#### **Research Article**

Algae 2020, 35(3): 263-275 https://doi.org/10.4490/algae.2020.35.8.20

Open Access



### Effects of temperature on the growth and ingestion rates of the newly described mixotrophic dinoflagellate *Yihiella yeosuensis* and its two optimal prey species

Hee Chang Kang<sup>1</sup>, Hae Jin Jeong<sup>1,2,\*</sup>, An Suk Lim<sup>3</sup>, Jin Hee Ok<sup>1</sup>, Ji Hyun You<sup>1</sup>, Sang Ah Park<sup>1</sup>, Sung Yeon Lee<sup>1</sup> and Se Hee Eom<sup>1</sup>

<sup>1</sup>School of Earth and Environmental Sciences, College of Natural Sciences, Seoul National University, Seoul 08826, Korea

Water temperature is known to affect the growth and feeding of marine dinoflagellates. Each dinoflagellate species grows well at a certain optimal temperature but dies at very cold and hot temperatures. Thus, changes in water temperatures driven by global warming and extremely high or low temperatures can affect the distribution of dinoflagellates. Yihiella yeosuensis is a mixotrophic dinoflagellate that can feed on only the cryptophyte Teleaulax amphioxeia and the chlorophyte *Pyramimonas* sp. Furthermore, it grows fast mixotrophically but rarely grows photosynthetically. We explored the direct and indirect effects of water temperature on the growth and ingestion rates of Y. yeosuensis feeding on *T. amphioxeia* and the growth rates of *T. amphioxeia* and *Pyramimonas* sp. under 7 different water temperatures (5-35°C). Both the autotrophic and mixotrophic growth rates of Y. yeosuensis on T. amphioxeia were significantly affected by temperature. Under the mixotrophic and autotrophic conditions, Y. yeosuensis survived at 10-25°C, but died at 5°C and ≥30°C. The maximum mixotrophic growth rate of Y. yeosuensis on T. amphioxeia (1.16 d¹) was achieved at 25°C, whereas the maximum autotrophic growth rate (0.16 d<sup>-1</sup>) was achieved at 15°C. The maximum ingestion rate of Y. yeosuensis on T. amphioxeia (0.24 ng C predator 1 d 1) was achieved at 25°C. The cells of T. amphioxeia survived at 10-25°C, but died at 5 and ≥30°C. The cells of *Pyramimonas* sp. survived at 5-25°C, but died at 30°C. The maximum growth rate of *T. amphioxeia* (0.72 d<sup>-1</sup>) and *Pyramimonas* sp. (0.75 d<sup>-1</sup>) was achieved at 25°C. The abundance of *Y. yeosuensis* is expected to be high at 25°C, at which its two prey species have their highest growth rates, whereas Y. yeosuensis is expected to be rare or absent at 5°C or ≥30°C at which its two prey species do not survive or grow. Therefore, temperature can directly or indirectly affect the population dynamics and distribution of *Y. yeosuensis*.

Key Words: Chlorophyte; cryptophyte; feeding; harmful algal bloom; protist; red tide

#### INTRODUCTION

Marine dinoflagellates are ubiquitous and a major component of marine ecosystems (Hallegraeff 1993, Taylor et al. 2008, Klueter et al. 2017, Kang et al. 2019b, Lee

et al. 2019*b*). They play diverse roles in marine food webs (Hansen 1991, Jeong et al. 2010, Anderson and Menden-Deuer 2017, You et al. 2020*a*); autotrophic dinoflagellates



This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Com-

mercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Received July 7, 2020, Accepted August 20, 2020

\*Corresponding Author

E-mail: hjjeong@snu.ac.kr Tel: +82-2-880-6746, Fax: +82-2-874-9695

<sup>&</sup>lt;sup>2</sup>Research Institute of Oceanography, Seoul National University, Seoul 08826, Korea

<sup>&</sup>lt;sup>3</sup>Division of Life Science, Gyeongsang National University, Jinju 52828, Korea

are important primary producers, whereas heterotrophic dinoflagellates are important predators of phytoplankton and bacteria (Levinsen and Nielsen 2002, Montero et al. 2017, Kang et al. 2018, 2019a, Spilling et al. 2018). Mixotrophic dinoflagellates, which perform both photosynthesis and predation, are also important primary producers, prey for diverse predators, and predators of phytoplankton and bacteria (Skovgaard et al. 2000, Seong et al. 2006, Glibert et al. 2009, Ok et al. 2017, Stoecker et al. 2017). Many mixotrophic dinoflagellates often form red tides or harmful algal blooms that cause great losses in diverse industries (Jeong et al. 2017, López-Cortés et al. 2019, Roselli et al. 2020, Sakamoto et al. 2020). The population dynamics and distribution of mixotrophic dinoflagellates are of primary concern to scientists, government officials, and aquafarmers (Li et al. 2000, Heil et al. 2005, Lee et al. 2013, 2019a). Predicting the population dynamics of a mixotrophic dinoflagellate is critical for science and the industry.

In population dynamics models of phytoplankton species, growth and mortality due to predation are two critical parameters (Jeong et al. 2015). The growth rate of a mixotrophic dinoflagellate is affected primarily by prey concentration, temperature, and light intensity (Hansen and Nielsen 1997, Jeong et al. 2005, Baek et al. 2008, Laabir et al. 2011). Dinoflagellate species grow well at an optimal temperature but die at very cold and hot temperatures (Matsubara et al. 2007, Kibler et al. 2012, Jeong et al. 2018b, Lim et al. 2019, Ok et al. 2019, You et al. 2020b). Therefore, changes in water temperature and extremely high or low temperature can critically affect distribution of dinoflagellates. Extremely high water temperature events worldwide are expected to be more intense, frequent, and long-lasting as a consequence of global warming (Belkin 2009, Hansen et al. 2012), and have altered the structure and function of marine ecosystems (McClanahan et al. 2007, Wernberg et al. 2013, Ruthrof et al. 2018). However, the effects of extremely high water temperatures on marine dinoflagellates are still poorly understood. To understand the population dynamics of a mixotrophic dinoflagellate at present and in the near future, the effects of a wide range of water temperatures on the eco-physiological characteristics of dinoflagellates should be determined.

Yihiella yeosuensis was established as a new species in a new genus in 2017 (Jang et al. 2017b). This species is a mixotrophic dinoflagellate that can feed on only the cryptophyte *Teleaulax amphioxeia* and the chlorophyte *Pyramimonas* sp. among the tested algal prey species (Jang et al. 2017a). It grows fast mixotrophically but rarely

grows photosynthetically (Jang et al. 2017a). Among the 11 heterotrophic protists explored, there were no potential predators that were able to feed on actively swimming Y. yeosuensis cells, which escaped via rapid jumps (Jeong et al. 2018a). Furthermore, the results of the transcriptomic analysis showed that when Y. yeosuensis cells changed from the vegetative cells to cysts, polyketide synthase and cell-wall biogenesis genes related to antipredation were highly up-regulated (Jang et al. 2019). Thus, predators may not critically affect the population dynamics of the vegetative cells and cysts of Y. yeosuensis. In its population dynamics, its growth rate is likely to be more important than mortality due to predation. Prey concentration, a critical biotic factor, is known to significantly affect the growth rates of Y. yeosuensis (Jang et al. 2017a). As a critical abiotic factor, temperature is likely to affect the growth and feeding of Y. veosuensis on optimal prey species (direct effect) and also the growth of the prey species (i.e., prey availability, indirect effect). Thus, it is worth exploring the direct and indirect effects of temperature on the growth rates and ingestion rates of Y. yeosuensis and its prey.

We explored the effects of water temperature on the growth and ingestion rates of *Y. yeosuensis* feeding on *T. amphioxeia* and also the growth rates of *T. amphioxeia* and *Pyramimonas* sp. under 7 different water temperatures (5-35°C). Under the mixotrophic (with prey) and autotrophic (without prey) conditions, the optimal temperatures for the maximum mixotrophic and autotrophic growth rates of *Y. yeosuensis*, as well as the lowest and highest temperatures for its survival, were determined. We also investigated the optimal temperature and temperature range for survival of *T. amphioxeia* and *Pyramimonas* sp. The results of the present study provide a basis for understanding the effects of water temperature on the eco-physiological characteristics and population dynamics of *Y. yeosuensis* and its prey species.

#### **MATERIALS AND METHODS**

#### **Culture of organisms**

A clonal culture of *Y. yeosuensis* YYYS1405, which was isolated from the coastal waters of Yeosu, Korea, in May 2014 (Jang et al. 2017*b*), was used. A dense culture (approximately 20,000 cells mL<sup>-1</sup>) of *Y. yeosuensis* was transferred every 5 d to a 270-mL polycarbonate (PC) bottle that contained a fresh culture of *T. amphioxeia* CR-MAL01 (approximately 100,000 cells mL<sup>-1</sup>). The bottle was placed

on a shelf at 20°C under illumination of 20  $\mu mol$  photons  $m^{\text{-}2}$  s  $^{\text{-}1}$  cool-white fluorescent light with a 14 : 10 h light-dark cycle.

The cells of *T. amphioxeia* CR-MAL01 were isolated from the surface waters of Gomso Bay, Korea (Yih et al. 2004, Jang and Jeong 2020). The cells of *Pyramimonas* sp. PSSH1204 were isolated from the surface waters of Shiwha Bay, Korea (Jang and Jeong 2020). The cultures of *T. amphioxeia* and *Pyramimonas* sp. were maintained in an enriched f/2-Si seawater medium. The flasks that contained *T. amphioxeia* CR-MAL01 or *Pyramimonas* sp. PSSH1204 were filled to capacity, capped, and placed on a shelf at 20°C, illuminated with an irradiance of 20 µmol photons m<sup>-2</sup> s<sup>-1</sup> provided by cool white fluorescent light, under a 14:10 h light-dark cycle.

The carbon content of *Y. yeosuensis*, *T. amphioxeia*, and *Pyramimonas* sp. were obtained from Jang et al. (2017*a*) and Lee et al. (2014).

#### Temperature effects on autotrophic and mixotrophic growth and ingestion of *Yihiella yeosuensis*

Experiment (Expt) 1 was designed to determine the autotrophic growth rate of *Y. yeosuensis* YYYS1405 (i.e., without prey) and the mixotrophic growth and ingestion rates of *Y. yeosuensis* on *T. amphioxeia* as a function of water temperature (Table 1). The initial single high prey concentration at which the growth and ingestion rates of *Y. yeosuensis* on *T. amphioxeia* were saturated was chosen (Table 1). Expt 1 was designed with appropriate acclimation periods as shown in Fig. 1. In this experiment, the autotrophic growth rate of *T. amphioxeia* as a function of water temperature was determined.

A dense culture of *Y. yeosuensis* YYYS1405 (ca. 20,000 cells mL<sup>-1</sup>) growing on *T. amphioxeia* was transferred to each of the seven 270-mL PC bottles. A dense culture of *T. amphioxeia* (ca. 100,000 cells mL<sup>-1</sup>) growing in f/2-Si medium was also transferred to each of the seven 270-mL flasks. The target temperatures were established in seven temperature-controlled chambers. Bottles, each containing *Y. yeosuensis*, and flasks, each containing *T. amphioxeia*, were placed in one of the seven chambers inside which a target temperature was established.

In the preliminary tests, Y. yeosuensis YYYS1405 did not grow at 5, 30, and 35°C. Thus, in preparation for 5°C in Expt 1, Y. yeosuensis cells were subsequently acclimated at 15°C for 2 d, at 10°C for 5 d, and at 5°C for 2 d at 20 µmol photons m<sup>-2</sup> s<sup>-1</sup> on a 14:10 h light-dark cycle (Fig. 1A). For 30 and 35°C, Y. yeosuensis cells were subsequently acclimated at 25°C for 7 d, and at 30°C for 2 d. Furthermore, for 10°C, Y. yeosuensis cells were subsequently acclimated at 15°C for 2 d and at 10°C for 7 d. This gradual acclimation was conducted to avoid any shock that may occur when a large temperature change occurs rapidly. For 15, 20, and 25°C, Y. yeosuensis cells were incubated at each target temperature for 9 d (Fig. 1A). In preliminary tests, T. amphioxeia also did not grow at 5, 30, and 35°C. Thus, T. amphioxeia cells were also acclimated in a manner similar to the acclimation of Y. yeosuensis cells (Fig. 1B). At 1-3 d intervals after this pre-incubation started, 5-mL aliquots were obtained from each bottle and flask incubated at the target temperature and fixed with 5% acidic Lugol's solution; subsequently, the abundances of Y. yeosuensis and T. amphioxeia were measured.

In Expt 1, the initial concentrations of *Y. yeosuensis* YYYS1405 and *T. amphioxeia* were obtained using an autopipette with the predetermined volume of culture hav-

Table 1. Design of the experiments

Expt No.	Temperature (°C)	Prey	Prey concentration	Predator	Predator concentration
1	5, 10, 15, 20, 25, 30, 35	Teleaulax amphioxeia	31,347, 34,592, 33,829, 37,029, 37,587, 33,975, 35,590	Yihiella yeosuensis	258, 265, 278, 307, 367, 304, 305
	5, 10, 15, 20, 25, 30, 35	Teleaulax amphioxeia	32,448, 31,979, 33,608, 35,297, 37,352, 35,238, 34,680		0
	5, 10, 15, 20, 25, 30, 35		0	Yihiella yeosuensis	246, 186, 236, 284, 295, 298, 344
2	5, 10, 15, 20, 25, 30	Pyramimonas sp.	20,960, 17,922, 19,740, 19,029, 21,105, 16,218		0

The numbers in the prey (*Teleaulax amphioxeia*) and predator (*Yihiella yeosuensis*) columns in Experiment (Expt) and that of *Pyramimonas* sp. in Expt 2 are the actual initial concentrations (cells mL<sup>-1</sup>) of the prey and predator. The possible effects of prey concentration on the growth and ingestion rates were avoided by providing high prey concentrations at which the growth and ingestion rates of *Y. yeosuensis* on prey were saturated (Jang et al. 2017*a*).

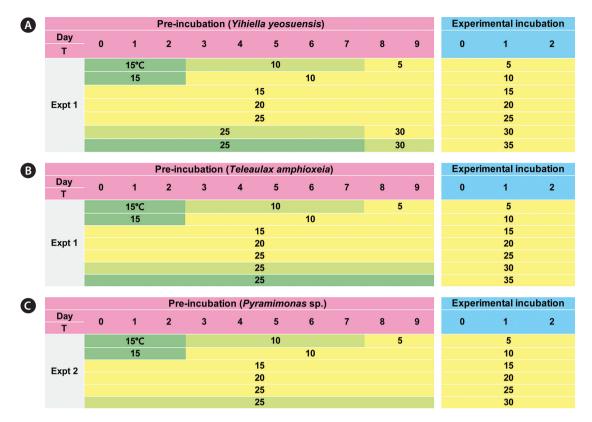


Fig. 1. Data obtained during the pre-incubation and experimental incubation periods when testing the effects of temperature on the growth of *Yihiella yeosuensis* (A), *Teleaulax amphioxeia* (B), and *Pyramimonas* sp. (C). T, temperature (°C). Pink and blue indicate the pre-incubation and experimental incubation periods, respectively. Yellow indicates the period of maintaining the cultures at a target temperature. Bright and dark green indicate the periods of temporarily maintaining the cultures to avoid possible inhibition at certain target temperatures.

ing a known cell density to the experimental PC bottles (Table 1). Triplicate 42-mL experimental PC bottles (mixtures of Y. yeosuensis and T. amphioxeia), triplicate prey control bottles (*T. amphioxeia* only), and triplicate predator control bottles (Y. yeosuensis only) were set up at each temperature. Similar water conditions were ensured by filtering each culture of the predator through a 0.2-µm disposable membrane filter (ADVANTEC; Toyo Rhoshi Kaisha, Ltd., Tokyo, Japan) and then adding this filtered water to the prey control bottles at the same water volume as that of the predator culture added to the experiment and predator control bottles (You et al. 2020b). The prey cultures were also filtered in the same manner and then added to the predator control bottles. Next, 10-mL of f/2-Si medium was added to all the experimental and control bottles; they were then filled to capacity with freshly-filtered seawater, capped, and then placed on a shelf at 20 umol photons m<sup>-2</sup> s<sup>-1</sup> on a 14:10 h light-dark cycle for 2 d at each target temperature. The actual predator and prey densities (cells mL-1) at the beginning of the experiment and after a two-day incubation period were determined The specific growth rate of Y. yeosuensis ( $\mu$ ,  $d^{-1}$ ) was calculated following Heinbokel (1978):

$$\mu (d^{-1}) = [Ln (C_t / C_0)] / t$$

, where  $C_0$  is the initial concentration of Y. yeosuensis and  $C_t$  is the final concentration after time t (2 d).

The ingestion rates of *Y. yeosuensis* on *T. amphioxeia* were calculated following Lim et al. (2018) by using the modified equations of Frost (1972) and Heinbokel (1978) because diluting the cultures by refilling them with sea water after subsampling was considered in the growth and ingestion rate calculations. The incubation time for

calculating the ingestion rates was the same as that for calculating the growth rates. The specific growth rate of *T. amphioxeia* ( $\mu$ , d<sup>-1</sup>) was also calculated as described above.

Three milliliter aliquots were removed from each bottle after 2-d incubations and transferred into confocal dishes (SPL100350; SPL Life Sciences Co., Ltd., Pocheon, Korea). The cells of *Y. yeosuensis* and *T. amphioxeia* were photographed on the confocal dishes using a digital camera (Zeiss AxioCam HRc 5; Carl Zeiss Ltd., Göttingen, Germany) and a microscope at 1,000× magnification.

## Temperature effects on autotrophic growth of *Pyramimonas* sp.

Expt 2 was designed to determine the autotrophic growth rate of *Pyramimonas* sp. PSSH1204 as a function of water temperature (Table 1). The initial single high concentration at which the growth and ingestion rates of *Y. yeosuensis* on *Pyramimonas* sp. were saturated was chosen (Table 1). In the preliminary tests, the *Pyramimonas* sp. strain did not grow at 5 and 30°C. Expt 2 was designed accordingly, with appropriate acclimation periods as shown in Fig. 1C.

In preparation for 5°C in Expt 2, the *Pyramimonas* sp. cells were subsequently acclimated at 15°C for 2 d, at 10°C for 5 d, and at 5°C for 2 d at 20 µmol photons m<sup>-2</sup> s<sup>-1</sup> on a 14:10 h light-dark cycle (Fig. 1C). For 30°C, the *Pyramimonas* sp. cells were acclimated at 25°C for 9 d because all the *Pyramimonas* sp. cells were dead after a 1-d incubation period. For 10°C, *Pyramimonas* sp. cells were subsequently acclimated at 15°C for 2 d and at 10°C for 7 d. For 15, 20, and 25°C, the *Pyramimonas* sp. cells were incubated at each target temperature for 9 d (Fig. 1C). At 1-3 d intervals after this pre-incubation period started, 5-mL aliquots were obtained from each flask incubated at the target temperature and fixed with 5% acidic Lugol's solution, and the abundance of *Pyramimonas* sp. was determined.

In Expt 2, the initial concentrations of *Pyramimonas* sp. were achieved as described above (Table 1). Triplicate 42-mL experimental PC bottles (*Pyramimonas* sp.) were set up at each temperature. The bottles were then filled to capacity with freshly-filtered seawater, capped, and then placed on a shelf at 20 µmol photons m<sup>-2</sup> s<sup>-1</sup> on a 14:10 h light-dark cycle for 2 d at each target temperature. The actual densities (cells mL<sup>-1</sup>) at the beginning of the experiment and after a 2-d incubation period were determined by obtaining 5- and 10-mL aliquots from each bottle and fixing them with 5% acidic Lugol's solu-

tion;  $\geq$ 300 *Pyramimonas* sp. cells were enumerated using a Sedgewick-Rafter counting chamber. The bottles were refilled again to capacity with freshly-filtered seawater after subsampling at the beginning of the experiment, capped, and then incubated under the same conditions described above. The specific growth rate of *Pyramimonas* sp. ( $\mu$ , d<sup>-1</sup>) was calculated as described above.

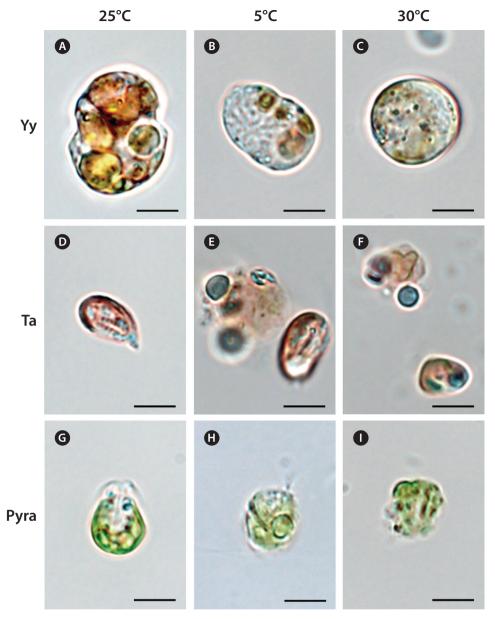
Three milliliter aliquots were removed from each bottle after 2-d incubations and transferred into confocal dishes, and *Pyramimonas* sp. cells were photographed on the confocal dishes using a digital camera and a microscope at 1,000× magnification.

#### Statistical analysis

Univariate analyses were used to investigate the effects of water temperature on the autotrophic growth rates of Y. yeosuensis, mixotrophic growth rates and ingestion rates of Y. yeosuensis on T. amphioxeia, and the growth rates of prey T. amphioxeia and Pyramimonas sp. Two assumptions, such as normality and homogeneity of variance, were tested using Shapiro-Wilk's W and Levene's test, respectively. A parametric one-way analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD) post-hoc test was conducted if the data satisfied those two assumptions. If the data satisfied only the normality assumption, a Welch's one-way ANOVA and Games-Howell post-hoc test were used. If the data did not satisfy the normality assumption, a non-parametric Kruskal-Wallis test and a Mann-Whitney U comparison with the Bonferroni correction was performed.

A multivariate analysis of variance (MANOVA, Pillai's trace statistics) was performed to investigate the differential effects of water temperature on the autotrophic and mixotrophic growth rates of *Y. yeosuensis*. The assumptions of normality and homogeneity for the MANOVA were checked using Shapiro-Wilk's W and Box's M test, respectively.

An independent-samples t-test was conducted to assess the significant differences between the autotrophic and mixotrophic growth rates of *Y. yeosuensis*, and between the ingestion rates of *Y. yeosuensis* on *T. amphioxeia* and zero at each water temperature. The data between the ingestion rates at 35°C and zero were not assessed because the ingestion rates at the temperature were zero. All statistical analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). The criterion for statistical significance was p < 0.05.



**Fig. 2.** Light micrographs of *Yihiella yeosuensis* (Yy) (A-C), *Teleaulax amphioxeia* (Ta) (D-F), and *Pyramimonas* sp. (Pyra) (G-I) cells incubated for 2 d at 25 (A, D, G), 5 (B, E, H), and  $30^{\circ}$ C (C, F, I) at 20 µmol photons  $m^{-2}$  s<sup>-1</sup>. The cells of *Y. yeosuensis* swelled at 5 and  $30^{\circ}$ C and, therefore, the sharply depressed cingulum was seen in (A), but not in (B) and (C). Many *T. amphioxeia* cells decomposed at 5 and  $30^{\circ}$ C (E, F). Many *Pyramimonas* sp. cells decomposed at 5 and  $30^{\circ}$ C (H, I). Scale bars represent: A-I, 5 µm.

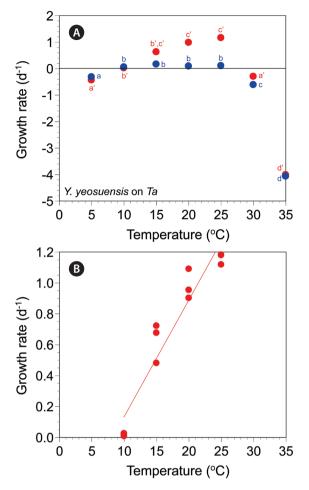
#### **RESULTS**

# Temperature effects on autotrophic and mixotrophic growth and ingestion of *Yihiella yeosuensis*

The cells of *Y. yeosuensis* YYYS1405 had normal shapes at 10-25°C, with a distinct cingulum, but they were swollen and had a spherical shape without a distinct cingu-

lum at 5°C or  $\geq$ 30°C (Fig. 2A-C).

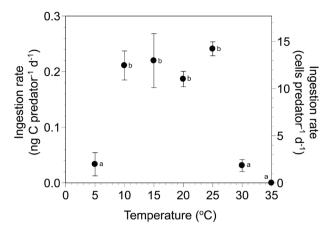
The autotrophic growth rates of *Y. yeosuensis* YYYS1405 increased from -0.32 d<sup>-1</sup> at 5°C to 0.16 d<sup>-1</sup> at 15°C, slightly decreased to 0.09-0.10 d<sup>-1</sup> at 20-25°C, and greatly decreased to -0.61 d<sup>-1</sup> at 30°C and -4.07 d<sup>-1</sup> at 35°C (Fig. 3A). The autotrophic growth rates were significantly affected by water temperature (one-way ANOVA,  $F_{6,14}$  = 1,130.27, p < 0.001) and were divided into four different temperature groups (Tukey's HSD *post-hoc* test, p < 0.05) (Fig. 3A).



**Fig. 3.** Specific autotrophic growth rates of *Yihiella yeosuensis* (blue circles) and mixotrophic growth rates of *Y. yeosuensis* on *Teleaulax amphioxeia* (Ta, red circles) as a function of water temperature (T, °C) at 5-35°C (A) and 10-25°C (B). Symbols represent treatment means  $\pm$  1 standard error. The linear regression equation in (B) is growth rate ( $d^{-1}$ ) = 0.0756 × (T) - 0.627,  $r^2$  = 0.917. Significantly different groups based on *post-hoc* tests of ANOVAs: autotrophic growth rate using the Tukey's honestly significant difference *post-hoc* test, 5 (a), 10-25 (b), 30 (c), 35°C (d); mixotrophic growth rate using the Games-Howell *post-hoc* test, 5 (a'), 10 (b'), 15 (b', c'), 20-25 (c'), 30 (a'), 35°C (d').

The mixotrophic growth rates of *Y. yeosuensis* YYYS1405 on *T. amphioxeia* at 5-35°C ranged from -4.01 to 1.16 d<sup>-1</sup> (Fig. 3A). The rates were positive at 10-25°C, but negative at 5°C and 30-35°C. The maximum mixotrophic growth rate was achieved at 25°C. The rates were significantly affected by temperature (Welch's one-way ANOVA,  $F_{6, 5.91}$  = 30,300.48, p < 0.001); the rates were subdivided into four different temperature groups (Games-Howell *post-hoc* test, p < 0.05) (Fig. 3A).

The effects of temperature were significantly different between the autotrophic and mixotrophic growth rates

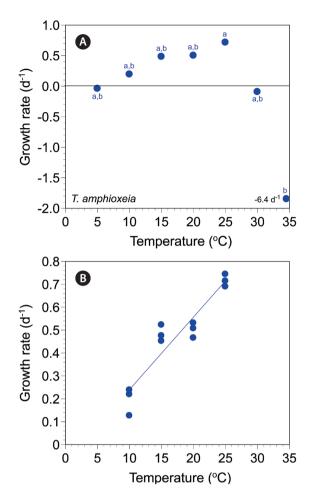


**Fig. 4.** Ingestion rates of *Yihiella yeosuensis* on *Teleaulax amphioxeia* as a function of water temperature. Symbols represent treatment means  $\pm$  1 standard error. Significantly different groups based on the Tukey's honestly significant difference *post-hoc* test of a one-way ANOVA: 5 and 30-35 (a); 10-25°C (b).

of *Y. yeosuensis* YYYS1405 (MANOVA, Pillai's Trace = 0.99,  $F_{2.13}$  = 741.17, p < 0.001) (Fig. 3A).

At 10 and 35°C, the autotrophic and mixotrophic growth rates of *Y. yeosuensis* YYYS1405 were not significantly different (two-tailed t test, t2.02 = -0.48, p = 0.678 at  $10^{\circ}$ C; t4 = 1.94, p = 0.125 at 35°C) (Fig. 3A). However, at 5°C and from 15 to 30°C, the autotrophic and mixotrophic growth rates of *Y. yeosuensis* were significantly different (two-tailed t test, t4 = -3.19, p = 0.033 at 5°C; t4 = 5.88, p = 0.004 at 15°C; t4 = 10.76, p < 0.001 at 20°C; t4 = 21.75, p < 0.001 at 25°C; t4 = 7.18, p = 0.002 at 30°C) (Fig. 3A). The slope of the linear regression line for the mixotrophic growth rates of *Y. yeosuensis* YYYS1405 at 10-25°C was 0.0756 (Fig. 3B).

The ingestion rates of *Y. yeosuensis* YYYS1405 on *T. amphioxeia* at 5-35°C ranged from 0 to 0.24 ng C predator¹ d¹ (Fig. 4), and the maximum rate was at 25°C. The ingestion rates at 5-35°C were significantly affected by water temperature (one-way ANOVA,  $F_{6, 14} = 19.64$ , p < 0.001) and were divided into two different temperature groups (Tukey's HSD *post-hoc* test, p < 0.05) (Fig. 4). However, the ingestion rates at 10-25°C were not significantly affected by water temperature (one-way ANOVA,  $F_{3,8} = 0.63$ , p = 0.633). The ingestion rates of *Y. yeosuensis* on *T. amphioxeia* were significantly higher than zero at 10-30°C (one-tailed t test, t4 = 1.61, p = 0.091 at 5°C; t2 = 8.10, p = 0.008 at 10°C; t4 = 4.54, p = 0.005 at 15°C; t2 = 13.70, p = 0.003 at 20°C; t2 = 18.80, p = 0.002 at 25°C; t4 = 2.92, p = 0.022 at 30°C).

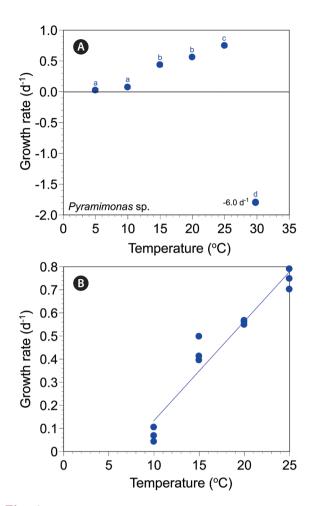


**Fig. 5.** Specific growth rates of *Teleaulax amphioxeia* as a function of water temperature (T, °C) at 5-35°C (A) and 10-25°C (B). Symbols represent treatment means  $\pm$  1 standard error. The linear regression equation in (B) is growth rate (d¹¹) = 0.0316 × (T) - 0.0796, r² = 0.882. Significantly different groups based on Mann-Whitney U comparisons with the Bonferroni correction of the Kruskal-Wallis test: 5-20 and 30 (a, b); 25 (a); 35°C (b).

## Temperature effects on autotrophic growth of *Teleaulax amphioxeia* and *Pyramimonas* sp.

The cells of *T. amphioxeia* CR-MAL01 at 10-25°C had normal shapes, but many cells decomposed at 5°C and  $\geq$ 30°C (Fig. 2D-F).

The autotrophic growth rates of T. amphioxeia CR-MAL01 at 5-35°C ranged from -6.38 to 0.72 d<sup>-1</sup> (Fig. 5A). The rates were positive at 10-25°C, but negative at 5°C and 30-35°C. The maximum autotrophic growth rate was achieved at 25°C. The rates were significantly affected by temperature (Kruskal-Wallis test,  $H_6$  = 19.33, p = 0.004); the rates were subdivided into two different temperature groups (Mann-Whitney U test with Bonferroni's correc-



**Fig. 6.** Specific growth rates of *Pyramimonas* sp. as a function of water temperature (T, °C) at 5-30°C (A) and 10-25°C (B). Symbols represent treatment means  $\pm$  1 standard error. The linear regression equation in (B) is growth rate (d¹¹) = 0.0430 × (T) - 0.299, r² = 0.934. Significantly different groups based on the Tukey's honestly significant difference *post-hoc* test of a one-way ANOVA: 5-10 (a); 15-20 (b); 25 (c); 30°C (d).

tion, p < 0.05) (Fig. 5A). The slope of the linear regression line for the growth rates of T. amphioxeia at 10-25°C was 0.0316 (Fig. 5B).

The cells of *Pyramimonas* sp. PSSH1204 at 10-25°C had normal shapes, but some cells decomposed at 5°C and many cells at 30°C (Fig. 2G-I).

The autotrophic growth rates of *Pyramimonas* sp. PSSH1204 at 5-30°C ranged from -5.99 to 0.75 d<sup>-1</sup> (Fig. 6A). The rates were positive at 5-25°C, but negative at 30°C. The maximum autotrophic growth rate was achieved at 25°C. The rates were significantly affected by temperature (one-way ANOVA,  $F_{5,12} = 7,580.15$ , p < 0.001); the rates were subdivided into four different temperature groups (Tukey's HSD *post-hoc* test, p < 0.05) (Fig. 6A). The

slope of the linear regression line for the growth rates of *Pyramimonas* sp. at 10-25°C was 0.0430 (Fig. 6B).

#### **DISCUSSION**

The present study showed that *Y. yeosuensis* YYYS1405 grew at 10-25°C regardless of the presence of prey cells, but did not grow at 5°C or 30-35°C. However, the mixotrophic growth rates of *Y. yeosuensis* on *T. amphioxeia* at 15-30°C were much greater than the autotrophic growth rates at the same temperatures, whereas both rates were similar at 10 and 35°C. Thus, the growth rate of *Y. yeosuensis* was affected by both prey (i.e., indirect effect) and water temperature (direct effect).

In the water samples that were seasonally collected from 28 stations in the East, West, and South Seas of Korea and Jeju Island from April 2015 to October 2018, *Y. yeosuensis* were present when water temperatures were 2.3-28.0°C, but the highest abundance of *Y. yeosuensis* was found when the water temperature was 25.0°C (Jang and Jeong 2020). In the present study, some cells of *Y. yeosuensis* were alive at 5°C, although the growth rate was negative. Therefore, in natural environments, some *Y. yeosuensis* cells may tolerate a temperature of 2.3°C or cysts suspended in water columns might be detected.

Interestingly, the optimal temperatures for the maximum growth rates of T. amphioxeia and Pyramimonas sp. as well as the lowest and highest temperatures for the survival of these two prey species are almost same as for Y. yeosuensis. Furthermore, in Jang and Jeong (2020), the water temperatures at which T. amphioxeia and Pyramimonas sp. were present (1.1-28.0 and 3.5-27.0°C, respectively) and also those at which the highest abundances of T. amphioxeia and Pyramimonas sp. were found (23.3 and 25.0°C, respectively) were very similar to those of Y. yeosuensis. The cells of Y. yeosuensis grew fast when fed on T. amphioxeia or Pyramimonas sp., but did not grow well without these prey (Jang et al. 2017a). Thus, Y. yeosuensis may have evolved to survive in the range of the water temperatures in which T. amphioxeia or Pyramimonas sp. survive. Furthermore, Y. yeosuensis may have evolved to be abundant at the water temperature at which T. amphioxeia or Pyramimonas sp. are abundant. Similarly, the mixotrophic dinoflagellate Alexandrium pohangense is known to grow at 15-30°C, at which its only prey, Margalefidinium (Cochlodinium) polykrikoides, can grow (Kim et al. 2004, Lim et al. 2019). It may be a survival strategy for a predator to adapt to the range of water temperatures in which its prey species survives.

In the order Suessiales, there have been several studies on the effects of water temperature on the survival and growth of Polarella glacialis and several dinoflagellate species in the genus Symbiodinium, the name of which has recently been changed to Breviolum, Cladocopium, Durusdinium, Effrenium, Fugacium, and Gerakladium, but few studies on those in other genera (Supplementary Table S1). Cells of *P. glacialis* can survive at 4°C, at which Y. yeosuensis does not survive (Zheng et al. 2012). Meanwhile, many species in the genera Symbiodinium, Breviolum, Cladocopium, Durusdinium, Effrenium, and Fugacium can survive at 30°C, unlike Y. yeosuensis (Robison and Warner 2006, Rogers and Davis 2006, McBride et al. 2009, Karim et al. 2015, Nitschke et al. 2015, Díaz-Almeyda et al. 2017, Grégoire et al. 2017). Furthermore, some species such as Symbiodinium microadriaticum, S. necroappetens, S. linuchae, and S. pilosum can survive at ≥32°C. Most of these species are symbiotic partners of corals, sea anemone, or jellyfish (Robison and Warner 2006, Díaz-Almeyda et al. 2017). Thus, the water temperatures at which they survive may be affected by the distribution of their hosts. Effrenium voratum (Symbiodinium voratum and S. californium) is a free-living species and a mixotrophic dinoflagellate (Jeong et al. 2014, LaJeunesse et al. 2018). This species can survive at 5-30°C (McBride et al. 2009). Thus, E. voratum has a temperature range for survival slightly wider than that of *Y. yeosuensis*.

The ingestion rates of *Y. yeosuensis* on *T. amphioxeia* at 10-25°C were not significantly affected by water temperature, although those at 5-35°C were. The mixotrophic growth rates of *Y. yeosuensis* on *T. amphioxeia* at 10-25°C continuously increased. Therefore, the gross growth efficiency of *Y. yeosuensis* at 10-25°C is likely to increase. No major differences in the ingestion rates of mixotrophic dinoflagellates were found in *A. pohangense, Gymnodinium smaydae*, and *Takayama helix*, though there was a continuous increase in their mixotrophic growth rates (Lim et al. 2019, Ok et al. 2019, You et al. 2020*b*). The activity of enzymes related to the conversion of ingested prey carbon to predator body carbon is likely to increase with increasing temperatures in a range supporting positive growth.

The slope of the regression line with the growth rate of *Y. yeosuensis* as a function of temperature (0.076) is similar to that of the dinoflagellates *Paragymnodinium shiwhaense* (0.077) and *Prorocentrum hoffmannianum* (0.071), but greater than that of the other dinoflagellates (0.006-0.048) (Jeong et al. 2018*b*). Therefore, the growth rate of *Y. yeosuensis* is more sensitive to a change in water temperature than the other dinoflagellates, except for *P.* 

shiwhaense and P. hoffmannianum.

From 1965 to 2010, the water temperature in the coastal waters of Yeosu from which *Y. yeosuensis* YYYS1405 was isolated has increased at a rate of 0.0305 y¹ (Seong et al. 2014). From 2015-2019, the water temperature in the coastal waters of Yeosu ranged from 3.65 to 29.77°C (Korea Hydrographic and Oceanographic Agency 2020). Theoretically, the water temperature is likely to reach 30°C within 8 y. Thus, global warming may limit the distribution of *Y. yeosuensis* during summers in the near future. It is worth testing whether the abundance of *Y. yeosuensis* reduces or whether its cells are absent when water temperatures largely increase during the global warming period.

#### **ACKNOWLEDGEMENTS**

This research was supported by the Useful Dinoflagellate program of Korea Institute of Marine Science and Technology Promotion (KIMST) funded by the Ministry of Oceans and Fisheries (MOF) and the National Research Foundation (NRF) funded by the Ministry of Science and ICT (NRF-2017R1E1A1A01074419) award to HJJ.

#### **SUPPLEMENTARY MATERIALS**

**Supplementary Table S1.** Effect of water temperature (T, °C) on the growth rate (GR) of the dinoflagellate species in the order Suessiales (https://e-algae.org).

#### **REFERENCES**

- Anderson, S. R. & Menden-Deuer, S. 2017. Growth, grazing, and starvation survival in three heterotrophic dinoflagellate species. J. Eukaryot. Microbiol. 64:213-225.
- Baek, S. H., Shimode, S. & Kikuchi, T. 2008. Growth of dinoflagellates, *Ceratium furca* and *Ceratium fusus* in Sagami Bay, Japan: the role of temperature, light intensity and photoperiod. Harmful Algae 7:163-173.
- Belkin, I. M. 2009. Rapid warming of large marine ecosystems. Prog. Oceanogr. 81:207-213.
- Díaz-Almeyda, E. M., Prada, C., Ohdera, A. H., Moran, H., Civitello, D. J., Iglesias-Prieto, R., Carlo, T. A., LaJeunesse, T. C. & Medina, M. 2017. Intraspecific and interspecific variation in thermotolerance and photoacclimation in *Symbiodinium* dinoflagellates. Proc. R. Soc. B Biol. Sci. 284:20171767.

- 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.
- 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.
- Grégoire, V., Schmacka, F., Coffroth, M. A. & Karsten, U. 2017. Photophysiological and thermal tolerance of various genotypes of the coral endosymbiont *Symbiodinium* sp. (Dinophyceae). J. Appl. Phycol. 29:1893-1905.
- Hallegraeff, G. M. 1993. A review of harmful algal blooms and their apparent global increase. Phycologia 32:79-99.
- Hansen, J., Sato, M. & Ruedy, R. 2012. Perception of climate change. Proc. Natl. Acad. Sci. U. S. A. 109:E2415-E2423.
- 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. & Nielsen, T. G. 1997. Mixotrophic feeding of Fragilidium subglobosum (Dinophyceae) on three species of Ceratium: effects of prey concentration, prey species and light intensity. Mar. Ecol. Prog. Ser. 147:187-196.
- Heil, C. A., Glibert, P. M. & Fan, C. 2005. *Prorocentrum minimum* (Pavillard) Schiller: a review of a harmful algal bloom species of growing worldwide importance. Harmful Algae 4:449-470.
- 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. 47:177-189.
- Jang, S. H. & Jeong, H. J. 2020. Spatio-temporal distributions of the newly described mixotrophic dinoflagellate *Yihi-ella yeosuensis* (Suessiaceae) in Korean coastal waters and its grazing impact on prey populations. Algae 35:45-59.
- Jang, S. H., Jeong, H. J. & Chon, J. K. 2019. De novo transcriptome of the newly described phototrophic dinoflagel-late Yihiella yeosuensis: comparison between vegetative cells and cysts. Mar. Biol. 166:104.
- Jang, S. H., Jeong, H. J., Kwon, J. E. & Lee, K. H. 2017a. 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., Moestrup, Ø., Kang, N. S., Lee, S. Y., Lee, K. H. & Seong, K. A. 2017b. Yihiella yeosuensis gen. et sp. nov. (Suessiaceae, Dinophyceae), a novel dinoflagellate isolated from the coastal waters of Korea. J. Phy-

- col. 53:131-145.
- 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., Lee, K. H., Yoo, Y. D., Kang, N. S., Song, J. Y., Kim, T. H., Seong, K. A., Kim, J. S. & Potvin, E. 2018b. Effects of light intensity, temperature, and salinity on the growth and ingestion rates of the red-tide mixotrophic dinoflagellate *Paragymnodinium shiwhaense*. Harmful Algae 80:46-54.
- Jeong, H. J., Lee, S. Y., Kang, N. S., Yoo, Y. D., Lim, A. S., Lee, M. J., Kim, H. S., Yih, W., Yamashita, H. & LaJeunesse, T. C. 2014. Genetics and morphology characterize the dinoflagellate *Symbiodinium voratum*, n. sp., (Dinophyceae) as the sole representative of *Symbiodinium* clade E. J. Eukaryot. Microbiol. 61:75-94.
- 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., 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., Shin, K., Jang, P. K., Ryu, J. -H., Kim, S. Y., Park, J. Y. & Kim, K. Y. 2017. 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., Yoo, Y. D., Kim, J. S., Seong, K. A., Kang, N. S. & Kim, T. H. 2010. 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., Seong, K. A., Kim, J. H., Park, J. Y., Kim, S., Lee, S. H., Ha, J. H. & Yih, W. H. 2005. Feeding by the mixotrophic red-tide dinoflagellate *Gonyaulax polygramma*: mechanisms, prey species, effects of prey concentration, and grazing impact. Aquat. Microb. Ecol. 38:249-257
- Kang, H. C., Jeong, H. J., Jang, S. H. & Lee, K. H. 2019*a*. Feeding by common heterotrophic protists on the phototrophic dinoflagellate *Biecheleriopsis adriatica* (Suessiaceae) compared to that of other suessioid dinoflagellates. Algae 34:127-140.
- 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, H. C., Jeong, H. J., Ok, J. H., You, J. H., Jang, S. H., Lee, S. Y., Lee, K. H., Park, J. Y. & Rho, J. -R. 2019b. Spatial and seasonal distributions of the phototrophic dinoflagellate *Biecheleriopsis adriatica* (Suessiaceae) in Korea: quantification using qPCR. Algae 34:111-126.
- Karim, W., Nakaema, S. & Hidaka, M. 2015. Temperature effects on the growth rates and photosynthetic activities of *Symbiodinium* cells. J. Mar. Sci. Eng. 3:368-381.
- Kibler, S. R., Litaker, R. W., Holland, W. C., Vandersea, M. W. & Tester, P. A. 2012. Growth of eight *Gambierdiscus* (Dinophyceae) species: effects of temperature, salinity and irradiance. Harmful Algae 19:1-14.
- Kim, D.-I., Matsuyama, Y., Nagasoe, S., Yamaguchi, M., Yoon, Y.-H., Oshima, Y., Imada, N. & Honjo, T. 2004. Effects of temperature, salinity and irradiance on the growth of the harmful red tide dinoflagellate *Cochlodinium polykrikoides* Margalef (Dinophyceae). J. Plankton Res. 26:61-66.
- Klueter, A., Trapani, J., Archer, F. I., McIlroy, S. E. & Coffroth, M. A. 2017. Comparative growth rates of cultured marine dinoflagellates in the genus *Symbiodinium* and the effects of temperature and light. PLoS ONE 12:e0187707.
- Korea Hydrographic and Oceanographic Agency (KHOA). 2020. Available from: http://www.khoa.go.kr. Accessed Jul 9, 2020.
- Laabir, M., Jauzein, C., Genovesi, B., Masseret, E., Grzebyk, D., Cecchi, P., Vaquer, A., Perrin, Y. & Collos, Y. 2011. Influence of temperature, salinity and irradiance on the growth and cell yield of the harmful red tide dinoflagellate *Alexandrium catenella* colonizing Mediterranean waters. J. Plankton Res. 33:1550-1563.
- LaJeunesse, T. C., Parkinson, J. E., Gabrielson, P. W., Jeong, H. J., Reimer, J. D., Voolstra, C. R. & Santos, S. R. 2018. Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. Curr. Biol. 28:2570-2580.
- Lee, C. -K., Park, T. -G., Park, Y. -T. & Lim, W. -A. 2013. Monitoring and trends in harmful algal blooms and red tides in Korean coastal waters, with emphasis on *Cochlodinium polykrikoides*. Harmful Algae 30(Suppl. 1):S3-S14.
- Lee, K. H., Jeong, H. J., Kang, H. C., Ok, J. H., You, J. H. & Park, S. A. 2019a. Growth rates and nitrate uptake of cooccurring red-tide dinoflagellates *Alexandrium affine* and *A. fraterculus* as a function of nitrate concentration under light-dark and continuous light conditions. Algae 34:237-251.
- Lee, S. K., Jeong, H. J., Jang, S. H., Lee, K. H., Kang, N. S., Lee, M. J. & Potvin, É. 2014. Mixotrophy in the newly

- described dinoflagellate *Ansanella granifera*: feeding mechanism, prey species, and effect of prey concentration. Algae 29:137-152.
- Lee, S. Y., Jeong, H. J., Kwon, J. E., You, J. H., Kim, S. J., Ok, J. H., Kang, H. C. & Park, J. Y. 2019b. First report of the photosynthetic dinoflagellate *Heterocapsa minima* in the Pacific Ocean: morphological and genetic characterizations and the nationwide distribution in Korea. Algae 34:7-21.
- 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.
- Li, A., Stoecker, D. K. & Coats, D. W. 2000. Spatial and temporal aspects of *Gyrodinium galatheanum* in Chesapeake Bay: distribution and mixotrophy. J. Plankton Res. 22:2105-2124.
- Lim, A. S., Jeong, H. J., Ok, J. H. & Kim, S. J. 2018. Feeding by the harmful phototrophic dinoflagellate *Takayama tasmanica* (Family Kareniaceae). Harmful Algae 74:19-29.
- Lim, A. S., Jeong, H. J., Ok, J. H., You, J. H., Kang, H. C. & Kim, S. J. 2019. Effects of light intensity and temperature on growth and ingestion rates of the mixotrophic dinoflagellate *Alexandrium pohangense*. Mar. Biol. 166:98.
- 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* (a.k.a. *Cochlodinium polykrikoides*) in Latin America. Front. Mar. Sci. 6:463.
- Matsubara, T., Nagasoe, S., Yamasaki, Y., Shikata, T., Shimasaki, Y., Oshima, Y. & Honjo, T. 2007. Effects of temperature, salinity, and irradiance on the growth of the dinoflagellate *Akashiwo sanguinea*. J. Exp. Mar. Biol. Ecol. 342:226-230.
- McBride, B. B., Muller-Parker, G. & Jakobsen, H. H. 2009. Low thermal limit of growth rate of *Symbiodinium californium* (Dinophyta) in culture may restrict the symbiont to southern populations of its host anemones (*Anthopleura* spp.; Anthozoa, Cnidaria). J. Phycol. 45:855-863.
- McClanahan, T. R., Ateweberhan, M., Muhando, C. A., Maina, J. & Mohammed, M. S. 2007. Effects of climate and seawater temperature variation on coral bleaching and mortality. Ecol. Monogr. 77:503-525.
- Montero, P., Pérez-Santos, I., Daneri, G., Gutiérrez, M. H., Igor, G., Seguel, R., Purdie, D. & Crawford, D. W. 2017. A winter dinoflagellate bloom drives high rates of primary production in a Patagonian fjord ecosystem. Estuar. Coast. Shelf Sci. 199:105-116.

- Nitschke, M. R., Davy, S. K., Cribb, T. H. & Ward, S. 2015. The effect of elevated temperature and substrate on free-living *Symbiodinium* cultures. Coral Reefs 34:161-171.
- 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.
- Robison, J. D. & Warner, M. E. 2006. Differential impacts of photoacclimation and thermal stress on the photobiology of four different phylotypes of *Symbiodinium* (Pyrrhophyta). J. Phycol. 42:568-579.
- Rogers, J. E. & Davis, R. H. 2006. Application of a new microculturing technique to assess the effects of temperature and salinity on specific growth rates of six *Symbiodinium* isolates. Bull. Mar. Sci. 79:113-126.
- Roselli, L., Vadrucci, M. R., Belmonte, M., Ciciriello, P., Rubino, F., Ungaro, N. & Caroppo, C. 2020. Two-stages bloom of *Margalefidinium* cf. *polykrikoides* in a Mediterranean shallow bay (Ionian Sea, Italy). Mar. Pollut. Bull. 151:110825.
- Ruthrof, K. X., Breshears, D. D., Fontaine, J. B., Froend, R. H., Matusick, G., Kala, J., Miller, B. P., Mitchell, P. J., Wilson, S. K., van Keulen, M., Enright, N. J., Law, D. J., Wernberg, T. & Hardy, G. E. S. J. 2018. Subcontinental heat wave triggers terrestrial and marine, multi-taxa responses. Sci. Rep. 8:13094.
- Sakamoto, S., Lim, W. A., Lu, D., Dai, X., Orlova, T. & Iwataki, M. 2020. Harmful algal blooms and associated fisheries damage in East Asia: current status and trends in China, Japan, Korea and Russia. Harmful Algae. Advanced online publication. https://doi.org/10.1016/j. hal.2020.101787.
- Seong, K. A., Jeong, H. J., Kim, S., Kim, G. H. & Kang, J. H. 2006. Bacterivory by co-occurring red-tide algae, heterotrophic nanoflagellates, and ciliates. Mar. Ecol. Prog. Ser. 322:85-97.
- Seong, K.-T., Choi, Y.-H., Koo, J. H. & Jeon, S.-B. 2014. Fluctuations and time series forecasting of sea surface temperature at Yeosu coast in Korea. J. Korean Soc. Mar. Environ. Energy 17:122-130.
- Skovgaard, A., Hansen, P. J. & Stoecker, D. K. 2000. Physiology of the mixotrophic dinoflagellate *Fragilidium sub-globosum*. I. Effects of phagotrophy and irradiance on photosynthesis and carbon content. Mar. Ecol. Prog. Ser. 201:129-136.

- Spilling, K., Olli, K., Lehtoranta, J., Kremp, A., Tedesco, L., Tamelander, T., Klais, R., Peltonen, H. & Tamminen, T. 2018. Shifting diatom: dinoflagellate dominance during spring bloom in the Baltic Sea and its potential effects on biogeochemical cycling. Front. Mar. Sci. 5:327.
- Stoecker, D. K., Hansen, P. J., Caron, D. A. & Mitra, A. 2017. Mixotrophy in the marine plankton. Annu. Rev. Mar. Sci. 9:311-335.
- Taylor, F. J. R., Hoppenrath, M. & Saldarriaga, J. F. 2008. Dinoflagellate diversity and distribution. Biodivers. Conserv. 17:407-418.
- Wernberg, T., Smale, D. A., Tuya, F., Thomsen, M. S., Langlois, T. J., De Bettignies, T., Bennett, S. & Rousseaux, C. S. 2013. An extreme climatic event alters marine ecosystem structure in a global biodiversity hotspot. Nat. Clim. Change 3:78-82.

- Yih, W., Kim, H. S., Jeong, H. J., Myung, G. & Kim, Y. G. 2004. Ingestion of cryptophyte cells by the marine photosynthetic ciliate *Mesodinium rubrum*. Aquat. Microb. Ecol. 36:165-170.
- You, J. H., Jeong, H. J., Kang, H. C., Ok, J. H., Park, S. A. & Lim, A. S. 2020a. Feeding by common heterotrophic protist predators on seven *Prorocentrum* species. Algae 35:61-78
- You, J. H., Jeong, H. J., Lim, A. S., Ok, J. H. & Kang, H. C. 2020b. Effects of irradiance and temperature on the growth and feeding of the obligate mixotrophic dinoflagellate *Gymnodinium smaydae*. Mar. Biol. 167:64.
- Zheng, S., Wang, G. & Lin, S. 2012. Heat shock effects and population survival in the polar dinoflagellate *Polarella glacialis*. J. Exp. Mar. Biol. Ecol. 438:100-108.