incubated to create a shallow depth in the flasks for increasing the encounter rates between *O. marina* and *K. japonica*, because *K. japonica* swam near the bottom of the flask.

The specific growth rate of *O. marina* was calculated as follows:

$$\mu(d^{-1}) = [\mathrm{Ln}\ (P_t/P_0)]/t \qquad (1)$$

, where C_0 is the initial concentration of *O. marina* and C_t is the final concentration after time t. The period was 2 days. The mean prey concentration was calculated using the equation of Frost (1972). The ingestion and clearance rates were calculated using the equations of Frost (1972) and Heinbokel (1978).

Table 4. 2. Experimental design

Exp. No.	Species	Prey density	Species	Predator density
1	*Katablepharis japonica* (living cells)	8,000	*Gyrodinium dominans*	2,000
		8,000	*Gyrodinium moestrupii*	800
		8,000	*Noctiluca scintillans*	15
		8,000	*Oblea rotunda*	1,000
		8,000	*Oxyrrhis marina*	3,000
		8,000	*Polykrikos kofoidii*	150
		8,000	*Pfiesteria piscicida*	2,000
		8,000	*Luciella masanensis*	2,000
		8,000	*Miamiensis* sp.	5,000
		5,000	*Strombidium* sp.	100
		7,000	*Pelagostrobilidium* sp.	100

2	*Katablepharis japonica* (heat-killed cells)	8,000	*Gyrodinium dominans*	2,000
		8,000	*Gyrodinium moestrupii*	800
		8,000	*Noctiluca scintillans*	15
		8,000	*Oblea rotunda*	1,000
		8,000	*Oxyrrhis marina*	3,000
		8,000	*Polykrikos kofoidii*	150
		8,000	*Pfiesteria piscicida*	2,000
		8,000	*Luciella masanensis*	2,000
		8,000	*Miamiensis* sp.	5,000
		5,000	*Strombidium* sp.	100
		7,000	*Pelagostrobilidium* sp.	100
3	*Katablepharis japonica* (living cells)	998, 1,913, 2,948, 3,517, 5,818, 9,650, 14,417, 23,475	*Oxyrrhis marina*	37, 58, 76, 97, 146, 203, 269, 329 (408)

The numbers in the prey and predator columns are the target initial densities (cells mL^{-1}) of prey and predator for Exp. 1 and 2, and the actual initial densities for Exp. 3. The values within parentheses in the predator column in Exp. 3 are the predator densities in the control bottles.

Distribution and abundance of *Katablepharis japonica*

Water samples and samples for qPCR were collected and filtered as mentioned in Chapter 2 (Fig 2.1) and the filters were preserved under −20℃. DNA extraction from the filter of each samples were conducted as described in Chapter 2.

To develop species−specific probe and primer set of *K. japonica*, DNA from approximately 15−mL of a dense culture of *K. japonica* (KJMS1610) was extracted as described in Chapter 2. The following primers were used to amplify small subunit ribosomal DNA (SSU rDNA), internal transcribed spacer (ITS) region, and large subunit ribosomal DNA (LSU rDNA): EukA, EukB (Stoeck et al. 2005), ITSF2, ITSR2 (Litaker et al. 2003), D1RF (Scholin et al. 1994), and LSUB (Litaker et al. 2003) (Table 4.3). The DNA was amplified in a Mastercycler ep gradient (Eppendorf, Hamburg, Germany) and PCR products were purified using an AccuPrep® DNA Purification Kit (Bioneer Cooperation, Daejeon, Korea) as same method in Chapter 2. To obtain species−specific region of *K. japonica* (KJMS1610), the sequences of the other strains of *K. japonica*, heterotrophic nanoflagellates, and related dinoflagellate ITS rDNA region available from GenBank were aligned using MEGA v.4 (Tamura et al. 2007). In addition, to improve species−specific probe and primer set of *K. japonica* used in Kwon et al. (2017), *K. remigera* (KRJH1707) isolated at Jinhae Bay, Korea ITS rDNA region was added when aligned the ITS rDNA sequence as mentioned above. Manual curation of the alignments was conducted to identify unique sequences and develop a *K. japonica* species−

specific qPCR assay. The sequences for the primer and probe set were selected from the region that were conserved within *K. japonica* strains, but differentiated *K. japonica* from other dinoflagellates (Table 4.3). The primer and probe sequences of *K. japonica* were analyzed and synthesized as described in Chapter 2.

Table 4. 3. Oligonucleotide primers used to amplify the SSU, ITS, and LSU regions of ribosomal DNA and the species-specific primers and probes of *Katablepharis japonica* (KJMS1610).

Name	Type	Primer region	5′ −3′
EUKA	Forward	SSU	AAC CTG GTT GAT CCT GCC AGT
EUKB	Reverse	SSU	TGA TCC TTC TGC AGG TTC ACC TAC
ITSF2	Forward	SSU −ITS	TAC GTC CCT GCC CTT TGT AC
ITSR2	Reverse	ITS	TCC CTG TTC ATT CGC CAT TAC
D1RF	Forward	LSU	ACC CGG TGA ATT TAA GCA TA
LSUB	Reverse	LSU	ACG AAC GAT TTG CAC GTC AG
Kjaponica_F	Forward	ITS	AAG CTC GTA GTT GGA TTT TGG A
Kjaponica_R	Reverse	ITS	TGA TTC CCA ACA CTC CTC AG
Kjaponica_P	Probe	ITS	TCT TCT TTT CTG GGG ACC ACA TTG CTC

For specificity test of *K. japonica*, associated other eukaryotes, including *K. remigera* (KRJH1707), and *K. japonica* (KJMS1610) were conducted as described in Chapter 2. Furthermore, to establish the standard curve, DNA was extracted from a dense monoclonal culture of *K. japonica*, targeting 100,000 cells in the final elution volume of 100 μL, using the identical method as stated above. The extracted DNA of *K. japonica* was

then diluted to 1, 10, 100, 1,000, 10,000, 100,000 cells by adding deionized sterile water (DDW) (Bioneer) to the 1.5-mL tubes. The samples were stored under −20℃ in the freezer. The qPCR assays were performed in quadruplicate to improve the accuracy of the results as described in Chapter 2. Samples using DDW as the template were used as the negative control, whereas the DNA used to construct standard curve was used as positive and standard control.

4.3. Results

Interactions between *Katablepharis japonica* and common heterotrophic protists

For 24 h, none of the heterotrophic protists tested in this study fed on actively swimming cells of *K. japonica* successfully (Table 4.4). In video recording for ca. 1 h, there were many encounters between *K. japonica* and each of the heterotrophic protists studied, but feeding by the heterotrophic protists on *K. japonica* were not observed (Fig 4.1-2). Interestingly, *O. marina*, *G. dominans*, *G. moestrupii*, *L. masanensis*, *P. piscicida*, *Pelagostrobilidium* sp., *Strombidium* sp., and *Miamiensis* sp. attempted to feed on them, but *N. scintillans*, *O. rotunda*, and *P. kofoidii* did not attempt to feed on *K. japonica* cells (Table 4.4).

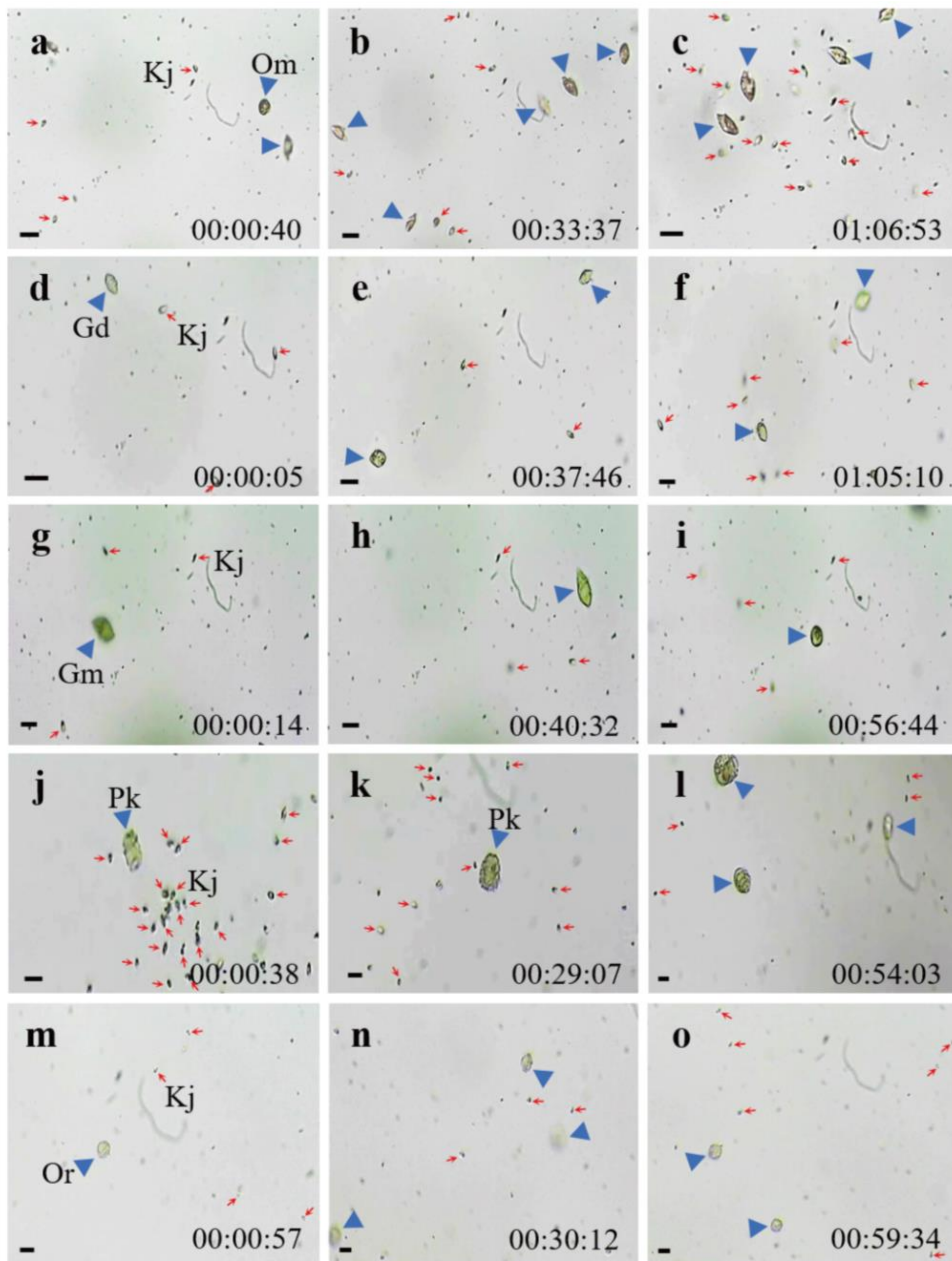


Fig 4. 1. No feeding by engulfment feeding heterotrophic dinoflagellates on *Katablepharis japonica* recorded using video microscopy for approximately 1 h. (a–c) No feeding by *Oxyrrhis marina* (Om) on *K. japonica* (Kj) cells. (d–f) No feeding by *Gyrodinium dominans* (Gd) on Kj cells. (g–i) No feeding by *Gyrodinium moestrupii* (Gm) on Kj cells. (j–l) No feeding by *Polykrikos kofoidii* (Pk) on Kj cells. (m–o) No feeding by *Oblea rotunda* (Or) on Kj cells. Red arrows and blue arrowheads indicate Kj cells and heterotrophic protists. The numbers indicate h : min : s. Scale bars represent: a–o, 25 μm.

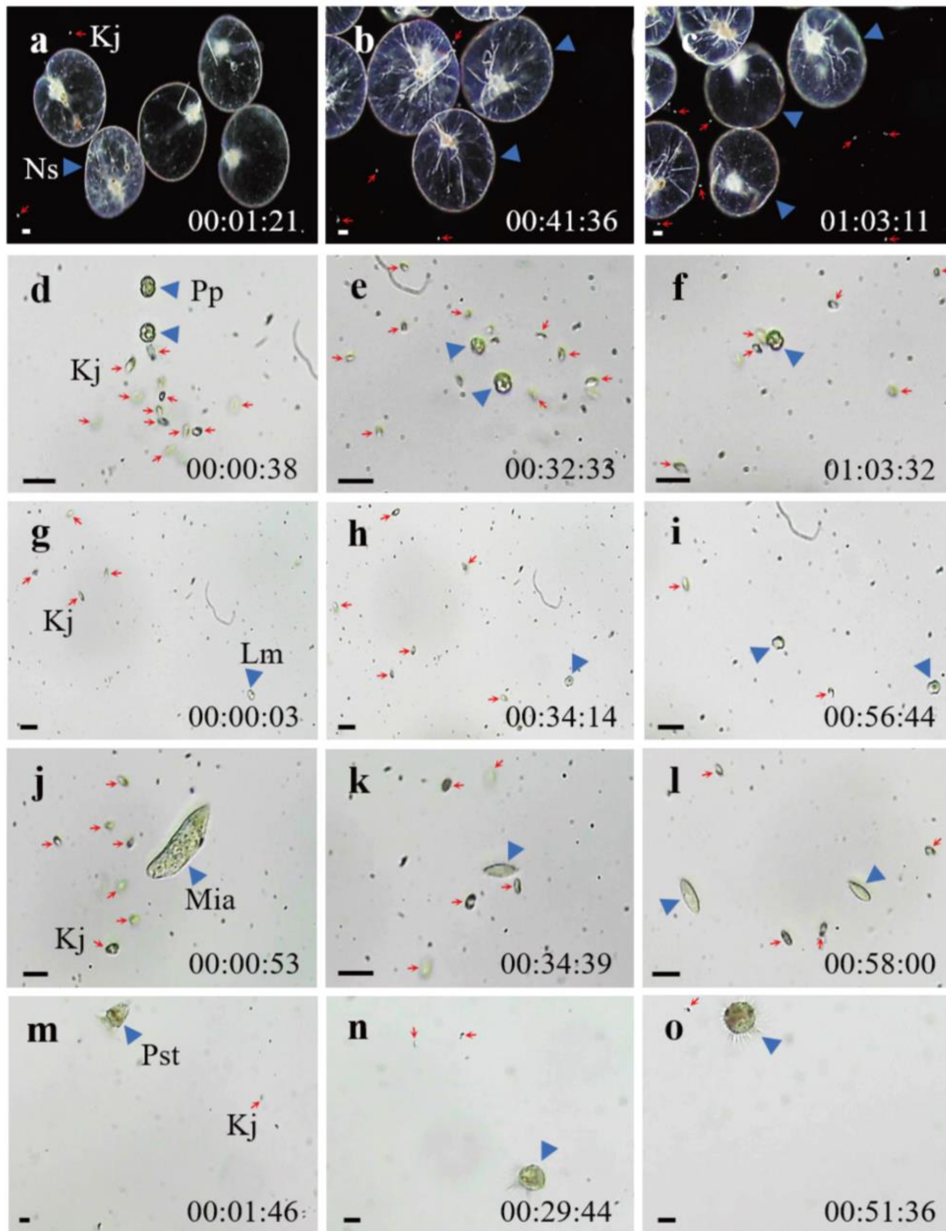


Fig 4. 2. No feeding by tentacle (a−c) and peduncle feeding heterotrophic dinoflagellates (d−i) and ciliates (j−o) on *Katablepharis japonica* recorded using video microscopy for approximately 1 h. (a−c) No feeding by *Noctiluca scintillans* (Ns) on *K. japonica* (Kj) cells. (d−f) No feeding by *Pfiesteria piscicida* (Pf) on Kj cells. (g−i) No feeding by *Luciella masanensis* (Lm) on Kj cells. (j−l) No feeding by *Miamiensis* sp. (Mia) on Kj cells. (m−o) No feeding by *Pelagostrobilidium* sp. (Pst) on Kj cells. Red arrows and blue arrowheads indicate Kj cells and heterotrophic protists. The numbers indicate h : min : s. Scale bars represent: a−c, 50 μm; d−o, 25 μm.

When heat-killed *K. japonica* cells were provided, *O. marina*, *G. dominans*, *L. masanensis*, and *P. piscicida* were able to feed on them, but the other heterotrophic protists studied did not feed on the heat-killed cells (Table 4.4, Fig 4.3).

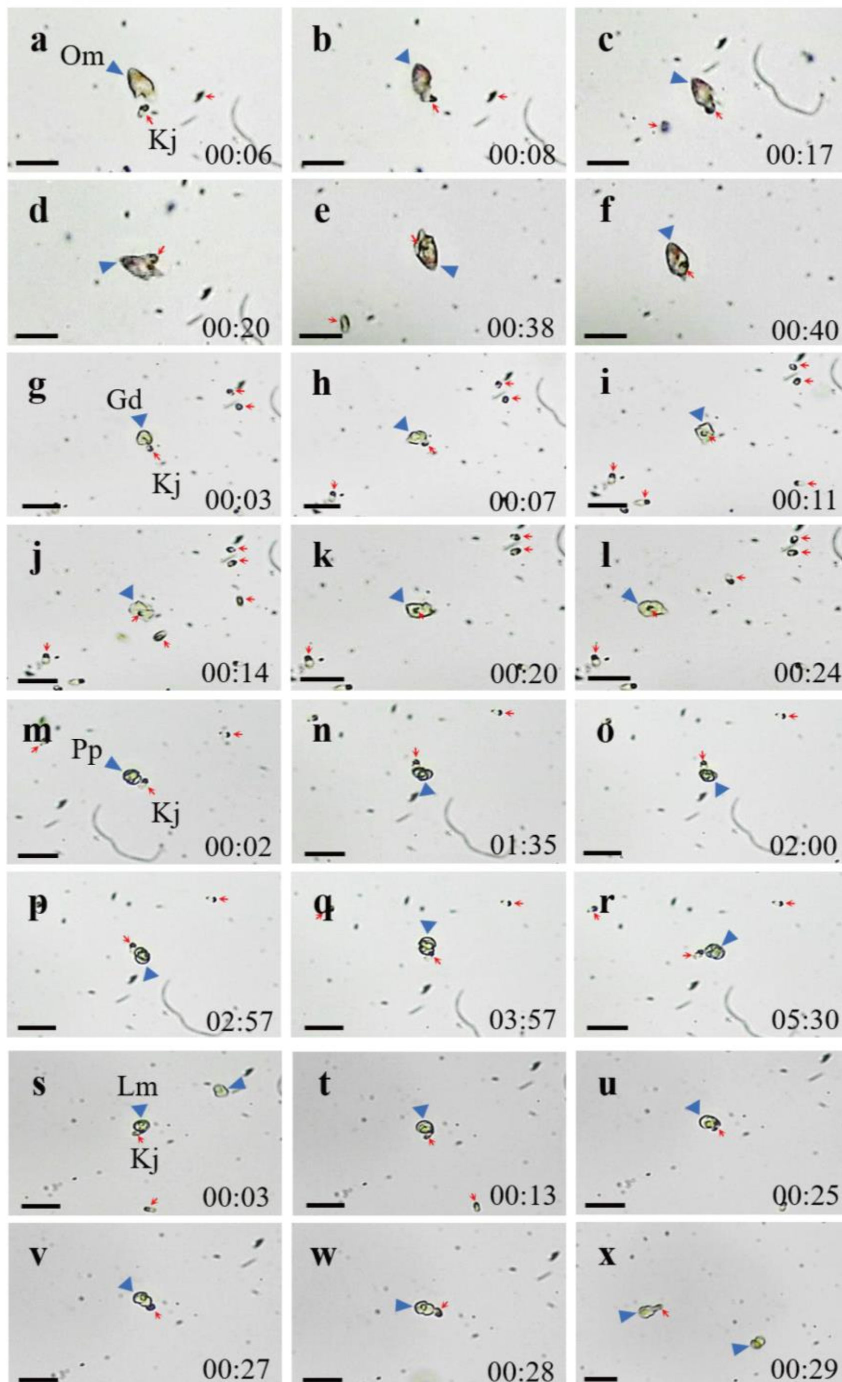


Fig 4. 3. The feeding process of heterotrophic dinoflagellates on heat−killed *Katablepharis japonica* (Kj) cells recorded using video microscopy. (a−f) Feeding by *Oxyrrhis marina* (Om) on a heat−killed Kj cell. (g−l) Feeding by *Gyrodinium dominans* (Gd) on a heat−killed Kj cell. (m−r) Feeding by *Pfiesteria piscicida* (Pp) on a heat−killed Kj cell. (s−x) Feeding by *Luciella masanensis* (Lm) on a heat−killed Kj cell. Red arrows and blue arrowheads indicate Kj cells and heterotrophic protists. The numbers indicate min : s. Scale bars represent: a−x, 25 μm.

To the contrary, *K. japonica* were able to feed on actively swimming cells of *O. marina*, *P. kofoidii*, *O. rotunda*, *Miamiensis* sp., *Pelagostrobilidium* sp., and *Strombidium* sp., but it did not feed on *G. dominans*, *G. moestrupii*, *L. masanensis*, *N. scintillans*, and *P. piscicida* (Table 4.4, Fig 4.4−5). Only a few *K. japonica* cells first attacked a HTD prey cell or a ciliate cell, but many *K. japonica* cells approached and attacked together (Fig 4.4−5). After a few *K. japonica* cells attacked a heterotrophic protist cell, the attacked cells almost disappeared within ca. 60 s for *O. marina*, 360 s for *P. kofoidii*, 280−285 s for *Miamiensis* sp. and *Strombidium* sp., 159 s for *Pelagostrobilidium* sp. and 1,200 s for *O. rotunda* (Fig 4.4− 5).

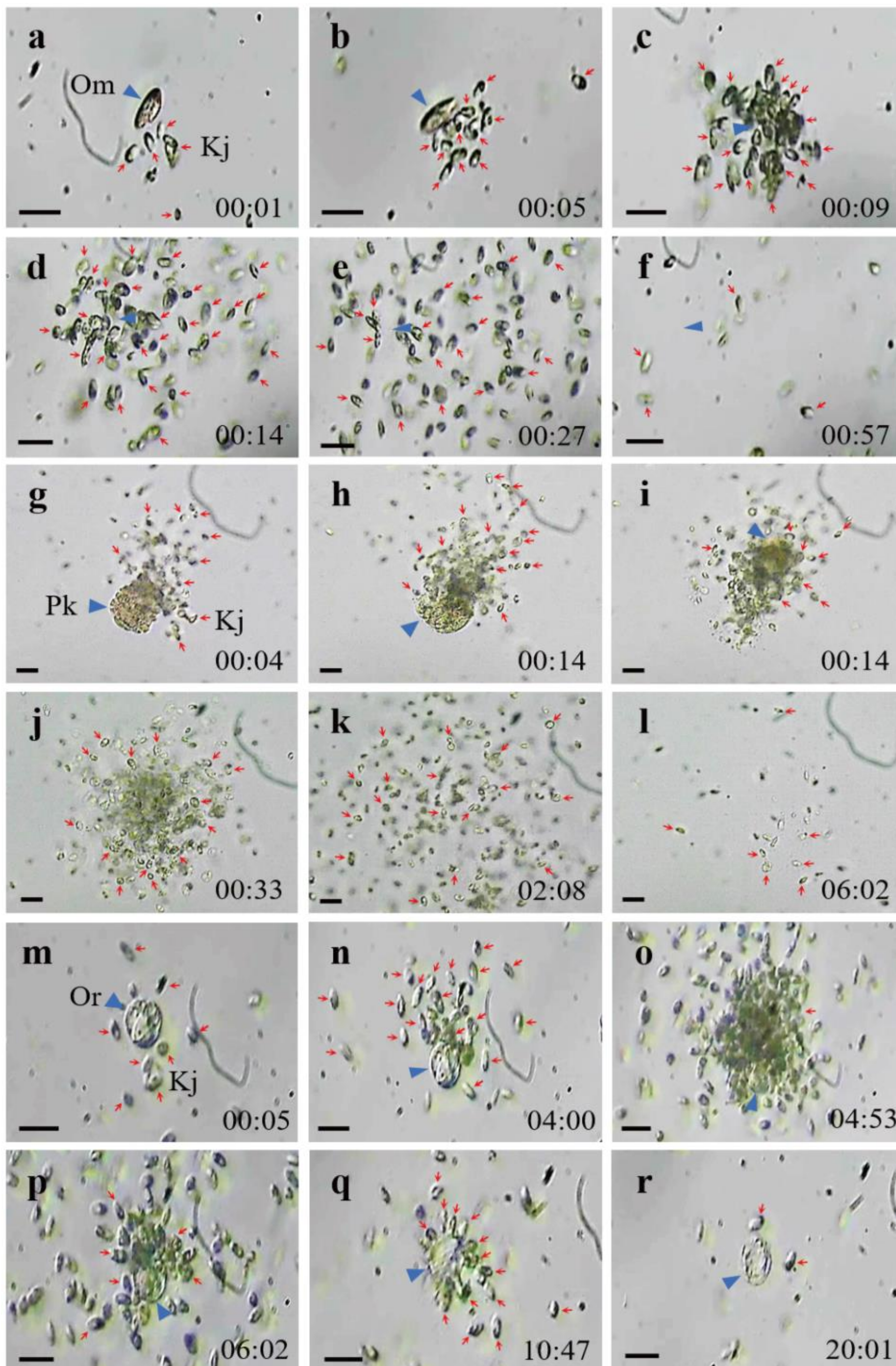


Fig 4. 4. The feeding process of *Katablepharis japonica* (Kj) on heterotrophic dinoflagellates recorded using video microscopy. (a–f) Kj cells feeding an *Oxyrrhis marina* (Om) cell. (g–l) Kj cells feeding on a *Polykrikos kofoidii* (Pk) cell. (m–r) Kj cells feeding an *Oblea rotunda* (Or) cell. Red arrows and blue arrowheads indicate Kj cells and heterotrophic protists. The numbers indicate min : s. Scale bars represent: 25 μm.

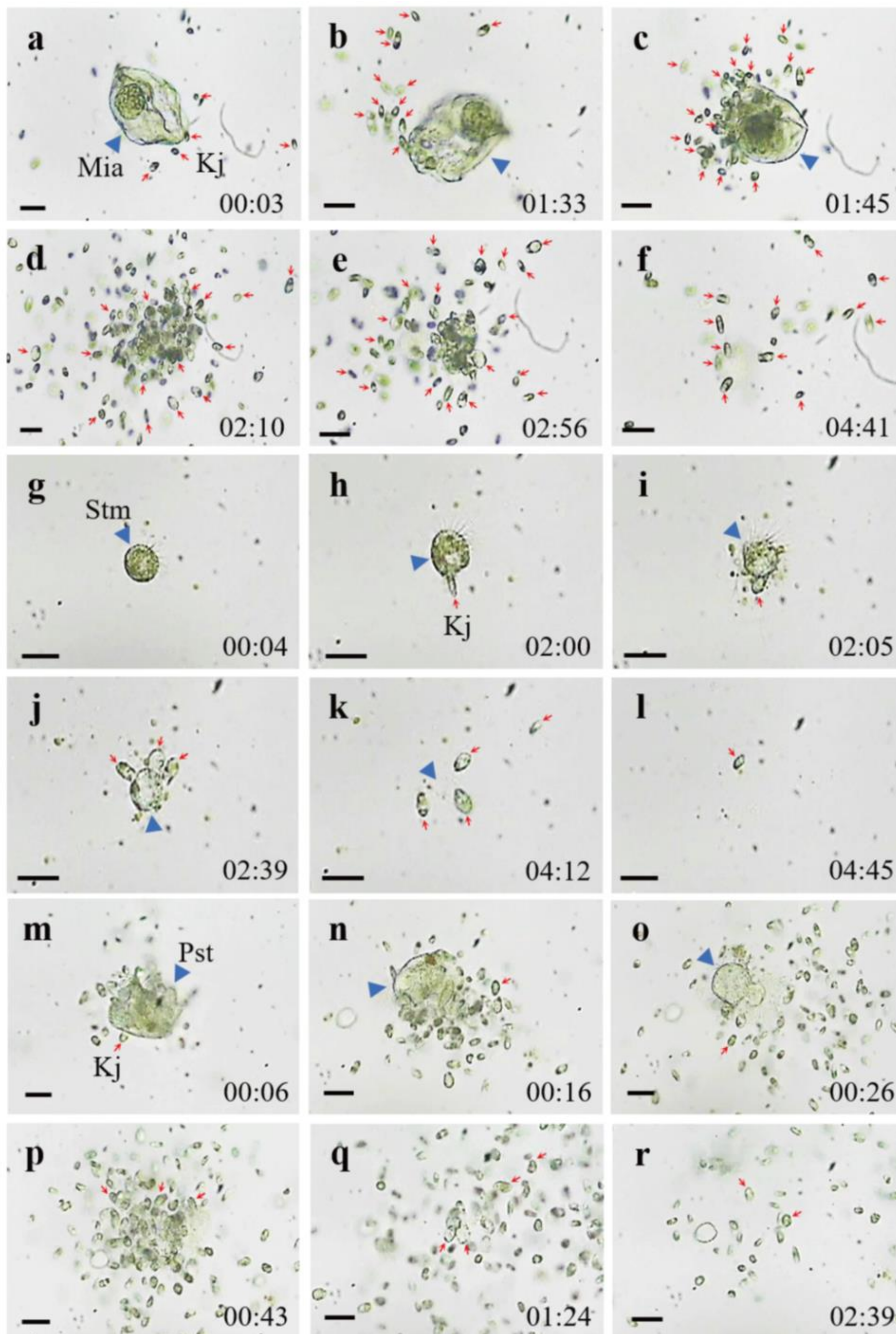


Fig 4. 5. The feeding process of *Katablepharis japonica* (Kj) on ciliates recorded using video microscopy. (a−f) Kj cells feeding a *Miamiensis* sp. (Mia) cell. (g−l) Kj cells feeding on a *Strombidium* sp. (Stm) cell. (m−r) Kj cells feeding an *Pelagostrobilidium* sp. (Pst) cell. Red arrows and blue arrowheads indicate Kj cells and heterotrophic protists. The numbers indicate min : s. Scale bars represent: 25 μm.

Table 4. 4. Taxa, size, and concentration of potential heterotrophic dinoflagellate and naked ciliate predators offered to *Katablepharis japonica*.

Potential predators	ESD (μm)	Initial predator concentration (cell mL^{-1})	By potential predators			By *K. japonica*	
			Physical attack	Successful capture		Physical attack	Successful capture
				Actively swimming *K. japonica*	Heat killing		
Heterotrophic dinoflagellate							
Luciella masanensis	13.5	2,000	O	X	O	O	X
Pfiesteria piscicida	13.5	2,000	O	X	O	O	X
Oxyrrhis marina	15.6	3,000	O	X	O	O	O
Gyrodinium moestrupii	18.4	800	O[a]	X	X	O[a]	X
Gyrodinium dominans	20.0	2,000	O	X	O	O	X
Oblea rotunda	21.6	1,000	X	X	X	O	O
Polykrikos kofoidii	43.5	150	X	X	X	O	O
Noctiluca scintillans	422.2	15	X	X	X	O	X
Naked ciliate							
Miamiensis sp.	79.4	5,000	O	X	X	O	O
Strombidium sp.	21.7	100	O	X	X	O	O
Pelagostrobilidium sp.	42.4	100	O	X	X	O	O

The initial concentrations of *K. japonica* were 5,000−8,000 cells mL^{-1}. Mean equivalent spherical diameter (ESD, μm).

O, observed, X, not observed.

O[a], Rarely attacked.

Effects of the *Katablepharis japonica* concentration on the growth rate of *Oxyrrhis marina*

When nine different *K. japonica* concentrations were provided, the specific growth rates of *O. marina* were not significantly different from one another ($p > 0.1$, ANOVA) (Fig 4.6).

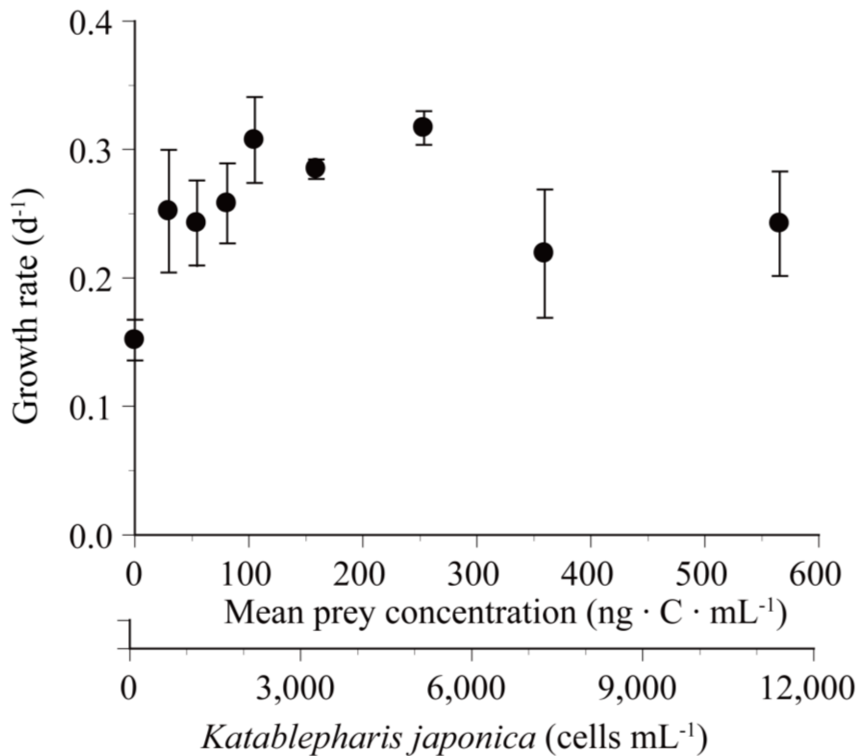


Fig 4. 6. Specific growth rates of *Oxyrrhis marina* as a function of mean *Katablepharis japonica* (Kj) concentration. Symbols represent treatment means ± 1 standard error. No difference in the growth rates was observed at the different Kj concentrations ($p > 0.1$, ANOVA).

Distribution and abundance of *Katablepharis japonica* in Korean coastal waters using qPCR

Among the 28 stations, *K. japonica* was detected more than 1 cells mL^{-1} at 20 stations in the East, West, and South Sea of Korea from January 2016 to October 2017 (Fig 4.7; Table 4.5). The ranges of temperature and salinity at the stations where *K. japonica* was detected were 5.2–28.0℃ (avr 19℃) and 9.9–35.6 (avr 28.6), respectively (Table 4.6). Cells of *K. japonica* were not detected in Sokcho, Donghae, Uljin, Yeosu, Gosan, Wimi, Seongsan, and Gimnyeong. In July 2016, the highest abundance of *K. japonica* (44,963 cells L^{-1}) was detected in Dadaepo, and the second-highest abundance (27,150 cells mL^{-1}) was measured in Mokpo. The water temperature and salinity was 21.4°C, 26.3 in Dadaepo, and 25.9℃, 27.7 in Mokpo. In addition, *K. japonica* was detected all around Korean peninsula in July 2016, 2017. Furthermore, *K. japonica* cells were observed all 8 months, January, March, July, October, and December in 2016 and March, July, and October in 2017 (Fig 4.8; Table 4.5).

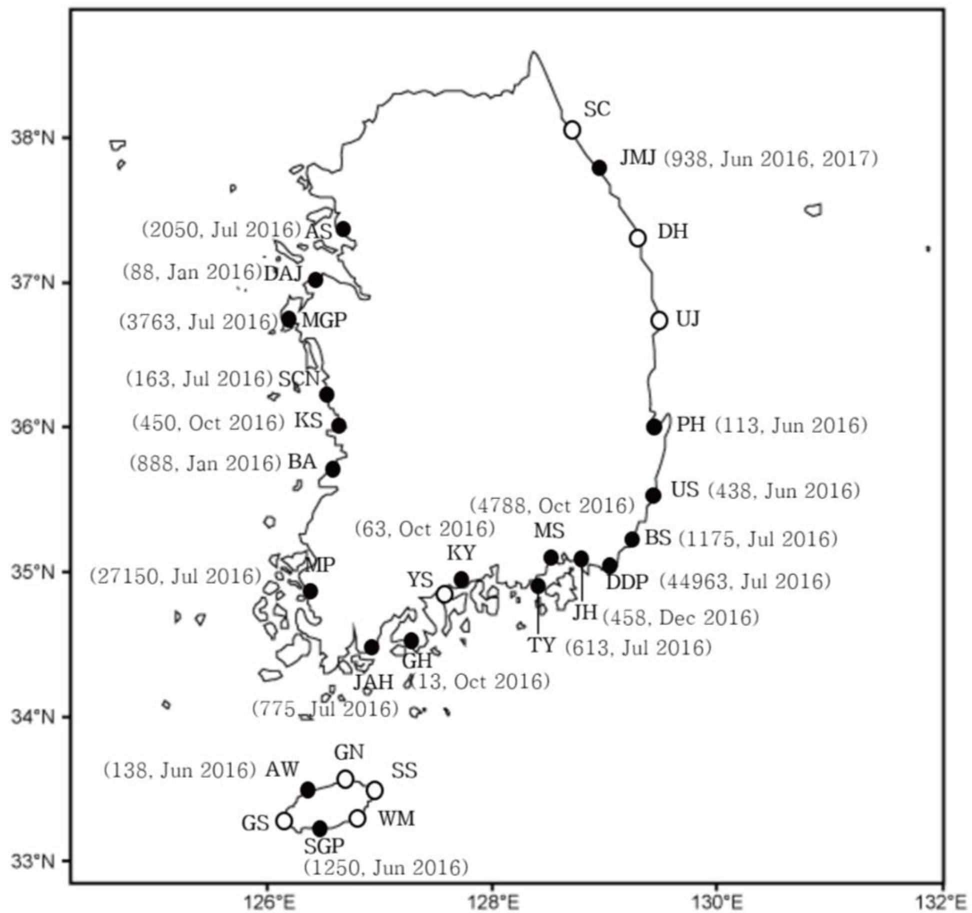


Fig 4. 7. Map of the sampling stations of the study area in Korea. The circles indicate the sampling stations. The closed circles (●) indicate the stations where cells of *Katablepharis japonica* was detected. The numbers in the parenthesis are the highest abundance at a station and the date when the highest abundance was observed at the station. SC, Sokcho; JMJ, Jumunjin; DH, Donghae; UJ, Uljin; PH, Pohang; US, Ulsan; BS, Busan; DDP, Dadaepo; MS, Masan; JH, Jinhae; TY, Tongyoung; YS, Yeosu; KY, Kwangyang; GH, Goheung; JAH, Jangheung; AS, Ansan; DAJ, Dangjin; MGP, Mageompo; SCN, Seocheon; KS, Kunsan; BA, Buan; MP, Mokpo; AW, Aewol; GS, Gosan; SGP, Seogwipo; WM, Wimi; SS, Seongsan; GN, Gimnyeong.

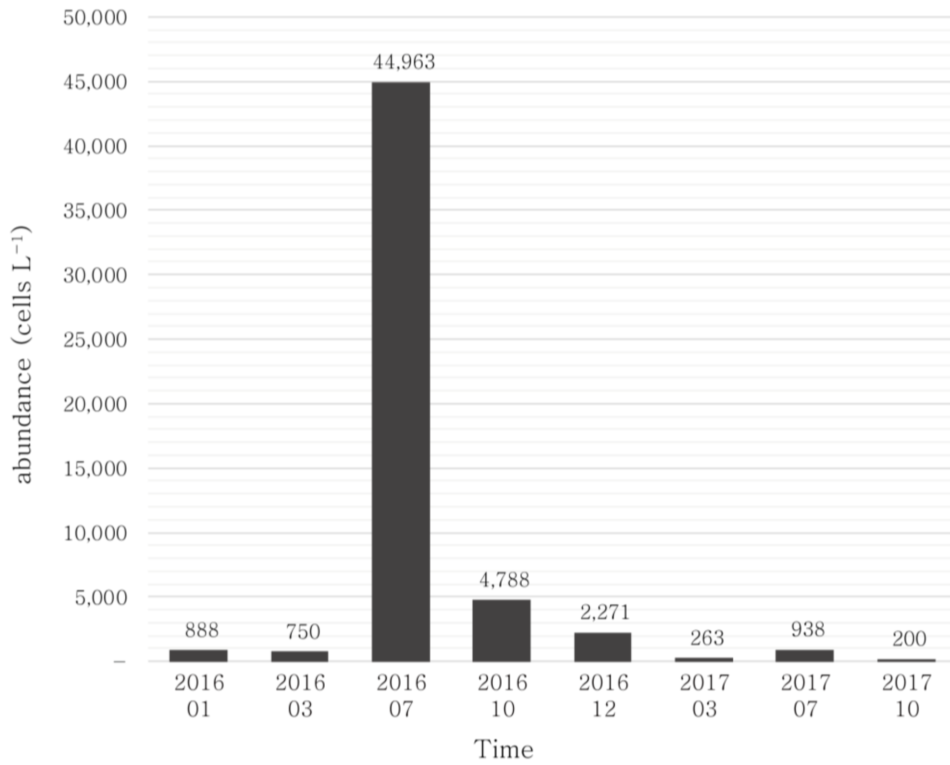


Fig 4. 8. The highest abundance of *Katablepharis japonica* from January 2016 to October 2017. The numbers on the bar indicate density (cells L^{-1}) of *K. japonica*.

Table 4. 5. The abundance of *Katablepharis japonica* (cells L^{-1}) in the surface water of Korean coasts.

	2016 01	2016 03	2016 07	2016 10	2016 12	2017 03	2017 07	2017 10	Max
SC	–	–	–	–	NA	–	–	–	–
JMJ	–	–	938	–	–	–	938	–	938
DH	–	–	–	–	–	–	–	–	–
UJ	–	–	–	–	–	–	–	–	–
PH	–	–	113	–	–	–	–	–	113
US	–	–	438	–	–	–	–	–	438
BS	–	–	1,175	–	–	–	–	–	1,175
DDP	3	–	44,963	–	–	138	38	–	44,963
JH	–	–	425	238	458	188	125	–	458
MS	–	–	2,075	4,788	2,271	–	–	–	4,788
TY	–	–	613	38	–	–	38	200	613
YS	–	–	–	–	–	–	–	–	–
KY	–	–	–	63	–	–	–	–	63
GH	–	–	–	13	–	–	1	–	13
JAH	–	–	775	–	–	263	–	–	775
AS	–	1	2,050	–	–	–	201	–	2,050
DAJ	88	–	–	13	–	–	25	–	88
MGP	–	–	3,763	50	–	–	–	–	3,763
SCN	–	–	163	–	–	–	–	–	163
KS	–	363	75	450	–	–	–	–	450
BA	888	750	725	125	850	–	50	–	888
MP	–	–	27,150	–	–	163	38	–	27,150
AW	–	–	138	–	–	–	–	–	138
GS	–	NA	NA	–	–	–	–	–	–
SGP	125	363	1,250	100	–	–	50	–	1,250
WM	–	–	–	–	–	–	–	–	–
SS	–	–	–	–	–	–	–	–	–
GN	–	NA	NA	–	–	–	–	–	–

Max, maximum abundance (cells L^{-1}); NA, not available; –, no detection of *Katablepharis japonica*.

Table 4. 6. Temperature, salinity, number of appeared months, and maximum density of *Katablepharis japonica*.

	T_{max}	T_{min}	T_{avr}	S_{max}	S_{min}	S_{avr}	appeared months/total months	D_{max}
K. japonica	28.0	5.2	19.0	35.6	9.9	28.6	8/8	44,963

T_{max}, T_{min}, and T_{avr}, maximum, minimum, and average of temperature of the waters collected at the stations where *K. japonica* was detected; S_{max}, S_{min}, and S_{avr}, maximum, minimum, and average of salinity of the waters collected at the stations where *K. japonica* was detected; D_{max}, maximum density of *K. japonica*.

4.4. Discussion

The present study clearly showed that common HTDs and ciliates were not able to feed on actively swimming *Katablepharis japonica* cells. Recently, *K. japonica* has been known to be an effective grazer on red-tide species (Kwon et al. 2017). There are many dinoflagellate grazers that feed on red-tide species (Stoecker 1999, Tillmann 2004, Jeong et al. 2005a, 2010b, 2012, 2015, Burkholder et al. 2008, Lee et al. 2014b, 2016, Johnson 2015, Kim et al. 2015, Lim et al. 2015, 2017a, Jang et al. 2017). However, some of these dinoflagellate grazers are eventually fed by other dinoflagellates and ciliates, unlike *K. japonica*, (Hansen 1992, Jeong and Latz 1994, Jacobson and Anderson 1996, Buskey 1997, Jeong et al. 1999a, 2004c, Naustvoll 2000, Kim and Jeong 2004, Park et al. 2006, Adolf et al. 2007, Berge et al. 2008, Jang et al. 2016). Thus, *K. japonica* may have an advantage and outcompete some

dinoflagellate grazers. Furthermore, since mortality rate of *K. japonica* due to predation by heterotrophic protists is negligible, it may contribute to grazing on red-tide species.

Of the HTDs included in the present study, *O. marina*, *G. dominans*, *L. masanensis*, and *P. piscicida* fed on heat-killed *K. japonica* cells, whereas *G. moestrupii*, *N. scintillans*, *O. rotunda*, *P. kofoidii* did not feed on even heat-killed cells. Furthermore, *O. marina*, *G. dominans*, *L. masanensis*, and *P. piscicida* attempted to feed on them. Therefore, the active swimming behavior of *K. japonica* is likely to be the primary cause for the lack of feeding by these four HTDs.

The HTDs *N. scintillans*, *O. rotunda*, and *P. kofoidii* did not feed on heat-killed *K. japonica* cells or attempt to feed on actively swimming *K. japonica* cells. Before ingesting prey cells, *N. scintillans* uses a tentacle to capture prey cells. *O. rotunda* uses a tow filament, and *P. kofoidii* uses a nematocyst-taeniocyst complex (Strom and Buskey 1993, Kiϕrboe and Titelman 1998, Matsuoka et al. 2000, Jeong et al. 2001b). Therefore, *K. japonica* cells may not draw a use of the tentacle or deployment of a two filament or a nematocyst-taeniocyst complex.

Interestingly, the ciliates *Pelagostrobilidium* sp., *Strombidium* sp., and *Miamiensis* sp. attempted to feed on actively swimming *K. japonica* cells, but they did not feed on heat-killed cells. Therefore, these ciliates may need a stimulus from prey cells to attack them. Feeding by some ciliates is known to be affected by mechanical or chemical stimuli of prey cells (e.g., Buskey and

Stoecker 1989, Jakobsen et al. 2006).

Cells of *K. japonica* are able to feed on actively swimming cells of *O. marina*, *P. kofoidii*, *O. rotunda*, *Miamiensis* sp., *Pelagostrobilidium* sp., and *Strombidium* sp. The duration from the time when a few *K. japonica* cells attacked a heterotrophic protist cell to that when the attacked cells almost disappeared was the shortest for the naked dinoflagellate *O. marina* (60 s), intermediate for the naked dinoflagellate *P. kofoidii* and naked ciliates *Miamiensis* sp., *Pelagostrobilidium* sp., and *Strombidium* sp. (159−360 s), but the longest for the thecate dinoflagellate *O. rotunda* (1,200 s). The cell volumes of *P. kofoidii* and the ciliates are much greater than that of *O. rotunda*. Therefore, the presence of the theca of *O. rotunda* may be partially responsible for the greater duration, as compared to that of the protists that do not have theca.

O. marina does not feed on actively swimming *K. japonica*, but it is able to feed on heat-killed *K. japonica* cells. Thus, *O. marina* may feed on dead *K. japonica* cells in natural environments. However, *K. japonica* is able to feed on *O. marina*. Thus, determining growth rate (or survival rate) of *O. marina* as a function of *K. japonica* is needed. When suitable prey species are provided, the specific growth rates of *O. marina* usually increase rapidly and then become saturated with increasing prey concentration. The phototrophic dinoflagellate prey *Amphidinium carterae*, *Azadinium* cf. *poporum*, *Biecheleria cincta*, *Gymnodinium aureolum*, *Effrenium* (*Symbiodinium*) *voratum*, the HTD *Pfiesteria piscicida*, the haptophyte *Isochrysis galbana*, the euglenophyte

Eutreptiella gymnastica, and the raphidophyte *Heterosigma akashiwo* cause this positive trend (Jeong et al. 2001a, 2003, 2007b, 2011, 2014, Kimmance et al. 2006, Yoo et al. 2010, 2013c, Potvin et al. 2013). However, when inhibitory species are provided, with increasing prey concentration, the specific growth rates of *O. marina* usually decrease; the phototrophic dinoflagellates *Alexandrium pohangense*, *Alexandrium tamarense*, *Paragymnodinium shiwhaense*, and *Takayama helix* cause this negative trend (Tillmann and John 2002, Kim et al. 2016, Jeong et al. 2017, Ok et al. 2017). However, in the present study, the specific growth rates of *O. marina* were not significantly different among the nine different *K. japonica* concentrations. Thus, *K. japonica* may neither markedly support nor inhibit the growth of *O. marina*.

The results of the present study and those of our previous study (Kwon et al. 2017) suggest that the HNF *K. japonica* could be an effective grazer on diverse red-tide organisms with negligible mortality due to heterotrophic protist predators. Thus, when establishing models for predicting the process of red tide formation, grazing impact by *K. japonica* on the red-tide organisms and low mortality of *K. japonica* should be considered. Furthermore, in the microbial loop, flagellates are positioned between bacteria or algal prey and ciliates (Azam et al. 1983). However, *K. japonica* is not fed on by ciliates. Thus, *K. japonica* may not be a link between bacteria or algal prey and ciliates. Moreover, the maximum growth rate of *K. japonica* is lower than that of the other HNFs (Kwon et al.

2017). Thus, the low mortality rate due to predation by common heterotrophic protists may compensate the relatively low growth rate of *K. japonica*.

Prior to this study, *K. japonica* was detected 600 cells L^{-1}, and 119,600 cells L^{-1} in Masan Bay, Korea in January and October 2016 (Kwon et al. 2017). However, in this study, there was no detection in January 2016 and 4,788 cells L^{-1} were detected in October 2016 at the same location (Table 4.5). Therefore, the higher density in the previous data could be other *Katablepharis* species or related dinoflagellates. In addition, cells of *K. japonica* detected at 20 locations in the East, West, and South Sea of Korea during 2016–2017. Thus, *K. japonica* is nationwide distributed in Korean waters. Furthermore, cells of *K. japonica* appeared in the spring, summer, fall, and even winter in 2016–2017, when the ranges of water temperature and salinity were 5.2–28.0℃ and 9.9–35.6 (Table 4.6). Therefore, *K. japonica* has wide ranges of temperature and salinity tolerance. Besides, according to the results of the present study, *K. japonica* has low mortality due to predation by common heterotrophic protists. Consequently, cells of *K. japonica* have been found in all seasons around Korean peninsula.

Chapter 5. Conclusion

In this thesis, differential responses of heterotrophic protists feeding on four *Scrippsiella* species (*Scrippsiella acuminata*, *Scrippsiella donghaiensis*, *Scrippsiella lachrymosa*, *Scrippsiella masanensis*) isolated in the Korean coast and their eco-physiological characteristics were investigated. In addition, I explored the interactions between heterotrophic nanoflagells, *Katablepharis japonica*, and heterotrophic protists that might co-exist because they could feed on same red-tide organisms. Furthermore, the temporal and spatial distribution of *K. japonica* and four *Scrippsiella* species that could affect heterotrophic protists were analyzed.

To analyze distribution and abundance of four *Scrippsiella* species, species-specific primer and probe set of each *Scrippsiella* speices was designed and examined using qPCR. Three *Scrippsiella* species were detected more than 1 cells L^{-1} around Korea peninsula, but *S. lachrymosa* was not detected on the coast of Jeju. In addition, *S. lachrymosa* and *S. donghaiensis* showed high abundance and wide distribution in March and July, respectively. Furthermore, *S. masanensis* has the broadest ranges of water temperature and salinity among the four *Scrippsiella* species, but its density was low (1-25 cells L^{-1}), and the highest density of *S. acuminata* was observed in January 2016, but it was rarely observed and also appeared only Ansan, Mageompo, Ulsan, Awal,

and Dadaepo. These four *Scrippsiella* species are distributed globally, but the information about temperature or salinity where the other strains were found is not specifically indicated. The environmental data provide a basis on understanding their ecophysiological characteristics. However, although *S. donghaiensis* and *S. lachrymosa* have a seasonality, there is no correlation with temperature and salinity. If additional analysis is conducted, it will be possible to better understand the distribution characteristics according to their environmental factors. In addition, besides environmental factors, mortality due to predators may affect their distributions.

Consequently, to explore the interactions between four *Scrippsiella* species and heterotrophic protists, feeding occurrence of predators and growth and ingestion rates of *Polykrikos kofoidii*, *Gyrodinium dominans*, and *Oxyrrhis marina* feeding on four *Scrippsiella* species were compared. Thus, predators tested in this study showed different growth and ingestion rates for four *Scrippsiella* species (Fig 5.1). The growth and ingestion rates of predators feeding on *S. acuminata* and *S. lachrymosa* were higher than feeding on *S. donghaiensis* and *S. masanensis*. In addition, cells and filtrate of *S. donghaiensis* was able to lyse *P. kofoidii* cells and the growth rate of predators feeding on *S. masanensis* is considerably lower than ingestion rate. Moreover, toxicity test of four *Scrippsiella* speices was conducted using *Artemia* nauplius to test whether feeding rate of heterotrophic protists was affected, and none of *Scrippsiella* species affect nauplius. Conclusively,

although *Scrippsiella* species have a little morphological and genetic differences, they have a considerably different impact on predators. Therefore, differential growth and ingestion rates of heterotrophic protists tested in this study on four *Scrippsiella* species may cause different ecological niches and have different eco-physiological characteristics.

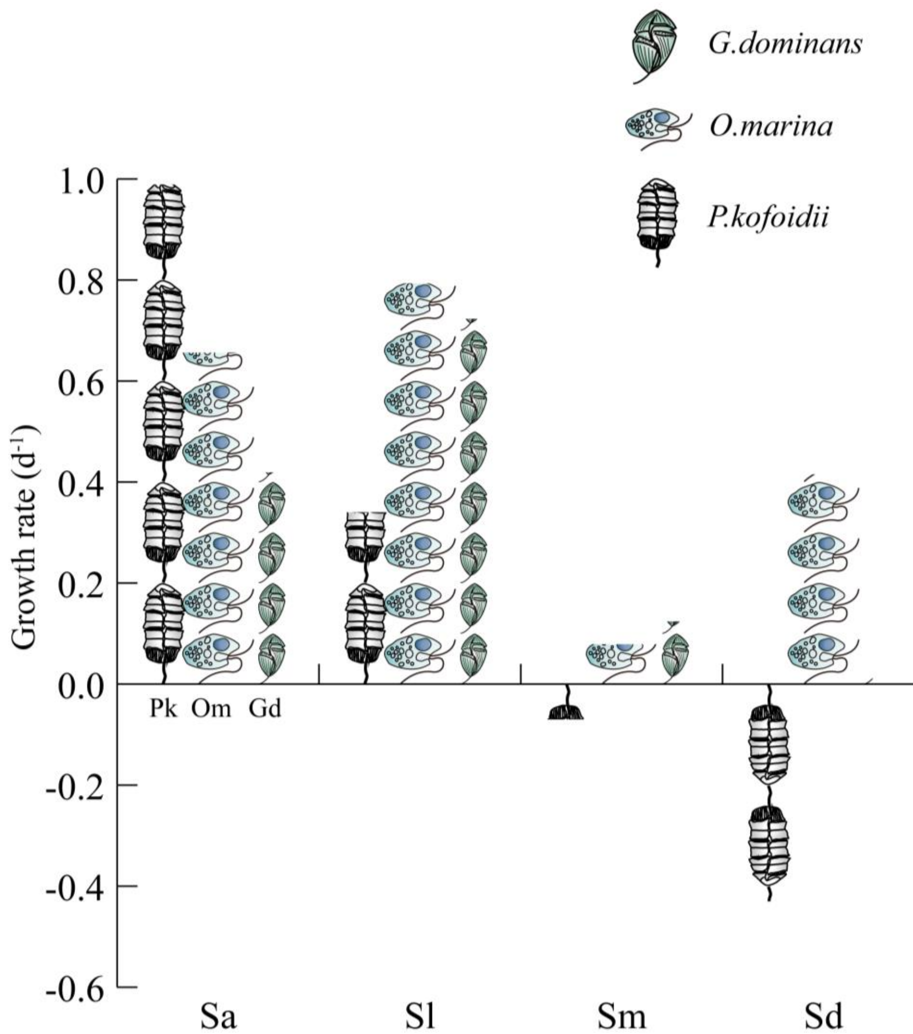


Fig 5. 1. Different growth rates of *Polykrikos kofoidii*, *Oxyrrhis marina*, and *Gyrodinium dominans* feeding on *Scrippsiella acuminata* (Sa), *S. lachrymosa* (Sl), *S. masanensis* (Sm), *S. donghaiensis* (Sd) at single high prey concentrations.

Moreover, to investigate the interactions between heterotrophic protists and *K. japonica*, their feeding occurrence was studied. Consequently, none of heterotrophic protists feed on actively swimming *K. japonica* cells, but *K. japonica* was able to feed on six heterotrophic protists. Thus, mortality rate of *K. japonica* due to predation is negligible as it is considerably low. According to this study, since *K. japonica* is able to feed on diverse phytoplankton and has low mortality rate, they may appear frequently in the field. To analyze distribution and abundance of *K. japonica*, I developed improved species-specific primer and probe set of *K. japonica*. Cells of *K. japonica* were distributed nationwide ranged from 1 to 1,750 cells L^{-1} and detected at 20 stations in 8 months. Consequently, *K. japonica* cells are frequently found in the Korean waters and they may affect distribution of heterotrophic protists and diverse phytoplankton. In conclusion, these results suggest a newly discovered pathways in marine planktonic food web when focusing on heterotrophic protists, *Scrippsiella*, and *K. japonica* (Fig 5.2).

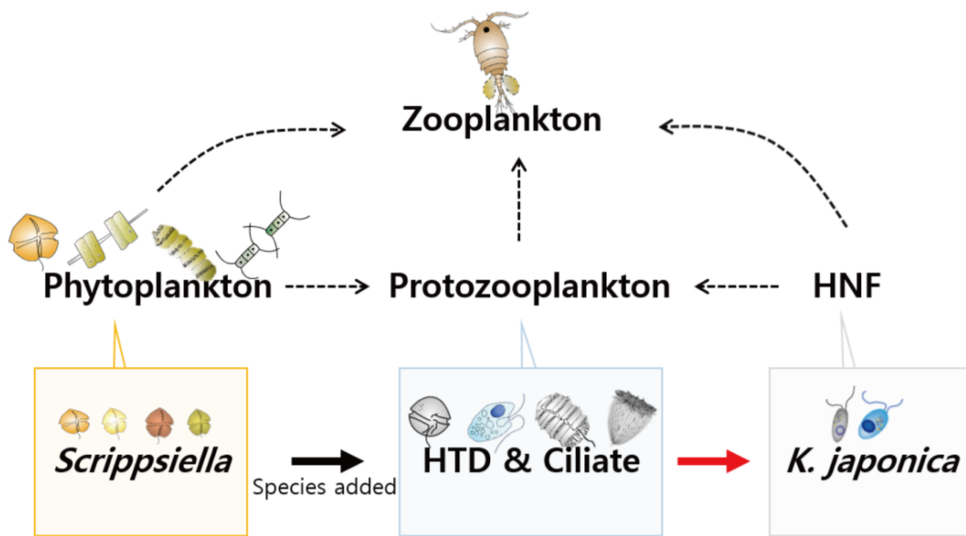


Fig 5. 2. A diagram of marine planktonic food web focusing on phytoplankton, protozooplankton, and heterotrophic nanoflagellates(HNF). Black arrow indicates a modified pathway and red arrow indicates newly discovered pathway.

Reference

Adolf, J. E., Krupatkina, D., Bachvaroff, T. & Place, A. R. 2007. Karlotoxin mediates grazing by *Oxyrrhis marina* on strains of *Karlodinium veneficum*. Harmful Algae. 6:400−412.

Anderson, S. R. & Menden-Deuer, S. 2017. Growth, grazing, and starvation survival in three heterotrophic dinoflagellate species. J. Eukaryot. Microbiol. 64:213−225.

Asakawa, M., Miyazawa, K., Takayama, H. & Noguchi, T. 1995. Dinoflagellate *Alexandrium tamarense* as the source of paralytic shellfish poison (PSP) contained in bivalves from Hiroshima Bay, Hiroshima Prefecture, Japan. Toxicon 33:691−697.

Attaran−Fariman, G. & Bolch, C. J. 2007. *Scrippsiella irregularis* sp. nov.(Dinophyceae), a new dinoflagellate from the southeast coast of Iran. Phycologia 46:572−582.

Azam, F., Fenchel, T., Field, J. G., Gray, J. S., Meyer−Reil, L. A. & Thingstad, F. 1983. The ecological role of water−column microbes in the sea. Mar. Ecol. Prog. Ser. 10:257−263.

Balech, E. 1959. Two new genera of dinoflagellates from California. Biol Bull. 116:195−203.

Balkis, N., Balci, M., Giannakourou, A., Venetsanopoulou, A. & Mudie, P. 2016. Dinoflagellate resting cysts in recent marine sediments from the Gulf of Gemlik (Marmara Sea, Turkey) and seasonal harmful algal blooms. Phycologia 55:187−209.

Bardouil, M., Bohec, M., Cormerais, M., Bougrier, S. & Lassus, P. 1993. Experimental study of the effects of a toxic microalgal diet on feeding of the oyster *Crassostrea gigas* Thunberg. J. Shellfish Res. 12:417−422.

Bauerfeind, E., Elbrächter, M., Steiner, R. & Throndsen, J. 1986. Application of Laser Doppler Spectroscopy (LDS) in determining swimming velocities of motile phytoplankton. Mar. Biol. 93:323−327.

Berge, T., Hansen, P. J. & Moestrup, Ø. 2008. Feeding mechanism, prey specificity and growth in light and dark of the plastidic dinoflagellate *Karlodinium armiger*. Aquat. Microb. Ecol. 50:279−288.

Blanco, J. 1995. The distribution of dinoflagellate cysts along the Galician (NW Spain) coast. J. Plankton Res. 17:283−302.

Burkholder, J. M. & Glasgow, H. B. 1997. *Pfiesteria piscicida* and other *Pfiesreria*-like dinoflagellates: Behavior, impacts, and environmental controls. Limnol. Oceanogr. 42:1052−1075.

Burkholder, J. M., Glibert, P. M. & Skelton, H. M. 2008. Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. Harmful Algae. 8:77−93.

Buskey, E. J. & Stoecker, D. K. 1989. Behavioral responses of the marine tintinnid *Favella* sp. to phytoplankton: influence of chemical, mechanical and photic stimuli. J. Exp. Mar. Biol. Ecol. 132:1−16.

Buskey, E. J. 1997. Behavioral components of feeding selectivity of the heterotrophic dinoflagellate *Protoperidinium pellucidum*.

Mar. Ecol. Prog. Ser. 153:77−89.

Calbet, A., Isari, S., Martínez, R. A., Saiz, E., Garrido, S., Peters, J., Borrat, R. M. & Alcaraz, M. 2013. Adaptations to feast and famine in different strains of the marine heterotrophic dinoflagellates *Gyrodinium dominans* and *Oxyrrhis marina*. Mar. Ecol. Prog. Ser. 483:67−84.

Ciminiello, P., Dell'Aversano, C., Fattorusso, E., Forino, M., Tartaglione, L., Grillo, C. & Melchiorre, N. 2008. Putative palytoxin and its new analogue, ovatoxin−a, in *Ostreopsis ovata* collected along the Ligurian coasts during the 2006 toxic outbreak. J. Am. Soc. Mass. Spectr. 19:111−120.

Daugbjerg, N., Hansen, G., Larsen, J., & Moestrup, Ø. (2000). Phylogeny of some of the major genera of dinoflagellates based on ultrastructure and partial LSU rDNA sequence data, including the erection of three new genera of unarmoured dinoflagellates. Phycologia. 39:302−317.

Edwards, E. S. & Burkill, P. H. 1995. Abundance, biomass and distribution of microzooplankton in the Irish Sea. J. Plankton Res. 17:771−782.

Eker, E., Georgieva, L., Senichkina, L. & Kideys, A. E. 2000. Phytoplankton distribution in the western and eastern Black Sea in spring and autumn 1995. Ices. J. Mar. Sci. 56:15−22.

Estrada, M., 1984. Phytoplankton distribution and composition off the coast of Galicia (northwest of Spain). J. Plankton Res. 6:417−34.

Fenchel, T. 1980. Relation between particle size selection and

clearance in suspension-feeding ciliates. Limnol. Oceanogr. 25:733–738.

Fenchel, T. 1982. Ecology of heterotrophic microflagellates. IV. Quantitative occurrence and importance as bacterial consumers. Mar. Ecol. Prog. Ser. 9:35–42.

Frost, B. W. 1972. Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. Limnol. Oceanogr. 17:805–815.

Fuhrman, J. A., Ammerman, J. W. & Azam, F. 1980. Bacterioplankton in the coastal euphotic zone: distribution, activity and possible relationships with phytoplankton. Mar. Biol. 60:201–207.

Fuller, A. K. R. 1990. The grazing and growth rates of some marine protozoa measured in batch and continuous culture with particular reference to the heterotrophic dinoflagellate *Oxyrrhis marina*. Ph. D. dissertation, University of London, Royal Holloway, pp. 57–129.

Gárate–Lizárraga, I., Band–Schmidt, C. J., López–Cortés, D. J. & Muñetón–Gómez, M. D. S. 2009. Bloom of *Scrippsiella trochoidea* (Gonyaulacaceae) in a shrimp pond in the southwestern Gulf of California, Mexico. Mar. Pollut. Bull. 58:145–149.

Giannakourou, A., Orlova, T. Y., Assimakopoulou, G. & Pagou, K. 2005. Dinoflagellate cysts in recent marine sediments from Thermaikos Gulf, Greece: effects of resuspension events on vertical cyst distribution. Cont Shelf Res. 25:2585–2596.

Giovannoni, S. J., DeLong, E. F., Olsen, G. J. & Pace, N. R. 1988. Phylogenetic group-specific oligodeoxynucleotide probes for identification of single microbial cells. J Bacteriol. 170:720-726.

Gottschling, M., Knop, R., Plötner, J., Kirsch, M., Willems, H. & Keupp, H. 2005. A molecular phylogeny of *Scrippsiella sensu lato* (Calciodinellaceae, Dinophyta) with interpretations on morphology and distribution. Eur. J. Phycol. 40:207-220.

Grzebyk, D., Audic, S., Lasserre, B., Abadie, E., de Vargas, C. & Bec, B. 2017. Insights into the harmful algal flora in northwestern Mediterranean coastal lagoons revealed by pyrosequencing metabarcodes of the 28S rRNA gene. Harmful Algae. 68:1-16.

Gu, H., Luo, Z., Liu, T. & Lan, D. 2013. Morphology and phylogeny of *Scrippsiella enormis* sp. nov. and *S.* cf. *spinifera* (Peridiniales, Dinophyceae) from the China Sea. Phycologia. 52:182-190.

Gu, H., Sun, J., Kooistra, W. H. & Zeng, R. 2008. Phylogenetic position and morphology of thecae and cysts of *Scrippsiella* (dinophyceae) species in the east China Sea 1. J. Phycol. 44:478-494.

Guillard, R. R. L. & Ryther, J. H. 1962. Studies of marine planktonic diatoms. I. Cyclotella nana Hustedt and Detonula confervacea (Cleve) Grun. Can. J. Microbiol. 8:229-239.

Guilloux, L., Rigaut-Jalabert, F., Jouenne, F., Ristori, S., Viprey, M., Not, F., Vaulot, D. & Simon, N. 2013. An annotated checklist

of Marine Phytoplankton taxa at the SOMLIT−Astan time series off Roscoff (Western English Channel, France): data collected from 2000 to 2010. Cah. Biol. Mar. 54:247−256.

Guiry, M. D. & Guiry, G. M. 2018. AlgaeBase. World−wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; (last accessed 30 July 2018).

Guzmán, H. M., Cortés, J., Glynn, P. W. & Richmond, R. H. 1990. Coral mortality associated with dinoflagellate blooms in the eastern Pacific (Costa Rica and Panama). Mar. Ecol. Prog. Ser. 299−303.

Hallegraeff, G. M. 1992. Harmful algal blooms in the Australian region. Mar. Pollut. Bull. 25:186−190.

Hansen, P. J. & Calado, A. J. 1999. Phagotrophic mechanisms and prey selection in free-living dinoflagellates. J. Eukaryot. Microbiol. 46:382−389.

Hansen, P. J. 1989. The red tide dinoflagellate *Alexandrium tamarense*: Effects on behaviour and growth of a tintinnid ciliate. Mar. Ecol. Prog. Ser. 53:105−116.

Hansen, P. J. 1991. Quantitative importance and trophic role of heterotrophic dinoflagellates in a coastal pelagial food web. Mar. Ecol. Prog. Ser. 73:253−261.

Hansen, P. J. 1992. Prey size selection, feeding rates and growth dynamics of heterotrophic dinoflagellates with special emphasis on *Gyrodinium spirale*. Mar. Biol. 114:327−334.

Hansen, P. J., Bjørnsen, P. K. & Hansen, B. W. 1997. Zooplankton grazing and growth: Scaling within the 2-2,000-μm body size

range. Limnol. Oceanogr. 42:687–704.

Heinbokel, J. F. 1978. Studies on the functional role of tintinnids in the Southern California Bight. I. Grazing and growth rates in laboratory cultures. Mar. Biol. 47:177–89.

Hold, G. L., Smith, E. A., Rappë, M. S., Maas, E. W., Moore, E. R., Stroempl, C., Stephen, J. R., Prosser, J. I., Birkbeck, T. H. & Gallacher, S. 2001. Characterisation of bacterial communities associated with toxic and non-toxic dinoflagellates: *Alexandrium* spp. and *Scrippsiella trochoidea*. FEMS Microbiol. Ecol. 37:161–173.

Hoppenrath, M. 2004. A revised checklist of planktonic diatoms and dinoflagellates from Helgoland (North Sea, German Bight). Helgol Mar Res. 58:243–251.

Hwang, B. S., Yoon, E. Y., Kim, H. S., Yih, W., Park, J. Y., Jeong, H. J. & Rho, J. R. 2013. Ostreol A: a new cytotoxic compound isolated from the epiphytic dinoflagellate *Ostreopsis* cf. *ovata* from the coastal waters of Jeju Island, Korea. Bioorg. Med. Chem. Lett. 23:3023–3027.

Ishikawa, A. & Taniguchi, A. 1993. Some cysts of the genus *Scrippsiella* (Dinophyceae) newly found in Japanese waters. Bull. Plankton. Soc. Japan.

Jacobson, D. M. & Anderson, D. M. 1986. Thecate heterophic dinoflagellates: feeding behavior and mechanisms 1. J. Phycol. 22:249–258.

Jacobson, D. M. & Anderson, D. M. 1996. Widespread phagocytosis of ciliates and other protists by marine mixotrophic and

heterotrophic thecate dinoflagellates. J. Phycol. 32:279−285.

Jacobson, D. M. 1987. The ecology and feeding biology of thecate heterotrophic dinoflagellates. Ph.D. thesis. Woods Hole Oceanographic Institution/Massachusetts Institute of Technology Joint Program, Flamouth, MA, pp.210

Jakobsen, H. H., Everett, L. M. & Strom, S. L. 2006. Hydromechanical signaling between the ciliate *Mesodinium pulex* and motile protist prey. Aquat. Microb. Ecol. 44:197−206.

Jang, S. H., Jeong, H. J., Kwon, J. E. & Lee, K. H. 2017. Mixotrophy in the newly described dinoflagellate *Yihiella yeosuensis*: a small, fast dinoflagellate predator that grows mixotrophically, but not autotrophically. Harmful algae. 62:94−103.

Jang, S. H., Jeong, H. J., Lim, A. S., Kwon, J. E. & Kang, N. S. 2016. Feeding by the newly described heterotrophic dinoflagellate *Aduncodinium glandula*: having the most diverse prey species in the family Pfiesteriaceae. Algae 31:17−31.

Janofske, D. 2000. *Scrippsiella trochoidea* and *Scrippsiella regalis*, nov. comb. (Peridiniales, Dinophyceae): a comparison. J. Phycol. 36:178−189.

Jeone, H. J., Lim, A. S., Lee, K., Lee, M. J., Seong, K. A., Kang, N. S., Jang, S. H., Lee, K. H., Lee, S. Y., Kim, M. O., Kim, J. H., Kwon, J. E., Kang, H. C., Kim, J. S., Yih, W. H., Shin, K. S., Jang, P. K., Ryu, J. H., Kim, J. H., Kim, S. Y., Park, J. Y. & Kim, K. Y. 2017b. Ichthyotoxic *Cochlodinium polykrikoides* red tides offshore in the South Sea, Korea in 2014: I.

Temporal variations in three-dimensional distributions of red-tide organisms and environmental factors. Algae 32:101-130.

Jeong, H. J. & Latz, M. I. 1994. Growth and grazing rates of the heterotrophic dinoflagellates *Protoperidinium* spp. on red tide dinoflagellates. Mar. Ecol. Prog. Ser. 106:173-185.

Jeong, H. J. 1994. Predation by the heterotrophic dinoflagellate *Protoperidinium* cf. *divergens* on copepod eggs and early naupliar stages. Mar. Ecol. Prog. Ser. 114:203-208.

Jeong, H. J. 1999a. The ecological roles of heterotrophic dinoflagellates in marine planktonic community. J. Eukaryot. Microbiol. 46:390-396.

Jeong, H. J., Ha, J. H., Park, J. Y., Kim, J. H., Kang, N. S., Kim, S. H., Kim, J. S., Yoo, Y. D. & Yih, W. H. 2006. Distribution of the heterotrophic dinoflagellate *Pfiesteria piscicida* in Korean waters and its consumption of mixotrophic dinoflagellates, raphidophytes and fish blood cells. Aquat. Microb. Ecol. 44:263-278.

Jeong, H. J., Ha, J. H., Yoo, Y. D., Park, J. Y., Kim, J. H., Kang, N. S., Kim, T. H., Kim, H. S. & Yih, W. H. 2007a. Feeding by the *Pfiesteria*-Like Heterotrophic Dinoflagellate *Luciella masanensis*. J. Eukaryot. Microbio. 54:231-241.

Jeong, H. J., Kang, H. C., You, J. H. & Jang, S. H. 2018a. Interactions between the Newly Described Small-and Fast-Swimming Mixotrophic Dinoflagellate *Yihiella yeosuensis* and Common Heterotrophic Protists. J. Eukaryot. Microbiol.

65:612–626

Jeong, H. J., Kang, H., Shim, J. H., Park, J. K., Kim, J. S., Song, J. Y. & Choi, H. –J. 2001a. Interactions among the toxic dinoflagellate *Amphidinium carterae*, the heterotrophic dinoflagellate *Oxyrrhis marina*, and the calanoid copepods *Acartia* spp. Mar. Ecol. Prog. Ser. 218:77–86.

Jeong, H. J., Kim, J. S., Kim, J. H., Kim, S. T., Seong, K. A., Kim, T. H., Song, J. Y. & Kim, S. K. 2005a. Feeding and grazing impact of the newly described heterotrophic dinoflagellate *Stoeckeria algicida* on the harmful alga *Heterosigma akashiwo*. Mar. Ecol. Prog. Ser. 295:69–78.

Jeong, H. J., Kim, J. S., Kim, S., Song, J. Y., Lee, I. & Lee, G. H. 2004a. *Strombidinopsis* jeokjo n. sp. (Ciliophora: Choreotrichida) from the coastal waters off western Korea: morphology and small subunit ribosomal DNA gene sequence. J. Eukaryot. Microbiol. 51:451–455.

Jeong, H. J., Kim, J. S., Lee, K. H., Seong, K. A., Yoo, Y. D., Kang, N. S., Kim, T. H., Song, J. Y. & Kwon, J. E. 2017a. Differential interactions between the nematocyst–bearing mixotrophic dinoflagellate *Paragymnodinium shiwhaense* and common heterotrophic protists and copepods: killer or prey. Harmful Algae. 62:37–51.

Jeong, H. J., Kim, J. S., Song, J. Y., Kim, J. H., Kim, T. H., Kim, S. K. & Kang, N. S. 2007b. Feeding by protists and copepods on the heterotrophic dinoflagellates *Pfiesteria pisicicida*, *Stoeckeria algicida*, and *Luciella masanensis*. Mar. Ecol. Prog.

Ser. 349:199−211.

Jeong, H. J., Kim, J. S., Yoo, Y. D., Kim, S. T., Kim, T. H., Park, M. G., Lee, C. H., Seong, K. A., Kang, N. S. & Shim, J. H. 2003. Feeding by the heterotrophic dinoflagellate *Oxyrrhis marina* on the red-tide raphidophyte *Heterosigma akashiwo*: a potential biological method to control red tides using mass-cultured grazers. J. Eukaryot. Microbio. 50:274−282.

Jeong, H. J., Kim, J. S., Yoo, Y. D., Kim, S. T., Song, J. Y., Kim, T. H., Seong, K. A., Kang, N. S., Kim, M. S., Kim, J. H., Kim, S., Ryu, J., Lee, H. M. & Yih, W. H. 2008a. Control of the harmful alga *Cochlodinium polykrikoides* by the naked ciliate *Strombidinopsis jeokjo* in mesocosm enclosures. Harmful Algae. 7:368−377.

Jeong, H. J., Kim, S. K., Kim, J. S., Kim, S. T., Yoo, Y. D. & Yoon, J. Y. 2001b. Growth and grazing rates of the heterotrophic dinoflagellate *Polykrikos kofoidii* on red−tide and toxic dinoflagellates. J. Eukaryot. Microbiol. 48:298−308.

Jeong, H. J., Kim, T. H., Yoo, Y. D., Yoon, E. Y., Kim, J. S., Seong, K. A., Kim, K. Y. & Park, J. Y. 2011. Grazing impact of heterotrophic dinoflagellates and ciliates on common red−tide euglenophyte *Eutreptiella gymnastica* in Masan Bay, Korea. Harmful Algae. 10:576−588.

Jeong, H. J., Lim, A. S., Franks, P. J. S., Lee, K. H., Kim, J. H., Kang, N. S., Lee, M. J., Jang, S. H., Lee, S. Y., Yoon, E. Y., Park, J. Y., Yoo, Y. D., Seong, K. A., Kwon, J. E. & Jang, T. Y. 2015. A hierarchy of conceptual models of red−tide generation:

nutrition, behavior, and biological interactions. Harmful Algae. 47:97−115.

Jeong, H. J., Lim, A. S., Yoo, Y. D., Lee, M. J., Lee, K. H., Jang, T. Y. & Lee, K. 2014. Feeding by heterotrophic dinoflagellates and ciliates on the free-living dinoflagellate *Symbiodinium* sp. (Clade E). J. Eukaryot. Microbiol. 61:27−41.

Jeong, H. J., Park, K. H., Kim, J. S., Kang, H. J., Kim, C. H., Choi, H. J., Kim, Y. S., Park, J. Y. & Park, M. G. 2003. Reduction in the toxicity caused by a toxic dinoflagellate *Gymnodinium catenatum* by the feeding of the heterotrophic dinoflagellate *Polykrikos kofoidii*. Aquat. Microb. Ecol. 31:307−312.

Jeong, H. J., Seong, K. A., Yoo, Y. D., Kim, T. H., Kang, N. S., Kim, S., Park, J. Y., Kim, J. S., Kim, K. H. & Song, J. Y. 2008b. Feeding and grazing impact by small marine heterotrophic dinoflagellates on heterotrophic bacteria. J. Eukaryot. Microbiol. 55:271−288.

Jeong, H. J., Shim, J. H., Kim, J. S., Park, J. Y., Lee, C. W. & Lee, Y. 1999b. Feeding by the mixotrophic thecate dinoflagellate *Fragilidium* cf. *mexicanum* on red−tide and toxic dinoflagellates. Mar. Ecol. Prog. Ser. 176:263−277.

Jeong, H. J., Shim, J. H., Lee, C. W., Kim, J. S., Koh, S. M., 1999c. Growth and grazing rates of the marine planktonic ciliate *Strombidinopsis* sp. on red-tide and toxic dinoflagellates. J. Eukaryot. Microbiol. 46:69−76.

Jeong, H. J., Song, J. E., Kang, N. S., Kim, S., Yoo, Y. D. & Park, J. Y. 2007c. Feeding by heterotrophic dinoflagellates on the

common marine heterotrophic nanoflagellate *Cafeteria* sp. Mar. Ecol. Prog. Ser. 333:151–160.

Jeong, H. J., Song, J. Y., Lee, C. H. & Kim, S. T. 2004b. Feeding by larvae of the mussel *Mytilus galloprovincialis* on red–tide dinoflagellates. J. Shellfish Res. 23:185–196.

Jeong, H. J., Yoo, Y. D., Kang, N. S., Lim, A. S., Seong, K. A., Lee, S. Y., Lee, M. J., Lee, K. H., Kim, H. S., Shin, W., Nam, S. W., Yih, W. & Lee, K. 2012. Heterotrophic feeding as a newly identified survival strategy of the dinoflagellate *Symbiodinium*. Proc. Natl. Acad. Sci. U. S. A. 109:12604–12609.

Jeong, H. J., Yoo, Y. D., Kang, N. S., Rho, J. R., Seong, K. A., Park, J. W., Kang, N. S. & Yih, W. H. 2010a. Ecology of *Gymnodinium aureolum*. I. Feeding in western Korean waters. Aquat. Microb. Ecol. 59:239–255.

Jeong, H. J., Yoo, Y. D., Kim, J. S., Kang, N. S., Kim, T. H. & Kim, J. H. 2004c. Feeding by the marine planktonic ciliate *Strombidinopsis jeokjo* on common heterotrophic dinoflagellates. Aquat. Microb. Ecol. 36:181–187.

Jeong, H. J., Yoo, Y. D., Kim, J. S., Seong, K. A., Kang, N. S. & Kim, T. H. 2010b. Growth, feeding and ecological roles of the mixotrophic and heterotrophic dinoflagellates in marine planktonic food webs. Ocean Sci. J. 45:65–91.

Jeong, H. J., Yoo, Y. D., Lee, K. H., Kim, T. H., Seong, K. A., Kang, N. S., Lee, S. Y., Kim, J. S., Kim, S. & Yih, W. H. 2013. Red tides in Masan Bay, Korea in 2004–2005: I. Daily variations in the abundance of red–tide organisms and environmental

factors. Harmful Algae. 30:75−88.

Jeong, H. J., Yoo, Y. D., Park, J. Y., Song, J. Y., Kim, S. T., Lee, S. H., Kim, K. Y. & Yih, W. H. 2005b. Feeding by phototrophic red−tide dinoflagellates: five species newly revealed and six species previously known to be mixotrophic. Aquat. Microb. Ecol. 40:133−150.

Jeong, H. J., Yoon, J. Y., Kim, J. S., Yoo, Y. D. & Seong, K. A. 2002. Growth and grazing rates of the prostomatid ciliate *Tiarina fusus* on red−tide and toxic algae. Aquat. Microb. Ecol. 28:289−297.

Jeong, H. J., You, J. H., Lee, K. H., Kim, S. J. & Lee, S. Y. 2018b. Feeding by common heterotrophic protists on the mixotrophic alga *Gymnodinium smaydae* (Dinophyceae), one of the fastest growing dinoflagellates. J. Phycol.54:734−743

Johnson, M. D. 2015. Inducible mixotrophy in the dinoflagellate *Prorocentrum minimum*. J. Eukaryot. Microbiol. 62:431−443.

Jürgens, K., Wickham, S. A., Rothhaupt, K. O. & Santer, B. 1996. Feeding rates of macro-and microzooplankton on heterotrophic nanoflagellates. Limnol. Oceanogr. 41:1833−1839.

Kahn, P., Herfort, L., Peterson, T. D. & Zuber, P. 2014. Discovery of a *Katablepharis* sp. in the Columbia River estuary that is abundant during the spring and bears a unique large ribosomal subunit sequence element. MicrobiologyOpen 3:764−776.

Kamiyama, T. & Matsuyama, Y. 2005. Temporal changes in the ciliate assemblage and consecutive estimates for their grazing

effect during the course of a *Heterocapsa circularisquama* bloom. J. Plankton Res. 27:303−311.

Kang, H. C., Jeong, H. J., Kim, S. J., You, J. H. & Ok, J. H. 2018. Differential feeding by common heterotrophic protists on 12 different *Alexandrium* species. Harmful algae. 78:106−117.

Kang, Y. J., Ko, T. H., Lee, J. A., Lee, J. B., & Chung, I. K. (1999). The community dynamics of phytoplankton and distribution of dinoflagellate cysts in Tongyoung Bay, Korea. Algae, 14:43−54.

Ke, Z., Tan, Y., Huang, L., Zhang, J. & Lian, S. 2012. Relationship between phytoplankton composition and environmental factors in the surface waters of southern South China Sea in early summer of 2009. Acta. Oceanol. Sin. 31:109−19.

Kibbe, WA. 2007. OligoCalc: an online oligonucleotide properties calculator. Nucleic Acids Res. 35:43–46.

Kim, J. H., Jeong, H. J., Lim, A. S., Kwon, J. E., Lee, K. H., Park, K. H. & Kim, H. S. 2017. Removal of two pathogenic scuticociliates *Miamiensis avidus* and *Miamiensis* sp. using cells or culture filtrates of the dinoflagellate *Alexandrium andersonii*. Harmful Algae. 63:133−145.

Kim, J. H., Jeong, H. J., Lim, A. S., Rho, J. R. & Lee, S. B. 2016. Killing potential protist predators as a survival strategy of the newly described dinoflagellate *Alexandrium pohangense*. Harmful Algae. 55:41−55.

Kim, J. S. & Jeong, H. J. 2004. Feeding by the heterotrophic dinoflagellates *Gyrodinium dominans* and *G. spirale* on the

red-tide dinoflagellate *Prorocentrum minimum*. Mar. Ecol. Prog. Ser. 280:85-94

Kim, J. S., Jeong, H. J., Yoo, Y. D., Kang, N. S., Kim, S. K., Song, J. Y., Lee, M. J., Kim, S. T., Kang, J. H., Seong, K. A. & Yih, W. H. 2013. Red tides in Masan Bay, Korea, in 2004-2005: III. Daily variation in the abundance of mesozooplankton and their grazing impacts on red-tide organisms. Harmful Algae. 30:102-113.

Kim, S., Yoon, J. & Park, M. G. 2015. Obligate mixotrophy of the pigmented dinoflagellate *Polykrikos lebourae* (Dinophyceae, Dinoflagellata). Algae. 30:35-47.

Kim, Y. O. & Han, M. S. 2000. Seasonal relationships between cyst germination and vegetative population of *Scrippsiella trochoidea* (Dinophyceae). Mar. Ecol. Prog. Ser. 204:111-118.

Kimmance, S. A., Atkinson, D. & Montagnes, D. J. S. 2006. Do temperature-food interactions matter? Responses of production and its components in the model heterotrophic flagellate *Oxyrrhis marina*. Aquat. Microb. Ecol. 42:63-73.

Kiφrboe, T. & Titelman, J. 1998. Feeding, prey selection and prey encounter mechanisms in the heterotrophic dinoflagellate *Noctiluca scintillans*. J. Plankton Res. 20:1615-1636.

Kretschmann, J., Elbrächter, M., Zinssmeister, C., Soehner, S., Kirsch, M., Kusber, W. H. & Gottschling, M. 2015. Taxonomic clarification of the dinophyte *Peridinium acuminatum* Ehrenb.,≡*Scrippsiella acuminata*, comb. nov.

(Thoracosphaeraceae, Peridiniales). Phytotaxa. 220:239–256.

Kuylenstierna, M. & Karlson, B. 2000. Checklist of phytoplankton in the Skagerrak–Kattegat.

Kwon, J. E., Jeong, H. J., Kim, S. J., Jang, S. H., Lee, K. H. & Seong, K. A. 2017. Newly discovered role of the heterotrophic nanoflagellate *Katablepharis japonica*, a predator of toxic or harmful dinoflagellates and raphidophytes. Harmful Algae. 68:224–239.

LaJeunesse, T. C., Lee, S. Y., Gil–Agudelo, D. L., Knowlton, N. & Jeong, H. J. 2015. *Symbiodinium necroappetens* sp. nov. (Dinophyceae): an opportunist 'zooxanthella' found in bleached and diseased tissues of Caribbean reef corals. Eur. J. Phycol. 50:223–238.

Lee, J. B., Kim, D. Y., & Lee, J. (1998). Community dynamics and distribution of dinoflagellates and their cysts in Masan–Chinhae Bay, Korea. Fish. Aquatic. Sci. 1:283–292.

Lee, K. H., Jeong, H. J., Jang, T. Y., Lim, A. S., Kang, N. S., Kim, J. H., Kim, K. Y., Park, K–T. & Lee, K. 2014a. Feeding by the newly described mixotrophic dinoflagellate *Gymnodinium smaydae*: feeding mechanism, prey species, and effect of prey concentration. J. Exp. Mar. Biol. Ecol. 459:114–125.

Lee, K. H., Jeong, H. J., Kwon, J. E., Kang, H. C., Kim, J. H., Jang, S. H., Park, J. Y., Yoon, E. Y. & Kim, J. S. 2016. Mixotrophic ability of the phototrophic dinoflagellates *Alexandrium andersonii*, *A. affine*, and *A. fraterculus*. Harmful Algae. 59:67–81.

Lee, K. H., Jeong, H. J., Yoon, E. Y., Jang, S. H., Kim, H. S. & Yih, W. 2014b. Feeding by common heterotrophic dinoflagellates and a ciliate on the red-tide ciliate *Mesodinium rubrum*. Algae. 29:153-163.

Lee, M. J., Jeong, H. J., Jang, S. H., Lee, S. Y., Kang, N. S., Lee, K. H., Kim, H. S., Wham, D. C. & LaJeunesse, T. C. 2016. Most low-abundance "background" *Symbiodinium* spp. are transitory and have minimal functional significance for symbiotic corals. Microb. Ecol. 71:771-783.

Lee, M. J., Jeong, H. J., Kim, J. S., Jang, K. K., Kang, N. S., Jang, S. H., Lee, H. B., Lee, S. B., Kim, H. S. & Choi, C. H. 2017. Ichthyotoxic *Cochlodinium polykrikoides* red tides offshore in the South Sea, Korea in 2014: III. Metazooplankton and their grazing impacts on red-tide organisms and heterotrophic protists. Algae. 32:285-308.

Lee, M. J., Jeong, H. J., Lee, K. H., Jang, S. H., Kim, J. H. & Kim, K. Y. 2015. Mixotrophy in the nematocyst-taeniocyst complex-bearing phototrophic dinoflagellate *Polykrikos hartmannii*. Harmful Algae. 49:124-134.

Lee, S. Y., Jeong, H. J., Kim, S. J., Lee, K. H. & Jang, S. H. *Scrippsiella masanensis* sp. nov. (Thoracosphaerales, Dinophyceae), a phototrophic dinoflagellate from the coastal waters of southern Korea. Phycologia. In revision

Lee, S. Y., Jeong, H. J., Seong, K. A., Lim, A. S., Kim, J. H., Lee, K. H., Lee, M. J. & Jang, S. H. 2017. Improved real-time PCR method for quantification of the abundance of all known

ribotypes of the ichthyotoxic dinoflagellate *Cochlodinium polykrikoides* by comparing 4 different preparation methods. Harmful Algae. 63:23–31.

Lee, S. Y., Jeong, H. J., You, J. H. & Kim, S. J. 2018. Morphological and genetic characterization and the nationwide distribution of the phototrophic dinoflagellate *Scrippsiella lachrymosa* in the Korean waters. Algae. 33:21−35.

Leverone, J. R., Blake, N. J., Pierce, R. H. & Shumway, S. E. 2006. Effects of the dinoflagellate *Karenia brevis* on larval development in three species of bivalve mollusc from Florida. Toxicon. 48:75−84.

Levinsen, H. & Nielsen, T. G. 2002. The trophic role of marine pelagic ciliates and heterotrophic dinoflagellates in arctic and temperate coastal ecosystems: a cross-latitude comparison. Limnol. Oceanogr. 47:427−439.

Lewis, J. 1991. Cyst−theca relationships in *Scrippsiella* (Dinophyceae) and related orthoperidinioid genera. Bot. Mar. 34:91−106.

Licea, S., Zamudio, M. E., Ruth Luna, Y. B. O. & Gómez−Aguirre, S. 2002. Toxic and Harmful Dinonagelates in the southern Gulf of Mexico.

Lim, A. S., Jeong, H. J., Jang, T. Y., Yoo, Y. D, Kang, N. S., Yoon, E. Y. & Kim, G. H. 2014. Feeding by the newly described heterotrophic dinoflagellate *Stoeckeria changwonensis*: a comparison with other species in the family Pfiesteriaceae. Harmful Algae. 36:11−21.

Lim, A. S., Jeong, H. J., Kim, J. H. & Lee, S. Y. 2017a. Control of ichthyotoxic *Cochlodinium polykrikoides* using the mixotrophic dinoflagellate *Alexandrium pohangense*: a potential effective sustainable method. Harmful Algae. 63:109–118.

Lim, A. S., Jeong, H. J., Kim, J. H., Jang, S. H., Lee, M. J. & Lee, K. 2015. Mixotrophy in the newly described dinoflagellate *Alexandrium pohangense*: a specialist for feeding on the fast-swimming ichthyotoxic dinoflagellate *Cochlodinium polykrikoides*. Harmful Algae. 49:10–18.

Lim, A. S., Jeong, H. J., Seong, K. A., Lee, M. J., Kang, N. S., Jang, S. H., Lee, K. H., Park, J. Y., Jang, T. Y. & Yoo, Y. D. 2017b. Ichthyotoxic *Cochlodinium polykrikoides* red tides offshore in the South Sea, Korea in 2014: II. Heterotrophic protists and their grazing impacts on red-tide organisms. Algae. 32:199–222.

Litaker, R. W., Vandersea, M. W., Kibler, S. R., Reece, K. S., Stokes, N. A., Steidinger, K. A., Millie, D. F., Bendis, B. J., Pigg, R. J. & Tester, P. A. 2003. Identification of *Pfiesteria piscicida* (Dinophyceae) and *Pfiesteria*-like organisms using internal transcribed spacer-specific PCR assays. J. Phycol. 39:754–761.

Liu, L. R. 2008. Checklist of marine biota of China Seas. China: Institute of Oceanography, Chinese Academy of Science.

Löder, M. G. J., Boersma, M., Kraberg, A. C., Aberle, N. & Wiltshire, K. H. 2014. Microbial predators promote their competitors:

commensalism within an intra-guild predation system in microzooplankton. Ecosphere. 5:1−23.

Lu, S. & Hodgkiss, I. J. 2004. Harmful algal bloom causative collected from Hong Kong waters. In Asian Pacific Phycology in the 21st Century: Prospects and Challenges. Springer, Dordrecht, pp. 231−238.

Luo, Z., Mertens, K. N., Bagheri, S., Aydin, H., Takano, Y., Matsuoka, K., McCarthy, F. M. & Gu, H. 2016. Cyst−theca relationship and phylogenetic positions of *Scrippsiella plana* sp. nov. and *S. spinifera* (Peridiniales, Dinophyceae). Eur. J. Phycol. 51:188−202.

Matsuoka, K., Cho, H. J. & Jacobson, D. M. 2000. Observations of the feeding behavior and growth rates of the heterotrophic dinoflagellate *Polykrikos kofoidii* (Polykrikaceae, Dinophyceae). Phycologia. 39:82−86.

Matsuyama, Y., Miyamoto, M. & Kotani, Y. 1999. Grazing impacts of the heterotrophic dinoflagellate *Polykrikos kofoidii* on a bloom of *Gymnodinium catenatum*. Aquat. Microb. Ecol. 17:91−98.

McCarthy, P. M. 2013. Census of Australian Marine Dinoflagellates.

Menden−Deuer, S. & Lessard, E. J. 2000. Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnol. Oceanogr. 45:569−579.

Moestrup, Ø., Hansen, G., Daugbjerg, N., Lundholm, N., Overton, J., Vestergård, M., Steenfeldt, S. J., Calado, A. J. & Hansen, P. J. 2014. The dinoflagellates *Pfiesteria shumwayae* and *Luciella masanensis* cause fish kills in recirculation fish farms in

Denmark. Harmful Algae. 32:33−39.

Montagnes, D. J. S., Berger, J. D. & Taylor, F. J. R. 1996. Growth rate of the marine planktonic ciliate *Strombidinopsis cheshiri* Snyder and Ohman as a function of food concentration and interclonal variability. J. Exp. Mar. Biol. Ecol. 206:121−132.

Montresor, M. & Marino, D. 1989. Reproduction and cyst formation in *Scrippsiella precaria* (Dinophyceae). G. Bot. Ital. 123:157−167.

Montresor, M., Sgrosso, S., Procaccini, G. & Kooistra, W. H. 2003. Intraspecific diversity in *Scrippsiella trochoidea* (Dinopbyceae): evidence for cryptic species. Phycologia 42:56−70.

Nakamura, Y., Suzuki, S. & Hiromi, J. 1995a. Population dynamics of heterotiophic dinoflagellates during a *Gymnodinium mikimotoi* red tide in the Seto Inland Sea. Mar. Ecol. Prog. Ser. 125:269−277.

Nakamura, Y., Suzuki, S. Y. & Hiromi, J. 1995b. Growth and grazing of a naked heterotrophic dinoflagellate, *Gyrodinium dominans*. Aquat. Microb. Ecol. 9:157−164.

Nakamura, Y., Yamazaki, Y. & Hiromi, J. 1992. Growth and grazing of a heterotrophic dinoflagellate, *Gyrodinium dominans*, feeding on a red tide flagellate, *Chattonella antiqua*. Mar. Ecol. Prog. Ser. 275−279.

National institute of fisheries science, 2018. “red tide information system”, Busan, Republic of Korea. http://www.nifs.go.kr/red/main.red; (last accessed 21 Nov

2018)

Naustvoll, L. -J. 2000. Prey size spectra and food preferences in thecate heterotrophic dinoflagellates. Phycologia. 39:187-198.

Nehring, S. 1994. *Scrippsiella* spp. resting cysts from the German Bight (North Sea): A tool for more complete check-lists of dinoflagellates. J. Sea Res. 33:57-63.

Ok, J. H., Jeong, H. J., Lim, A. S. & Lee, K. H. 2017. Interactions between the mixotrophic dinoflagellate *Takayama helix* and common heterotrophic protists. Harmful algae. 68:178-191.

Okamoto, N. & Inouye, I. 2005. The katablepharids are a distant sister group of the Cryptophyta: a proposal for Katablepharidophyta divisio nova/Kathablepharida phylum novum based on SSU rDNA and beta-tubulin phylogeny. Protist. 156:163-179.

Olli, K. & Anderson, D. M. 2002. High encystment success of the dinoflagellate *Scrippsiella* cf. *lachrymosa* in culture experiments 1. J. Phycol. 38:145-156.

Olli, K. & Trunov, K. 2010. Abundance and distribution of vernal bloom dinoflagellate cysts in the Gulf of Finland and Gulf of Riga (the Baltic Sea). Deep Sea Research Part II: Topical Studies in Oceanography, 57:235-242.

Orlova, T. Y. & Morozova, T. V. 2003. The morphology of cysts and vegetative cells of *Scrippsiella crystallina* Lewis (Dinophyta) from Far-Eastern seas of Russia. Intern. J. Algae. 5:41-46.

Oshima, Y., Hasegawa, M., Yasumoto, T., Hallegraeff, G. &

Blackburn, S. 1987. Dinoflagellate *Gymnodinium catenatum* as the source of paralytic shellfish toxins in Tasmanian shellfish. Toxicon 25:1105-1111.

Park, J., Jeong, H. J., Du Yoo, Y. & Yoon, E. Y. 2013. Mixotrophic dinoflagellate red tides in Korean waters: distribution and ecophysiology. Harmful Algae. 30:28-40.

Park, M. G., Kim, S., Kim, H. S., Myung, G., Kang, Y. G. & Yih, W. 2006. First successful culture of the marine dinoflagellate *Dinophysis acuminata*. Aquat. Microb. Ecol. 45:101-106.

Park, T. G., Lim, W. A., Park, Y. T., Lee, C. K. & Jeong, H. J. 2013. Economic impact, management and mitigation of red tides in Korea. Harmful Algae. 30:131-143.

Patterson, D. J. & Larsen, J. 1991. The biology of free-living heterotrophic flagellates. Oxford University Press, New York, NY, 505 pp.

Paz, B., Riobó, P., Fernández, M. L., Fraga, S. & Franco, J. M. 2004. Production and release of yessotoxins by the dinoflagellates *Protoceratium reticulatum* and *Lingulodinium polyedrum* in culture. Toxicon 44: 251-258.

Pomeroy, L. R. 1974. The ocean's food web, a changing paradigm. Bioscience 24:499-504.

Potvin, É., Hwang, Y. J., Yoo, Y. D., Kim, J. S. & Jeong, H. J. 2013. Feeding by heterotrophic protists and copepods on the photosynthetic dinoflagellate *Azadinium* cf. *poporum* from western Korean waters. Aquat. Microb. Ecol. 68:143-158.

Rahmadyani, R. F., Widiarti, R. & Hendrayanti, D. 2017. Distribution

analysis on cyst abundance of HAB causing dinoflagellates at green mussel (*Perna viridis*) culture area in Kamal Muara, North Jakarta. Integrated Coastal Management and Marine Biotechnology, 14, 14.

Robineau, B., Gagné, J. A., Fortier, L. & Cembella, A. D. 1991. Potential impact of a toxic dinoflagellate (*Alexandrium excavatum*) bloom on survival of fish and crustacean larvae. Mar. Biol. 108:293–301.

Saiz, E., Griffell, K., Calbet, A. & Isari, S. 2014. Feeding rates and prey: predator size ratios of the nauplii and adult females of the marine cyclopoid copepod *Oithona davisae*. Limnol. Oceanogr. 59:2077–2088.

Satta, C. T., Anglès, S., Garcés, E., Sechi, N., Pulina, S., Padedda, B. M., Stacca, D. & Lugliè, A. 2014. Dinoflagellate cyst assemblages in surface sediments from three shallow Mediterranean lagoons (Sardinia, North Western Mediterranean Sea). Estuar. Coast. 37:646–663.

Scholin, C. A., Herzog, M., Sogin, M. & Anderson, D. M. 1994. Identification of group– and strain–specific genetic markers for globally distributed *Alexandrium* (Dinophyceae). II. Sequence analysis of a fragment of the LSU rRNA gene. J Phycol. 30:999–1011.

Scholz, B. & Einarsson, H. 2015. Microphytobenthic community composition of two sub–Arctic intertidal flats in Huna Bay (Northern Iceland). Eur. J. Phycol. 50:182–206.

Shears, N. T. & Ross, P. M. 2009. Blooms of benthic dinoflagellates

of the genus *Ostreopsis*; an increasing and ecologically important phenomenon on temperate reefs in New Zealand and worldwide. Harmful algae. 8:916–925.

Sherr, E. B. & Sherr, B. F. 2007. Heterotrophic dinoflagellates: a significant component of microzooplankton biomass and major grazers of diatoms in the sea. Mar. Ecol. Prog. Ser. 352:187–197.

Shin, H. H., Yoon, Y. H. & Matsuoka, K. 2007. Modern dinoflagellate cysts distribution off the eastern part of Geoje Island, Korea. Ocean. Sci. J. 42:31–39.

Sieburth, J. M. 1984. Protozoan bacterivory in pelagic marine waters. In Hobbie, J. E. & Williams, P. J. (Eds.) Heterotrophic Activity in the Sea. Plenum Press, New York, NY, pp. 405–444.

Silke, J., O'Beirn, F. X. & Cronin, M. 2005. *Karenia mikimotoi*: an exceptional dinoflagellate bloom in western Irish waters, summer 2005. Marine Environment and Health Series No. 21, Marine Institute 2005.

Smayda, T. J. 1997. Harmful algal blooms: their ecophysiology and general relevance to phytoplankton blooms in the sea. Limnol. Oceanogr. 42:1137–1153.

Soehner, S., Zinssmeister, C., Kirsch, M., & Gottschling, M. 2012. Who am I—and if so, how many? Species diversity of calcareous dinophytes (Thoracosphaeraceae, Peridiniales) in the Mediterranean Sea. Org. Divers. Evol. 12: 339–348.

Solis, P. N., Wright, C. W., Anderson, M. M., Gupta, M. P. &

Phillipson, J. D. 1993. A microwell cytotoxicity assay using *Artemia salina* (brine shrimp). Planta Med. 59:250−252.

Sommer, U. 1988. Some size relationships in phytoflagellate motility. Hydrobiologia. 161:125–131.

Stentiford, G. D., Green, M., Bateman, K., Small, H. J., Neil, D. M. & Feist, S. W. 2002. Infection by a *Hematodinium*−like parasitic dinoflagellate causes Pink Crab Disease (PCD) in the edible crab Cancer pagurus. J. Invertebr. Pathol. 79:179−191.

Stoeck, T., Schwarz, M. V. J., Boenigk, J., Schweikert, M., von der Heyden, S. & Behnke, A. 2005. Cellular identity of an 18S rRNA gene sequence clade within the class Kinetoplastea: the novel genus *Actuariola* gen. nov. (Neobodonida) with description of the type species *Actuariola framvarensis* sp. nov. Int J Syst Evol Microbiol. 55:2623–2635.

Stoecker, D. K. 1999. Mixotrophy among dinoflagellates. J. Eukaryot. Microbiol. 46:397−401.

Stoecker, D. K., Davis, L. H. & Anderson, D. M. 1984. Fine scale spatial correlations between planktonic ciliates and dinoflagellates. J. Plankton Res. 6:829−842.

Strom, S. L. & Buskey, E. J. 1993. Feeding, growth, and behavior of the thecate heterotrophic dinoflagellate *Oblea rotunda*. Limnol. Oceanogr. 38:965−977.

Taira, H., Aoki, S., Yamanoha, B. & Taguchi, S. 2004. Daily variation in cellular content of UV−absorbing compounds mycosporine−like amino acids in the marine dinoflagellate *Scrippsiella sweeneyae*. J. Photochem. Photobiol. B, Biol.

75:145–155.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol. 24:1596–1599.

Tang, Y. Z. & Gobler, C. J. 2012. Lethal effects of Northwest Atlantic Ocean isolates of the dinoflagellate, *Scrippsiella trochoidea*, on Eastern oyster (*Crassostrea virginica*) and Northern quahog (*Mercenaria mercenaria*) larvae. Mar. Biol. 159:199–210.

Throndsen, J. 1973. Motality in some marine nanoplankton flagellates. Norw. J. Zool. 21:193–200.

Tillmann, U. & John, U. 2002. Toxic effects of *Alexandrium* spp. on heterotrophic dinoflagellates: an allelochemical defence mechanism independent of PSP–toxin content. Mar. Ecol. Prog. Ser. 230:47–58.

Tillmann, U. & Reckermann, M. 2002. Dinoflagellate grazing on the raphidophyte *Fibrocapsa japonica*. Aquat. Microb. Ecol. 26:247–257.

Tillmann, U. 2004. Interactions between planktonic microalgae and protozoan grazers. J. Eukaryot. Microbiol. 51:156–168.

Turner, J. T. 2006. Harmful algae interactions with marine planktonic grazers. In Granéli, E. & Turner, J. T. (Eds.) Ecology of Harmful Algae. Springer–Verlag, Berlin, pp. 259–270.

Waggett, R. J., Tester, P. A. & Place, A. R. 2008. Anti–grazing properties of the toxic dinoflagellate *Karlodinium veneficum*

during predator-prey interactions with the copepod *Acartia tonsa*. Mar. Ecol. Prog. Ser. 366:31-42.

Wang, Z., Matsuoka, K., Qi, Y. & Chen, J. 2004. Dinoflagellate cysts in recent sediments from Chinese coastal waters. Mar. Ecol. 25:289-311.

Wang, Z. H., Qi, Y. Z. & Yang, Y. F. 2007. Cyst formation: an important mechanism for the termination of *Scrippsiella trochoidea* (Dinophyceae) bloom. J. Plankton Res. 29:209-218.

Watts, P. C., Martin, L. E., Kimmance, S. A., Montagnes, D. J. & Lowe, C. D. 2010. The distribution of *Oxyrrhis marina*: a global disperser or poorly characterized endemic. J. Plankton Res. 33:579-589.

Widdows, J., Moore, M. N., Lowe, D. M. & Salkeld, P. N. 1979. Some effects of a dinoflagellate bloom (*Gyrodinium aureolum*) on the mussel, *Mytilus edulis*. J. Mar. Biolog. Assoc. U.K. 59:522-524.

Wong, C. K. & Wong, C. K. 2004. Study of phytoplankton characteristics in Tolo Harbour, Hong Kong, by HPLC analysis of chemotaxonomic pigments. J. Appl. Phycol. 16:469-476.

Xiao, Y., Wang, Z., Chen, J., Lu, S., & Qi, Y. 2003. Seasonal dynamics of dinoflagellate cysts in sediments from daya bay, the south china sea its relation to the bloom of *Scrippsiella trochoidea*. Acta. Hydrobiol. Sin. 27:372-377.

Yamochi, S. & Abe, T. 1984. Mechanisms to initiate a *Heterosigma*

akashiwo red tide in Osaka Bay. Mar. Biol. 83:255–261.

Yan, T., Zhou, M. J. & Zou, J. Z. 2002. A national report on harmful algal blooms in China. Harmful algal blooms in the PICES region of the North Pacific, 21.

Yoo, Y. D., Jeong, H. J., Kang, N. S., Kim, J. S., Kim, T. H. & Yoon, E. Y. 2010. Ecology of *Gymnodinium aureolum*. II. Predation by common heterotrophic dinoflagellates and a ciliate. Aquat. Microb. Ecol. 59:257–272.

Yoo, Y. D., Jeong, H. J., Kim, J. S., Kim, T. H., Kim, J. H., Seong, K. A., Lee, S. H., Kang, N. S., Park, J. W., Park, J., Yoon, E. Y. & Yih, W. H. 2013a. Red tides in Masan Bay, Korea in 2004–2005: II. Daily variations in the abundance of heterotrophic protists and their grazing impact on red-tide organisms. Harmful Algae. 30:89–101.

Yoo, Y. D., Jeong, H. J., Lee, S. Y., Yoon, E. Y., Kang, N. S., Lim, A. S., Lee, K. H., Jang, S. H., Park, J. Y. & Kim, H. S. 2015. Feeding by heterotrophic protists on the toxic dinoflagellate *Ostreopsis* cf. *ovata*. Harmful Algae. 49:1–9.

Yoo, Y. D., Yoon, E. Y., Jeong, H. J., Lee, K. H., Hwang, Y. J., Seong, K. A., Kim, J. S. & Park, J. Y. 2013b. The newly described heterotrophic dinoflagellate *Gyrodinium moestrupii*, an effective protistan grazer of toxic dinoflagellates. J. Eukaryot. Microbiol. 60:13–24.

Yoo, Y. D., Yoon, E. Y., Lee, K. H., Kang, N. S. & Jeong, H. J. 2013c. Growth and ingestion rates of heterotrophic dinoflagellates and a ciliate on the mixotrophic dinoflagellate *Biecheleria*

cincta. Algae. 28:343−354.

Yoon, E. Y., Kang, N. S. & Jeong, H. J. 2012. *Gyrodinium moestrupii* n. sp., a new planktonic heterotrophic dinoflagellate from the coastal waters of western Korea: morphology and ribosomal DNA gene sequence. J. Eukaryot. Microbiol. 59:571−586.

Yu−Zao, Q., Ying, H., Lei, Z., Kullis, D. & Anderson, D. M. 1996. Dinoflagellate cysts from recent marine sediments of the South and East China Seas. Asian Mar. Biol. 13:87−103.

Zhuo−Ping, C., Wei−Wei, H., Min, A. & Shun−Shan, D. 2009. Coupled effects of irradiance and iron on the growth of a harmful algal bloom−causing microalga *Scrippsiella trochoidea*. Acta Ecol. Sin. 29:297−301.

Zinssmeister, C., Soehner, S., Facher, E., Kirsch, M., Meier, K. S. & Gottschling, M. 2011. Catch me if you can: the taxonomic identity of *Scrippsiella trochoidea* (F. Stein) AR Loebl. (Thoracosphaeraceae,Dinophyceae). Syst. Biodivers. 9:145−157.

국문초록

와편모류인 스크립시엘라 4종에 대한 종속영양성 포식자의 서로 다른 반응 및 종속영양성 미세편모류 카타블레페리스 자포니카와의 상호작용 연구

와편모류 속인 *Scrippsiella*는 독성은 없지만 적조를 발생시킨다고 알려져 있다. *Scrippsiella*속에서 가장 흔한 종인 *S. acuminata*는 한국의 남해안에서 때때로 적조를 발생시키기도 한다. 이들은 동중국 해에 종종 분포하는 것으로 알려져 있지만 주로 휴면포자(cyst)형태로 발견된다. 최근 한국연안 표층수에 운동성 형태(motile form)의 4종의 *Scrippsiella* (*S. acuminata*, *S. donghaiensis*, *S. lachrymosa*, *S. masanensis*) 가 종종 발견되며 우리나라 연안에도 다양한 *Scrippsiella* 종들이 흔히 존재하고 있다는 것을 알 수 있었다. 따라서 *Scrippsiella* 종들은 한국 연안의 침전물에 휴면포자(cyst)형태로 존재함으로서 적조를 발생시킬수 있는 잠재성을 가지고 있기 때문에 이들의 분포에 대한 연구가 필요하다. 본 연구에서는 각 종들의 종 특이적 프라이머를 제작하여 qPCR을 통해 한국연안에서 4종의 *Scrippsiella*분포를 파악하였다. 그 결과 네 종의 *Scrippsiella*는 우리나라 연안에 각각 다르게 분포하고 있으며, 다양한 수온과 염분 범위를 가지고 있었다. 그 중 *S. donghaiensis*는 7월, *S. lachrymosa*는 3월에 유독 많이 출현하여 계절성을 가지고 있는것으로 보이는 반면 *S. masanensis*는 실험 기간 중

2017년 10월을 제외한 모든 달에서 비교적 낮은 농도로 출현하였으며 *S. acuminata*는 다른 종들에 비해 비교적 드물게 나타났다.

이와 더불어 *Scrippsiella*종의 개체군 동태를 이해하기 위해서는 포식자들의 포식에 의한 사망률을 알아야한다. *Scrippsiella*종에 대한 종속영양 원생생물에 의한 포식을 연구하기 위해, 4종의 *Scrippsiella*와 해양환경에서 흔하게 존재하는 종속영양 와편모류와 섬모충 사이의 상호작용 연구를 진행하였다. 실험에 사용된 모든 종속영양 원생생물들은 4종의 *Scrippsiella*를 섭식할 수 있었으나, 4종의 *Scrippsiella*를 섭식한 *Oxyrrhis marina*, *Polykrikos kofoidii*, *Gyrodinium dominans*의 성장률과 섭식률은 종마다 매우 다른 것으로 나타났다. 따라서 이러한 선택적 섭식은 적조종을 먹이로 하는 포식자의 개체수를 줄임으로써 적조종의 증가를 야기할 수 있다. 게다가 포식자들에 의한 4종의 *Scrippsiella*에 대한 차별적인 반응은 포식자와 먹이종 모두 서로 다른 생태학적 지위를 야기할 수 있다.

최근 종속영양 미세편모류인 *Katablepharis japonica*는 다양한 적조생물 종들을 섭식함에 따라 적조생물의 효과적인 포식자가 될 수도 있다고 밝혀졌다. 하지만, *K. japonica*를 잘 섭식하는 포식자들이 있다면 이들의 grazing impact는 감소할 수도 있다. *K. japonica*에 대한 잠재적인 포식자를 연구하기 위해 다양한 종속영양 와편모류와 섬모충에 의한 포식률을 연구하였다. 그 결과 활발하게 움직이는 *K. japonica*를 섭식하는 종속영양 원생생물은 없었다. 반면, *K. japonica*는 여섯종의 종속영양 원생생물을 섭식할 수 있었다. 이 연구결과에 따르면 *K. japonica*의 종속영양 원생생물에 의한 사망률은 무시해도 될 정도로 낮기때문에 *K. japonica*의 적조 생물 개체군 grazing impact는 포식에 의한 사망률에 의해 감소되지 않을 수 있음을 시사한다. 또한, *K. japonica*의 개체군역학을 이해하기 위해서, qPCR 정량분석을 통해 이들의 분포를 연구한 결

과 *K. japonica*는 한국연안에 널리 분포하고 있으며 실험 기간동안 매계절마다 출현하였다. 이는 *K. japonica*의 포식자에 의한 낮은 사망률과 적조 생물의 grazing impact로 우리나라 전 해역에서 상시 존재하고 있으며 다양한 환경요인에서도 생존할 수 있다는 것을 알 수 있다.

본 연구에서는 다양한 생태계 먹이망(microbial loop)의 상호작용 중 식물성 플랑크톤의 주요한 속 중 하나인 독립영양 와편모류 *Scrippsiella*와 종속영양성 원생생물간의 상호작용, 그리고 공통된 적조 생물을 섭식하는 종속영양성 원생생물과 종속영양성 미세편모류 *Katablepharis japonica*간의 상호작용을 연구하였다.

Keywords: **분포, 섭식, 유해적조, 와편모류, 원생생물, 종속영양성, 종속영양성 미세편모류**

Student Number: 2016-26602