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Feeding by heterotrophic protists and copepods on the photosynthetic dinoflagellate *Azadinium* cf. *poporum* from western Korean waters

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ABSTRACT: We explored the interactions between the photosynthetic dinoflagellate *Azadinium* cf. *poporum* isolated from Korean waters and potential predators, including engulfment feeders, a pallium feeder, peduncle feeders, and filter feeders. We measured the growth and/or ingestion rates of *Oxyrrhis marina*, *Strobilidium* sp., and *Acartia* spp. on *A.* cf. *poporum* as a function of prey concentrations. We also calculated grazing coefficients by using field data on abundance of *Strobilidium* sp.-sized naked ciliates co-occurring with *A.* cf. *poporum* and laboratory data on ingestion rates obtained in this study. Most of the tested organisms were able to feed on *A.* cf. *poporum*, but only *O. marina*, *Strobilidium* sp., and *Acartia* spp. showed sustained growth and/or ingestion on *A.* cf. *poporum*. Thus, some heterotrophic dinoflagellates using engulfment and filter feeders, such as ciliates and copepods, are likely to be optimal predators, while peduncle-feeding heterotrophic dinoflagellates are unlikely to efficiently feed due to the handling of the theca. The predators had low ratios of maximum growth rate to maximum ingestion rate on *A.* cf. *poporum*, as well as low gross growth efficiencies. Therefore, *A.* cf. *poporum* appears to be a low-quality prey for the predators tested. Grazing coefficients ranged between 0.052 and 0.446 d^{-1}, suggesting that *Strobilidium* sp.-sized naked ciliates may sometimes have a high impact on *A.* cf. *poporum* populations, leading to the removal of up to 36% of the population in 1 d. However, the low quality of the prey and predator selectivity in a more complex microbial community may reduce this impact.

KEY WORDS: *Azadinium* · Azaspiracids · Toxins · Seasonal dynamics · Grazing · Predation · Ingestion · Growth

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INTRODUCTION

The azaspiracids (AZAs) are the most recently discovered group of lipophilic marine biotoxins of microalgal origin associated with cases of shellfish poisoning in humans (Twiner et al. 2008). The production of AZAs was previously associated with a small photosynthetic dinoflagellate (Krock et al. 2009), designated as *Azadinium spinosum* Elbrächter et Tillmann and assigned to a new genus (Tillmann et al. 2009). The genus *Azadinium* is now composed of 4 well defined species, namely *A. spinosum* Elbrächter et Tillmann (Tillmann et al. 2009), *A. obesum* Tillmann et Elbrächter (Tillmann et al. 2010), *A. poporum* Tillmann et Elbrächter (Tillmann et al. 2011), and *A. caudatum* (Halldal) Nézan et Chomérat (Nézan et al. 2012). Undefined species were also described as *A.* cf. *poporum* (Potvin et al. 2012) and *A.* cf. *spinosum* (Akselman & Negri 2012). Until recently, *A. spinosum* was the only known planktonic source of AZAs. However, new AZAs have since been discovered in *A. poporum*, *A.* cf. *poporum*, and the closely related species *Amphidoma languida* Tillmann, Salas et Elbrächter (Krock et al. 2012, Tillmann et al. 2012). *A.*

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obesum and *A. caudatum* are the only members of the genus *Azadinium* established in culture for which no AZA has been detected. While the AZAs produced by *A. spinosum* have been shown empirically to be toxic (Ito et al. 2002, Román et al. 2002, Colman et al. 2005, Twiner et al. 2005, Alfonso et al. 2006, Kulagina et al. 2006, Vale et al. 2007), research is pending for the new AZAs.

The population dynamics of the species of the genus *Azadinium* are not well known. These species apparently seem to either remain at low density, such as *A. caudatum*, which was not observed to exceed 1.3 cells ml^{-1} (Nézan et al. 2012), or are able to bloom (e.g. *A.* cf. *spinosum*) and can reach up to 9.03×10^3 cells ml^{-1} (Akselman & Negri 2012).

Grazing pressure sometimes plays an important role in population dynamics (Watras et al. 1985). Heterotrophic dinoflagellates (HTDs), ciliates, and copepods are integral parts of marine planktonic food webs (Jeong et al. 2010). HTDs and ciliates can be found everywhere and can sometimes dominate in abundance and/or biomass, while copepods can similarly dominate the mesozooplankton (Brownlee & Jacobs 1987, Lessard 1991, Jeong 1999, Turner et al. 2005). These 3 groups sometimes have considerable grazing impact on populations of diverse prey (Painting et al. 1993, Strom et al. 1993, Sherr & Sherr 2007). In particular, grazing by heterotrophic protists is believed to contribute to the decline of blooms (Kim & Jeong 2004). In order to investigate grazing pressure by predators, growth and ingestion need to be studied. No study extensively exploring predation on species of the genus *Azadinium* is currently available.

In order to determine which predators are more likely to grow and feed actively on *Azadinium* spp., we explored feeding of HTDs (*Polykrikos kofoidii*, *Gyrodinium dominans*, *G. moestrupii*, *Oxyrrhis marina*, *Oblea rotunda*, *Stoeckeria algicida*, *Pfiesteria piscicida*, and *Gyrodiniellum shiwhaense*), a ciliate (*Strobilidium* sp.) and the copepods *Acartia* spp. on *Azadinium* cf. *poporum*. We found that *O. marina*, *Strobilidium* sp., and *Acartia* spp. fed well on *A.* cf. *poporum*. Therefore, we measured their growth and/or ingestion rates on *A.* cf. *poporum* as a function of prey concentration. Furthermore, we calculated grazing coefficients by using field data on abundance of *Strobilidium* sp.-sized naked ciliates co-occurring with *A.* cf. *poporum* and laboratory data on ingestion rate obtained in this study. Our results provide a basis for understanding interactions between *Azadinium* spp. and common heterotrophic protists and copepods.

MATERIALS AND METHODS

Preparation of experimental organisms

For the isolation and culture of *Azadinium* cf. *poporum* (GenBank accession number = FR877580), surface sediment samples from Shiwha Bay, a highly eutrophic bay in Korea (37° 18′ N, 126° 36′ E), were incubated in F/2-Si medium (Guillard & Ryther 1962) in a growth chamber at 20°C under an illumination of 20 µE m^{-2} s^{-1} of cool white fluorescent light on a 14:10 h light:dark cycle. The monoclonal culture was established as described in detail by Potvin et al. (2012).

For the isolation and culture of potential protistan predators, plankton samples collected with water samplers were taken from the Korean coastal waters off Shiwha, Masan, Saemankeum, Karorim and Jinhae between 2007 and 2011 (Table 1). A clonal culture of each predator was established as in Kim & Jeong (2004), Jeong et al. (2003a,b, 2005a, 2006,

Table 1. Isolation and maintenance conditions of the experimental organisms. Sampling location in Korea and time, field water temperature (T, °C), and salinity (S) from which species were isolated, and prey species and concentrations (cells ml^{-1}) for maintenance. All organisms are heterotrophic dinoflagellates, except *Strobilidium* sp., a ciliate

Organism	Location	Year (month)	T	S	Prey species	Concentration
Gyrodiniellum shiwhaense	Shiwha Bay	2009 (09)	24.5	24.0	*Amphidinium carterea*	20 000
Gyrodinium dominans	Masan Bay	2007 (04)	15.1	33.4	*Amphidinium carterea*	8000
Gyrodinium moestrupii	Saemankeum Bay	2009 (10)	21.2	31.0	*Alexandrium minutum*	3000–5000
Oblea rotunda	Shiwha Bay	2010 (08)	26.8	23.7	*Prorocentrum minimum*	30 000
Oxyrrhis marina	Karorim	2010 (05)	19.5	33.0	*Amphidinium carterea*	8000
Pfiesteria piscicida	Jinhae	2010 (02)	24.5	12.6	*Amphidinium carterea*	20 000–30 000
Polykrikos kofoidii	Shiwha Bay	2010 (03)	9.2	23.4	*Scrippsiella trochoidea*	8000
Stoeckeria algicida	Masan Bay	2007 (08)	24.5	29.7	*Heterosigma akashiwo*	30 000
Strobilidium sp.	Shiwha Bay	2011 (08)	27.0	15.0	*Heterocapsa rotundata*	50 000–60 000

2011b), Kang et al. (2011), and Yoon et al. (2012). *Oblea rotunda* was brought into culture as *Gyrodinium* spp. in Kim & Jeong (2004; Table 1). The cultures were maintained on a wheel rotating at 0.9 rpm in a growth chamber at 20°C under an illumination of 20 µE m^{-2} s^{-1} of cool white fluorescent light on a 14:10 h light:dark cycle. Fresh prey cells were provided every 1 to 3 d.

The copepods *Acartia* spp. were collected from Kunsan port with a 303 µm mesh net in May 2011 when water temperature and salinity were 16.8°C and 18.9, respectively. The copepods were acclimated in a room at 20°C, with *Prorocentrum minimum* provided as prey. Species of the genus *Acartia*, which co-occur in coastal waters off western Korea, can be very similar and impossible to distinguish from each other when they are alive (e.g. Soh & Suh 2000). Therefore, we had to use a mixture of adult *Acartia* spp.

The mean equivalent spherical diameter (ESD) of live *Azadinium* cf. *poporum* was measured (ESD = 10.0 µm) by an electronic particle counter and size analyzer (model Z2, Beckman Coulter). The shape of a sphere was used to estimate the volume of *A.* cf. *poporum* based on the ESD. *Oxyrrhis marina* and *Strobilidium* sp. were measured in order to estimate their volume in the different predator–prey combinations at the end of the incubation. Measurements were made with specimens fixed in 5% acid Lugol's solution using a transmitted light inverted microscope (Zeiss Axiovert 200M, Carl Zeiss) at a magnification of 400× with a Zeiss AxioCam MRc5 digital camera (Carl Zeiss). The shape of *O. marina* was estimated as 2 cones joined at their bases. The shape of *Strobilidium* sp. was estimated as a sphere, cylinder, or cone depending of the shape of the specimen. Carbon content was estimated from the cell volume according to Menden-Deuer & Lessard (2000).

Feeding

Expt 1 was designed to test whether *Polykrikos kofoidii*, *Gyrodinium dominans*, *G. moestrupii*, *Oxyrrhis marina*, *Oblea rotunda*, *Stoeckeria algicida*, *Pfiesteria piscicida*, *Gyrodiniellum shiwhaense*, *Strobilidium* sp., and *Acartia* spp. were able to feed on *Azadinium* cf. *poporum* (Table 2).

Azadinium cf. *poporum* (10 000 cells ml^{-1} final concentration) was added to 80 ml polycarbonate (PC) bottles, followed by the addition of each of the other HTDs (100 to 3000 cells ml^{-1} final concentration), the ciliates (20 cells ml^{-1} final concentration), or the copepods (0.025 ind. ml^{-1} final concentration). Duplicates were established for each predator put into contact with *A.* cf. *poporum*. One control bottle (without prey) was set up for each experiment. The bottles were placed on a plankton wheel rotating at 0.9 rpm in a growth chamber at 20°C under an illumination of 20 µE m^{-2} s^{-1} of cool white fluorescent light on a 14:10 h light:dark cycle.

Aliquots (5 ml) were removed from each bottle after 1, 2, 6, and 24 h of incubation and then transferred into 6-well plate chambers or onto microscopic

Table 2. Experimental design to assess predation by various planktonic predator species on the prey species *Azadinium* cf. *poporum*. The numbers in prey and predator columns are the actual initial concentrations (cells or ind. ml^{-1}) of prey and predator. In each experiment, the lowest *A.* cf. *poporum* concentration was tested against the lowest predator concentration, the next lowest against the next lowest, etc. Values within parentheses in the prey and predator columns are the corresponding prey and predator concentrations in the predator-only control bottles. Feeding occurrence of each predator on *A.* cf. *poporum* in Expt 1 is represented by Y (feeding observed) or N (no feeding observed)

Expt	Prey concentration	Species	Predator concentration	Feeding
1	10 000	*Gyrodiniellum shiwhaense*	1000	Y
		Gyrodinium dominans	500	Y
		Gyrodinium moestrupii	500	Y
		Oblea rotunda	500	N
		Oxyrrhis marina	3000	Y
		Pfiesteria piscicida	3000	Y
		Polykrikos kofoidii	100	N
		Stoeckeria algicida	1000	N
		Strobilidium sp.	20	Y
		Acartia spp.	0.025	Y
2	44, 282, 1223, 2991, 9600, 22 758 (0)	*Oxyrrhis marina*	5, 12, 24, 36, 53, 106 (7, 261)	
3	62, 558, 2415, 5536, 11 771, 22 919, 59 267 (0)	*Strobilidium* sp.	6, 6, 6, 15, 13, 13, 29 (7, 28)	
4	52, 151, 492, 1895, 6553, 27 070, 52 332 (0)	*Acartia* spp.	0.02, 0.02, 0.02, 0.02, 0.02, 0.02, 0.02 (0.02)	

slides. Approximately 200 cells of each predator were observed using a transmitted light inverted microscope (Zeiss Axiovert 200M, Carl Zeiss) at a magnification of 100× to 630× to determine whether the predators were able to feed on *Azadinium* cf. *poporum*. Cells of predators containing ingested *A.* cf. *poporum* cells were photographed using a Zeiss AxioCam MRc5 digital camera at a magnification of 630×. To confirm the absence of prey ingestion, higher prey concentrations were provided.

Growth, ingestion, and gross growth efficiency

Expts 2 and 3 were designed to measure the growth and ingestion rates of *Oxyrrhis marina* and *Strobilidium* sp. as a function of the prey concentration when fed on *Azadinium* cf. *poporum* (Table 2). Only this HTD and this ciliate were shown to feed and to have sustained growth on *A.* cf. *poporum* among the protists tested.

Two weeks before the experiments were conducted, dense cultures of *Oxyrrhis marina* and *Strobilidium* sp. growing on algal prey listed in Table 1 were transferred into 500 ml PC bottles containing *Azadinium* cf. *poporum* (ca. 20 000 cells ml^{-1} final concentration). These predator cultures were transferred to 500 ml PC bottles of fresh prey (ca. 20 000 cells ml^{-1} final concentration) every 1 to 3 d. The bottles were filled to capacity with freshly filtered seawater, capped, and placed on plankton wheels rotating at 0.9 rpm and incubated under the conditions described above. To monitor the conditions and interactions between the predator and prey species, the cultures were periodically removed from the rotating wheels, examined through the surface of the capped bottles by using a stereomicroscope (Olympus, SZX-12), and then returned to the rotating wheels. Once the target prey cells in ambient water were no longer detectable, predators were routinely inspected until the prey cells were no longer observed in the cytoplasm. This was carried out to minimize possible residual growth resulting from the ingestion of prey during batch culture. Once the predators were starved, the cultures were then used to conduct the experiments.

For each experiment, the initial concentrations of protists were established using an autopipette to deliver predetermined volumes of known cell concentrations to the bottles. Triplicate 42 ml (for the HTD) or 80 ml (for the ciliate) experimental bottles (mixtures of predator and prey) and triplicate control bottles (prey only) were set up for each predator–prey combination. Triplicate control bottles containing only predators were also established at 2 predator concentrations. F/2-Si medium (5 ml for the HTD, 10 ml for the ciliate) was added to all PC bottles. To obtain similar water conditions, the water of the predator culture was filtered through a 0.7 µm GF/F filter and then added to the prey control bottles in the same amount as the volume of the predator culture added to the experiment bottles for each predator–prey combination. Culture of *Azadinium* cf. *poporum* was filtered and added to the predator control bottles in the same way as for the prey control bottles. All bottles were then filled to capacity with filtered seawater and capped. To determine the actual protist concentrations at the beginning of the experiment, a 5 or 6 ml aliquot was taken from each bottle for the HTD and ciliate, respectively. The bottles were refilled to capacity with freshly filtered seawater, capped, and placed on rotating wheels under the conditions described above. Dilution of the cultures associated with refilling was considered when calculating growth and ingestion rates. Another aliquot of the same volume as before was taken from each bottle after 48 or 24 h for the HTD and ciliate, respectively. The aliquots were fixed with 5% acid Lugol's solution (final concentration). The abundances of predator and prey were determined by counting all or >300 cells in 3 Sedgwick-Rafter chambers (SRCs, 1 ml). The conditions of the predator and its prey were assessed using a stereomicroscope as described above before subsampling.

Expt 4 was designed to measure only the ingestion and clearance rates of *Acartia* spp. on *Azadinium* cf. *poporum* as a function of the prey concentration (Table 2). Adult female *Acartia* spp. were used.

The initial concentration of *Acartia* spp. was determined by the individual transfer of the copepods using a Pasteur pipette with a stereomicroscope (Olympus, SZX-12). Triplicate 500 ml experimental bottles (mixtures of predator and prey) and triplicate control bottles (prey only) were set up for each predator–prey combination. Triplicate control bottles containing only predators were also established at 1 predator concentration. F/2-Si medium (50 ml) was added to all PC bottles. To obtain similar water conditions, the same procedures described above were followed. Furthermore, the actual protist concentrations at the beginning and end of the experiment were determined as before by taking a 10 ml aliquot. The final concentration of *Acartia* spp. was determined by direct counting with a stereomicroscope (Olympus, SZX-12).

The specific growth rate of predators, μ (d^{-1}), was calculated as:

$$\mu = [\text{Ln}\ (P_t/P_0)]/t \quad (1)$$

where P_0 and P_t are the concentrations of predators at 0 and 48 h for the HTD, and at 0 and 24 h for the ciliate, and t is the time elapsed during the experiment.

Growth rates were fit to the Michaelis-Menten model (Michaelis & Menten 1913). The equation for growth rate data is:

$$\mu = \mu_{max}\ (X - X')/[K_{GR}+(X - X')] \quad (2)$$

where μ_{max} is the maximum growth rate (d^{-1}); X is the prey concentration (cells ml^{-1} or ng C ml^{-1}), X' is the threshold prey concentration (the prey concentration where $\mu = 0$), K_{GR} is the prey concentration sustaining half of μ_{max}. Data were iteratively fitted to this regression type using DeltaGraph® (Delta Point) and the Levenberg-Marquardt algorithm, which minimizes the sum of squares of differences between the dependent variables in the equation and the observed data.

Ingestion and clearance rates were calculated using the equations of Frost (1972) and Heinbokel (1978). The incubation times for calculating ingestion and clearance rates were the same as those for estimating growth rates. The data on the ingestion rates were fit to the Michaelis-Menten model or a simple linear regression model depending of the functional response. The Michaelis-Menten equation for ingestion rate (IR) data is:

$$\text{IR} = I_{max}\ (X)/[K_{IR}+(X)] \quad (3)$$

and the simple linear regression equation is:

$$\text{IR} = C_1(X) + C_2 \quad (4)$$

where I_{max} is the maximum ingestion rate (cells predator^{-1} d^{-1} or ng C predator^{-1} d^{-1}); X is the prey concentration (cells ml^{-1} or ng C ml^{-1}), K_{IR} is the half saturation constant (the prey concentration sustaining half of I_{max}), and C_1 and C_2 are constants.

Gross growth efficiency (GGE), defined as predator biomass produced (+) or lost (−) per prey biomass ingested, was calculated from estimates of carbon content per cell based on cell volume for each mean prey concentration.

Field data

Water samples were obtained at least at monthly intervals in ice-free conditions from January 2009 to December 2011 in Shiwha Bay, Korea, at different stations from the surface and 1 m above the bottom (Fig. 1). To quantify cells based on optical microscopy, samples were fixed with 5% acid Lugol's solution (final concentration) and used to determine the abundance of *Oxyrrhis marina* and *Strobilidium* sp.-sized naked ciliates. *Azadinium* cf. *poporum* cannot be differentiated easily from species of its own genus and other species highly similar morphologically based on optical microscopy. Therefore, real-time PCR was used to estimate its concentration in the field. Field samples were concentrated by the filtration of 100 ml of seawater with a 25 mm GF/C glass microfiber filter (Whatman™) and immediately frozen on dry ice. Filters were subsequently stored at −20°C until further processing.

Fixed field samples were settled for 48 h. They were then concentrated by gently removing the surface water. To determine the concentration of *Oxyrrhis marina* and *Strobilidium* sp.-sized naked ciliates, an aliquot was removed from each concentrated field sample and examined with a compound microscope

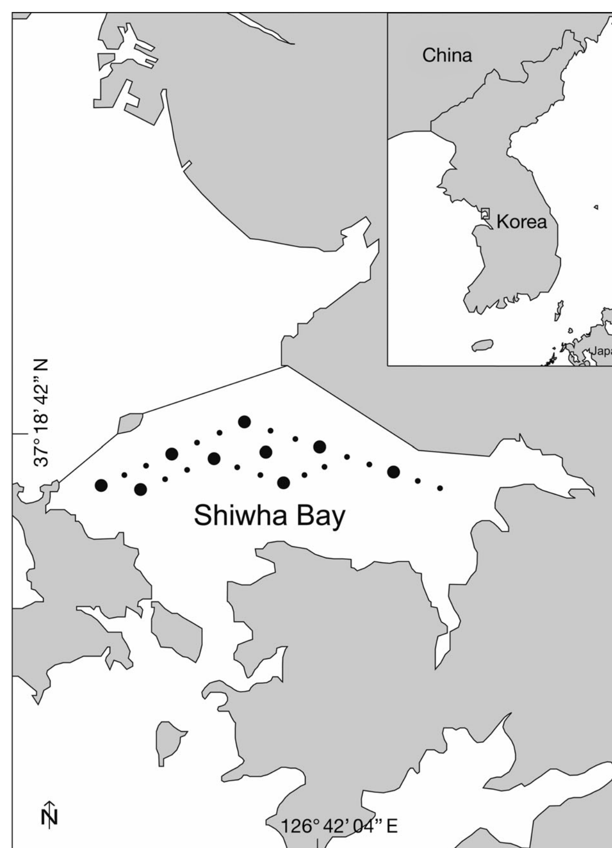

Fig. 1. Shiwha Bay, Korea, and fixed stations where water samples were collected. Surface water samples used for protistan determination and real-time PCR were taken at every station, while bottom water samples were only taken at stations represented by enlarged circles

to determine their abundances by enumerating the cells in three 1 ml SRCs. The protists were enumerated at 40× to 200× magnification.

After the filters stored at −20°C had defrosted, cells were resuspended by adding TE buffer (Tris HCl pH 8.0 10 mM, EDTA 1 mM, Bioneer) and grinding the filters in 2 ml tubes (GenePole). The suspension was subsequently boiled at 100°C for 5 min and cooled at room temperature for 20 min. We added 850 µl of 25:24:1 phenol:chloroform: isoamyl alcohol (Bioneer) and mixed by vortexing. The samples were subsequently centrifuged (Hanil Science, model MICRO 17TR) at 17 640 × *g* (30 min at 20°C). The supernatant was transferred to a DNA binding column (Bioneer) and subsequently centrifuged at 2250 × *g* (1 min at 20°C). We then added 700 µl of 99.5% ethanol (Merck) at −20°C and 30 µl 3M sodium acetate (pH 7.0, Bioneer) to the binding column. The mixture was subsequently incubated at −20°C for 1 h. The mixture was then centrifuged at 2250 × *g* (1 min at 4°C). The pellet was then washed twice by the addition of 70% ethanol (Merck) followed by centrifugation at 2250 × *g* (1 min at 4°C). The pellet contained in the binding column was subsequently dried at 50°C for 1 h. TE buffer (200 µl) was added to the binding column. The binding column was left to stand for more than 10 min in order to resuspend the DNA. Finally, the binding column was centrifuged at 1440 × *g* (5 min) followed by 17 640 × *g* (5 min). The crude extracts and 1:10 dilutions were stored at −20°C until further use in real-time PCR assays. A preliminary survey was done with the undiluted and diluted extracts for each sample collected. Each positive assay of this survey was measured in triplicate.

The design of the primers and the probe was based on the ITS rDNA region sequences of all species of the genus *Azadinium* available from GenBank. Sequences were aligned using the program Clustal X2 (Larkin et al. 2007). Manual searches of the alignment were conducted to determine unique sequences and to develop an *A.* cf. *poporum* specific real-time PCR assay. The primers and probe sequences as well as their general properties were determined with Primer 3 (Rozen & Skaletsky 2000). Their secondary structures were analyzed with OligoCalc (Kibbe 2007). Subsequently, the primers and probe were synthesized by Biosearch Technologies (Novato, CA). The probe was dual-labeled with the fluorescent dye FAM and the BHQ-1 quencher at the 5′ and 3′ ends, respectively. The sequences of the primers and probe were checked against published sequences in GenBank by BLAST homology search. BLAST searches showed that the sequences of the selected primers and probe matched only with the sequences of *A.* cf. *poporum*. The specificity was further assessed with the DNA from 22 dinoflagellate cultures (Table 3) extracted as in Potvin et al. (2012). Agarose gel analysis of the PCR products showed only amplicons of the expected size (94 bp) for *A.* cf. *poporum* and no product for other species. Real-time PCR experiments showed that the primers–probe set was specific to the target for which it was designed.

To achieve optimal performance, series of primers and probe concentrations as well as annealing–extension times and temperatures were tested by real-time PCR assays. The tested primers and probe concentrations ranged from 200 to 1000 and 100 to 500 nM, respectively. The tested annealing–ex-

Table 3. Isolation conditions of the dinoflagellates used in the real-time PCR specificity test. Sampling location and time, field water temperature (T, °C), and salinity (S) are shown. na: not available

Dinoflagellate	Location	Year (month)	T	S
Azadinium cf. *poporum*	Shiwha Bay, Korea	2010 (06)	24.1[a]	26.7[a]
Bysmatrum caponii	Karorim, Korea	2010 (05)	19.5	33.0
Cryptoperidiniopsis brodyi	Pamlico River, USA	1992 (07)	na	na
Dinophysis acuminata	Masan Bay, Korea	2005 (12)	na	na
Gymnodinium aureolum	Kunsan, Korea	2008 (03)	10.0	30.5
Gymnodinium simplex	Shiwha Bay, Korea	2009 (09)	25.1	24.0
Gyrodiniellum shiwhaense	Shiwha Bay, Korea	2009 (09)	24.5	24.0
Gyrodinium moestrupii	Saemangum Bay, Korea	2009 (10)	21.2	31.0
Heterocapsa rotundata	Kunsan, Korea	2002 (05)	16.6	22.0
Heterocapsa triquetra	Masan Bay, Korea	2008 (06)	20.2	29.0
Karenia brevis	Gulf of Mexico, USA	1999 (09)	na	na
Karlodinium veneficum	Shiwha Bay, Korea	2011 (08)	27.1	7.2
Oxyrrhis marina	Karorim, Korea	2010 (05)	19.5	33.0
Paragymnodinium shiwhaense	Shiwha Bay, Korea	2006 (05)	18.8	30.4
Pfiesteria piscicida	Jinhae, Korea	2010 (02)	24.5	12.6
Prorocentrum donghaiense	Jeju, Korea	2010 (06)	na	na
Prorocentrum micans	Shiwha Bay, Korea	2009 (10)	16.8	27.0
Prorocentrum minimum	Shiwha Bay, Korea	2009 (01)	1.2	30.3
Scrippsiella precaria	Shiwha Bay, Korea	2009 (06)	22.8	27.6
Scrippsiella sweeneyae	Masan Bay, Korea	2009 (08)	27.0	31.5
Stoeckeria algicida	Masan Bay, Korea	2007 (08)	24.5	29.7
Woloszynskia cincta	Shiwha Bay, Korea	2009 (06)	22.0	29.3

[a]Isolated from sediment

tension times and temperatures ranged from 45 to 85 s and 56 to 64°C, respectively. The conditions that provided the lowest cycle threshold (CT) value and the highest fluorescence were selected. PCR assays were performed on a Rotor-Gene 6000 (Corbett Research). The following reagents were added in the reaction mixture: 5 µl of 2× SensiMix II Probe (GenePole), forward (5′-GGG AAC CTT CGC ATC AAT CAA C -3′) and reverse (5′-CAC GAA GCA GCC TTG GGT TT-3′) primers each at a final concentration of 0.2 µM, probe (5′-TGA GTG TCT TTG ATA CCA TCT GTT GCA-3′) at a final concentration of 0.15 µM, 1 µl of template DNA, and nuclease-free water (GenePole) to a final volume of 10 µl. The thermal cycling conditions consisted of 10 min at 95°C followed by 50 cycles of 10 s at 95°C and 65 s at 60°C. Fluorescence data were collected at the end of each cycle, and determination of the cycle threshold line was carried out automatically by the instrument. PCR products that were positive were further analyzed by gel electrophoresis to confirm the amplicon size. Three samples that gave positive real-time PCR results were reamplified without the probe. The PCR products were purified using the AccuPrep® PCR purification kit (Bioneer) according to the manufacturer's instructions. The purified DNA was sent to the Genome Research Facility (School of Biological Science, Seoul National University, Korea) and sequenced with a ABI PRISM® 3700 DNA Analyzer (Applied Biosystems). All sequences obtained corresponded to *Azadinium* cf. *poporum*.

A standard curve using cell numbers was constructed from *Azadinium* cf. *poporum* culture. Cell numbers (100 000 cells) were estimated by counting 3 SRCs, and genomic DNA was extracted the same way as for the field samples. Ten-fold serial dilutions of the DNA extract were used to construct the standard curve. A strong linear relationship between the CT values and the log of the cell numbers was established, with a correlation coefficient (r^2) of 0.999. The cell number of *A.* cf. *poporum* in field samples was determined from CT values and comparison with the standard curve.

Grazing impact

We calculated grazing coefficients by using field data on the abundance of *Strobilidium* sp.-sized naked ciliates (25 to 60 µm in length) co-occurring with *Azadinium* cf. *poporum* and laboratory data on ingestion rate. We assumed that the ingestion rates of *Strobilidium* sp.-sized naked ciliates on *A.* cf. *poporum* were the same as those obtained in this study.

The grazing coefficients (g, d^{-1}) were calculated as:

$$g = CR \times GC \times 24 \quad (5)$$

where CR is the clearance rate (ml predator^{-1} h^{-1}) of a predator on *Azadinium* cf. *poporum* at a given prey concentration, and GC is the grazer concentration (cells ml^{-1}). CRs were calculated as:

$$CR = IR/X \quad (6)$$

where IR is the ingestion rate (cells eaten predator^{-1} h^{-1}) of the predator on the prey and X is the prey concentration (cells ml^{-1}). CRs were corrected using Q_{10} = 2.8 (Hansen et al. 1997) because *in situ* water temperatures and the temperature used in the laboratory for the experiments (20°C) were sometimes different.

Swimming speed

A culture of *Azadinium* cf. *poporum* (ca. 20 000 cells ml^{-1}) growing at 20°C under an illumination of 20 µE m^{-2} s^{-1} of cool white fluorescent light on a 14:10 h light:dark cycle in F/2-Si medium was added to a 50 ml cell culture flask and allowed to acclimate for 30 min. The video camera focused on 1 field seen as 1 circle in a cell culture flask under a stereomicroscope (Olympus, SZX-12) at 20°C, and swimming of *A.* cf. *poporum* cells was then recorded at a magnification of 40× using a video analyzing system (Samsung, SV-C660) and a CCD camera (Hitachi, KP-D20BU). The swimming speed was calculated based on the linear displacement of cells in 1 s. The average swimming speed was calculated based on the measures of 30 cells during single-frame playback. The swimming of individual cells was interrupted by short and high-speed 'jumps' in various directions typical of the genus *Azadinium* (Tillmann et al. 2009). These 'jumps' were excluded from the measurements.

RESULTS

Among the predators tested, *Gyrodinium dominans*, *G. moestrupii*, *Oxyrrhis marina*, *Pfiesteria piscicida*, *Gyrodiniellum shiwhaense*, *Strobilidium* sp., and *Acartia* spp. were able to feed on *Azadinium* cf. *poporum* (Fig. 2), while *Polykrikos kofoidii*, *Oblea rotunda*, and *Stoeckeria algicida* were not. Only the HTD *O. marina* and the ciliate *Strobilidium* sp. showed sustained growth on *A.* cf. *poporum*.

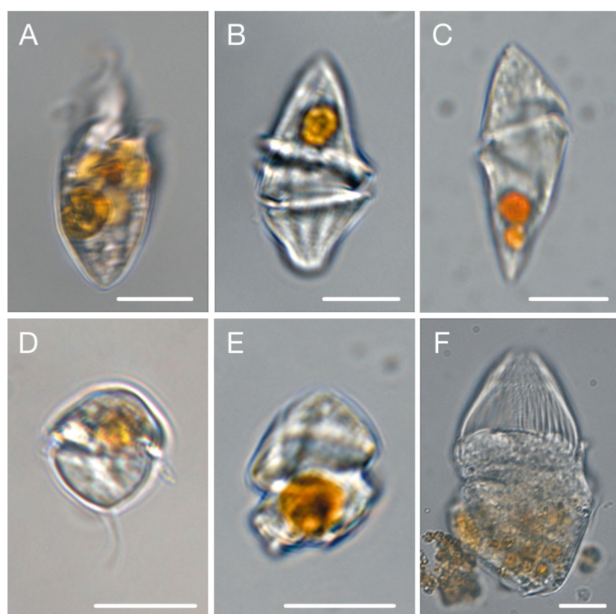

Fig. 2. Feeding by (A–E) heterotrophic dinoflagellates and (F) a ciliate on *Azadinium* cf. *poporum*. (A) *Oxyrrhis marina* with several ingested *A.* cf. *poporum* cells, (B) *Gyrodinium dominans* with an ingested *A.* cf. *poporum* cell, (C) *Gyrodinium moestrupii* with 2 ingested *A.* cf. *poporum* cells, (D) *Pfiesteria piscicida* with an ingested *A.* cf. *poporum* cell, (E) *Gyrodiniellum shiwhaense* with an ingested *A.* cf. *poporum* cell, (F) *Strobilidium* sp. with several ingested *A.* cf. *poporum* cells. All photographs were taken by means of an inverted microscope using Zeiss AxioCam MRc5 digital camera at a magnification of 630×. Scale bars = 10 µm

Growth rate

The growth rate of *Oxyrrhis marina* on *Azadinium* cf. *poporum* rapidly increased up to ca. 5.2×10^3 cells ml^{-1} (669 ng C ml^{-1}), but became saturated at the higher prey concentrations (Fig. 3A). When the data were fitted to Eq. (2), the maximum growth rate (μ_{max}) of *O. marina* on *A.* cf. *poporum* was 0.497 d^{-1} and the threshold prey concentration for the growth of the predator was 4 cells ml^{-1} (0.509 ng C ml^{-1}). The prey concentration sustaining half of μ_{max} was 619 cells ml^{-1} (79.4 ng C ml^{-1}).

The growth rate of *Strobilidium* sp. on *Azadinium* cf. *poporum* rapidly increased up to ca. 4.6×10^3 cells ml^{-1} (590 ng C ml^{-1}), but became saturated at the higher prey concentrations (Fig. 3B). When the data were fitted to Eq. (2), the μ_{max} of *Strobilidium* sp. on *A.* cf. *poporum* was 0.636 d^{-1} and the threshold prey concentration for the growth of the predator was 1.4×10^3 cells ml^{-1} (185 ng C ml^{-1}). The prey concentration sustaining half of μ_{max} was 2.1×10^3 cells ml^{-1} (268 ng C ml^{-1}).

Ingestion rate

The ingestion rate of *Oxyrrhis marina* on *Azadinium* cf. *poporum* increased rapidly with mean prey concentration up to ca. 5.2×10^3 cells ml^{-1} (669 ng

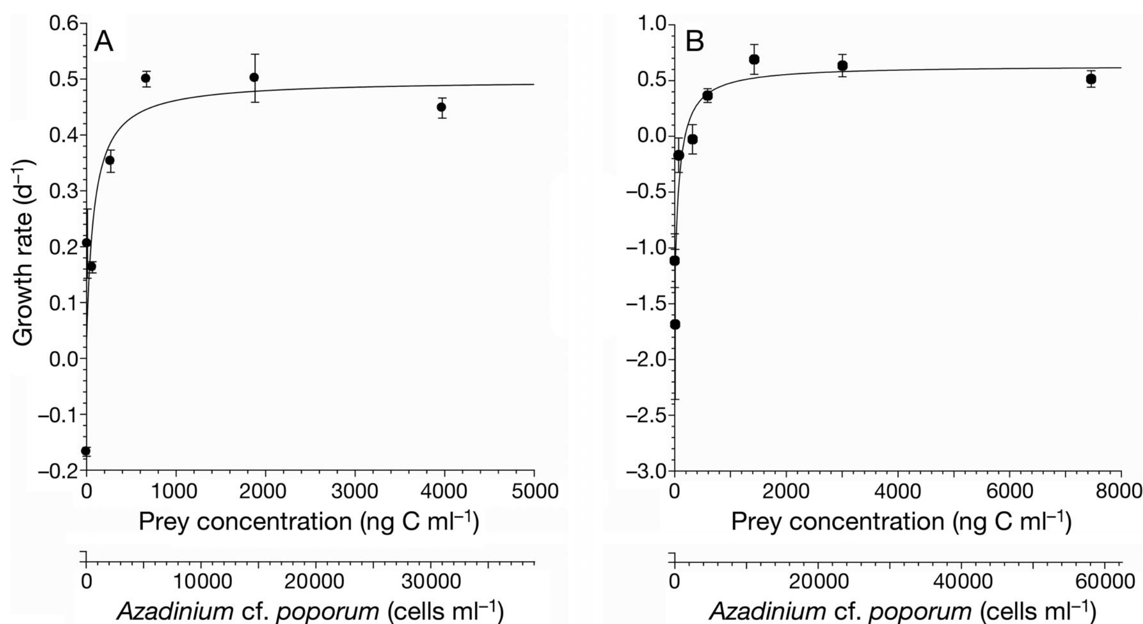

Fig. 3. *Oxyrrhis marina* and *Strobilidium* sp. Specific growth rate (μ, d^{-1}) of (A) the heterotrophic dinoflagellate and (B) a ciliate feeding on the dinoflagellate *Azadinium* cf. *poporum* as a function of mean prey concentration (X). Symbols represent treatment means ± 1 SE. A Michaelis-Menten equation (Eq. 2) was used to produce curves for (A) and (B) for all treatments in the experiments. (A) $\mu = 0.497(X - 0.509)/[79.4 + (X - 0.509)]$, $r^2 = 0.776$; (B) $\mu = 0.636(X - 185)/[268 + (X - 185)]$, $r^2 = 0.744$

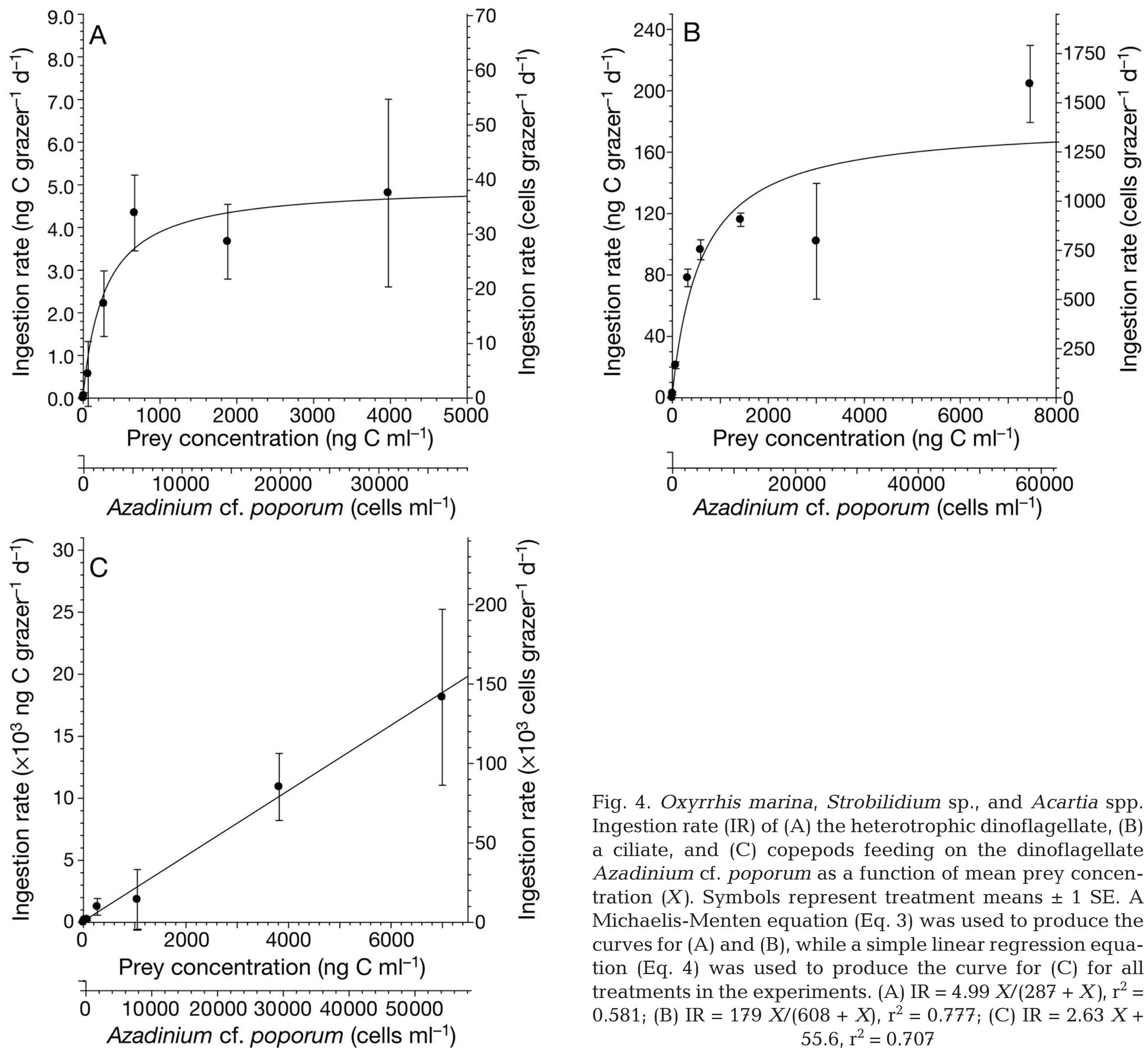

Fig. 4. *Oxyrrhis marina*, *Strobilidium* sp., and *Acartia* spp. Ingestion rate (IR) of (A) the heterotrophic dinoflagellate, (B) a ciliate, and (C) copepods feeding on the dinoflagellate *Azadinium* cf. *poporum* as a function of mean prey concentration (X). Symbols represent treatment means ± 1 SE. A Michaelis-Menten equation (Eq. 3) was used to produce the curves for (A) and (B), while a simple linear regression equation (Eq. 4) was used to produce the curve for (C) for all treatments in the experiments. (A) IR = 4.99 X/(287 + X), r^2 = 0.581; (B) IR = 179 X/(608 + X), r^2 = 0.777; (C) IR = 2.63 X + 55.6, r^2 = 0.707

C ml^{-1}), but became saturated at higher concentrations (Fig. 4A). When the data were fitted to Eq. (3), the maximum ingestion rate (I_{max}) of *O. marina* on *A.* cf. *poporum* was 39 cells predator^{-1} d^{-1} (4.99 ng C predator^{-1} d^{-1}). The prey concentration sustaining half of I_{max} was 2.2 × 10^3 cells ml^{-1} (287 ng C ml^{-1}). The maximum clearance rate of *O. marina* on *A.* cf. *poporum* was 8.85 µl predator^{-1} d^{-1}.

The ingestion rate of *Strobilidium* sp. on *Azadinium* cf. *poporum* increased rapidly with mean prey concentration up to ca. 4.6 × 10^3 cells ml^{-1} (590 ng C ml^{-1}). The ingestion rate continued to increase until the maximum prey concentration was reached at 5.8 × 10^4 cells ml^{-1} (7.5 × 10^3 ng C ml^{-1}), but at a slower rate (Fig. 4B). When the data were fitted to Eq. (3), the I_{max} of *Strobilidium* sp. on *A.* cf. *poporum* was 1.4 × 10^3 cells predator^{-1} d^{-1} (179 ng C predator^{-1} d^{-1}). The prey concentration sustaining half of I_{max} was 4.8 × 10^3 cells ml^{-1} (608 ng C ml^{-1}). The maximum clearance rate of *Strobilidium* sp. on *A.* cf. *poporum* was 346 µl predator^{-1} d^{-1} .

The ingestion rate of *Acartia* spp. on *Azadinium* cf. *poporum* increased linearly up to ca. 5.5 × 10^4 cells ml^{-1} (7.0 × 10^3 ng C ml^{-1}), the maximum prey concentration used (Fig. 4C). When the data were fitted to Eq. (4), the I_{max} of *Acartia* spp. at the maximum prey concentration tested was 1.4 × 10^5 cells predator^{-1} d^{-1} (1.9 × 10^4 ng C predator^{-1} d^{-1}). The maximum clearance rate of *Acartia* spp. on *A.* cf. *poporum* was 8.4 ml predator^{-1} d^{-1}.

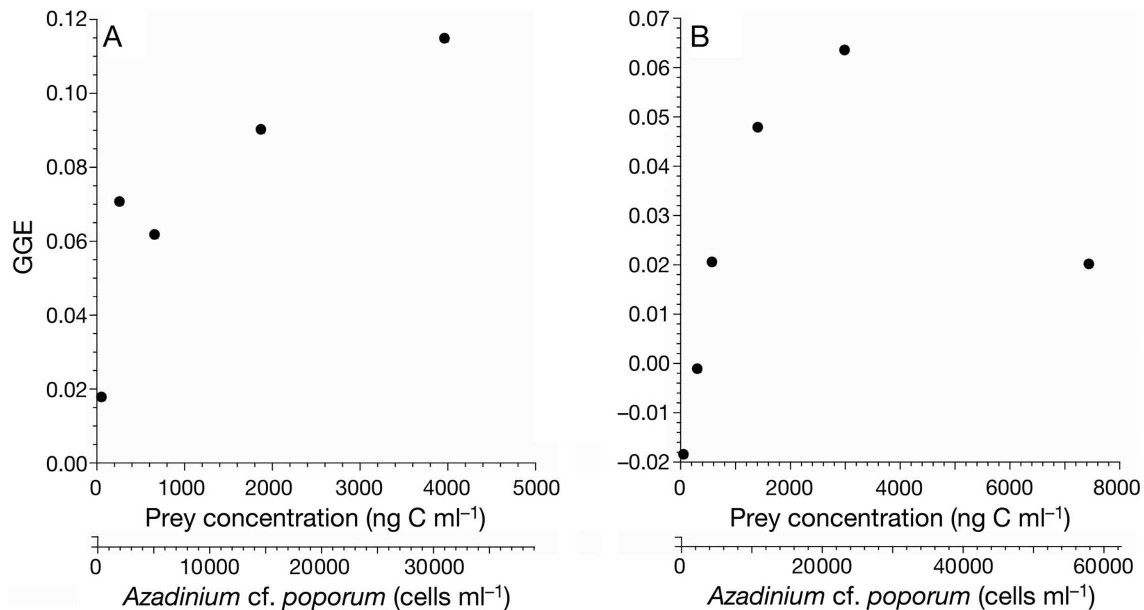

Fig. 5. *Oxyrrhis marina* and *Strobilidium* sp. Mean gross growth efficiencies (GGEs) of (A) the heterotrophic dinoflagellate and (B) a ciliate on *Azadinium* cf. *poporum* as a function of mean prey concentration. The GGEs at the first mean prey concentration are not illustrated since they were highly negative

Gross growth efficiency

The mean GGEs by predator–prey combination of *Oxyrrhis marina* on *Azadinium* cf. *poporum* generally increased with the mean prey concentrations. The GGEs were 6 to 11% at the prey concentrations for which ingestion rate was saturated (Fig. 5A). The mean GGEs by predator–prey combination of *Strobilidium* sp. on *A.* cf. *poporum* generally increased from the first mean prey concentration to 2.4×10^4 cells ml^{-1} (3.01×10^3 ng C ml^{-1}), the mean prey concentration preceding the last. However, *Strobilidium* sp. underwent a decrease of GGE at the last mean prey concentration (5.8×10^4 cells ml^{-1}, 7.5×10^3 ng C ml^{-1}), likely caused by incomplete digestion, a phenomenon referred as 'superfluous feeding' (Straile 1997). The GGEs were 2 to 6% at prey concentrations $\geq 4.6 \times 10^3$ cells ml^{-1} (590 ng C ml^{-1}; Fig. 5B).

Dynamics of *Azadinium* cf. *poporum*

The 36 mo field survey conducted in Shiwha Bay showed that *Azadinium* cf. *poporum* cell concentrations were generally low (Fig. 6). The DNA of *A.* cf. *poporum* was detected each year of the survey. *A.* cf. *poporum* reached its highest concentration in September 2009 at 5.2 cells ml^{-1}.

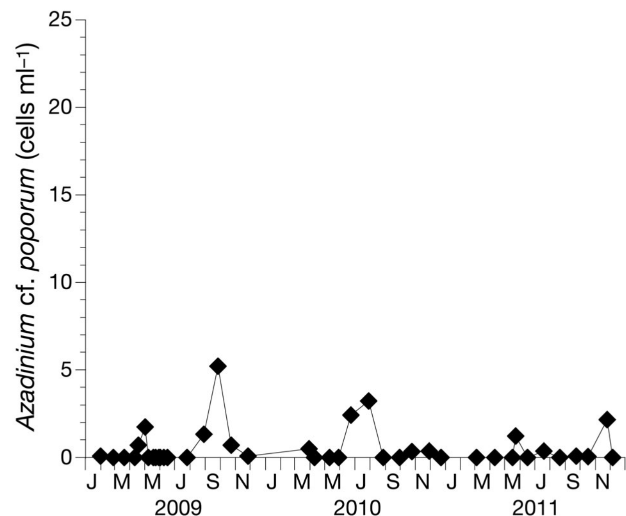

Fig. 6. *Azadinium* cf. *poporum.* Dynamics of abundances quantified by real-time PCR in Shiwha Bay, Korea, from 2009 to 2011. The highest values obtained at each sampling date are reported. The presence of ice prevented sampling in some months

Grazing impact

The grazing impact of *Oxyrrhis marina* was not possible to assess with the data provided by this study, since *Azadinium* cf. *poporum* and *O. marina* were not found to co-occur. However, *Strobilidium*

sp.-sized naked ciliates (25 to 60 µm in length) were observed to co-occur with *A.* cf. *poporum* in Shiwha Bay, Korea, between January 2009 and December 2011. Grazing coefficients were variable in a narrow range of concentrations for *A.* cf. *poporum* (0.2 to 3.2 cells ml^{-1}) and *Strobilidium* sp.-sized naked ciliates (0.1 to 1.0 cells ml^{-1}). Assuming that the ingestion rates of all *Strobilidium* sp.-sized naked ciliates on *A.* cf. *poporum* were the same as that of *Strobilidium* sp. obtained in this study, the grazing coefficients ranged between 0.052 and 0.446 d^{-1} (n = 7; Fig. 7).

Swimming speed

The average (±SE, n = 30) and maximum swimming speeds of *Azadinium* cf. *poporum*, excluding the short and high-speed 'jumps' in various directions, at the given conditions were 416 (± 13) and 550 µm s^{-1}, respectively.

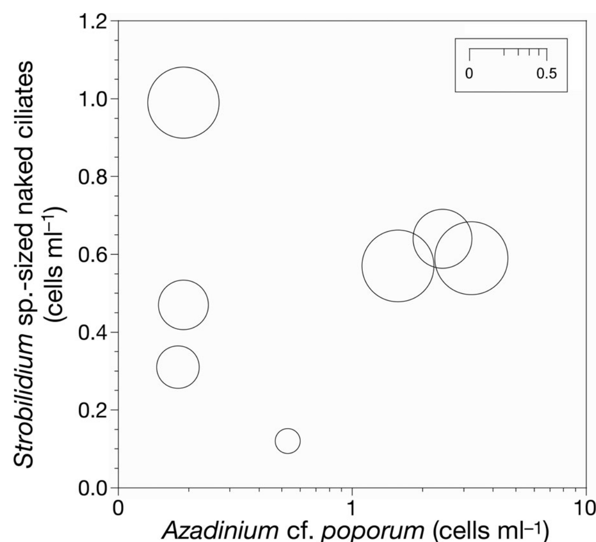

Fig. 7. *Strobilidium* sp.-sized naked ciliates (25 to 60 µm in length). Calculated grazing coefficients of the ciliates (n = 7) in relation to the concentration of co-occurring *Azadinium* cf. *poporum* (see Eqs. 5 & 6 for calculation). Clearance rates were corrected with a Q_{10} = 2.8 (Hansen et al. 1997) because *in situ* water temperatures and the experimental temperature used in the laboratory (20°C) were sometimes different. Inset shows the scale of the circles that represent grazing coefficients (g, d^{-1})

DISCUSSION

Feeding

This study is the first extensive report of feeding by HTDs, ciliates, and copepods on a species of the genus *Azadinium*. The engulfment feeders used in this study are raptorial feeders (Fenchel 1987, Sleigh 1989). These predators usually prefer prey approaching their size (Hansen et al. 1994). While the biggest engulfment feeder, *Polykrikos kofoidii*, did not feed on *Azadinium* cf. *poporum*, *Gyrodinium* spp. were of intermediate size and occasionally fed on *A.* cf. *poporum*. *Oxyrrhis marina* was the smallest engulfment feeder used in this study and revealed to be the most efficient on *A.* cf. *poporum*. Therefore, size is likely to be an important factor affecting the feeding of predators using engulfment on *Azadinium* spp. A particular case is *G. fusus*, which was previously suggested to be an important grazer on *A.* cf. *spinosum* (Akselman & Negri 2012). Akselman & Negri (2012) claimed that *G. fusus* is able to develop a hyaline cytoplasmic extension or feeding veil from its hypocone that reaches up to twice its length. This particularity was not observed with *Gyrodinium* spp. in our study and might explain the feeding efficiency of *G. fusus* on small prey such as *A.* cf. *spinosum* despite the difference in size. While size appears to be a relevant factor in feeding on *A.* cf. *poporum* for engulfment feeders, speed is unlikely to be a critical factor. For example, *P. kofoidii* is known to feed on prey of similar speed (e.g. *Gymnodinium catenatum* mean speed: 450 µm s^{-1}, maximum speed: 615 µm s^{-1}; Yoo et al. 2010, this study).

Peduncle feeders can be highly specialized, such as *Stoeckeria algicida* which is only known to feed on *Heterosigma akashiwo* among algal prey (Jeong et al. 2005b, 2011a, this study). However, these predators are usually generalists and are able to feed on a wide range of dinoflagellates, but are restricted in size regarding thecal dinoflagellates. Peduncle feeders do not seem to have peduncles that are strong enough to efficiently penetrate the theca of dinoflagellates. Therefore, the theca might explain the absence of growth by both *Pfiesteria piscicida* and *Gyrodiniellum shiwhaense* despite the occurrence of feeding. Peduncle feeders are therefore unlikely to be effective predators on *Azadinium* spp.

The pallium feeder *Oblea rotunda* was not able to feed on *Azadinium* cf. *poporum*. *O. rotunda* responds to chemosensory, but not to mechanosensory, stimulation (Strom & Buskey 1993). Therefore, the lack of chemosensory stimulation from *A.* cf. *poporum* might explain the lack of feeding by *O. rotunda*. *P. crassipes*, for which AZA-1, AZA-2, and AZA-3 have been detected (James et al. 2003), is likely to feed on *Azadinium* spp. or closely related species. Therefore,

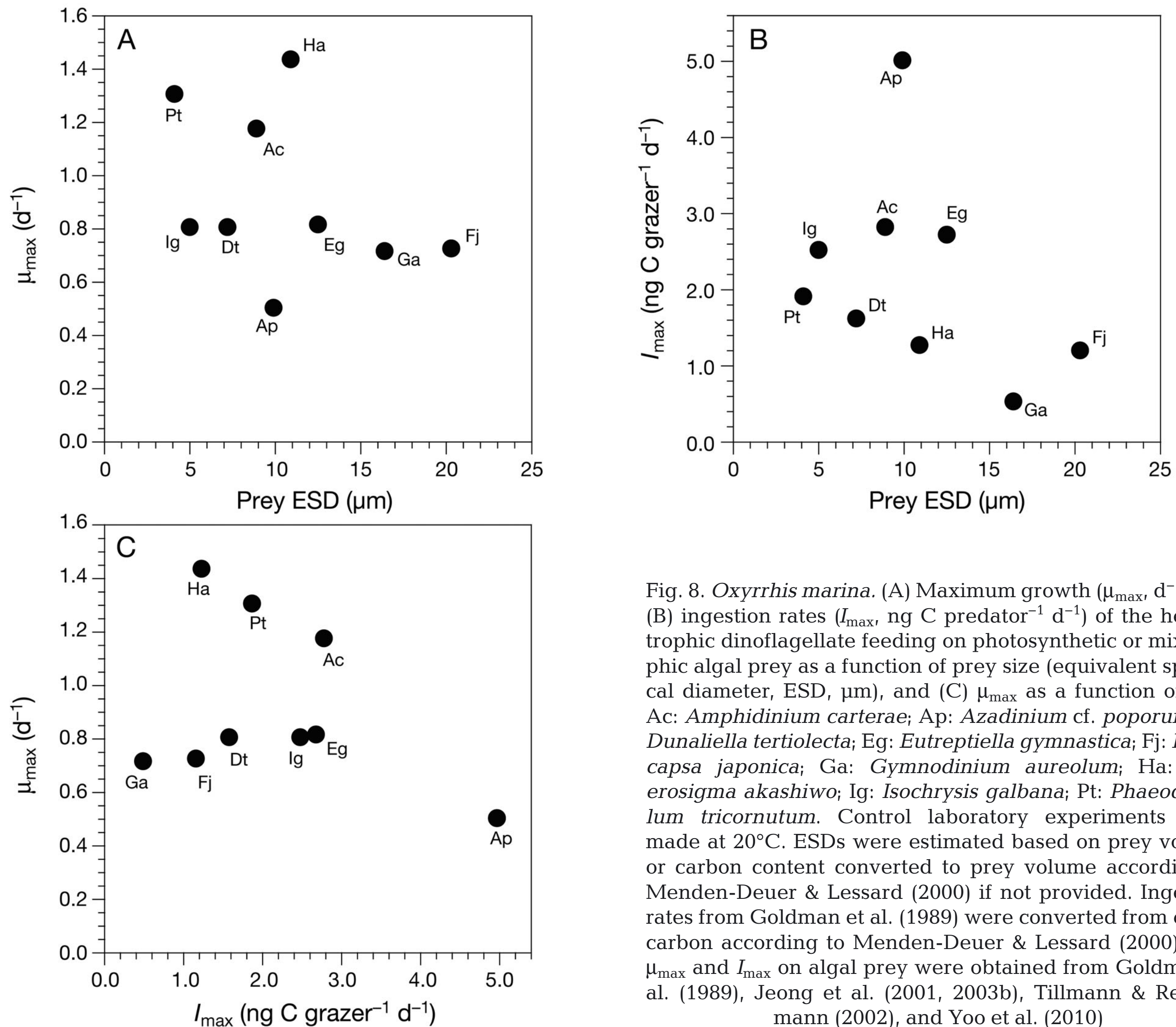

Fig. 8. *Oxyrrhis marina.* (A) Maximum growth (μ_{max}, d^{-1}) and (B) ingestion rates (I_{max}, ng C predator^{-1} d^{-1}) of the heterotrophic dinoflagellate feeding on photosynthetic or mixotrophic algal prey as a function of prey size (equivalent spherical diameter, ESD, µm), and (C) μ_{max} as a function of I_{max}. Ac: *Amphidinium carterae*; Ap: *Azadinium* cf. *poporum*; Dt: *Dunaliella tertiolecta*; Eg: *Eutreptiella gymnastica*; Fj: *Fibrocapsa japonica*; Ga: *Gymnodinium aureolum*; Ha: *Heterosigma akashiwo*; Ig: *Isochrysis galbana*; Pt: *Phaeodactylum tricornutum*. Control laboratory experiments were made at 20°C. ESDs were estimated based on prey volume or carbon content converted to prey volume according to Menden-Deuer & Lessard (2000) if not provided. Ingestion rates from Goldman et al. (1989) were converted from cell to carbon according to Menden-Deuer & Lessard (2000). The μ_{max} and I_{max} on algal prey were obtained from Goldman et al. (1989), Jeong et al. (2001, 2003b), Tillmann & Reckermann (2002), and Yoo et al. (2010)

it is worthwhile to study feeding by pallium feeders on *Azadinium* spp. further in order to assess the potential of such predators.

The filter feeders *Strobilidium* sp. and *Acartia* spp. were both able to feed on *Azadinium* cf. *poporum*. However, not all filter feeders are likely to feed on *Azadinium* spp. The ciliate *Laboea strobila* as well as 3 unidentified oligotrich ciliate species were previously observed to co-occur with *A.* cf. *spinosum*. Among them, only 1 oligotrich ciliate was suggested to feed (Akselman & Negri 2012). The facility of filter feeders to capture prey of smaller size (Hansen et al. 1994) might partly explain their success. However, more factors are apparently implicated in their capacity to feed on *Azadinium* spp.

Based on the results of the present study, the dynamics of HTDs that engulf their prey and are not disproportionately larger than *Azadinium* spp., as well as ciliates which use filtration, are more likely to be affected by the occurrence of *Azadinium* spp. than HTDs that feed using their peduncle.

Growth and ingestion rates

The μ_{max} of the predators of *Azadinium* cf. *poporum* were the lowest among known photosynthetic or mixotrophic prey enabling growth (Figs. 8A & 9A; Goldman et al. 1989, Montagnes 1996, Jeong et al. 2001, 2003b, Tillmann & Reckermann 2002, Chen et al. 2010, Yoo et al. 2010), while the I_{max} values were the highest (Figs. 8B, 9B, & 10; Houde & Roman 1987, Goldman et al. 1989, Jeong et al. 2001, 2003b, Besiktepe & Dam 2002, Tillmann & Reckermann 2002, Broglio et al. 2003, Cohen et al. 2007, Chen et al. 2010, Yoo et al. 2010). The low ratios of maximum

Fig. 9. *Strobilidium* spp. (A) Maximum growth (μ_{max}, d^{-1}) and (B) ingestion rates (I_{max}, ng C predator^{-1} d^{-1}) of the ciliates feeding on photosynthetic or mixotrophic algal prey as a function of prey size (equivalent spherical diameter, ESD, µm) and (C) μ_{max} as a function of I_{max}. Ap: *Azadinium* cf. *poporum*; Cs: *Chroomonas salina*; Ig: *Isochrysis galbana*; Ns: *Nannochloropsis* sp. Rates were corrected with a Q_{10} = 2.8 (Hansen et al. 1997) if control laboratory experiments were made at a temperature different than 20°C. ESDs were estimated based on carbon content converted to prey volume according to Menden-Deuer & Lessard (2000) if not provided. The μ_{max} and I_{max} on algal prey were obtained from Chen et al. (2010) and Montagnes (1996)

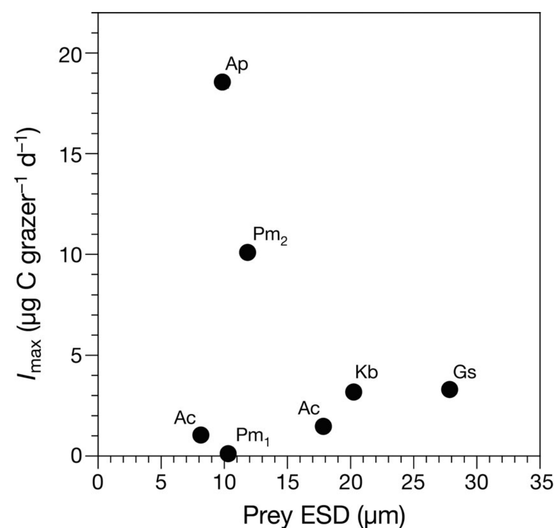

Fig. 10. *Acartia* spp. Maximum ingestion rate (I_{max}, µg C predator^{-1} d^{-1}) of the copepods on photosynthetic or mixotrophic algal prey as a function of prey size (equivalent spherical diameter, ESD, µm). Ac: *Amphidinium carterae**; Ap: *Azadinium* cf. *poporum**; Gs: *Gymnodinium sanguineum**; Kb: *Karenia brevis**; Pm_1* and Pm_2: *Prorocentrum minimum*. Rates were corrected with a Q_{10} = 2.8 (Hansen et al. 1997) if control laboratory experiments were unambiguously made at a temperature different than 20°C. ESDs were estimated based on carbon content converted to prey volume according to Menden-Deuer & Lessard (2000) if not provided. The I_{max} on algal prey were obtained from Houde & Roman (1987), Besiktepe & Dam (2002), Broglio et al. (2003), and Cohen et al. (2007). *Maximum value obtained

growth rate to maximum ingestion rate, or RMGI, and GGEs obtained by predators in this study suggest that *A.* cf. *poporum* is a low-quality prey. The energy necessary to capture, handle, and digest this prey is higher than for other prey species, or the conversion of ingested *A.* cf. *poporum* carbon to predator body carbon is low. This can lead the predators to compensate for the low quality of *A.* cf. *poporum* by feeding more and explains the high ingestion rates obtained in the laboratory experiments. *A.* cf. *poporum* is the only known photosynthetic or mixotrophic thecate prey species for *Oxyrrhis marina* and *Strobilidium* sp. for which we have quantitative data on growth and ingestion rates. The theca of *A.* cf. *poporum* might be partly responsible for its reduced quality as prey. It is also possible that the azaspiracid produced by *A.* cf. *poporum* (Krock et al. 2012) lowers its quality as prey.

Dynamics of *Azadinium* cf. *poporum*

The survey of *Azadinium* cf. *poporum* by real-time PCR revealed that the species is always in low concentration in Shiwha Bay. In this respect, it appears that *A.* cf. *poporum* acts similarly to *A. caudatum* (Nézan et al. 2012). The reasons explaining these observations are unknown. Predation on *A.* cf. *poporum* as well as the physiology of the species can result in such dynamics.

Grazing impact

The grazing coefficients obtained by using field data on abundance of *Strobilidium* sp.-sized naked ciliates (25 to 60 µm in length) co-occurring with *Azadinium* cf. *poporum* as well as laboratory data on ingestion rates suggest that up to 36% of the *A.* cf. *poporum* population could be removed by *Strobilidium* sp.-sized naked ciliates in 1 d. However, it is unclear whether such an impact is reflected in the field with a more complex microbial food web. The high ingestion rates obtained in laboratory experiments, as mentioned above, might be influenced by compensatory feeding. Furthermore, oligotrich ciliates are known to select food based on size (Kivi & Setälä 1995). However, ciliates have also shown selectivity at the species level (Stoecker et al. 1981, Verity 1988). Therefore, it is questionable to what extent the grazing impacts obtained in this study reflect what is actually happening in the field.

Ecological implications

Many predators are able to feed on *Azadinium* cf. *poporum*. However, only 2 predators used in this study responded with acute growth on *A.* cf. *poporum*. Furthermore, *A.* cf. *poporum* appears to be a low-quality prey for the planktonic predators tested here based on the RMGI and GGEs obtained. If this can be generalized at the genus level, it suggests that only a few predators are likely to feed efficiently on *Azadinium* spp. The abundances of *A.* cf. *poporum* in the field are revealed to be below or slightly above the threshold prey concentration of the predators. Therefore, *A.* cf. *poporum* is unlikely to affect the dynamics of these predators in the field. This is reflected by the absence or low co-occurrence of the protistan predators with *A.* cf. *poporum* over the 3 yr of monitoring. Factors other than predation are likely to be implicated in the determination of the abundance of *A.* cf. *poporum* in the field. Further research is required to determine these factors.

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