Research Article

Algae 2023, 38(1): 39-55
https://doi.org/10.4490/algae.2023.38.2.28
Open Access

ALGAE

Lack of mixotrophy in three *Karenia* species and the prey spectrum of *Karenia mikimotoi* (Gymnodiniales, Dinophyceae)

Jin Hee Ok[1,2], Hae Jin Jeong[1,3,*], An Suk Lim[4], Hee Chang Kang[1], Ji Hyun You[1], Sang Ah Park[1] and Se Hee Eom[1]

[1]*School of Earth and Environmental Sciences, College of Natural Sciences, Seoul National University, Seoul 08826, Korea*
[2]*Brain Korea 21, School of Earth and Environmental Sciences, College of Natural Sciences, Seoul National University, Seoul 08826, Korea*
[3]*Research Institute of Oceanography, Seoul National University, Seoul 08826, Korea*
[4]*Division of Life Science, Gyeongsang National University, Jinju 52828, Korea*

Exploring mixotrophy of dinoflagellate species is critical to understanding red-tide dynamics and dinoflagellate evolution. Some species in the dinoflagellate genus *Karenia* have caused harmful algal blooms. Among 10 *Karenia* species, the mixotrophic ability of only two species, *Karenia mikimotoi* and *Karenia brevis*, has been investigated. These species have been revealed to be mixotrophic; however, the mixotrophy of the other species should be explored. Moreover, although *K. mikimotoi* was previously known to be mixotrophic, only a few potential prey species have been tested. We explored the mixotrophic ability of *Karenia bicuneiformis*, *Karenia papilionacea*, and *Karenia selliformis* and the prey spectrum of *K. mikimotoi* by incubating them with 16 potential prey species, including a cyanobacterium, diatom, prymnesiophyte, prasinophyte, raphidophyte, cryptophytes, and dinoflagellates. Cells of *K. bicuneiformis*, *K. papilionacea*, and *K. selliformis* did not feed on any tested potential prey species, indicating a lack of mixotrophy. The present study newly discovered that *K. mikimotoi* was able to feed on the common cryptophyte *Teleaulax amphioxeia*. The phylogenetic tree based on the large subunit ribosomal DNA showed that the mixotrophic species *K. mikimotoi* and *K. brevis* belonged to the same clade, but *K. bicuneiformis*, *K. papilionacea*, and *K. selliformis* were divided into different clades. Therefore, the presence or lack of a mixotrophic ability in this genus may be partially related to genetic characterizations. The results of this study suggest that *Karenia* species are not all mixotrophic, varying from the results of previous studies.

Keywords: feeding; harmful algal bloom; Kareniaceae; lysis; protist; red tide; trophic mode

INTRODUCTION

Mixotrophic organisms conduct photosynthesis and uptake of external organic matters by phagotrophy or osmotrophy (Burkholder et al. 2008, Jeong et al. 2010*b*, Selosse et al. 2017, Stoecker et al. 2017). Exclusively photoautotrophic species are both primary producers and prey but mixotrophic species are primary producers, prey, and predators in food webs (Boraas et al. 1988, Jeong et al. 2010*b*, 2016, Ok et al. 2017). Mixotrophy elevates the growth rate of marine organisms and allows them to survive under inorganic nutrient depletion conditions (Park et al. 2006, Jeong et al. 2012, 2015, 2021, Kim et al. 2015). Therefore, determining the mixotrophic ability of

Received January 10, 2023, **Accepted** February 28, 2023
***Corresponding Author**
E-mail: hjjeong@snu.ac.kr
Tel: +82-2-880-6746, Fax: +82-2-874-9695

a photosynthetic organism is fundamental for predicting its population dynamics and bloom formation in marine ecosystems (Jeong et al. 2015). Furthermore, determination of the presence or lack of mixotrophic ability and prey items has greatly improved our understanding of evolution in photosynthetic organisms (Jones 2000, Mansour and Anestis 2021). Therefore, exploring the mixotrophic ability of an organism is a crucial step regarding its ecology and evolution.

Dinoflagellates are a major group of eukaryotes in marine ecosystems, and many dinoflagellate species have been revealed to be mixotrophic (Bockstahler and Coats 1993, Stoecker et al. 1997, Li et al. 1999, Jeong et al. 2005*b*, 2016, 2021, Lee et al. 2016, Lim and Jeong 2021, Park et al. 2021). Thus, mixotrophy is a major trophic mode of dinoflagellates (Stoecker 1999, Jeong et al. 2010*b*). Many mixotrophic dinoflagellates have caused red tides or harmful algal blooms (HABs) in the global ocean (López-Cortés et al. 2019, Eom et al. 2021, Ok et al. 2021*c*, 2023, Sakamoto et al. 2021, Yñiguez et al. 2021), and feeding on diverse prey species by some mixotrophic dinoflagellates has possibly resulted in their global dominance (Jeong et al. 2021). Therefore, to better understand the ecological roles of dinoflagellates and predict their red tides or HABs, it is necessary to determine whether they are mixotrophic.

The dinoflagellate family Kareniaceae has been of major interest to scientists, aquaculture farmers, and government officers because many species in the family have caused HABs (Kempton et al. 2002, Adolf et al. 2008, Sengco 2009, Steidinger 2009, Van Dolah et al. 2009, Calbet et al. 2011, Siswanto et al. 2013, Lin et al. 2018, Ok et al. 2019, 2022, Zhang et al. 2022). This family includes six genera: *Karenia*, *Karlodinium*, *Takayama*, *Asterodinium*, *Gertia*, and *Shimiella* (Daugbjerg et al. 2000, de Salas et al. 2003, Bergholtz et al. 2006, Benico et al. 2019, Takahashi et al. 2019, Ok et al. 2021*b*). The species in the genus *Karenia* are distributed globally and have often caused red tides and HABs (Brand et al. 2012, Li et al. 2019, Liu et al. 2022). Several *Karenia* species have toxins, such as brevetoxin, gymnocin, and gymnodimine, and thus, are harmful to fish, invertebrates, birds, mammals, and humans (Baden 1989, Miles et al. 2003, Pierce et al. 2003, Brand et al. 2012, Fowler et al. 2015). Thus, predicting red-tide or HAB outbreaks caused by these *Karenia* species is critical to minimize economic losses due to red tides or HABs. The growth rate of a *Karenia* species is one of the most important parameters for establishing prediction models, and is primarily affected by its trophic mode, which could be exclusively autotrophic or mixotrophic (Jeong et al. 2015). However, among the 10 formally described *Karenia* species (Guiry and Guiry 2023), the mixotrophic ability of only two species, *K. mikimotoi* and *K. brevis*, has been tested; these two species have been revealed to be mixotrophic (Jeong et al. 2005*a*, Glibert et al. 2009, Zhang et al. 2011). However, it is also necessary to analyze the mixotrophic ability of the remaining eight species in the genus *Karenia*. Furthermore, although *K. mikimotoi* and *K. brevis* have been revealed to be mixotrophic, their feeding occurrence has only been tested on a few potential prey species (Jeong et al. 2005*a*, Zhang et al. 2011). To understand interactions between *K. mikimotoi* or *K. brevis* and common microalgal species, feeding occurrence by *Karenia* species on a diversity of common microalgal species should be investigated.

In the present study, we explored the mixotrophic ability of *Karenia bicuneiformis* (= *Karenia bidigitata*), *Karenia papilionacea*, and *Karenia selliformis*. Moreover, we investigated the feeding occurrence of *K. mikimotoi* on a cyanobacterium and diverse microalgal prey species that have not previously been tested. The results of this study may contribute to a better understanding of mixotrophy in *Karenia*, interactions between *Karenia* species and common microalgal species, dynamics of red tides and HABs caused by *Karenia* species, and the ecological roles of *Karenia* species in marine ecosystems.

MATERIALS AND METHODS

Experimental organisms

Clonal cultures of *K. bicuneiformis* CAWD81 (= *K. bidigitata*), *K. papilionacea* CAWD91, *K. selliformis* NIES-4541, and *K. mikimotoi* NIES-2411 were obtained from the Cawthron Institute Culture Collection of Microalgae (New Zealand) and the National Institute for Environmental Studies (Japan). The cultures were transferred to 50 and 270-mL flasks containing L1 medium (Guillard and Hargraves 1993). These cultures were incubated at 20°C and 20 μmol photons $m^{-2} s^{-1}$ using cool white fluorescent lights on a 14 : 10 h light / dark cycle.

Diverse phytoplankton species, including a cyanobacterium, diatom, prymnesiophyte, prasinophyte, raphidophyte, cryptophytes, and dinoflagellates, were provided as potential prey (Table 1). All potential prey species, except *Synechococcus* sp., *Margalefidinium polykrikoides*, and *Lingulodinium polyedra*, were grown at 20°C and 20–50 μmol photons $m^{-2} s^{-1}$ on a 14 : 10 h light / dark cycle in enriched f/2 seawater medium (Guillard and Ryther 1962). *Synechococcus* sp. was incubated at 20°C under the dim light condition (≤10 μmol photons $m^{-2} s^{-1}$) on a 14 : 10 h light / dark cycle in enriched f/2 seawater medium. *M. polykrikoides* and *L. polyedra* were incubated in enriched f/2 and L1 seawater medium, respectively (Guillard and Ryther 1962, Guillard and Hargraves 1993), at 20°C and 50 μmol photons $m^{-2} s^{-1}$ under continuous illumination because they did not survive under a light / dark cycle (Lee et al. 2014).

Mixotrophic ability of *Karenia* species

Experiments were designed to explore whether *K. bicuneiformis* CAWD81, *K. papilionacea* CAWD91, *K. selliformis* NIES-4541, and *K. mikimotoi* NIES-2411 were able to feed on target potential prey species when a diversity of prey items was provided. Five milliliters were removed from dense cultures of *K. bicuneiformis*, *K. papilionacea*, *K. selliformis*, and *K. mikimotoi* (ca. 3,000, 3,000, 3,000, and 20,000 cells mL^{-1}, respectively) and then the cell density of the four *Karenia* species was determined using a compound microscope (BX53; Olympus, Tokyo, Japan). The initial cell density of the tested *Karenia* species and each target potential prey species were established using an autopipette to deliver a predetermined volume of each culture to experimental 42-mL polycarbonate (PC) bottles (Table 1). A 42-mL PC bottle with a mixture of one *Karenia* species and one potential prey species, control of a potential prey species, and control of a *Karenia* species was set up for each target potential prey species. The bottles were filled to capacity with filtered seawater, capped, and placed on a vertically rotating wheel (0.9 r min^{-1}). Each bottle, except that containing *Synechococcus* sp., *M. polykrikoides*, and *L. polyedra*, was incubated at 20°C and 20 μmol photons $m^{-2} s^{-1}$ illumination under a 14 : 10 h light / dark cycle. Each bottle containing *Synechococcus* sp. was incubated at 20°C and 10 μmol photons $m^{-2} s^{-1}$ illumination under a 14 : 10 h light / dark cycle. Each bottle containing *M. polykrikoides* and *L. polyedra* was incubated at 20°C and 50 μmol photons $m^{-2} s^{-1}$ under continuous illumination.

After 2, 24, and 48 h, a total of ≥30 cells of each *Karenia* species was tracked to determine physical contact, attack (attempt to capture), and feeding (successful capture) with a dissecting microscope (SZX2-ILLB; Olympus) at 20–63× magnification. In this process, the lysis of the target potential prey species was also observed. Photographs of the tested *Karenia* species and each target potential prey species were taken on confocal dishes using

Table 1. Initial target density of each potential predator *Karenia* species and prey species

Potential prey species	ESD (μm)	Initial target prey density (cells mL^{-1})			
		K. bicuneiformis CAWD81 (1,000)	*K. papilionacea* CAWD91 (150–1,000)	*K. selliformis* NIES-4541 (1,000)	*K. mikimotoi* NIES-2411 (2,000–3,000)
Cyanobacterium					
Synechococcus sp.	1.0	2,000,000	2,000,000	2,000,000	2,000,000
Prymnesiophyte					
Isochrysis galbana	4.8	150,000	150,000	1,000,000	150,000
Prasinophyte					
Pyramimonas sp.	5.6	100,000	100,000	300,000	150,000
Diatom					
Skeletonema costatum	5.9	150,000	150,000	150,000	150,000
Cryptophyte					
Teleaulax amphioxeia	5.6	100,000	100,000	300,000	100,000
Storeatula major	6.0	15,000	15,000	30,000	15,000
Rhodomonas salina	8.8	50,000	50,000	50,000	50,000
Raphidophyte					
Heterosigma akashiwo	11.5	30,000	10,000	30,000	30,000
Dinoflagellate					
Heterocapsa rotundata	5.8	10,000	10,000	20,000	50,000
Amphidinium carterae	9.7	30,000	30,000	50,000	30,000
Prorocentrum cordatum	12.1	15,000	15,000	30,000	15,000
Prorocentrum donghaiense	13.3	15,000	15,000	8,000	15,000
Margalefidinium polykrikoides	25.9	1,000	1,000	3,000	1,000
Prorocentrum micans	26.6	2,000	2,000	1,000	2,000
Akashiwo sanguinea	30.8	1,000	1,000	1,000	1,000
Lingulodinium polyedra	38.2	2,000	2,000	3,000	2,000

Values in parentheses below the name of each *Karenia* species indicate the initial target density.
ESD, equivalent spherical diameter.

a digital camera (Zeiss Axiocam 506; Carl Zeiss Ltd., Göttingen, Germany) on an inverted microscope (Zeiss Axiovert 200M; Carl Zeiss Ltd.) at 400–1,000× magnification.

To examine whether each *Karenia* species was able to feed on the cyanobacterium *Synechococcus* sp., the protoplasms of ≥100 *Karenia* cells were carefully observed after 2, 24, and 48-h incubation under the inverted epifluorescence microscope at a magnification of 1,000× (Zeiss Axiovert 200M; Carl Zeiss Ltd.). To observe whether *K. mikimotoi* NIES-2411 fed on the prymnesiophyte *Isochrysis galbana*, *I. galbana* cells were labeled with the fluorescent dye 5-([4,6-dichlorotriazin-2-yl]amino)fluorescein hydrochloride following the method of Rublee and Gallegos (1989).

Phylogenetic tree

Sequences of the large subunit ribosomal DNA (LSU rDNA) of *Karenia* species were obtained from GenBank. Sequences of the LSU rDNA of *Karlodinium* species as an outgroup were also obtained from GenBank. The sequences were aligned using MEGA v4 software (Tamura et al. 2007). The Bayesian and maximum likelihood analyses of the LSU rDNA region were conducted following Kang et al. (2010). The assumed empirical nucleotide frequencies of LSU rDNA comprised a substitution rate matrix with A–C substitutions = 0.0669, A–G = 0.1998, A–T = 0.0946, C–G = 0.0748, C–T = 0.4760, and G–T = 0.0879. Rates were assumed to follow a gamma distribution with a shape parameter of 0.5972 for variable sites. The proportion of sites assumed to be invariable was 0.4984.

RESULTS

Mixotrophic ability of three *Karenia* species

K. bicuneiformis CAWD81 did not feed on the cyanobacterium *Synechococcus* sp., prymnesiophyte *Isochrysis galbana*, prasinophyte *Pyramimonas* sp., diatom *Skeletonema costatum*, cryptophytes *Rhodomonas salina*, *Storeatula major*, and *Teleaulax amphioxeia*, raphidophyte *Heterosigma akashiwo*, and dinoflagellates *Akashiwo sanguinea*, *Amphidinium carterae*, *Heterocapsa rotundata*, *L. polyedra*, *M. polykrikoides*, *Prorocentrum cordatum*, *Prorocentrum donghaiense*, and *Prorocentrum micans* (Table 2, Fig. 1). Cells of *K. bicuneiformis* were observed to attack *I. galbana* and *R. salina* but did not feed

Table 2. Results of feeding occurrence test for four *Karenia* species in this study

Potential prey species	ESD (μm)	Predator							
		K. bicuneiformis CAWD81		*K. papilionacea* CAWD91		*K. selliformis* NIES-4541		*K. mikimotoi* NIES-2411	
		Attack	Feeding	Attack	Feeding	Attack	Feeding	Attack	Feeding
Cyanobacterium									
Synechococcus sp.	1.0	NA	×	NA	×	NA	×	NA	×
Prymnesiophyte									
Isochrysis galbana	4.8	○	×	×	×	○	×	○	○
Prasinophyte									
Pyramimonas sp.	5.6	×	×	×	×	×[a]	×	×[a]	×
Diatom									
Skeletonema costatum	5.9	×	×	×	×	×	×	×	×
Cryptophyte									
Teleaulax amphioxeia	5.6	×	×	×	×	×[a]	×	○	○
Storeatula major	6.0	×	×	×	×	○[a]	×	×	×
Rhodomonas salina	8.8	○	×	×	×	×	×	○	×
Raphidophyte									
Heterosigma akashiwo	11.5	×	×	×	×	○	×	○	×
Dinoflagellate									
Heterocapsa rotundata	5.8	×	×	×	×	○	×	×	×
Amphidinium carterae	9.7	×	×	×	×	×	×	○	×
Prorocentrum cordatum	12.1	×	×	×	×	×	×	×	×
Prorocentrum donghaiense	13.3	×	×	×	×	×	×	×	×
Margalefidinium polykrikoides	25.9	×	×	×	×	×	×	×	×
Prorocentrum micans	26.6	×	×	×	×	×	×	○	×
Akashiwo sanguinea	30.8	×	×	×	×	×	×	×[a]	×
Lingulodinium polyedra	38.2	×	×	×	×	×	×	×	×

ESD, equivalent spherical diameter; ○, attacked or fed by *Karenia* species; ×, not attacked or not fed by *Karenia* species; NA, not analyzed.
[a]Potential prey cells were lysed by *Karenia* species.

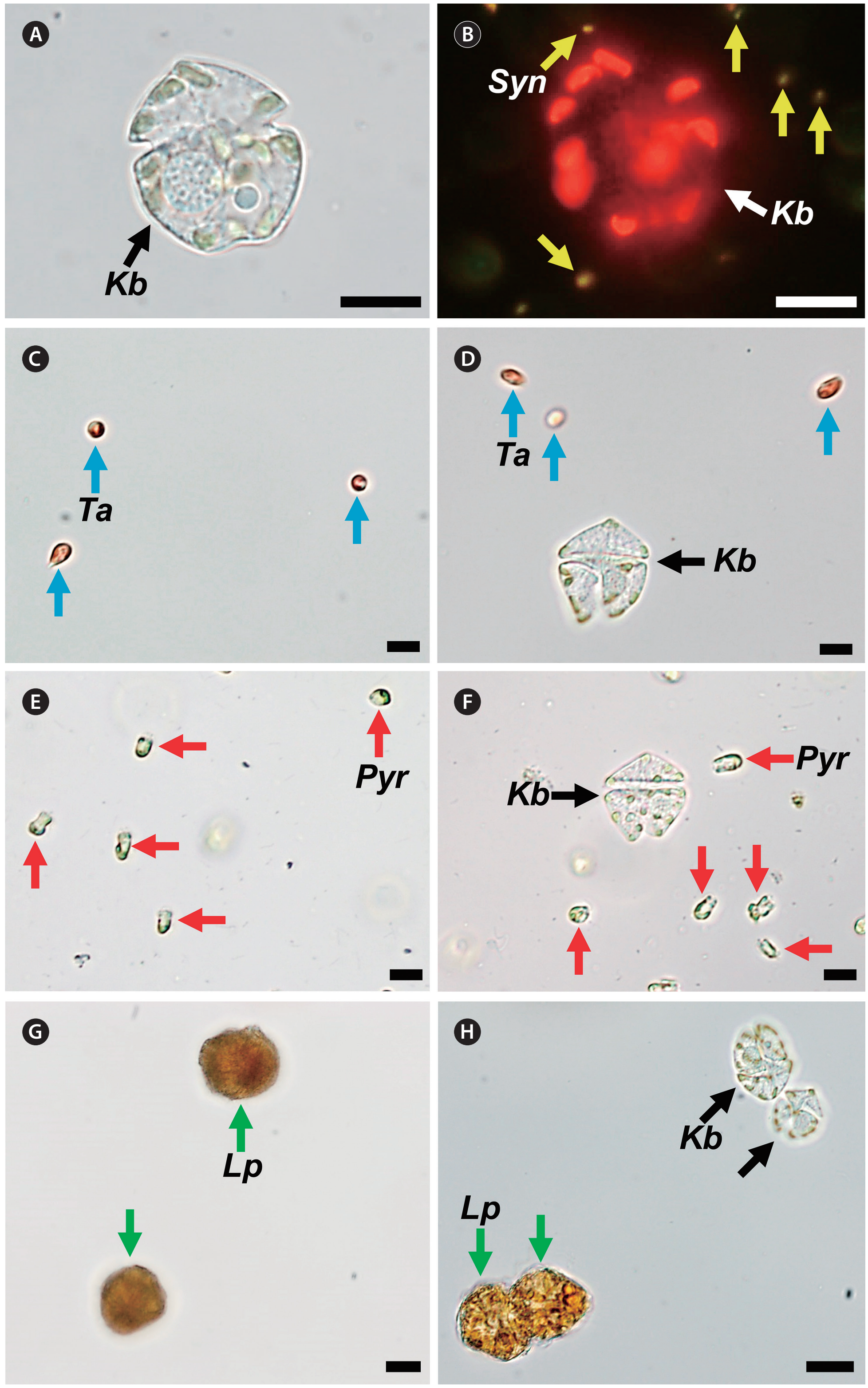

Fig. 1. Micrographs of *Karenia bicuneiformis* CAWD81 (*Kb*, black or white arrows) and potential prey species. *Kb* incubated with *Synechococcus* sp. (*Syn*, yellow arrows) taken under a light microscope (A) and epifluorescence microscope (B). (C) Intact *Teleaulax amphioxeia* (*Ta*, blue arrows). (D) *Kb* incubated with *Ta* (blue arrows). (E) Intact *Pyramimonas* sp. (*Pyr*, red arrows). (F) *Kb* incubated with *Pyr* (red arrows). (G) Intact *Lingulodinium polyedra* (*Lp*, green arrows). (H) *Kb* incubated with *Lp* (green arrows). No feeding of *Kb* on each potential prey species was observed. Scale bars represent: A–F, 10 μm; G & H, 20 μm.

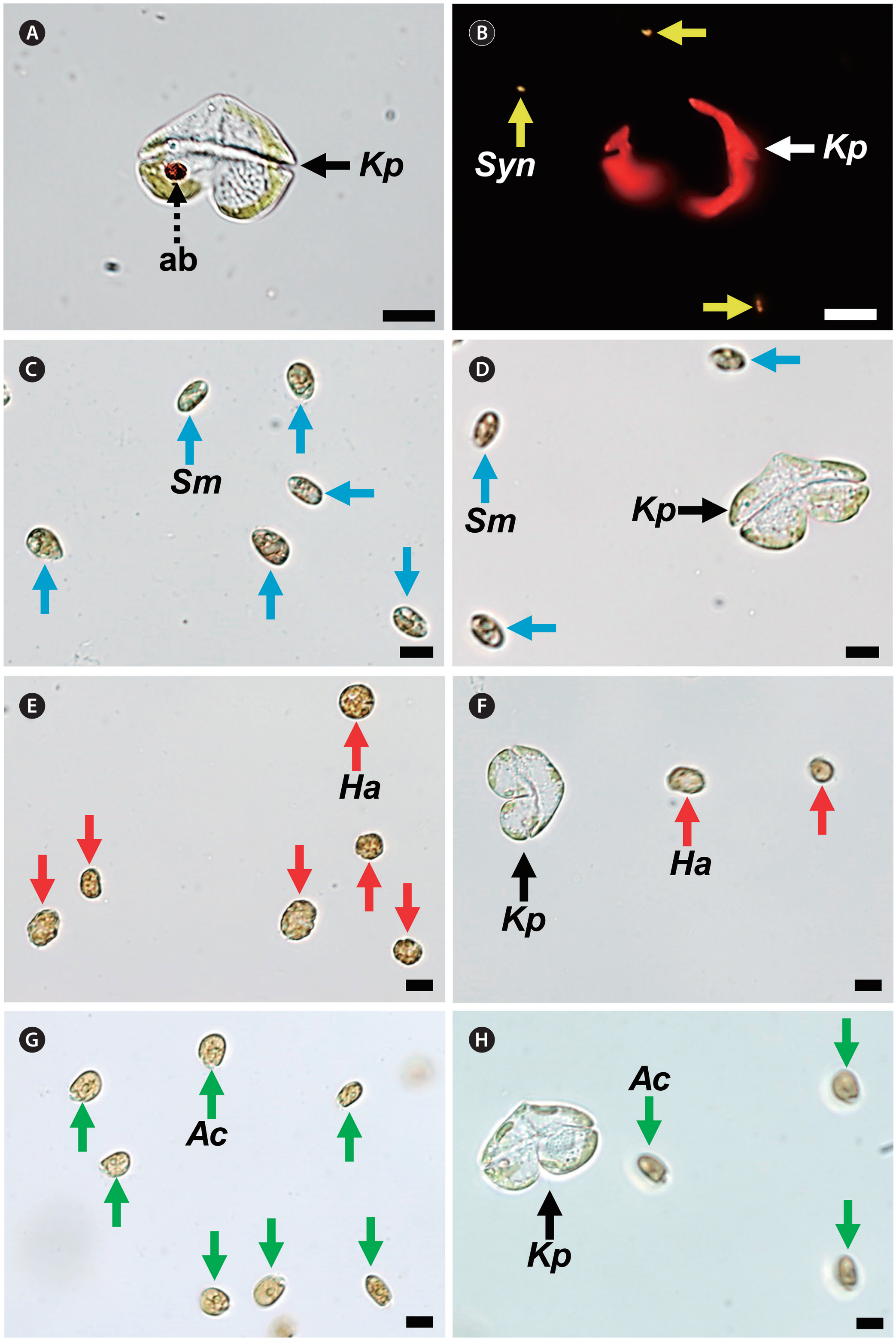

Fig. 2. Micrographs of *Karenia papilionacea* CAWD91 (*Kp*, black or white arrows) and potential prey species. *Kp* incubated with *Synechococcus* sp. (*Syn*, yellow arrows) taken under a light microscope (A) and epifluorescence microscope (B). A dashed arrow in (A) indicates the accumulation body (ab) of *Kp* (Haywood et al. 2004). (C) Intact *Storeatula major* (*Sm*, blue arrows). (D) *Kp* incubated with *Sm* (blue arrows). (E) Intact *Heterosigma akashiwo* (*Ha*, red arrows). (F) *Kp* incubated with *Ha* (red arrows). (G) Intact *Amphidinium carterae* (*Ac*, green arrows). (H) *Kp* incubated with *Ac* (green arrows). No feeding of *Kp* on each potential prey species was observed. Scale bars represent: A–H, 10 µm.

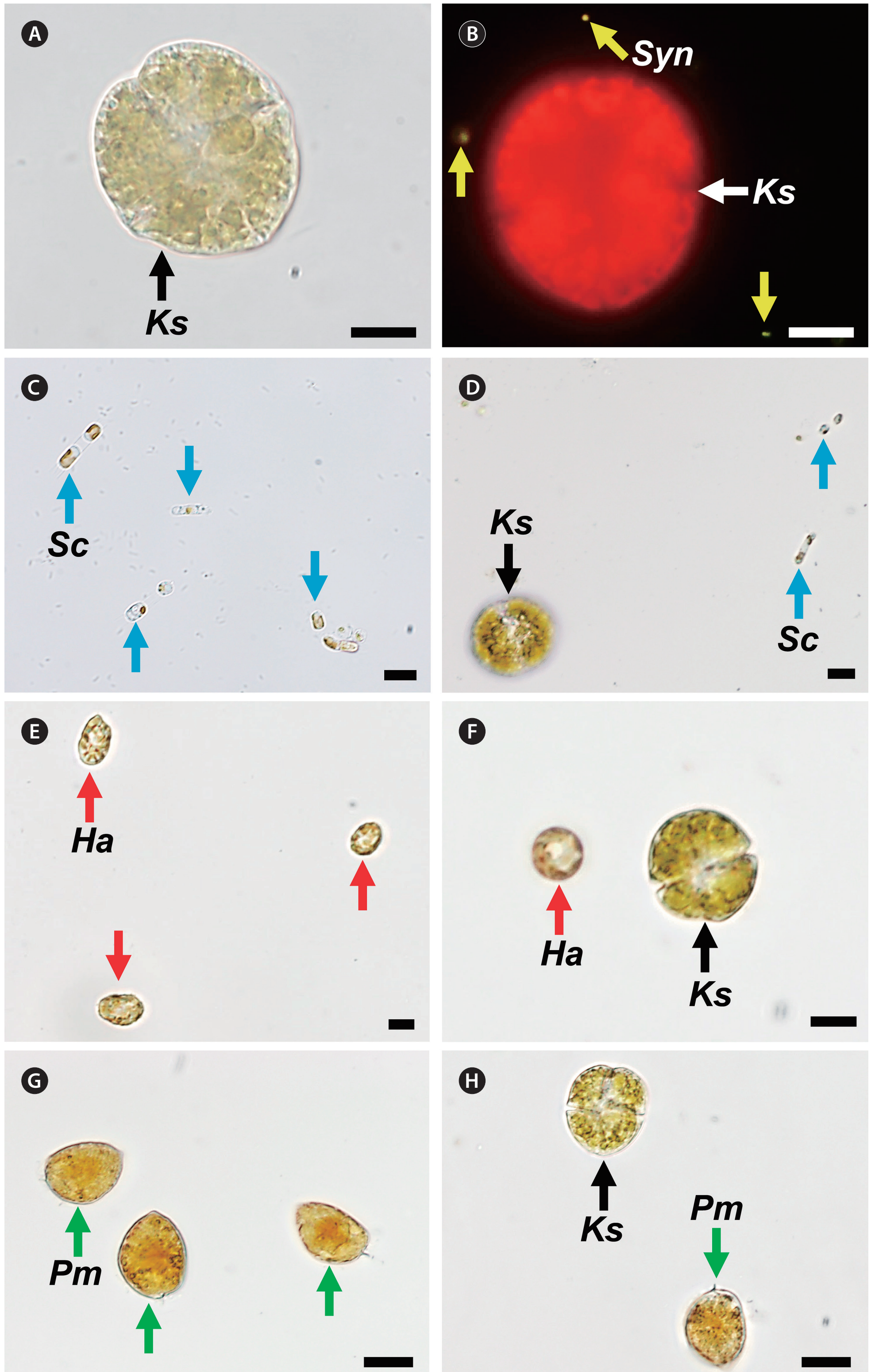

Fig. 3. Micrographs of *Karenia selliformis* NIES-4541 (*Ks*, black or white arrows) and potential prey species. *Ks* incubated with *Synechococcus* sp. (*Syn*, yellow arrows) taken under a light microscope (A) and epifluorescence microscope (B). (C) Intact *Skeletonema costatum* (*Sc*, blue arrows). (D) *Ks* incubated with *Sc* (blue arrows). (E) Intact *Heterosigma akashiwo* (*Ha*, red arrows). (F) *Ks* incubated with *Ha* (red arrow). (G) Intact *Prorocentrum micans* (*Pm*, green arrows). (H) *Ks* incubated with *Pm* (green arrow). No feeding of *Ks* on each potential prey species was observed. Scale bars represent: A–F, 10 µm; G & H, 20 µm.

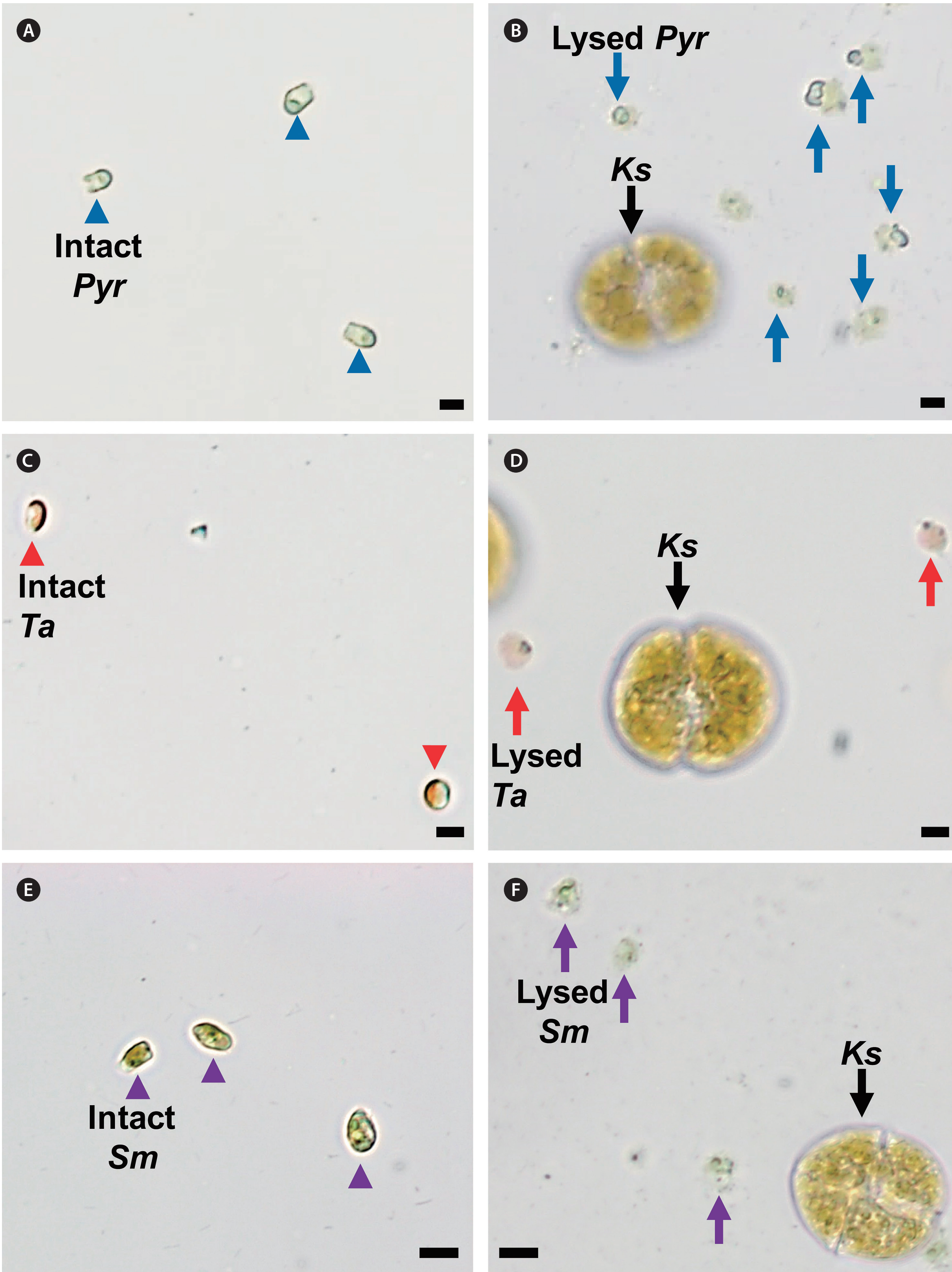

Fig. 4. Micrographs of lysed microalgal species when being incubated with *Karenia selliformis* NIES-4541 (*Ks*, black arrows). (A) Intact *Pyramimonas* sp. (*Pyr*, blue arrowheads). (B) *Ks* and lysed *Pyr* (blue arrows). (C) Intact *Teleaulax amphioxeia* (*Ta*, red arrowheads). (D) *Ks* and lysed *Ta* (red arrows). (E) Intact *Storeatula major* (*Sm*, purple arrowheads). (F) *Ks* and lysed *Sm* (purple arrows). Scale bars represent: A–D, 5 µm; E & F, 10 µm.

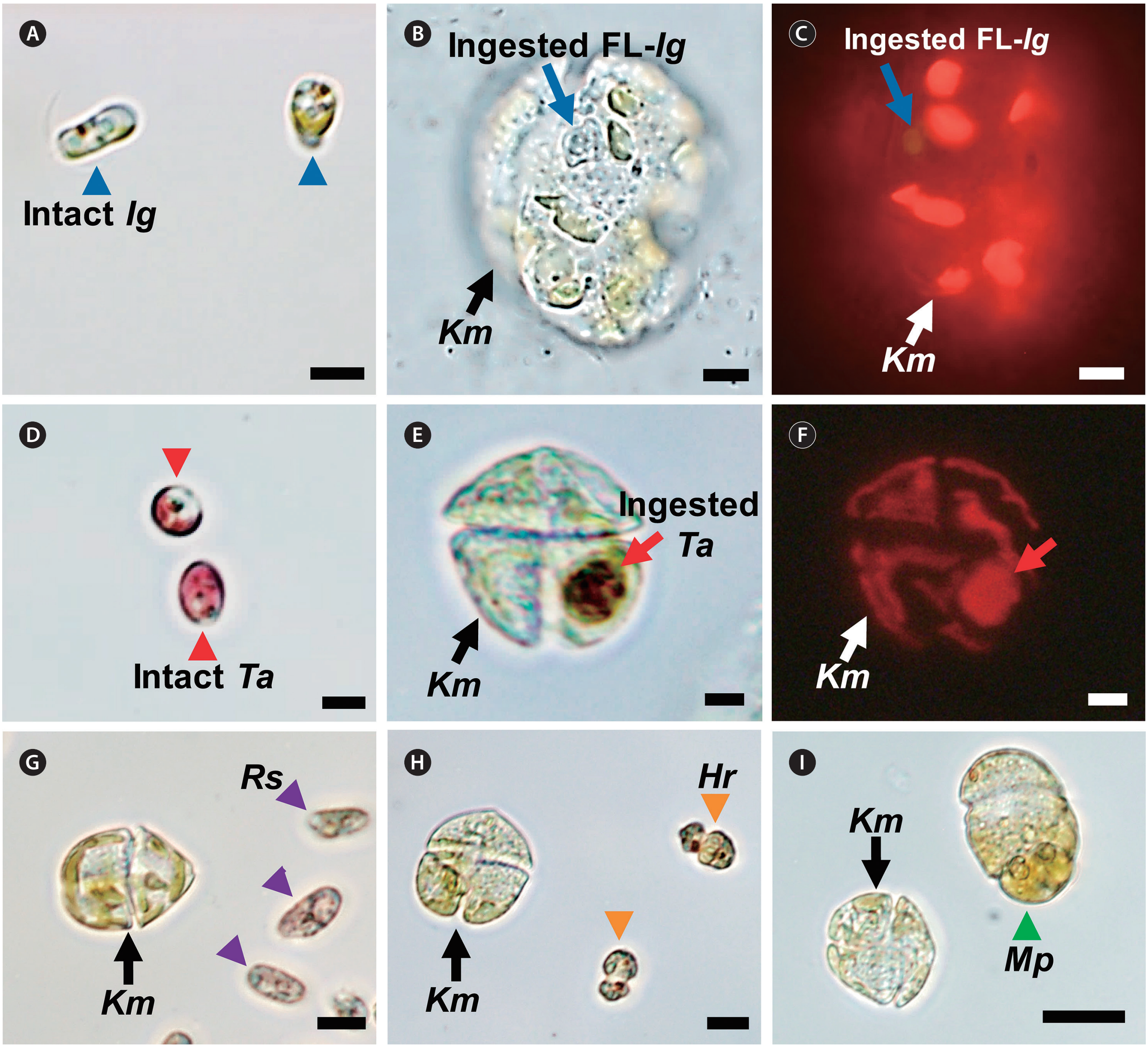

Fig. 5. Micrographs of *Karenia mikimotoi* NIES-2411 (*Km*, black or white arrows) and potential prey species. (A) Intact *Isochrysis galbana* (*Ig*, blue arrowheads). *Km* that fed on fluorescently-labeled *Ig* (FL-*Ig*, blue arrows) taken under a light (B) and epifluorescence microscope (C). (D) Intact *Teleaulax amphioxeia* (*Ta*, red arrowheads). *Km* that fed on a live *Ta* cell (red arrows) taken under a light (E) and epifluorescence microscope (F). (G) *Km* that did not feed on *Rhodomonas salina* (*Rs*, purple arrowheads). (H) *Km* that did not feed on *Heterocapsa rotundata* (*Hr*, orange arrowheads). (I) *Km* that did not feed on *Margalefidinium polykrikoides* (*Mp*, green arrowhead). Scale bars represent: A–F, 5 μm; G & H, 10 μm; I, 20 μm.

on them. No lysis of *K. bicuneiformis* to the potential prey species was observed.

Among 16 potential prey species, *K. papilionacea* CAWD91 did not feed on any of the target potential prey species (Table 2, Fig. 2). Moreover, *K. papilionacea* did not attack any of them. No lysis of *K. papilionacea* to the potential prey species was observed.

K. selliformis NIES-4541 did not feed on any of the target potential prey species (Table 2, Fig. 3). Cells of *K. selliformis* were observed to attack *I. galbana*, *S. major*, *Heterosigma akashiwo*, and *Heterocapsa rotundata* but did not feed on them. Moreover, *K. selliformis* lysed cells of *Pyramimonas* sp., *T. amphioxeia*, and *S. major* (Fig. 4).

Feeding occurrence of *Karenia mikimotoi*

K. mikimotoi NIES-2411 was able to feed on the fluorescent-labeled *I. galbana* and live *T. amphioxeia* (Table 2,

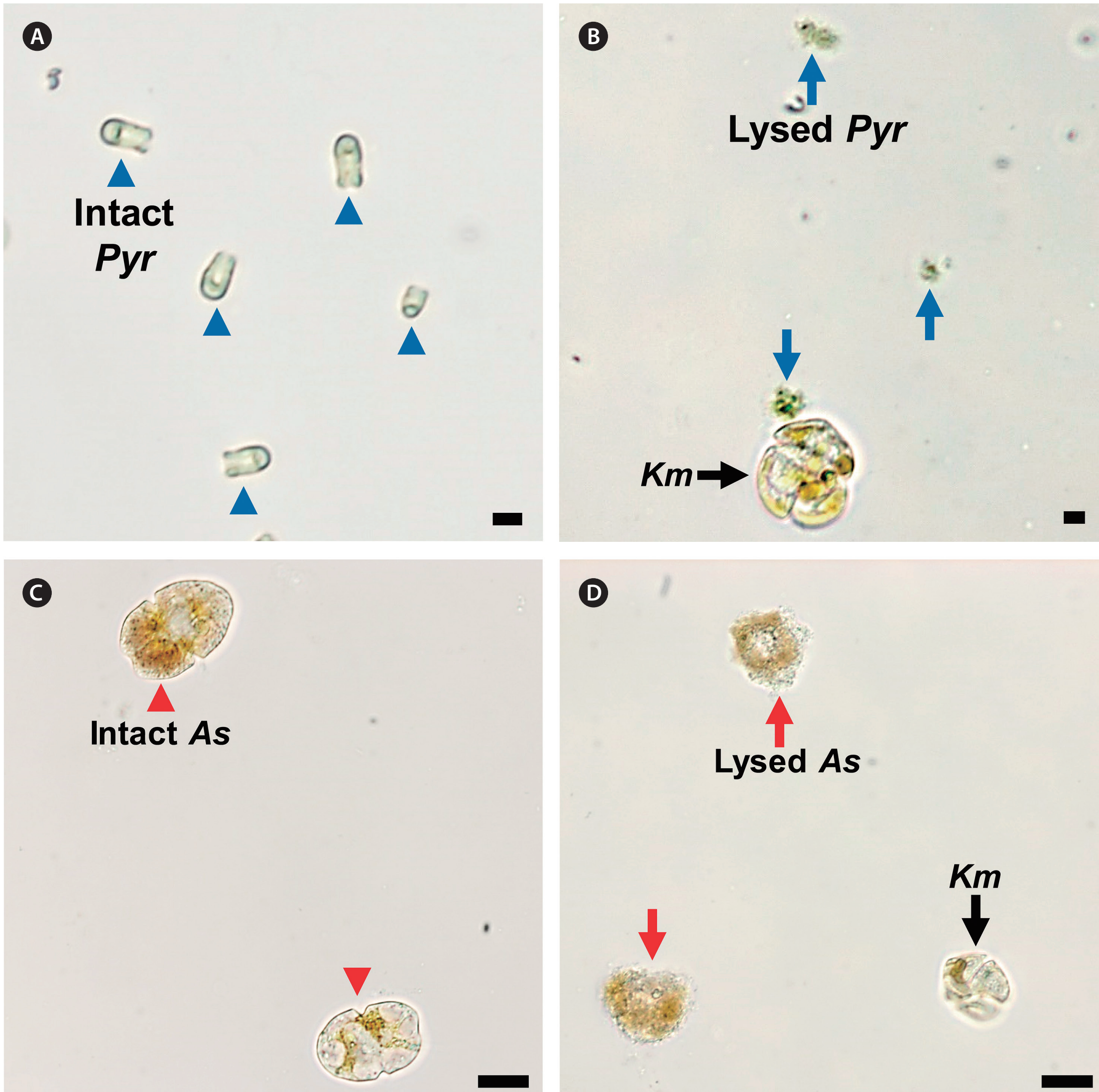

Fig. 6. Micrographs of lysed microalgal species by *Karenia mikimotoi* NIES-2411 (*Km*, black arrows). (A) Intact *Pyramimonas* sp. (*Pyr*, blue arrowheads). (B) *Km* and lysed *Pyr* (blue arrows). (C) Intact *Akashiwo sanguinea* (*As*, red arrowheads). (D) *Km* and lysed *As* (red arrows). Scale bars represent: A & B, 5 μm; C & D, 20 μm.

Fig. 5A–F). However, *K. mikimotoi* did not feed on the other potential prey species tested in this study (Table 2, Fig. 5G–I). Cells of *K. mikimotoi* were observed to attack *R. salina*, *Heterosigma akashiwo*, *Amphidinium carterae*, and *P. micans*, but did not feed on them. Moreover, *K. mikimotoi* lysed cells of *Pyramimonas* sp. and *Akashiwo sanguinea* (Fig. 6).

Phylogenetic analysis

In the phylogenetic tree based on the LSU rDNA of *Karenia* species, the mixotrophic species *K. brevis* and *K. mikimotoi* belonged to the same clade (Fig. 7). However, *K. bicuneiformis*, *K. papilionacea*, and *K. selliformis*, showing no mixotrophic ability, belonged to other clades in the phylogenetic tree.

DISCUSSION

Prior to the present study, all tested *Karenia* species were known to be mixotrophic; however, this included only two of the ten officially described species (Jeong et al. 2005*a*, Zhang et al. 2011, Guiry and Guiry 2023). The

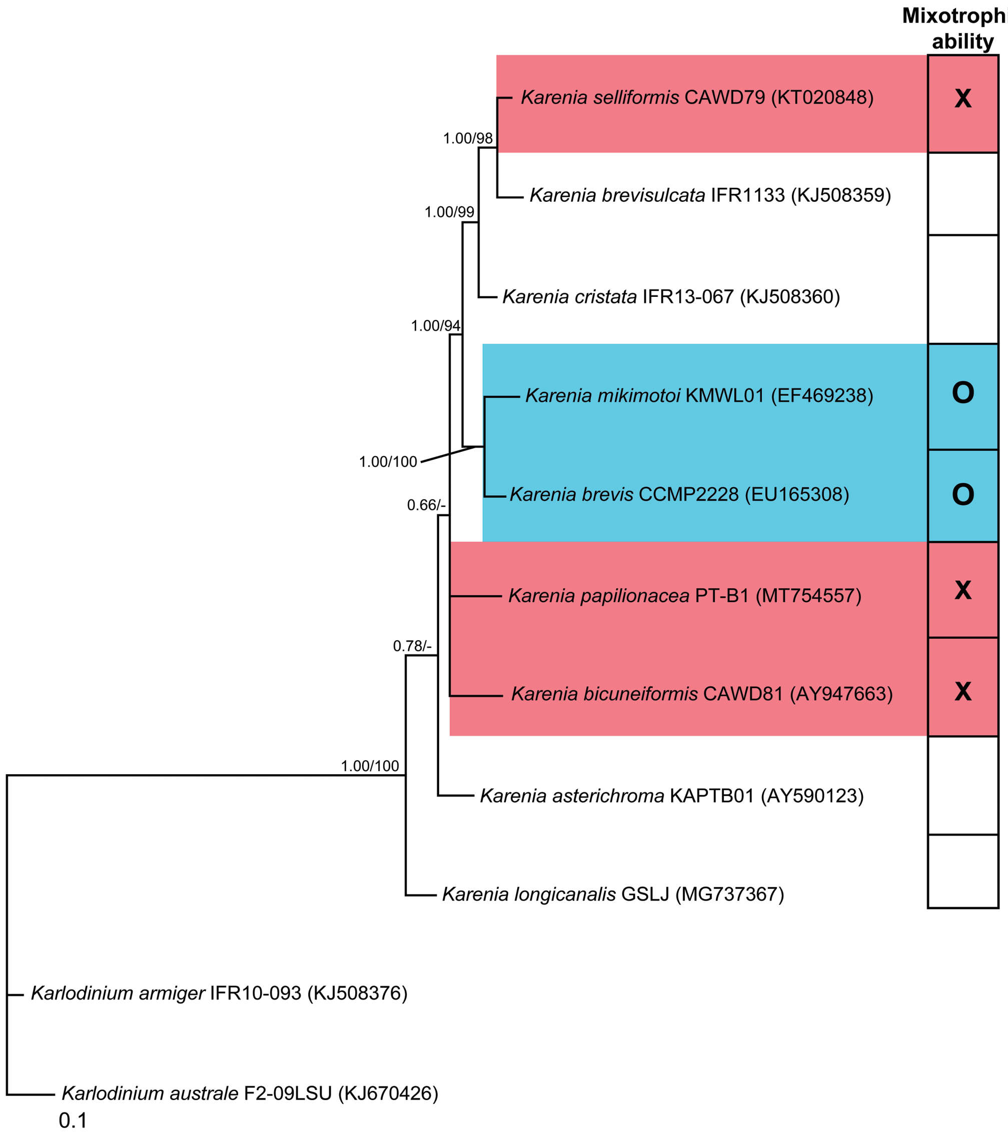

Fig. 7. Bayesian tree based on the large subunit ribosomal DNA region (847 bp), using a GTR + G + I model with the dinoflagellates *Karlodinium armiger* and *Karlodinium australe* as outgroup taxa. Numbers above or below the branches indicate the Bayesian posterior probability (left) and maximum likelihood bootstrap values (right). Posterior probabilities ≤0.5 are not shown. ○, presence of the mixotrophic ability; ×, lack of the mixotrophic ability.

results of the present study clearly showed that three *Karenia* species tested in this study lack the mixotrophic ability. Thus, among the five *Karenia* species tested so far, more than half the species lack the mixotrophic ability (Fig. 8). The presence or lack of mixotrophy among the species in the genus *Karenia* implies evolutionary and ecological divergence. The mixotrophic ability of the five remaining *Karenia* species (i.e., *K. asterichroma*, *K. brevisulcata*, *K. concordia*, *K. cristata*, and *K. longicanalis*) should be explored.

Previously, *K. mikimotoi* was reported to feed on fluorescent microspheres (0.5–2.0 μm), the heterotrophic bacterium *Marinobacter* sp., and *I. galbana* (Zhang et al. 2011). The results of the present study clearly showed that *K. mikimotoi* can feed on *T. amphioxeia* (5.6 μm in equivalent spherical diameter) but not larger microalgal prey species. Thus, we suggested that *K. mikimotoi* can feed on the prey species <6 μm but not on larger-sized prey species. *T. amphioxeia* is commonly found in many marine environments (Jeong et al. 2013, Johnson et al. 2013, Cloern 2018, Gran-Stadniczeñko et al. 2019, Jang and Jeong 2020), and *K. mikimotoi* has a global distribu-

Potential prey species	ESD (μm)	*Karenia bicuneiformis*	*Karenia papilionacea*	*Karenia selliformis*	*Karenia mikimotoi*	*Karenia brevis*
Marinobacter sp.					O	
Synechococcus sp.	1.0	X	X	X	X	O
Isochrysis galbana	4.8	X	X	X	O	
Pyramimonas sp.	5.6	X	X	X	X	
Teleaulax amphioxeia	5.6	X	X	X	O	
Heterocapsa rotundata	5.8	X	X	X	X	
Skeletonema costatum	5.9	X	X	X	X	
Storeatula major	6.0	X	X	X	X	
Rhodomonas salina	8.8	X	X	X	X	
Amphidinium carterae	9.7	X	X	X	X	
Heterosigma akashiwo	11.5	X	X	X	X	
Prorocentrum cordatum	12.1	X	X	X	X	
Prorocentrum donghaiense	13.3	X	X	X	X	
Margalefidinium polykrikoides	25.9	X	X	X	X	
Prorocentrum micans	26.6	X	X	X	X	
Akashiwo sanguinea	30.8	X	X	X	X	
Lingulodinium polyedra	38.2	X	X	X	X	
Reference		(1)	(1)	(1)	(1), (2)	(3), (4)

Fig. 8. Feeding by each *Karenia* species on diverse prey species. ○ in the blue box, fed by *Karenia* species; × in the red box, not fed by *Karenia* species. ESD, equivalent spherical diameter. 1, this study; 2, Zhang et al. (2011); 3, Jeong et al. (2005*a*); 4, Glibert et al. (2009).

tion (Jeong et al. 2021). Thus, they have a high chance of encountering each other, and *K. mikimotoi* feeds on *T. amphioxeia*. This cryptophyte is a prey for many dinoflagellates, such as the mixotrophic dinoflagellates *Biecheleria cincta*, *Gonyaulax polygramma*, *Gymnodinium aureolum*, *Heterocapsa steinii*, *Paragymnodinium shiwhaense*, *Prorocentrum cordatum*, *Prorocentrum donghaiense*, *Prorocentrum micans*, *M. polykrikoides*, and *Yihiella yeosuensis*, the kleptoplasitidic dinoflagellates *Pfiesteria piscicida* and *Shimiella gracilenta*, and the heterotrophic dinoflagellates *Gyrodiniellum shiwhaense* and *Luciella masanensis* (Skovgaard 1998, Jeong et al. 2004, 2005*b*, 2005*c*, 2006, 2007, 2010*a*, 2011, Yoo et al. 2010, Kang et al. 2011, Johnson 2015, Jang et al. 2017, Ok et al. 2021*a*). Thus, *K. mikimotoi* may compete with diverse predators feeding on *T. amphioxeia* in marine environments.

Among the four *Karenia* species tested in the present study, *K. selliformis* and *K. mikimotoi* lysed some microalgal species, whereas *K. bicuneiformis* and *K. papilionacea* did not lyse any microalgal species. *K. bicuneiformis*, *K. selliformis*, and *K. mikimotoi* were reported to form blooms (e.g., Botes et al. 2003, Davidson et al. 2009, Li et al. 2019, Baohong et al. 2021, Orlova et al. 2022, Boudriga et al. 2023). Thus, *K. selliformis* and *K. mikimotoi* are likely to eliminate several microalgal species by lysis but not by feeding when they form blooms. The results of the present study showed that both *K. selliformis* and *K. mikimotoi* lysed *Pyramimonas* sp. However, *K. selliformis* lysed *T. amphioxeia* and *Storeatula major* that *K. mikimotoi* did not lyse. On the contrary, *K. mikimotoi* lysed *Akashiwo sanguinea* that *K. selliformis* did not lyse. Thus, this differential lysis may cause different selections of co-occurring microalgal species. Many studies reported allelopathic effects of *K. mikimotoi* on microalgal species; previously, cells or filtrates of *K. mikimotoi* were reported to inhibit the growth of the dinoflagellates *Prorocentrum donghaiense*, and *Heterocapsa circularisquama*, the chlorophyte *Dunaliella salina*, and the diatom *Thalassiosira pseudonana* (Uchida et al. 1999, Shen et al. 2015, He et al. 2016, Zheng et al. 2021). The results of the present study added *Pyramimonas* sp. and *Akashiwo sanguinea* to the lysed species of *K. mikimotoi*.

In the phylogenetic tree, mixotrophic *K. mikimotoi* and *K. brevis* belong to the same clade, whereas *K. bicuneiformis*, *K. papilionacea*, and *K. selliformis*, belong to different clades. Thus, the presence or lack of mixotrophy of *Karenia* species may be related to their genetic characterizations such as LSU rDNA. In the dinoflagellate genus *Alexandrium*, the presence and lack of mixotrophy was found in the species belonging to the same clade in the phylogenetic tree based on the LSU rDNA of *Alexandrium* (Lim et al. 2019). However, the mixotrophic ability of only five species of ten formally described *Karenia* species has been tested and included in this phylogenetic tree, whereas that of 16 species of 32 formally described *Alexandrium* species has been tested (Lim et al. 2019, Guiry and Guiry 2023). Thus, further analyses of *Karenia* species that have not been explored yet are needed to confirm if mixotrophy is affected by genetic characterizations.

In conclusion, the present study showed that some species in the genus *Karenia* are mixotrophic, but others are not. They may have different ecological niches and strategies for bloom formation in marine ecosystems. To better understand the structure and function of marine ecosystems, the presence or lack of mixotrophy of species in other genera should be explored.

ACKNOWLEDGEMENTS

This research was supported by the National Research Foundation funded by the Ministry of Education (NRF-2022R1A6A3A01086348) award to JHO and the National Research Foundation by the Ministry of Science and ICT (NRF-2021M3I6A1091272; NRF-2021R1A2C1093379) award to HJJ.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

REFERENCES

Adolf, J. E., Bachvaroff, T. & Place, A. R. 2008. Can cryptophyte abundance trigger toxic *Karlodinium veneficum* blooms in eutrophic estuaries? Harmful Algae 8:119–128.

Baden, D. G. 1989. Brevetoxins: unique polyether dinoflagellate toxins. FASEB J. 3:1807–1817.

Baohong, C., Kang, W., Huige, G. & Hui, L. 2021. *Karenia mikimotoi* blooms in coastal waters of China from 1998 to 2017. Estuar. Coast. Shelf Sci. 249:107034.

Benico, G., Takahashi, K., Lum, W. M. & Iwataki, M. 2019. Morphological variation, ultrastructure, pigment composition and phylogeny of the star-shaped dinoflagellate *Asterodinium gracile* (Kareniaceae, Dinophyceae). Phycologia 58:405–418.

Bergholtz, T., Daugbjerg, N., Moestrup, Ø. & Fernández-Tejedor, M. 2006. On the identity of *Karlodinium veneficum* and description of *Karlodinium armiger* sp. nov. (Dinophyceae), based on light and electron microscopy, nuclear-encoded LSU rDNA, and pigment composition. J. Phycol. 42:170–193.

Bockstahler, K. R. & Coats, D. W. 1993. Spatial and temporal aspects of mixotrophy in Chesapeake Bay dinoflagellates. J. Eukaryot. Microbiol. 40:49–60.

Boraas, M. E., Estep, K. W., Johnson, P. W. & Sieburth, J. M. 1988. Phagotrophic phototrophs: the ecological significance of mixotrophy. J. Protozool. 35:249–252.

Botes, L., Sym, S. D. & Pitcher, G. C. 2003. *Karenia cristata* sp. nov. and *Karenia bicuneiformis* sp. nov. (Gymnodiniales, Dinophyceae): two new *Karenia* species from the South African coast. Phycologia 42:563–571.

Boudriga, I., Abdennadher, M., Khammeri, Y., Mahfoudi, M., Quéméneur, M., Hamza, A., Bel Haj Hmida, N., Zouari, A. B. & Hassen, M. B. 2023. *Karenia selliformis* bloom dynamics and growth rate estimation in the Sfax harbour (Tunisia), by using automated flow cytometry equipped with image in flow, during autumn 2019. Harmful Algae 121:102366.

Brand, L. E., Campbell, L. & Bresnan, E. 2012. *Karenia*: the biology and ecology of a toxic genus. Harmful Algae 14:156–178.

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.

Calbet, A., Bertos, M., Fuentes-Grünewald, C., Alacid, E., Figueroa, R., Renom, B. & Garcés, E. 2011. Intraspecific variability in *Karlodinium veneficum*: growth rates, mixotrophy, and lipid composition. Harmful Algae 10:654–667.

Cloern, J. E. 2018. Why large cells dominate estuarine phytoplankton. Limnol. Oceanogr. 63:S392–S409.

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.

Davidson, K., Miller, P., Wilding, T. A., Shutler, J., Bresnan, E., Kennington, K. & Swan, S. 2009. A large and prolonged bloom of *Karenia mikimotoi* in Scottish waters in 2006. Harmful Algae 8:349–361.

de Salas, M. F., Bolch, C. J. S., Botes, L., Nash, G., Wright, S. W. & Hallegraeff, G. M. 2003. *Takayama* gen. nov. (Gymnodiniales, Dinophyceae), a new genus of unarmored dinoflagellates with sigmoid apical grooves, including the description of two new species. J. Phycol. 39:1233–1246.

Eom, S. H., Jeong, H. J., Ok, J. H., Park, S. A., Kang, H. C., You, J. H., Lee, S. Y., Yoo, Y. D., Lim, A. S. & Lee, M. J. 2021. Interactions between common heterotrophic protists and the dinoflagellate *Tripos furca*: implication on the long duration of its red tides in the South Sea of Korea in 2020. Algae 36:25–36.

Fowler, N., Tomas, C., Baden, D., Campbell, L. & Bourdelais, A. 2015. Chemical analysis of *Karenia papilionacea*. Toxicon 101:85–91.

Glibert, P. M., Burkholder, J. M., Kana, T. M., Alexander, J., Skelton, H. & Shilling, C. 2009. Grazing by *Karenia brevis* on *Synechococcus* enhances its growth rate and may help to sustain blooms. Aquat. Microb. Ecol. 55:17–30.

Gran-Stadniczeñko, S., Egge, E., Hostyeva, V., Logares, R., Eikrem, W. & Edvardsen, B. 2019. Protist diversity and seasonal dynamics in Skagerrak plankton communities as revealed by metabarcoding and microscopy. J. Eukaryot. Microbiol. 66:494–513.

Guillard, R. R. L. & Hargraves, P. E. 1993. *Stichochrysis immobilis* is a diatom, not a chrysophyte. Phycologia 32:234–236.

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

Guiry, M. D. & Guiry, G. M. 2023. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Available from: http://www.algaebase.org. Accessed Jan 28, 2023.

Haywood, A. J., Steidinger, K. A., Truby, E. W., Bergquist, P. R., Bergquist, P. L., Adamson, J. & Mackenzie, L. 2004. Comparative morphology and molecular phylogenetic analysis of three new species of the genus *Karenia* (Dinophyceae) from New Zealand. J. Phycol. 40:165–179.

He, D., Liu, J., Hao, Q., Ran, L., Zhou, B. & Tang, X. 2016. Interspecific competition and allelopathic interaction between *Karenia mikimotoi* and *Dunaliella salina* in laboratory culture. Chin. J. Oceanol. Limnol. 34:301–313.

Jang, S. H. & Jeong, H. J. 2020. Spatio-temporal distributions of the newly described mixotrophic dinoflagellate *Yihiella yeosuensis* (Suessiaceae) in Korean coastal waters and its grazing impact on prey populations. Algae 35:45–59.

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.

Jeong, H. J., Ha, J. H., Park, J. Y., Kim, J. H., Kang, N. S., Kim, S., 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. 2007. Feeding by the *Pfiesteria*-like heterotrophic dinoflagellate *Luciella masanensis*. J. Eukaryot. Microbiol. 54:231–241.

Jeong, H. J., Kang, H. C., Lim, A. S., Jang, S. H., Lee, K., Lee, S. Y., Ok, J. H., You, J. H., Kim, J. H., Lee, K. H., Park, S. A., Eom, S. H., Yoo, Y. D. & Kim, K. Y. 2021. Feeding diverse prey as an excellent strategy of mixotrophic dinoflagellates for global dominance. Sci. Adv. 7:eabe4214.

Jeong, H. J., Lee, K. H., Yoo, Y. D., Kang, N. S. & Lee, K. 2011. Feeding by the newly described, nematocyst-bearing heterotrophic dinoflagellate *Gyrodiniellum shiwhaense*. J. Eukaryot. Microbiol. 58:511–524.

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., Ok, J. H., Lim, A. S., Kwon, J. E., Kim, S. J. & Lee, S. Y. 2016. Mixotrophy in the phototrophic dinoflagellate *Takayama helix* (family Kareniaceae): predator of diverse toxic and harmful dinoflagellates. Harmful Algae 60:92–106.

Jeong, H. J., Park, J. Y., Nho, J. H., Park, M. O., Ha, J. H., Seong, K. A., Jeng, C., Seong, C. N., Lee, K. Y. & Yih, W. H. 2005*a*. Feeding by red-tide dinoflagellates on the cyanobacterium *Synechococcus*. Aquat. Microb. Ecol. 41:131–143.

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., Nam, G. S. & Yih, W. 2010*a*. 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., Kim, T. H., Kim, J. H., Kang, N. S. & Yih, W. 2004. Mixotrophy in the phototrophic harmful alga *Cochlodinium polykrikoides* (Dinophycean): prey species, the effects of prey concentration, and grazing impact. J. Eukaryot. Microbiol. 51:563–569.

Jeong, H. J., Yoo, Y. D., Kim, J. S., Seong, K. A., Kang, N. S. & Kim, T. H. 2010*b*. 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(Suppl. 1):S75–S88.

Jeong, H. J., Yoo, Y. D., Park, J. Y., Song, J. Y., Kim, S. T., Lee, S. H., Kim, K. W. & Yih, W. H. 2005*b*. 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., Yoo, Y. D., Seong, K. A., Kim, J. H., Park, J. Y., Kim, S., Lee, S. H., Ha, J. H. & Yih, W. H. 2005*c*. Feeding by the mixotrophic dinoflagellate *Gonyaulax polygramma*: mechanisms, prey species, effects of prey concentration, and grazing impact. Aquat. Microb. Ecol. 38:249–257.

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

Johnson, M. D., Stoecker, D. K. & Marshall, H. G. 2013. Seasonal dynamics of *Mesodinium rubrum* in Chesapeake Bay. J. Plankton Res. 35:877–893.

Jones, R. I. 2000. Mixotrophy in planktonic protists: an overview. Freshw. Biol. 45:219–226.

Kang, N. S., Jeong, H. J., Moestrup, Ø., Shin, W., Nam, S. W., Park, J. Y., de Salas, M. F., Kim, K. W. & Noh, J. H. 2010. Description of a new planktonic mixotrophic dinoflagellate *Paragymnodinium shiwhaense* n. gen., n. sp. from the coastal waters off western Korea: morphology, pigments, and ribosomal DNA gene sequence. J. Eukaryot. Microbiol. 57:121–144.

Kang, N. S., Jeong, H. J., Yoo, Y. D., Yoon, E. Y., Lee, K. H., Lee, K. & Kim, G. 2011. Mixotrophy in the newly described phototrophic dinoflagellate *Woloszynskia cincta* from western Korean waters: feeding mechanism, prey species and effect of prey concentration. J. Eukaryot. Microbiol. 58:152–170.

Kempton, J. W., Lewitus, A. J., Deeds, J. R., Law, J. M. & Place, A. R. 2002. Toxicity of *Karlodinium micrum* (Dinophyceae) associated with a fish kill in a South Carolina brackish retention pond. Harmful Algae 1:233–241.

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

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. 2014. 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.

Li, A., Stoecker, D. K. & Adolf, J. E. 1999. Feeding, pigmentation, photosynthesis and growth of the mixotrophic dinoflagellate *Gyrodinium galatheanum*. Aquat. Microb. Ecol. 19:163–176.

Li, X., Yan, T., Yu, R. & Zhou, M. 2019. A review of *Karenia mikimotoi*: bloom events, physiology, toxicity and toxic mechanism. Harmful Algae 90:101702.

Lim, A. S. & Jeong, H. J. 2021. Benthic dinoflagellates in Korean waters. Algae 36:91–109.

Lim, A. S., Jeong, H. J. & Ok, J. H. 2019. Five *Alexandrium* species lacking mixotrophic ability. Algae 34:289–301.

Lin, C. -H. M., Lyubchich, V. & Glibert, P. M. 2018. Time series models of decadal trends in the harmful algal species *Karlodinium veneficum* in Chesapeake Bay. Harmful Algae 73:110–118.

Liu, C., Zhang, X. & Wang, X. 2022. DNA metabarcoding data reveals harmful algal-bloom species undescribed previously at the northern Antarctic Peninsula region. Polar Biol. 45:1495–1512.

López-Cortés, D. J., Núñez Vázquez, E. J., Dorantes-Aranda, J. J., Band-Schmidt, C. J., Hernández-Sandoval, F. E., Bustillos-Guzmán, J. J., Leyva-Valencia, I. & Fernández-Herrera, L. J. 2019. The state of knowledge of harmful algal blooms of *Margalefidinium polykrikoides* (aka *Cochlodinium polykrikoides*) in Latin America. Front. Mar. Sci. 6:463.

Mansour, J. S. & Anestis, K. 2021. Eco-evolutionary perspectives on mixoplankton. Front. Mar. Sci. 8:666160.

Miles, C. O., Wilkins, A. L., Stirling, D. J. & MacKenzie, A. L. 2003. Gymnodimine C, an isomer of gymnodimine B, from *Karenia selliformis*. J. Agric. Food Chem. 51:4838–4840.

Ok, J. H., Jeong, H. J., Kang, H. C., Park, S. A., Eom, S. H., You, J. H. & Lee, S. Y. 2021*a*. Ecophysiology of the kleptoplastidic dinoflagellate *Shimiella gracilenta*: I. spatiotemporal distribution in Korean coastal waters and growth and ingestion rates. Algae 36:263–283.

Ok, J. H., Jeong, H. J., Kang, H. C., Park, S. A., Eom, S. H., You, J. H. & Lee, S. Y. 2022. Ecophysiology of the kleptoplastidic dinoflagellate *Shimiella gracilenta*: II. Effects of temperature and global warming. Algae 37:49–62.

Ok, J. H., Jeong, H. J., Lee, S. Y., Park, S. A. & Noh, J. H. 2021*b*. *Shimiella* gen. nov. and *Shimiella gracilenta* sp. nov. (Dinophyceae, Kareniaceae), a kleptoplastidic dinoflagellate from Korean waters and its survival under starvation. J. Phycol. 57:70–91.

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.

Ok, J. H., Jeong, H. J., Lim, A. S., You, J. H., Kang, H. C., Kim, S. J. & Lee, S. Y. 2019. Effects of light and temperature on the growth of *Takayama helix* (Dinophyceae): mixotrophy as a survival strategy against photoinhibition. J. Phycol. 55:1181–1195.

Ok, J. H., Jeong, H. J., Lim, A. S., You, J. H., Yoo, Y. D., Kang, H. C., Park, S. A., Lee, M. J. & Eom, S. H. 2023. Effects of intrusion and retreat of deep cold waters on the causative species of red tides offshore in the South Sea of Korea. Mar. Biol. 170:6.

Ok, J. H., Jeong, H. J., You, J. H., Kang, H. C., Park, S. A., Lim, A. S., Lee, S. Y. & Eom, S. H. 2021*c*. Phytoplankton bloom dynamics in incubated natural seawater: predicting bloom magnitude and timing. Front. Mar. Sci. 8:681252.

Orlova, T. Y., Aleksanin, A. I., Lepskaya, E. V., Efimova, K. V., Selina, M. S., Morozova, T. V., Stonik, I. V., Kachur, V. A., Karpenko, A. A., Vinnikov, K. A., Adrianov, A. V. & Iwataki, M. 2022. A massive bloom of *Karenia* species (Dinophyceae) off the Kamchatka coast, Russia, in the fall of 2020. Harmful Algae 120:102337.

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, S. A., Jeong, H. J., Ok, J. H., Kang, H. C., You, J. H., Eom, S. H., Yoo, Y. D. & Lee, M. J. 2021. Bioluminescence capability and intensity in the dinoflagellate *Alexandrium* species. Algae 36:299–314.

Pierce, R. H., Henry, M. S., Blum, P. C., Lyons, J., Cheng, Y. S., Yazzie, D. & Zhou, Y. 2003. Brevetoxin concentrations in marine aerosol: human exposure levels during a *Karenia brevis* harmful algal bloom. Bull. Environ. Contam. Toxicol. 70:161–165.

Rublee, P. A. & Gallegos, C. L. 1989. Use of fluorescently labelled algae (FLA) to estimate microzooplankton grazing. Mar. Ecol. Prog. Ser. 51:221–227.

Sakamoto, S., Lim, W. A., Lu, D., Dai, X., Orlova, T. & Iwataki, M. 2021. Harmful algal blooms and associated fisheries damage in East Asia: current status and trends in China, Japan, Korea and Russia. Harmful Algae 102:101787.

Selosse, M. -A., Charpin, M. & Not, F. 2017. Mixotrophy everywhere on land and in water: the grand écart hypothesis. Ecol. Lett. 20:246–263.

Sengco, M. R. 2009. Prevention and control of *Karenia brevis* blooms. Harmful Algae 8:623–628.

Shen, A., Xing, X. & Li, D. 2015. Allelopathic effects of *Prorocentrum donghaiense* and *Karenia mikimotoi* on each other under different temperature. Thalassas 31:33–49.

Siswanto, E., Ishizaka, J., Tripathy, S. C. & Miyamura, K. 2013. Detection of harmful algal blooms of *Karenia mikimotoi* using MODIS measurements: a case study of Seto-Inland Sea, Japan. Remote Sens. Environ. 129:185–196.

Skovgaard, A. 1998. Role of chloroplast retention in a marine dinoflagellate. Aquat. Microb. Ecol. 15:293–301.

Steidinger, K. A. 2009. Historical perspective on *Karenia brevis* red tide research in the Gulf of Mexico. Harmful Algae 8:549–561.

Stoecker, D. K. 1999. Mixotrophy among Dinoflagellates. J. Eukaryot. Microbiol. 46:397–401.

Stoecker, D. K., Hansen, P. J., Caron, D. A. & Mitra, A. 2017. Mixotrophy in the marine plankton. Annu. Rev. Mar. Sci. 9:311–335.

Stoecker, D. K., Li, A., Coats, D. W., Gustafson, D. E. & Nannen, M. K. 1997. Mixotrophy in the dinoflagellate *Prorocentrum minimum*. Mar. Ecol. Prog. Ser. 152:1–12.

Takahashi, K., Benico, G., Lum, W. M. & Iwataki, M. 2019. *Gertia stigmatica* gen. et sp. nov. (Kareniaceae, Dinophyceae), a new marine unarmored dinoflagellate possessing the peridinin-type chloroplast with an eyespot. Protist 170:125680.

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

Uchida, T., Toda, S., Matsuyama, Y., Yamaguchi, M., Kotani, Y. & Honjo, T. 1999. Interactions between the red tide dinoflagellates *Heterocapsa circularisquama* and *Gymnodinium mikimotoi* in laboratory culture. J. Exp. Mar. Biol. Ecol. 241:285–299.

Van Dolah, F. M., Lidie, K. B., Monroe, E. A., Bhattacharya, D., Campbell, L., Doucette, G. J. & Kamykowski, D. 2009. The Florida red tide dinoflagellate *Karenia brevis*: new insights into cellular and molecular processes underlying bloom dynamics. Harmful Algae 8:562–572.

Yñiguez, A. T., Lim, P. T., Leaw, C. P., Jipanin, S. J., Iwataki, M., Benico, G. & Azanza, R. V. 2021. Over 30 years of HABs in the Philippines and Malaysia: what have we learned? Harmful Algae 102:101776.

Yoo, Y. D., Jeong, H. J., Kang, N. S., Song, J. Y., Kim, K. Y., Lee, G. & Kim, J. 2010. Feeding by the newly described mixotrophic dinoflagellate *Paragymnodinium shiwhaense*: feeding mechanism, prey species, and effect of prey concentration. J. Eukaryot. Microbiol. 57:145–158.

Zhang, Q. -C., Wang, Y. -F., Song, M. -J., Wang, J. -X., Ji, N. -J., Liu, C., Kong, F, -Z., Yan, T. & Yu, R. -C. 2022. First record of a *Takayama* bloom in Haizhou Bay in response to dissolved organic nitrogen and phosphorus. Mar. Pollut. Bull. 178:113572.

Zhang, Q., Yu, R., Song, J., Yan, T., Wang, Y. & Zhou, M. 2011. Will harmful dinoflagellate *Karenia mikimotoi* grow phagotrophically? Chin. J. Oceanol. Limnol. 29:849–859.

Zheng, J. -W., Mao, X. -T., Ye, M. -H., Li, H. -Y., Liu, J. -S. & Yang, W. -D. 2021. Allelopathy and underlying mechanism of *Karenia mikimotoi* on the diatom *Thalassiosira pseudonana* under laboratory condition. Algal Res. 54: 102229.