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ORIGINAL PAPER

Effects of irradiance and temperature on the growth and feeding of the obligate mixotrophic dinoflagellate *Gymnodinium smaydae*

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Abstract

Gymnodinium smaydae is a fast-growing mixotrophic dinoflagellate. This study investigated whether light intensity (0–346 µmol photons m^{-2} s^{-1}) and temperature (5–35 °C) affect the autotrophic or mixotrophic growth rate or ingestion rate of *Gymnodinium smaydae* GSSH1005. At all light intensities tested, *G. smaydae* GSSH1005 showed negative autotrophic growth rates, but positive mixotrophic growth rates when feeding on *Heterocapsa rotundata*. However, both autotrophic and mixotrophic growth rates were significantly affected by light intensity. The mixotrophic growth rates at 0–6 µmol photons m^{-2} s^{-1} were 0.67–0.72 day^{-1}; they increased up to 1.28 day^{-1} at 58 µmol photons m^{-2} s^{-1}, but became saturated at higher light intensities. The ingestion rates were also significantly affected by light intensity. The maximum ingestion rate of 2.3 ng C $predator^{-1}$ day^{-1} was achieved at 58 µmol photons m^{-2} s^{-1}. Although the autotrophic growth rates were negative at all temperatures tested, the mixotrophic growth rates were positive at 10–32 °C. Both autotrophic and mixotrophic growth rates were significantly affected by temperature. The maximum mixotrophic growth rate of 1.55 day^{-1} was noted at 25 °C. The ingestion rates were also significantly affected by temperature. The maximum ingestion rate of 4.2 ng C $predator^{-1}$ day^{-1} was noted at 32 °C. Therefore, both light intensity and temperature can affect the population dynamics of *G. smaydae* GSSH1005.

Introduction

Mixotrophic dinoflagellates are able to simultaneously conduct feeding and photosynthesis (Stoecker 1999; Jeong et al. 2010; Hansen 2011). Interest in mixotrophic dinoflagellates is increasing because they play diverse roles in marine ecosystems as primary producers, prey, predators, symbiotic partners, and parasites (Skovgaard 1996; Menden-Deuer et al. 2005; Adolf et al. 2006; Shumway et al. 2006; Skovgaard et al. 2012; Turner et al. 2012; Harvey et al. 2013; Jeong et al. 2015; Johnson 2015; LaJeunesse et al. 2018; Kang et al. 2019; Lee et al. 2019a) and have excessive DNA that may be attributed to the horizontal gene transfer by feeding (Holm-Hansen 1969; Allen et al. 1975; Fagan et al. 1998; Keeling and Palmer 2008; Johnson 2011). However, of approximately 1200 phototrophic dinoflagellates, < 10% have been assessed for mixotrophy (Bockstahler and Coats 1993; Jacobson and Anderson 1996; Stoecker et al. 1997; Jeong et al. 1999, 2004, 2005a, b, c, 2012, 2016; Burkholder et al. 2008; Yoo et al. 2009; Lim et al. 2018, 2019a). Furthermore, only a small portion of the mixotrophic dinoflagellates has been analyzed to determine whether environmental factors, such as temperature and light intensity, affect their growth and ingestion rates (Skovgaard 1996; Hansen and Nielsen 1997; Berge et al. 2008; Jeong et al. 2018a; Lim et al. 2019b; Ok et al. 2019). During the past decade, several new species and genera of mixotrophic dinoflagellates have been described (Yoo et al. 2010; Kang et al. 2014; Lee et al. 2014; Lim et al. 2015a, b; Jang et al. 2017a, b; Yokouchi et al. 2018). Understanding the role of a newly

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described mixotrophic dinoflagellate in marine ecosystems requires the determination of its prey and predators, effects of environmental factors on its growth and ingestion rates, and its distribution.

The phototrophic dinoflagellate *Gymnodinium smaydae* was described as a new species in 2014 (Kang et al. 2014). This dinoflagellate is one of the smallest *Gymnodinium* species reported thus far (Kang et al. 2014). This species can feed only on the thecate dinoflagellates *Heterocapsa rotundata*, *Heterocapsa steinii* (=*H. triquetra*), and *Scrippsiella acuminata* (=*S. trochoidea*), among the tested 19 algal prey species, and can divide approximately three times per day when fed on the optimal prey *H. rotundata* (Lee et al. 2014). Furthermore, *G. smaydae* was occasionally shown to have a considerable grazing impact on the population of co-occurring *H. rotundata* in Shiwha Bay (Lee et al. 2014). The heterotrophic dinoflagellates *Oxyrrhis marina*, *Gyrodinium dominans*, and *Gyrodinium moestrupii,* and the ciliate *Pelagostrobilidium* sp. are known to feed on *G. smaydae*, but the maximum growth and ingestion rates of *O. marina* on *G. smaydae* are lower than those on most other algal prey species (Jeong et al. 2018b). Therefore, understanding the population dynamics of *G. smaydae* requires determination of its autotrophic and mixotrophic growth and ingestion rates under diverse environmental conditions.

Light and water temperature are two major physical parameters affecting the growth and survival of phototrophic dinoflagellates (Ogata et al. 1987; Ono et al. 2000; Baek et al. 2008; López-Rosales et al. 2014). Light is the essential energy source for photosynthesis, but high light intensity can cause photoinhibition (Morton et al. 1992; Franklin et al. 2006; López-Rosales et al. 2014; Ok et al. 2019). Furthermore, temperature generally increases respiration which provides energy, but low or high temperature extremes often cause death in dinoflagellates (Baek et al. 2008; Xu et al. 2010; Kibler et al. 2012; Lim et al. 2019b). Light intensity and water temperature change seasonally and vertically in many marine environments (Richardson et al. 1983; Seip and Reynolds 1995; Lalli and Parsons 1997; Staehr and Sand-Jensen 2006). In general, migratory dinoflagellates experience a wide range of light intensities and water temperatures (Hasle 1950; Kamykowski and Zentara 1977; Blasco 1978; Kamykowski 1981; Whittington et al. 2000). The maximum swimming speed of *G. smaydae* is approximately 700 μm s^{-1}; thus, theoretically, it can descend to 25 m from the surface after travelling for 10 h (Lee et al. 2014). Thus, *G. smaydae* is also expected to experience a wide range of light intensities and water temperatures in a day. Global warming is known to directly or indirectly affect light intensity and seawater temperature (Levitus et al. 2005; Ding et al. 2007; IPCC 2007). A change in light intensity or water temperature due to global warming may affect the growth and survival of *G. smaydae* as well as its distribution.

In this study, the growth and ingestion rates of *G. smaydae* feeding on *H. rotundata* with (i.e., mixotrophic growth) and without added prey (autotrophic growth) were determined as a function of light intensity (0–346 μmol photons m^{-2} s^{-1}) and water temperature (5–35 °C). These data were used to determine whether the autotrophic or mixotrophic growth rate of *G. smaydae* is affected by light intensity and temperature, whether the ingestion rate of *G. smaydae* on *H. rotundata* is affected by light intensity and temperature, whether its growth or ingestion rate is inhibited by darkness or high light intensity, and whether a particular water temperature causes a negative growth rate in *G. smaydae*. In this study, the terminology "autotrophic" rather than "phototrophic" was used against "mixotrophic" because both "autotrophic" and "mixotrophic" are "phototrophic". The results of the present study provide a basis for understanding the effects of light intensity and water temperature on the eco-physiological characteristics and population dynamics of *G. smaydae*.

Materials and methods

Culture of organisms

A non-axenic clonal culture of *Gymnodinium smaydae* GSSH1005, which was isolated from Shiwha Bay, Korea, during May 2010 (Kang et al. 2014), was used. A dense culture (ca. 20,000 cells mL^{-1}) of *G. smaydae* was transferred every 3 days to a 270-mL flask containing fresh culture of *Heterocapsa rotundata* HRSH1201 (ca. 100,000 cells mL^{-1}). The flask was placed on a shelf at 20 °C under illumination of 20 μmol photons m^{-2} s^{-1} cool-white fluorescent light with a 14:10 h light–dark cycle. The mean equivalent spherical diameter (ESD) of *G. smaydae* GSSH1005 was obtained from Lee et al. (2014).

Light effects on autotrophic and mixotrophic growth and ingestion

Experiment (Expt) 1 was designed to determine the autotrophic growth rate of *G. smaydae* GSSH1005 (i.e., without prey) and the mixotrophic growth and ingestion rates of *G. smaydae* feeding on *H. rotundata* as a function of light intensity (Table 1). The initial single high prey concentration at which the growth and ingestion rates of *G. smaydae* on *H. rotundata* were saturated was chosen (Table 1).

In preparation for Expt 1, a culture of *G. smaydae* GSSH1005 (ca. 5,000–10,000 cells mL^{-1}) growing on *H. rotundata* was separately transferred to eight 250-mL polycarbonate (PC) bottles. A dense culture of *H. rotundata* (ca. 100,000 cells mL^{-1}) growing autotrophically in f/2-Si medium (Guillard and Ryther 1962) was also transferred

Table 1 Design of the experiments

Expt no	LI, *T*	Prey Concentration	Predator Concentration
1	0, 6, 15, 25, 58, 115, 247, 346 (μmol photons m^{-2} s^{-1})	22,177, 19,836, 20,280, 19,587, 18,395, 18,208, 20,106, 19,452	59, 57, 59, 60, 62, 64, 60, 58
2	5, 6, 8, 10 (°C)	17,699, 15,255, 17,459, 18,379	33, 44, 44, 48
3	15, 20, 25, 30 (°C)	19,611, 20,545, 24,501, 22,587	77, 68, 65, 62
4	32, 35 (°C)	16,085, 18,148	46, 43

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 *G. smaydae* on *H. rotundata* were saturated (Lee et al. 2014); both rates were saturated at ≥ 3500 cell mL^{-1} *H. rotundata* concentrations. The numbers in the prey (*Heterocapsa rotundata*) and predator (*Gymnodinium smaydae*) columns are the actual initial concentrations (cells mL^{-1}) of prey and predators

LI light intensity, *T* temperature

to each of the eight bottles. The target light intensities of 6, 15, 25, 58, 115, 247, and 346 μmol photons m^{-2} s^{-1} were established by adjusting the distances between the Light Emitting Diode lights (LED; FS-075MU, 6500K; Suram Inc., Suwon, Korea) and 0 μmol photons m^{-2} s^{-1} (i.e., darkness) was achieved by wrapping the bottles with aluminum foil and placing them in a completely dark culture room, as suggested by Ok et al. (2019). All bottles were placed on vertically rotating wheels. Light intensity was measured using an LI-COR Quantum Light Sensor attached to a data logger LI-1400 (LI-COR Inc.; Lincoln, Nebraska, USA). Accordingly, cultures of *G. smaydae* GSSH1005 feeding on *H. rotundata* were incubated for 10 days at the target light intensities except for those maintained at 0 and 346 μmol photons m^{-2} s^{-1} (Fig. 1a). The autotrophic and/or mixotrophic growth rates of *Alexandrium pohangense* at 0 μmol photons m^{-2} s^{-1} and *Takayama helix* at 346 μmol photons m^{-2} s^{-1} were negative (Lim et al. 2019b; Ok et al. 2019). Thus, these studies maintained cultures at light intensities close to 0 or 346 μmol photons m^{-2} s^{-1} for a week in the pre-incubation periods and then incubated the cultures at 0 or 346 μmol photons m^{-2} s^{-1} for 2 or 3 days. Similarly, in this study, for the darkness experiment, a culture of *G. smaydae* acclimated at 6 μmol photons m^{-2} s^{-1} for 7 days was transferred and incubated in the dark and acclimated for 3 days. Furthermore, possible photoinhibition at 346 μmol photons m^{-2} s^{-1} was avoided by maintaining a culture of *G. smaydae* at 247 μmol photons m^{-2} s^{-1} for 7 days and then incubating the culture at 346 μmol photons m^{-2} s^{-1} for 3 days. In the previous studies, the photoacclimation periods had been determined by considering the growth rate of a target experimental organism for at least two divisions at a target light intensity before the experiments began (Nielsen 1996; Skovgaard 1996; Li et al. 1999; Kim et al. 2008). Cells of *G. smaydae* GSSH1005 are known to divide more than once per day; thus, they could divide at least three times for 3 days in the dark and at 346 μmol photons m^{-2} s^{-1}. The abundances of *G. smaydae* and *H. rotundata* were measured by obtaining 5-mL aliquots from the bottles incubated at each target light intensity and fixing with 5% acidic Lugol's solution.

In Expt 1, the initial concentrations of *G. smaydae* GSSH1005 and *H. rotundata* were achieved using an autopipette with the predetermined volume of culture having a known cell density to the experimental PC bottles (Table 1). Triplicates each of 42-mL experimental PC bottles (mixtures of *G. smaydae* and *H. rotundata*), prey control bottles (*H. rotundata* only), and predator control bottles (*G. smaydae* only) were set up at each light intensity. 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 (Supplementary Fig. 1). The cultures of prey 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 experiment and control bottles; they were then filled to capacity with freshly filtered seawater, capped, and then placed at predetermined distances on a vertically rotating wheel from the light source to establish the targeted light intensities except darkness with a 14:10 h light–dark cycle for 2 days at 20 °C. The bottles for complete darkness were placed in another temperature-controlled chamber at 20 °C. To minimize photosynthesis by light stimulation, these bottles were handled in a room lit only by a 0.6 μmol photons m^{-2} s^{-1} red light. The actual predator and prey densities (cells mL^{-1}) at the beginning of the experiment and after a 2-day incubation were determined by obtaining 5- and 10-mL aliquots from each bottle and fixing with final 5% acidic Lugol's solution; all or ≥ 300 *G. smaydae* and *H. rotundata* cells were

a

Pre-incubation												Experimental incubation		
Day	0	1	2	3	4	5	6	7	8	9	10	0	1	2
LI														
Expt 1	6 µmol photons m^{-2} s^{-1}									0		0		
	6											6		
	15											15		
	25											25		
	58											58		
	115											115		
	247											247		
	247									346		346		

b

Pre-incubation											Experimental incubation		
Day	0	1	2	3	4	5	6	7	8	9	0	1	2
T													
Expt 2	15 °C					10				5	5		
	15					10				6	6		
	15					10				8	8		
	15						10				10		
Expt 3	15										15		
	20										20		
	25										25		
	25									30	30		
Expt 4	25					30				32	32		
	25					30				35	35		

Fig. 1 Data obtained during the pre-incubation and experimental incubation periods of the effects of light intensity (**a**) (Expt. 1) and temperature (**b**) (Expts. 2 – 4) on the growth of *Gymnodinium smaydae*. *LI* light intensity (µmol photons m^{-2} s^{-1}); *T* temperature (°C). Purple and blue indicate the pre-incubation and experimental incubation periods, respectively. Yellow indicates the period of maintaining the cultures at a target light intensity or temperature. Bright and dark green indicate the periods that temporarily maintain the cultures to avoid possible inhibition at certain target light intensities or temperatures

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 *G. smaydae* (µ, day^{-1}) was calculated as in Heinbokel (1978):

$$\mu = \frac{\mathrm{Ln}(C_t/C_0)}{t},$$

where C_0 is the initial concentration of *G. smaydae* and C_t is the final concentration after time *t* (2 days).

The ingestion rates of *G. smaydae* on *H. rotundata* were calculated following Lim et al. (2018) using the modified equations of Frost (1972) and Heinbokel (1978), because dilution of the cultures with refilling of 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.

Temperature effects on autotrophic and mixotrophic growth and ingestion

In preliminary tests, the *G. smaydae* strain did not grow at 5, 6, 8, and 35 °C. Expts 2–4 were designed accordingly, with appropriate acclimation periods as shown in Fig. 1b. The specific autotrophic and mixotrophic growth and ingestion rates of *G. smaydae* on *H. rotundata* were determined above described at different temperatures (Table 1).

A dense culture of *G. smaydae* (ca. 5,000–10,000 cells mL^{-1}) growing on *H. rotundata* was transferred to each of two or four 250 mL PC bottles. A dense culture of *H. rotundata* (ca. 100,000 cells mL^{-1}) growing in f/2-Si medium was also transferred to each of two or four 250-mL bottles. The target temperatures were established in two or four temperature-controlled chambers. Bottles each containing *G. smaydae* and *H. rotundata* were placed in one of the two or four chambers, inside which a target temperature was established. The light intensity was 58 µmol photons m^{-2} s^{-1} by LED on a 14:10 h light–dark cycle. These light conditions

supported the maximum mixotrophic growth rate of *G. smaydae* on *H. rotundata* in Expt 1.

Considering that preliminary tests had shown that *G. smaydae* did not grow at 5, 6, and 8 °C, in preparation for Expt 2, *G. smaydae* and *H. rotundata* were incubated each at 15 °C for 2 days and then acclimated at 10 °C for 5 days (Fig. 1b). Subsequently, the bottles for the 5, 6, and 8 °C experiments were acclimated at the target temperature for 2 days. This gradual acclimation was conducted to avoid any shock that may occur when a large temperature change occurs rapidly. In preparation for Expt 3, the cultures in the bottles were acclimated at each of 15, 20, and 25 °C for 9 days. For the experiments at 30 °C, the bottle containing *G. smaydae* cells maintained at 20 °C was gradually acclimated at 25 °C for 7 days and then at 30 °C for 2 days. The preliminary tests had also shown that *G. smaydae* cells died at 35 °C; therefore, shorter acclimation periods were used in Expt 4. For tests at 32 and 35 °C, bottles containing *G. smaydae* were first acclimated at 25 °C for 2 days, then at 30 °C for 5 days. Then the *G. smaydae* cultures were acclimated to the Expt 4 target temperatures of 32 or 35 °C for 2 days (Fig. 1b). Lim et al. (2006) measured the growth rates of *Alexandrium tamiyavanchii* and *Alexandrium minutum* at 15, 20, and 25 °C. For the experiment at 15 °C, the cultures were acclimated at 20 °C for 1 day and then incubated at 15 °C without further acclimation. At 2- or 3-day intervals after this pre-incubation started, 5-mL aliquots were obtained from each bottle incubated at the target temperature and fixed with 5% acidic Lugol's solution; subsequently, the abundance of *G. smaydae* and *H. rotundata* was measured.

For Expts 2–4, the initial concentrations of *G. smaydae* and *H. rotundata* were established as described above. Triplicated 42-mL experimental bottles, prey control bottles, and predator control bottles were set up for each target temperature. The experimental procedure was the same as that for Expt 1. The bottles were incubated for 2 days at each temperature in target chambers irradiated at 58 µmol photons m^{-2} s^{-1} by LED on a 14:10 h light–dark cycle. The specific growth and ingestion rates of *G. smaydae* were calculated as described above.

Statistical analysis

Univariate analyses were assessed using SPSS version 25.0 (IBM-SPSS Inc., New York, USA) to investigate the effects of light intensity or water temperature on the autotrophic and mixotrophic growth rates and ingestion rates of *G. smaydae* on *H. rotundata*. Before the analyses, normality and homogeneity of variance were checked using the Shapiro–Wilk's *W* and Levene's test, respectively (Levene 1961; Shapiro and Wilk 1965). When the data satisfied both normality and homogeneity assumptions, a parametric one-way analysis of variance (ANOVA) with the Tukey's honestly significant difference (HSD) post hoc test was performed (Tukey 1953). However, when the data satisfied only the normality assumption, but failed the homogeneity assumption, a Welch's one-way ANOVA and Games–Howell post hoc test were performed (Welch 1947; Games and Howell 1976). In contrast, when the data did not fulfil the normality assumption, a non-parametric Kruskal–Wallis test and Mann–Whitney *U* comparison with Bonferroni correction were conducted (Mann and Whitney 1947; Kruskal and Wallis 1952; Dunn 1961).

The differential effects of light intensity or water temperature on the autotrophic and mixotrophic growth rates of *G. smaydae* were assessed by performing multivariate analysis of variance (MANOVA). Before the analysis, the assumption of normality and homogeneity for MANOVA was checked using the Shapiro–Wilk's *W* and Box's *M* test, respectively (Box 1949; Shapiro and Wilk 1965). Pillai's trace statistics were used to assess the significance of differential effects on multivariate growth rates (Pillai 1955).

An independent samples *t* test was used to assess the significant differences between autotrophic and mixotrophic growth rates of *G. smaydae* at the same light intensity or water temperature and between ingestion rates of *G. smaydae* on *H. rotundata* and zero at each light intensity or water temperature. Before the analysis, the assumption of homogeneity for the parametric independent samples *t* test was checked using the Shapiro–Wilk's *W* test (Shapiro and Wilk 1965). Values with $P < 0.05$ were considered as statistically significant.

Results

Effects of light intensity

The autotrophic growth rates of *Gymnodinium smaydae* at 0–346 µmol photons m^{-2} s^{-1} ranged from − 0.41 to − 0.12 day^{-1}, and the maximum autotrophic growth rate was achieved in darkness (Fig. 2). The autotrophic growth rates of *G. smaydae* were significantly affected by light intensity [Welch's one-way ANOVA, F (7, 6.68) = 5.52, $P = 0.021$], and the Games–Howell post hoc test ($P < 0.05$) revealed that the autotrophic growth rates were divided into two different light intensity groupings (Fig. 2).

The mixotrophic growth rates in darkness and at 6 µmol photons m^{-2} s^{-1} were 0.67–0.72 day^{-1}, increased up to 1.28 day^{-1} at 58 µmol photons m^{-2} s^{-1}, but became saturated at the higher light intensities (Fig. 2). The mixotrophic growth rates were significantly affected by light intensity [one-way ANOVA, F (7, 16) = 22.97, $P < 0.001$]. The Tukey's HSD post hoc test ($P < 0.05$) revealed that the mixotrophic growth rates of *G. smaydae* were divided into three different light intensity groupings (Fig. 2).

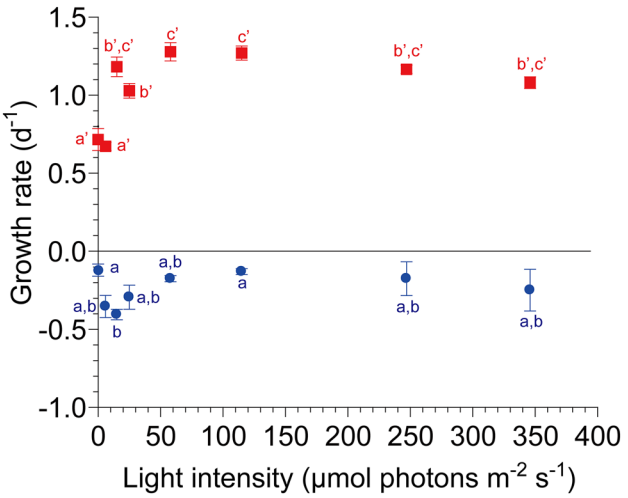

Fig. 2 Specific autotrophic growth rates of *Gymnodinium smaydae* (blue circles) and mixotrophic growth rates of *G. smaydae* on *Heterocapsa rotundata* (red squares) as a function of light intensity. Symbols represent treatment mean values ± 1 SE. Significantly different groups based on post hoc test of ANOVAs: autotrophic growth rate by Games–Howell post hoc test, darkness (a); 6 (ab); 15 (b); 25–58 (ab); 115 (a); and 247–346 µmol photons m^{-2} s^{-1} (ab); mixotrophic growth rate by Tukey's HSD post hoc test, darkness-6 (a′); 15 (b′c′); 25 (b′); 58–115 (c′); and 247–346 µmol photons m^{-2} s^{-1} (b′c′)

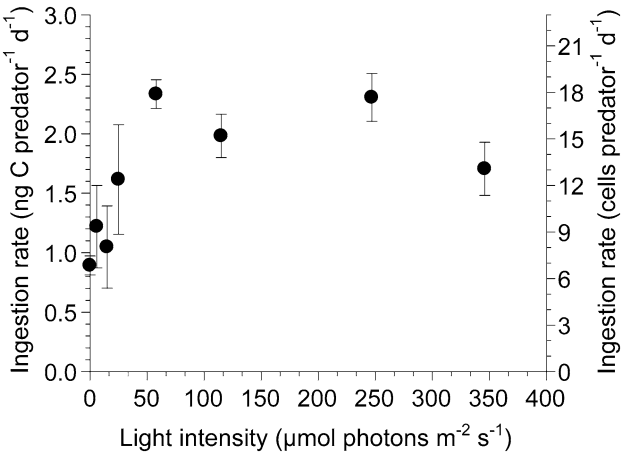

Fig. 3 Ingestion rates of *Gymnodinium smaydae* on *Heterocapsa rotundata* as a function of light intensity. Symbols represent treatment mean values ± 1 SE. No significant differences among ingestion rates based on Mann–Whitney *U* comparison with Bonferroni correction of Kruskal–Wallis test: 0–346 µmol photons m^{-2} s^{-1} (a)

The effects of light intensity were significantly different between the autotrophic and mixotrophic growth rates of *G. smaydae* [MANOVA, Pillai's Trace = 1.373, F (7, 16) = 5.01, $P < 0.001$].

At all light intensities, the autotrophic and mixotrophic growth rates of *G. smaydae* were significantly different (two-tailed t test, $t4 = 10.42$, $P < 0.001$ in darkness; $t4 = 13.54$, $P < 0.001$ at 6 µmol photons m^{-2} s^{-1}; $t4 = 22.30$, $P < 0.001$ at 15 µmol photons m^{-2} s^{-1}; $t4 = 14.66$, $P < 0.001$ at 25 µmol photons m^{-2} s^{-1}; $t4 = 23.43$, $P < 0.001$ at 58 µmol photons m^{-2} s^{-1}; $t4 = 28.52$, $P < 0.001$ at 115 µmol photons m^{-2} s^{-1}; $t4 = 12.00$, $P < 0.001$ at 247 µmol photons m^{-2} s^{-1}; and $t4 = 9.60$, $P = 0.001$ at 346 µmol photons m^{-2} s^{-1}).

The ingestion rates of *G. smaydae* feeding on *H. rotundata* at 0–346 µmol photons m^{-2} s^{-1} ranged from 0.9 to 2.3 ng C $predator^{-1}$ day^{-1} (Fig. 3); the lowest rate was achieved in darkness, whereas the highest rate was achieved at 58 µmol photons m^{-2} s^{-1}. The ingestion rates were significantly affected by light intensity (Kruskal–Wallis test, $H7 = 14.9$, $P = 0.037$). However, the Mann–Whitney *U* comparison with Bonferroni correction ($P < 0.05$) indicated that the ingestion rates of *G. smaydae* on *H. rotundata* at different light intensities were not divided into different light intensity grouping (Fig. 3). All the ingestion rates were significantly higher than zero at all light intensities (one-tailed t test, $t2 = 13.00$, $P = 0.003$ in darkness; $t4 = 3.50$, $P = 0.013$ at 6 µmol photons m^{-2} s^{-1}; $t2 = 3.20$, $P = 0.043$ at 15 µmol photons m^{-2} s^{-1}; $t2 = 3.64$, $P = 0.034$ at 25 µmol photons m^{-2} s^{-1}; $t4 = 16.06$, $P < 0.001$ at 58 µmol photons m^{-2} s^{-1}; $t2 = 11.80$, $P = 0.004$ at 115 µmol photons m^{-2} s^{-1}; $t2 = 11.05$, $P = 0.004$ at 247 µmol photons m^{-2} s^{-1}; and $t2 = 8.17$, $P = 0.008$ at 346 µmol photons m^{-2} s^{-1}).

Effects of water temperature

The autotrophic growth rates of *Gymnodinium smaydae* increased from − 0.54 day^{-1} at 5 °C to − 0.05 day^{-1} at 20 °C, but decreased to − 0.35 to − 0.52 day^{-1} at 30–35 °C (Fig. 4). The autotrophic growth rates were significantly affected by water temperature [one-way ANOVA, F (9, 20) = 5.87, $P < 0.001$] and were divided into three different temperature groupings (Tukey's HSD post hoc test, $P < 0.05$; Fig. 4).

The mixotrophic growth rates of *G. smaydae* at 5–35 °C ranged from − 0.64 to 1.55 day^{-1} (Fig. 4), and the maximum mixotrophic growth rate was achieved at 25 °C. The rates were significantly affected by temperature [Welch's one-way ANOVA, F (9, 7.66) = 742.03, $P < 0.001$]; the rates were subdivided into seven different temperature groupings (Games–Howell post hoc test, $P < 0.05$; Fig. 4).

The effects of temperature were significantly different between the autotrophic and mixotrophic growth rates of *G. smaydae* [MANOVA, Pillai's Trace = 1.635, F (9, 20) = 9.95, $P < 0.001$].

At 5, 8, and 35 °C, the autotrophic and mixotrophic growth rates of *G. smaydae* were not significantly different (two-tailed t test, $t4 = -1.974$, $P = 0.120$ at 5 °C; $t4 = 0.175$, $P = 0.870$ at 8 °C; $t4 = 2.717$, $P = 0.053$ at 35 °C). However, at 6 °C and from 10 to 32 °C, the autotrophic and mixotrophic growth rates of *G. smaydae* were significantly

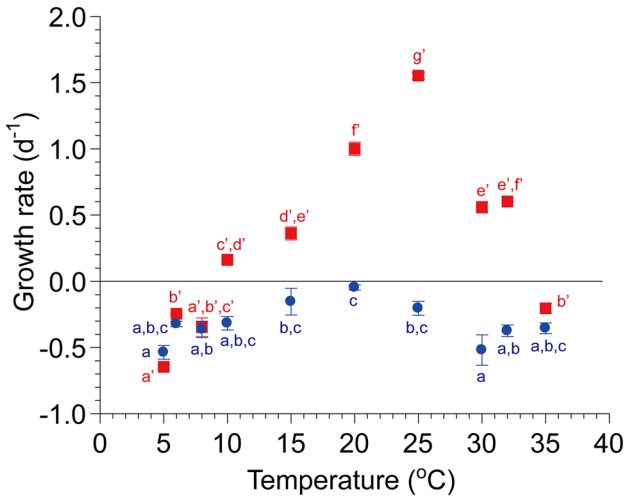

Fig. 4 Specific autotrophic growth rates of *Gymnodinium smaydae* (blue circles) and mixotrophic growth rates of *G. smaydae* on *Heterocapsa rotundata* (red squares) as a function of water temperature. Symbols represent treatment mean values ± 1 SE. Significantly different groups based on post hoc test of ANOVAs: autotrophic growth rate by Tukey's HSD post hoc test, 5 (a); 6 (abc); 8 (ab); 10 (abc); 15 (bc); 20 (c); 25 (bc); 30 (a); 32 (ab); and 35 ºC (abc); mixotrophic growth rate by Games–Howell post hoc test, 5 (a′); 6 (b′); 8 (a′b′c′); 10 (c′d′); 15 (d′e′); 20 (f′); 25 (g′); 30 (e′); 32 (e′f′); and 35 ºC (b′)

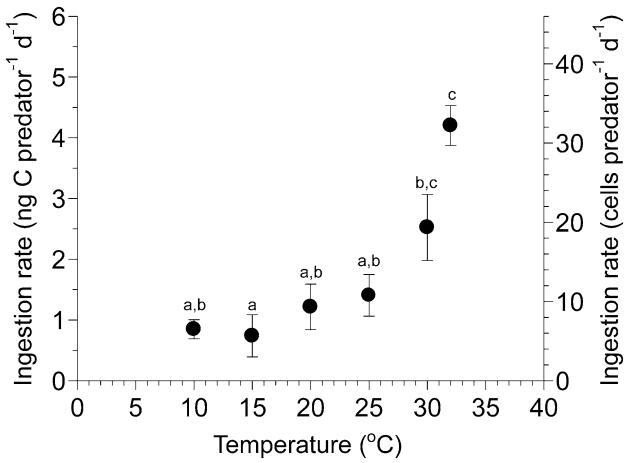

Fig. 5 Ingestion rates of *Gymnodinium smaydae* on *Heterocapsa rotundata* as a function of water temperature. Data at which the mixotrophic growth rates of *G. smaydae* were negative were omitted because of the overestimation of the ingestion rates. Symbols represent treatment mean values ± 1 SE. Significantly different groups based on Tukey's HSD post hoc test of one-way ANOVA: 10 (ab); 15 (a); 20 – 25 (ab); 30 (bc); and 32 °C (c)

different (two-tailed *t* test, $t4 = 3.163$, $P = 0.034$ at 6 °C; $t4 = 9.221$, $P = 0.001$ at 10 °C; $t4 = 4.590$, $P = 0.010$ at 15 °C; $t4 = 19.178$, $P < 0.001$ at 20 °C; $t4 = 30.368$, $P < 0.001$ at 25 °C; $t4 = 8.759$, $P = 0.001$ at 30 °C; and $t4 = 21.962$, $P < 0.001$ at 32 °C).

The ingestion rates of *G. smaydae* feeding on *H. rotundata* at 5–35 °C ranged from 0.7 to 4.2 ng C predator^{-1} day^{-1} (Fig. 5), and the maximum rate was at 32 °C. Ingestion rates at 5, 6, 8, and 35 °C have been omitted from the figure. They were unusually high because the cell concentrations of the predator at these temperatures were very low owing to cell death. The ingestion rates were significantly affected by water temperature [one-way ANOVA, $F(5, 12) = 13.81$, $P < 0.001$] and were divided into three different temperature groupings (Tukey's HSD post hoc test, $P < 0.05$; Fig. 5). The ingestion rates of *G. smaydae* on *H. rotundata* were significantly higher than those at zero at all water temperatures except at 15 °C (one-tailed *t* test, $t4 = 5.74$, $P = 0.003$ at 10 °C; $t2 = 1.99$, $P = 0.092$ at 15 °C; $t2 = 3.42$, $P = 0.038$ at 20 °C; $t4 = 4.04$, $P = 0.008$ at 25 °C; $t2 = 4.72$, $P = 0.021$ at 30 °C; $t4 = 13.07$, $P < 0.001$ at 32 °C).

Cells of *G. smaydae* at 8 and 35 °C had negative autotrophic and mixotrophic growth rates and were swollen. However, those at 25 °C had normal shapes with a distinct cingulum (Fig. 6).

Discussion

Effects of light intensity

In this study, the one strain (GSSH1005) of tested *Gymnodinium smaydae* grew at all tested light intensities, including darkness, when prey was added, but not when prey was not added. The data indicate that regardless of light intensity, *G. smaydae* can grow mixotrophically, but not with only autotrophy. Under these experimental conditions, *G. smaydae* GSSH1005 had a growth rate of 0.72 day^{-1}, equivalent to one division per day, in darkness. The ingestion rate of this strain feeding on *H. rotundata* in darkness was 0.9 ng C predator^{-1} day^{-1}; thus, this strain of *G. smaydae* was capable of acquiring 300% of its body carbon (0.3 ng C per cell) in a day. The data suggest that feeding is a survival strategy for *G. smaydae* GSSH1005 in the dark. Ok et al. (2019) suggested two types of growth rates among mixotrophic dinoflagellates in darkness: Darkness-types I and II, which include the mixotrophic dinoflagellates that do not grow and those that grow in complete darkness, respectively. Interestingly, *G. smaydae* GSSH1005 belongs to Darkness-Type I under autotrophic conditions, but to Darkness-Type II under mixotrophic conditions (Table 2, Fig. 7). The mixotrophic dinoflagellates *Barrufeta resplendens*, *Dinophysis acuminata* DA-MAL01, *Fragilidium subglobosum*, and *Karlodinium veneficum* GE also show this pattern (Skovgaard 1996, 2000; Li et al. 1999; Kim et al. 2008). However, among these dinoflagellates, the mixotrophic growth rate of *G. smaydae* was the greatest. Furthermore, the mixotrophic dinoflagellates *Alexandrium pohangense* APPH1409,

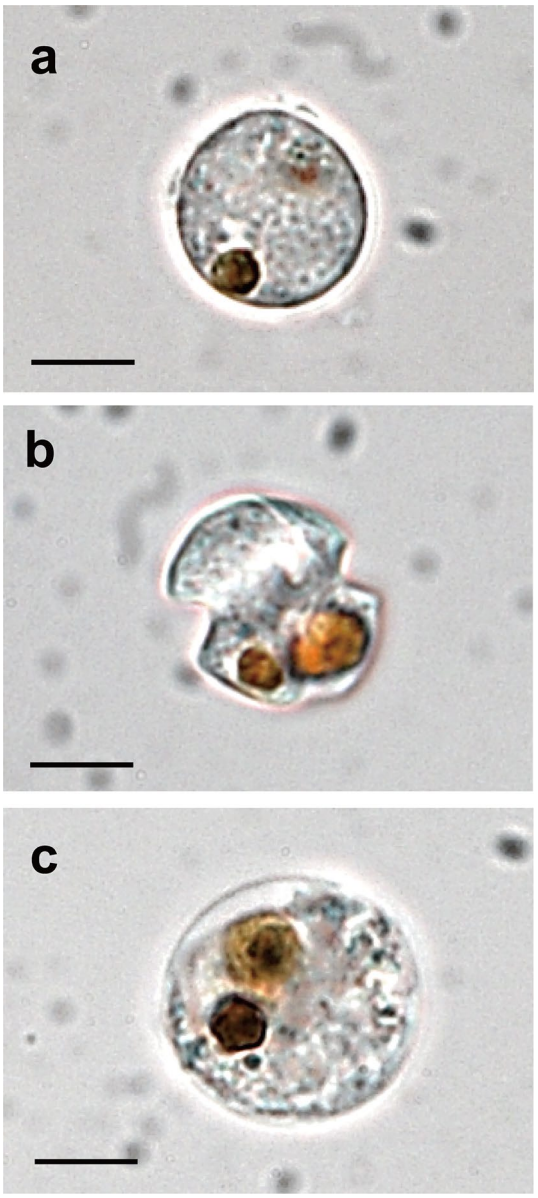

Fig. 6 Light micrographs of *Gymnodinium smaydae* cells incubated for 2 days at 8 (a), 25 (b), and 35 °C (c) at 58 µmol photons m^{-2} s^{-1}, taken under an epi-fluorescent microscope. Cells of *G. smaydae* swelled at 8 and 35 °C and thus the sharply depressed cingulum was seen in (b), but not in (a) and (c). Scale bars 5 µm

Paragymnodinium shiwhaense PSSW0605, and *Takayama helix* CCMP 3082 maintained Darkness-Type I under both autotrophic and mixotrophic conditions (Fig. 7; Jeong et al. 2018a; Lim et al. 2019b; Ok et al. 2019). Thus, *G. smaydae* may have an advantage to outgrow all these mixotrophic dinoflagellates at night. When the autotrophic or mixotrophic growth rates of all these mixotrophic dinoflagellates in darkness were pooled, they were not significantly correlated with their cell sizes (Fig. 7). Furthermore, when the ingestion rates of the mixotrophic dinoflagellates with positive mixotrophic growth rates were pooled, the ingestion rates in darkness were not significantly correlated with their cell sizes. Based on this strain, the ingestion rate of *G. smaydae* in darkness was greater than those of other mixotrophic dinoflagellates having similar sizes. Therefore, *G. smaydae* may feed on its optimal prey more effectively than the similar-sized mixotrophic dinoflagellates.

The mixotrophic growth rates of *G. smaydae* GSSH1005 were maintained as high as 1.1 day^{-1}, to almost two divisions per day, at the highest light intensity tested (i.e., 346 µmol photons m^{-2} s^{-1}), and unlike *G. smaydae*, the mixotrophic growth rate of *T. helix* at the same light intensity was reduced to as low as 0.02 day^{-1} (Ok et al. 2019). Thus, *G. smaydae* may also have an advantage in outgrowing *T. helix* at high light intensity.

The present study indicated that light intensity can significantly affect the mixotrophic growth and ingestion rates of *G. smaydae* GSSH1005. Ok et al. (2019) also suggested two types of mixotrophic dinoflagellates, based on their responses to different photon flux density (DPFD): DPFD-Type I includes species for which the mixotrophic growth and ingestion rates are affected by light intensity, whereas DPFD-Type II includes species for which the mixotrophic growth and ingestion rates are not affected by light intensity except in darkness. Thus, *G. smaydae* GSSH1005 belongs to DPFD-Type I, like *F. subglobosum*, *Karlodinium armiger* K0668, *K. veneficum* GE, and *T. helix* CCMP 3082 (Hansen and Nielsen 1997; Li et al. 1999; Berge and Hansen 2016; Ok et al. 2019; this study). In particular, the mixotrophic growth and ingestion rates of *G. smaydae* on *H. rotundata* largely changed at 0–58 µmol photons m^{-2} s^{-1}, indicating that *G. smaydae* GSSH1005 may be sensitive to a change in light intensity at low light intensities.

Shiwha Bay, where the *G. smaydae* strain was originally isolated, is part of the Yellow Sea and urban development lines its shores. The maximum depth of the bay is about 14 m, according to the Marine Environment Information System (MEIS at https://www.meis.go.kr/portal/main.do), but the tidal range is as high as 5 m (Kang et al. 2013). Secchi depth in the bay in 2016–2018 ranged from 0.2 to 4.0 m (MEIS). The light extinction coefficient, k, can be calculated by dividing 1.7 by the Secchi depth (Poole and Atkins 1929). When the incident light intensity is assumed to be 2,200 µmol photons m^{-2} s^{-1}, the calculated depth below which the light intensity is ≤ 50 µmol photons m^{-2} s^{-1} is 1–9 m in the bay. Thus, light intensity rapidly decreases with depth, and *G. smaydae* cells are likely to often experience low light intensity. The mixotrophic growth and ingestion rates of *G. smaydae* are likely to change readily owing to the light effects in this bay.

Table 2 Growth and ingestion rates of mixotrophic dinoflagellates when prey was added (mixotrophic) and not added (autotrophic) under darkness condition

Species	ESD	autotrophic (without prey)	Mixotrophic (with prey)		References
		GR	GR	IR	
Karlodinium veneficum	9.1	− 0.05	0.05	0.01	Li et al. (1999)
Gymnodinium gracilentum	9.8	NA	0.53	0.05	Jakobsen et al. (2000)
Gymnodinium smaydae	10.5	− 0.12	0.72	0.87	This study
Paragymnodinium shiwhaense	12.5	− 0.66	− 0.12	Not feed	Jeong et al. (2018a, b)
Amphidinium poecilochroum	12.8	NA	0.06	0.01	Jakobsen et al. (2000)
Takayama helix	27.4	− 0.04	− 0.09	0.27	Ok et al. (2019)
Alexandrium pohangense	32.0	− 0.08	− 0.12	2.10	Lim et al. (2019b)
Barrufeta resplendens	34.3	NA	0.11	0.99	Skovgaard (2000)
Dinophysis acuminata	35.0	− 0.01	0.01	0.13	Kim et al. (2008)
Fragilidium subglobosum	50.0	− 0.14	0.23	3.70	Skovgaard (1996)

NA not available, *GR* growth rates (μ, day^{-1}), *IR* ingestion rates (ng C $predator^{-1}$ day^{-1}), *ESD* equivalent spherical diameter (μm)

Effects of temperature

The present study showed that *G. smaydae* GSSH1005 grew at 10–32 °C when prey was added, but not without prey. Thus, the data from this strain indicate that *G. smaydae* can grow mixotrophically at a wide range of water temperatures, but not autotrophically.

The maximum autotrophic growth rate of *G. smaydae* GSSH1005 was achieved at 20 °C, whereas the maximum mixotrophic growth rate was noted at 25 °C (Fig. 4). Hence, 20 °C seems to be an optimal temperature for the growth of *G. smaydae* when prey cells are not available. However, the ingestion rate at 25 °C (1.4 ng C $predator^{-1}$ day^{-1}) was higher than that at 20 °C (1.2 ng C $predator^{-1}$ day^{-1}). Thus, higher carbon acquisition from prey cells at 25 °C is likely to cause a higher growth rate than that at 20 °C, and thus the optimal temperature for supporting the highest mixotrophic growth rate was observed at 25 °C. The range of mixotrophic growth rates at 10–32 °C (0.16–1.55 day^{-1}) was considerably wider than that of the autotrophic growth rates (− 0.05 to − 0.52 day^{-1}). Thus, mixotrophy may increase the sensitivity of *G. smaydae* to temperature changes. Enzymes related to feeding may be more sensitive to temperature changes than those related to photosynthesis.

Relatively few studies have investigated the survival or growth rates of mixotrophic dinoflagellates under both autotrophic and mixotrophic conditions as a function of temperature (Lim et al. 2019b; Ok et al. 2019), whereas many studies have conducted such investigations under autotrophic conditions (Matsuoka et al. 1989; Grzebyk and Berland 1996; Band-Schmidt et al. 2004; Kim et al. 2004; Magaña and Villareal 2006; Nagasoe et al. 2006; Matsubara et al. 2007; Baek et al. 2008; Laabir et al. 2011). At 30–32 °C, the growth rate of *G. smaydae* GSSH1005 was negative under autotrophic condition, but became positive under mixotrophic condition. This pattern is different from that of *Takayama helix* CCMP 3082 and *Alexandrium pohangense* APPH1409 (Lim et al. 2019b; Ok et al. 2019); the growth rates of *T. helix* at 25–28 °C under both autotrophic and mixotrophic conditions were positive, but became negative at 30 °C. Furthermore, the growth rates of *A. pohangense* at 20–30 °C under both autotrophic and mixotrophic conditions were positive, but became negative at 32–35 °C. Thus, among these three mixotrophic dinoflagellates, *G. smaydae* is the only one in which mixotrophic growth changes from negative to positive at a certain temperature. Thus, mixotrophy might be a survival strategy of *G. smaydae* at high temperatures. Furthermore, *G. smaydae* can survive at 32 °C, but *T. helix*, *A. pohangense*, and *Paragymnodinium shiwhaense* PSSW0605 cannot (Jeong et al. 2018a; Lim et al. 2019b; Ok et al. 2019). Thus, high temperature may affect the causative species of blooms and may also be a driving force for the succession of dominant mixotrophic dinoflagellates.

In 2008–2012, water temperatures at the surface of Shiwha Bay were 0.2–28.4 °C, but those from November to March were 0.2–8.9 °C (Kang et al. 2013). Thus, based on the data from this strain, *G. smaydae* can grow in April–November if *H. rotundata* or other suitable prey is available. However, global warming can cause the elevation

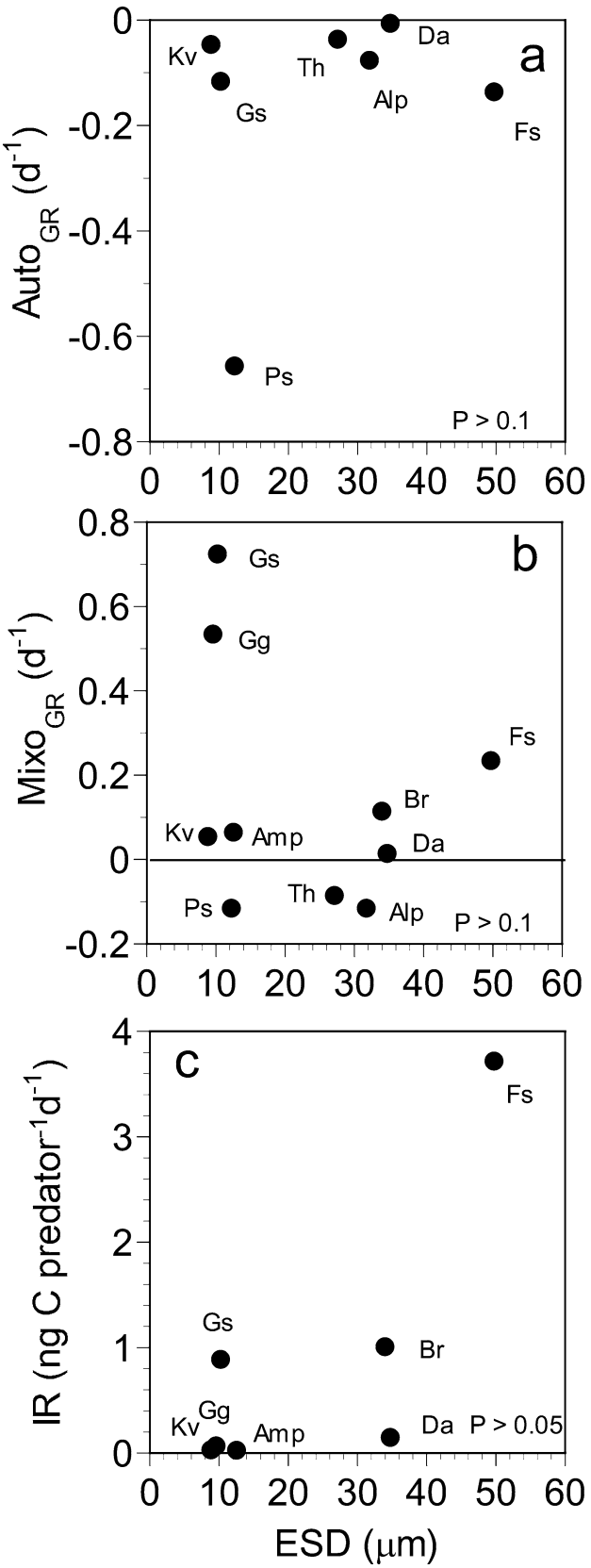

Fig. 7 Autotrophic (**a**) and mixotrophic (**b**) growth rates and ingestion rates (**c**) of mixotrophic dinoflagellates fed on the optimal prey as a function of predator sizes (Equivalent Spherical Diameter, ESD, μm) in darkness. Kv: *Karlodinium veneficum* GE, Gg: *Gymnodinium gracilentum*, Gs: *Gymnodinium smaydae* GSSH1005, Ps: *Paragymnodinium shiwhaense* PSSW0605, Amp: *Amphidinium poecilochroum*, Th: *Takayama helix* CCMP 3082, Alp: *Alexandrium pohangens* APPH1409, Br: *Barrufeta resplendens*, Da: *Dinophysis acuminata* DA-MAL01, Fs: *Fragilidium subglobosum*. Data were obtained from this study, Skovgaard (1996, 2000), Li et al. (1999), Jakobsen et al. (2000), Kim et al. (2008), Jeong et al. (2018a), Lim et al. (2019b), and Ok et al. (2019)

of water temperature in the bay, and in particular, heat waves in summer may accelerate the elevation of water temperature (Lee et al. 2019b). The water temperature at a depth of 1 m in the bay in the summer of 2018 increased up to 31.5 °C (MEIS). Therefore, *G. smaydae* may have an advantage in outgrowing *T. helix*, *A. pohangense*, and *P. shiwhaense* during the global warming period and/or heat wave year. Moreover, a maximum of 6 °C elevation by 2100 is expected, according to the Intergovernmental Panel on Climate Change report (IPCC 2013). The data from this study indicate that *G. smaydae* cannot survive at 35 °C. Thus, if the water temperature becomes ≥ 35 °C due to global warming or heat waves, *G. smaydae* may also not survive in the surface water. These data also indicate that *G. smaydae* can survive at depth, but its growth can be reduced because of light limitation. Over the coming several decades, water temperature at Shiwha Bay is expected to change largely because it is a small water body (Lee et al. 2019b). Therefore, the distribution of *G. smaydae* in the bay in the near future needs to be explored. Furthermore, to our knowledge, only one strain of *G. smaydae* has been reported. Responses by other strains of *G. smaydae* to light intensity and temperature may be different from those by *G. smaydae* GSSH1005. Thus, if another strain of *G. smaydae* is developed, it would be necessary to explore the differences in the responses.

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Data availability Data collected and analyzed during this current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical standards All applicable international, national, and/or institutional guidelines for the care and use of organisms were followed.

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