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Feeding by heterotrophic dinoflagellates on the common marine heterotrophic nanoflagellate *Cafeteria* sp.

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ABSTRACT: To investigate interactions between heterotrophic dinoflagellates (HTDs) and heterotrophic nanoflagellates (HNFs), we tested whether or not 5 HTDs (*Oxyrrhis marina*, *Gyrodinium* cf. *guttula*, *Pfiesteria piscicida*, *Luciella masanensis*, and *Stoeckeria algicida*) feed on the common HNF *Cafeteria* sp. and measured the growth and ingestion rates of *O. marina*, *G.* cf. *guttula*, and *P. piscicida* when feeding on *Cafeteria* sp. (equivalent spherical diameter = ca. 3.5 µm). We calculated grazing coefficients by combining field data on abundances of *O. marina* and *G.* cf. *guttula* and co-occurring HNFs with laboratory data on ingestion rates obtained in the present study. *O. marina*, *G.* cf. *guttula*, *P. piscicida*, and *L. masanensis* were able to ingest *Cafeteria* sp., while *S. algicida* did not feed on it. *Cafeteria* sp. supported positive growth in *O. marina* and *G.* cf. *guttula*, but not in *P. piscicida.* Specific growth rates of *O. marina* and *G.* cf. *guttula* on *Cafeteria* sp. increased with increasing mean prey concentration before becoming saturated at the mean prey concentration of ca. 100 ng C ml^{-1} (20 000 cells ml^{-1}). The maximum growth rates of *O. marina* and *G.* cf. *guttula* on *Cafeteria* sp. were 0.19 and 0.05 d^{-1}, respectively. With increasing mean prey concentration, the ingestion rates of *O. marina*, *G.* cf. *guttula*, and *P. piscicida* on *Cafeteria* sp. rapidly increased at mean prey concentrations <100 ng ml^{-1} (ca. 20 000 cells ml^{-1}), but slowly increased at higher mean prey concentrations. The maximum ingestion rate of *O. marina* on *Cafeteria* sp. (0.294 ng C $predator^{-1}$ d^{-1}) was much higher than that of *G.* cf. *guttula* (0.034 ng C $predator^{-1}$ d^{-1}) or *P. piscicida* (0.028 ng C $predator^{-1}$ d^{-1}). The maximum clearance rates were 0.18 to 0.54 µl $predator^{-1}$ h^{-1}. Mean grazing coefficients for *O. marina* and *G.* cf. *guttula* on co-occurring HNFs calculated in the present study were 2.88 and 0.42 d^{-1}, respectively. The results of the present study suggest that *O. marina* and *G.* cf. *guttula* sometimes have considerable grazing impacts on populations of HNFs.

KEY WORDS: Feeding · Food web · Growth · Ingestion · Protist · Protozoa

INTRODUCTION

Marine heterotrophic nanoflagellates (HNFs) are major components of marine planktonic food webs (e.g. Patterson & Larsen 1991). They are major predators of marine bacteria and occasionally control the populations of marine bacteria (Fenchel 1982, Azam et al. 1983, Sieburth 1984) and, in turn, they are prey for ciliates (e.g. Verity 1991). Therefore, they are known to play an important role in the transfer of bacteria to ciliates in marine microbial loops. However, feeding by heterotrophic protists other than ciliates on HNFs is poorly understood as yet.

Heterotrophic dinoflagellates (HTDs) are often abundant and ubiquitous protists in marine environments (e.g. Jeong 1999). They have diverse ecological

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roles in marine planktonic communities (Lessard 1991, Jeong 1995, 1999, Sherr & Sherr 2000, Tillmann & Reckermann 2002); HTDs are known to feed on a diverse array of prey species, such as heterotrophic bacteria, phytoplankton, mixotrophic dinoflagellates (Hansen 1992, Strom & Buskey 1993, Jeong & Latz 1994, Jeong et al. 2004b, 2005a, 2006, Kim & Jeong 2004, Tillmann 2004), heterotrophic protists, and metazoans through a variety of feeding mechanisms (Burkholder & Glasgow 1995, Hansen & Calado 1999, Jeong et al. 2005a). HTDs are also known to be important prey for several planktonic consumers such as ciliates (Jeong et al. 2004a) and copepods (Jeong et al. 2001a). They have also been found to participate in reciprocal predator–prey interactions with mixotrophic dinoflagellates (Jeong et al. 1997). Recently several HTD species such as *Pfiesteria piscicida*, *Pseudopfiesteria shumwayae*, *Stoeckeria algicida*, and *Luciella masanensis* (previously Lucy) have been newly discovered (Steidinger et al. 1996, Litaker et al. 2005, Jeong et al. 2005b, 2007, Mason et al. 2007). These heterotrophic dinoflagellates are harmful to finfish and shellfish due to their feeding on the blood cells and flesh of fish. Discovering new interactions among major components of marine plankton food webs is one of the most important steps in understanding the function of marine pelagic ecosystems. However, the feeding by HTDs on HNFs has not yet been explored, even though HTDs and HNFs often co-occur (e.g. Gonzalez et al. 1998).

To understand the interactions between HTDs and HNFs, we established monoclonal cultures of the HTDs *Oxyrrhis marina*, *Gyrodinium* cf. *guttula*, *Pfiesteria piscicida*, *Luciella masanensis*, and *Stoeckeria algicida* and the common HNF *Cafeteria* sp. and conducted a range of experiments. We (1) investigated whether or not the 5 HTDs, having a wide range of morphological properties (size, shape, thecate or naked, etc.) and feeding mechanisms, were able to feed on *Cafeteria* sp. and (2) measured the ingestion rates of *O. marina*, *G.* cf. *guttula*, and *P. piscicida* on the HNF as a function of the prey concentration. (3) The ingestion rates of HTDs on HNFs were compared to those of ciliates reported in the literature, and (4) they were also compared to HTDs on algal prey reported in the literature. (5) We also estimated the grazing coefficients attributable to *O. marina* and *G.* cf. *guttula* on co-occurring HNFs using our data for ingestion rates obtained from laboratory experiments and from the abundances of predators and prey in the field. The results of the present study provide a basis for understanding the interactions between HTDs and co-occurring HNFs and the potential for HTDs to influence the population dynamics of HNFs.

MATERIALS AND METHODS

Preparation of experimental organisms. For the isolation and culture of *Cafeteria* sp. (GenBank accession no. AM493687), plankton samples, collected with water samplers, were taken in Shiwha Bay, Korea, during October 2005, when the water temperature and salinity were 21.3°C and 24.5 psu, respectively. The samples were gently screened through a 5 µm Nitex mesh and placed in 80 ml polycarbonate (PC) bottles. Two barley seeds were added to each bottle to increase the abundance of bacteria that were potential prey for HNFs. The bottles were placed on plankton wheels rotating at 0.9 rpm and incubated at 20°C in darkness. A week later, aliquots of the enriched water were transferred to 6-well tissue culture plates and a monoclonal culture of *Cafeteria* sp. was established by 3 serial, single-cell isolations. Once dense cultures of *Cafeteria* sp. were obtained, they were transferred to 270 ml PC bottles of bacteria (density = ca. 10^6 cells ml^{-1}) and any aggregated materials were removed by screening every 3 d. Experiments were conducted when a large volume of *Cafeteria* sp. culture was available.

For the isolation and culture of *Oxyrrhis marina*, plankton samples, collected with a 25 cm diameter, 25 µm mesh plankton net, were taken from the mouth of the Keum Estuary, Kunsan, Korea, during May 2001, when the water temperature and salinity were 16.0°C and 27.7 psu, respectively. A monoclonal culture was established by 2 serial single-cell isolations, as in Jeong et al. (2003).

For the isolation and culture of *Gyrodinium* cf. *guttula*, plankton samples, collected with water samplers, were taken from coastal waters off Masan, Korea, during April 2003, when the water temperature and salinity were 18.5°C and 25 psu, respectively. A monoclonal culture of *G.* cf. *guttula* was established by 2 serial, single-cell isolations.

For the isolation and culture of *Pfiesteria piscicida*, plankton samples, collected with water samplers, were taken from coastal waters off Incheon, Korea, during July 2005, when the water temperature and salinity were 24.0°C and 25.4 psu, respectively. A monoclonal culture of *P. piscicida* was established by 2 serial, single-cell isolations, as in Jeong et al. (2006).

For the isolation and culture of *Stoeckeria algicida*, plankton samples, collected with water samplers, were taken from a pier in Masan Bay, Korea, during July 2004, when the water temperature and salinity were 24.8°C and 20.6 psu, respectively. A monoclonal culture of *S. algicida* was established by 2 serial, single-cell isolations, as in Jeong et al. (2005a).

For the isolation and culture of *Luciella masanensis*, plankton samples, collected with water samplers, were

taken from a pier in Masan Bay, Korea, during April 2005, when the water temperature and salinity were 16.0°C and 28.0 psu, respectively. A monoclonal culture of *L. masanensis* was established by 2 serial, single-cell isolations, as in Jeong et al. (2007).

The carbon contents for *Cafeteria* sp. (0.005 ng C $cell^{-1}$, n = 30) were estimated from cell volume according to Menden-Deuer & Lessard (2000).

Feeding occurrence. These experiments were designed to test whether or not each of the HTDs *Oxyrrhis marina*, *Gyrodinium* cf. *guttula*, *Pfiesteria piscicida*, *Luciella masanensis*, and *Stoeckeria algicida* was able to feed on an HNF.

HNFs were fluorescently labeled using dichlorotriazinylaminofluorescein (DTAF) (fluorescently labeled HNF [FLH]; Sherr et al. 1987). Approximately 8×10^6 FLH cells were added into each of two 80 ml PC bottles containing each HTD at 1000 cells ml^{-1}. One control bottle (without FLH) was set up for each experiment. The bottles were placed on a plankton wheel rotating at 0.9 rpm and incubated at 20°C under an illumination of 20 µE m^{-2} s^{-1} of cool white fluorescent light, on a 14 h light:10 h dark cycle.

After 30 min and 1, 6, and 24 h incubation, 5 ml aliquots were removed from each bottle, transferred into 20 ml vials, and then fixed with borate-buffered formalin (final conc. = 4%). The fixed samples were stained using DAPI (final conc. = 1 µM) and then filtered onto PC white membrane filters with a 5 µm pore size. Approximately 300 to 1000 concentrated cells on the membranes were observed using epifluorescence microscopy at a magnification of 1250×, and pictures were taken.

Growth and ingestion rates. These experiments were designed to measure growth, ingestion, and clearance rates of *Oxyrrhis marina*, *Gyrodinium* cf. *guttula*, and *Pfiesteria piscicida*, respectively, as functions of prey concentration, when feeding on *Cafeteria* sp.

One day before these experiments were conducted, a dense culture of *Oxyrrhis marina* (ca. 8000 cells ml^{-1}) growing on the raphidophyte *Heterosigma akashiwo* (ca. 30 000 cells ml^{-1}) was transferred into 270 ml PC bottles containing a low (5000 cells ml^{-1}) and a medium HNF concentration (15 000 cells ml^{-1}) of the target prey. This was done to acclimatize the predator to the target prey and to minimize possible residual growth resulting from ingestion of prey during batch culture. The bottles were filled to capacity with filtered seawater and placed on a rotating wheel to incubate as above. The abundances of *O. marina* and the prey were determined by enumerating cells in three 1 ml Sedgwick-Rafter counting chambers (hereafter SRCs).

The initial concentrations of *Oxyrrhis marina* and the HNF were established using an autopipette to deliver predetermined volumes of known cell concentrations to the bottles. Triplicate 80 ml PC experiment bottles (mixtures of predator and prey), triplicate prey control bottles (prey only), and triplicate predator control bottles (predator only) were set up for each predator–prey combination. To make the water conditions similar, some water from a predator culture was filtered through a GF/F filter (pore size = 0.7 µm) and then added into the prey control bottles in the same amount as the volume of the predator culture added into the predator control bottles and the experimental bottles for each predator–prey combination. Also, some water from a prey culture was filtered through a GF/F filter (pore size = 0.7 µm) and then added into the predator control bottles in the same amount as the volume of the prey culture added into the prey control bottles and the experimental bottles. Ten ml of *f*/2 medium were added to all bottles, which were then filled to capacity with freshly filtered seawater and capped. To determine the actual predator and prey densities (cells ml^{-1}) at the beginning of the experiment (*O. marina*/ *Cafeteria* sp. = 6/26, 17/59, 26/173, 41/609, 79/3730, 187/17 050, 236/44 690, 478/78 080; *Gyrodinium* cf. *guttula*/ *Cafeteria* sp. = 14/48, 40/153, 74/666, 103/1130, 274/4480, 459/11 120, 672/41 050, 713/67 210; *Pfiesteria piscicida*/*Cafeteria* sp. = 20/46, 66/145, 111/412, 271/943, 356/8300, 664/20 430, 862/42 980, 1537/63 630) and after 48 h incubation, a 3 ml aliquot for enumerating predator cells was removed from each bottle and fixed with 5% Lugol's solution, and a 7 ml aliquot for enumerating prey cells was removed from each bottle and fixed with 4% formalin. All (low predator concentration) or >300 (high predator concentrations) predator cells in the samples fixed with 5% Lugol's solution in two 1 ml SRCs were enumerated under a compound microscope. DAPI was added to the samples fixed with 4% formalin, which were then filtered by a 0.8 µm black PC membrane filter. Almost all or >300 prey cells on the filters were enumerated using epifluorescence microscopy. The bottles were filled again to capacity with freshly filtered seawater, capped, and placed on rotating wheels under the same environmental conditions as described above. Dilution of the cultures associated with refilling the bottles was considered in calculating growth and ingestion rates. The experiments for *Gyrodinium* cf. *guttula* and *Pfiesteria piscicida* were also conducted as described above after *G.* cf. *guttula* and *P. piscicida* were acclimated for 1 d as was done with *O. marina*.

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

$$\mu = \frac{\ln(L_t / L_0)}{t} \quad (1)$$

where L_0 is the initial concentration of the HTD; t is time in d; and L_t is the final concentration after 2 d.

Data for the growth rate of *Oxyrrhis marina* were fitted to a Michaelis-Menten equation:

$$\mu = \frac{\mu_{max}(x - x')}{K_{GR} + (x - x')} \quad (2)$$

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 $\mu = 0$), and K_{GR} is the prey concentration sustaining $\frac{1}{2}\,\mu_{max}$. Data were iteratively fitted to the model using DeltaGraph (Delta Point).

Ingestion and clearance rates were calculated using the equations by Frost (1972) and Heinbokel (1978). The incubation time for calculating ingestion and clearance rates was the same as for estimating the growth rate. Ingestion rate data for a HTD were fitted to a Michaelis-Menten equation:

$$IR = \frac{I_{max}(x)}{K_{IR} + (x)} \quad (3)$$

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}), and K_{IR} is the prey concentration sustaining $\frac{1}{2}\,I_{max}$.

Grazing impact. We estimated the grazing coefficients attributable to *Oxyrrhis marina* and *Gyrodinium* cf. *guttula* on HNFs by combining field data on the abundances of *O. marina* and *G.* cf. *guttula* and their prey with ingestion rates of the predators on *Cafeteria* sp. obtained in the present study. The data on the abundances of *O. marina* and HNFs (2 to 5 µm in size) used in this estimation were obtained from water samples collected from solar salterns near Shiwha, Korea (in 2006), while those for *G.* cf. *guttula* (plus *G. dominans*) and HNFs (2 to 5 µm in size) were obtained from the water samples taken in Masan Bay, Korea (in 2004 and 2005). For this estimation, we assumed that the ingestion rates of *O. marina* and *G.* cf. *guttula* on the HNFs (2 to 5 µm in size) were the same as those on *Cafeteria* sp. and that the ingestion rate of *G. dominans* was the same as that of *G.* cf. *guttula*.

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

$$g = CR \times PC \times 24 \quad (4)$$

where CR (ml HTD^{-1} h^{-1}) is the clearance rate of an HTD on HNF at a particular prey concentration and PC is the predator concentration (cells ml^{-1}). CRs were calculated as:

$$CR = IR/x \quad (5)$$

where IR (cells eaten HTD^{-1} h^{-1}) is the ingestion rate of the predator on the prey and x (cells ml^{-1}) is the prey concentration. 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 this experiment (20°C) were sometimes different.

RESULTS

HTD predators

Among the 5 HTDs tested, *Oxyrrhis marina*, *Gyrodinium* cf. *guttula*, *Luciella masanensis*, and *Pfiesteria piscicida* were able to ingest *Cafeteria* sp. (Fig. 1), but *Stoeckeria algicida* did not feed on the HNF. Up to 7 HNF cells were observed inside the protoplasm of *O. marina*.

Growth rates

Cafeteria sp. supported positive growth in *Oxyrrhis marina* and *Gyrodinium* cf. *guttula*, but not in *Pfiesteria piscicida* (Figs. 2 to 4).

Specific growth rates of *Oxyrrhis marina* on *Cafeteria* sp. increased with increasing mean prey concentration before becoming saturated at the mean prey concentration of ca. 100 ng C ml^{-1} (20 000 cells ml^{-1}; Fig. 2). The maximum specific growth rate of *O. marina* on *Cafeteria* sp. was 0.19 d^{-1} when the data were fitted to Eq. (2). The threshold prey concentration (where net growth = 0) for *O. marina* was 0.059 ng C ml^{-1} (12 cells ml^{-1}).

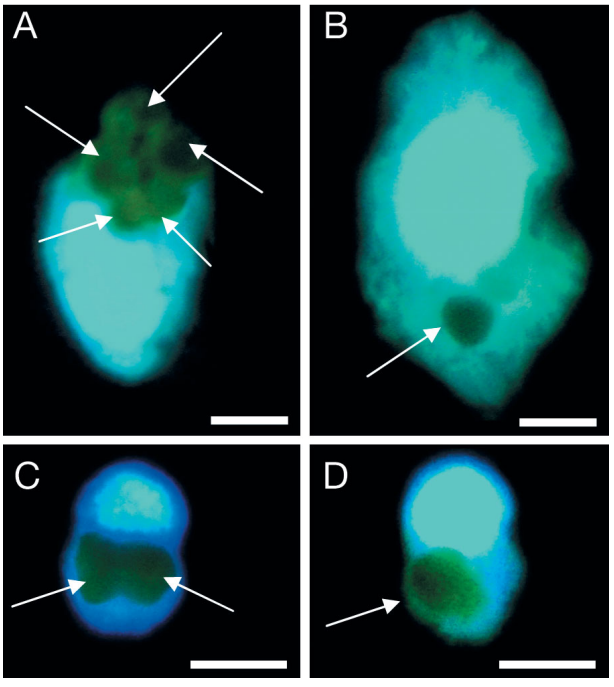

Fig. 1. Feeding by heterotrophic dinoflagellates on the heterotrophic nanoflagellate (HNF) *Cafeteria* sp. (A) *Oxyrrhis marina* with 5 ingested HNFs, (B) *Gyrodinium* cf. *guttula* with an ingested HNF, (C) *Pfiesteria piscicida* with 2 ingested HNFs, and (D) *Luciella masanensis* with an ingested HNF. Scale bars: 5 µm. Arrows indicate ingested prey cells, which are fluorescently labeled HNFs. All photographs were taken using epifluorescence microscopy

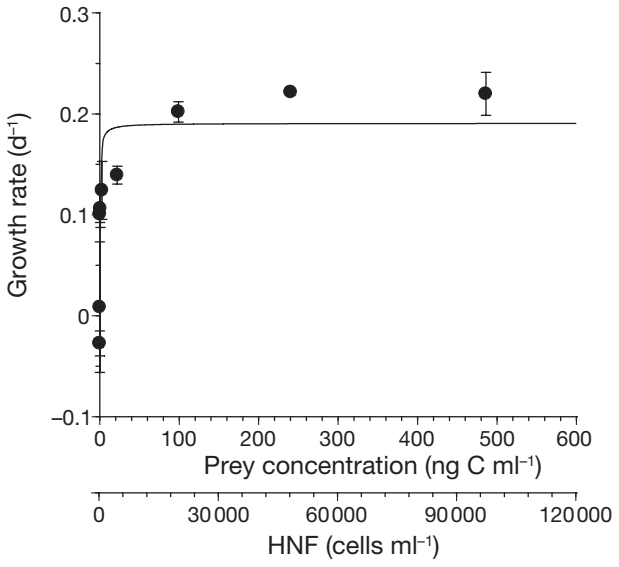

Fig. 2. *Oxyrrhis marina*. Specific growth rates of heterotrophic dinoflagellate (HTD) *O. marina* on heterotrophic nanoflagellate (HNF) *Cafeteria* sp. as a function of mean prey concentration (x). Symbols represent treatment means (±1 SE). The curve is fitted by a Michaelis-Menten equation (Eq. 2) using all treatments in the experiment. Growth rate (GR, d^{-1}) = 0.19 {(x – 0.059) / [0.48 + (x – 0.059)]}, r^2 = 0.744

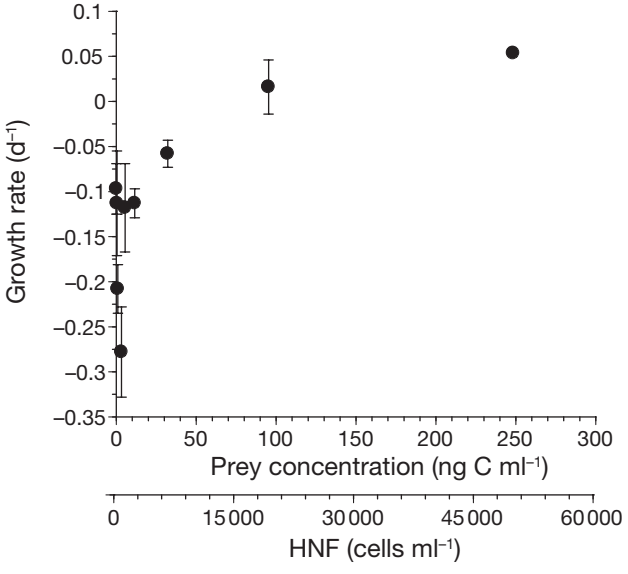

Fig. 3. *Gyrodinium* cf. *guttula*. Specific growth rates of *G.* cf. *guttula* (HTD) on *Cafeteria* sp. (HNF) as a function of mean prey concentration (x). Symbols represent treatment means (±1 SE)

Specific growth rates of *Gyrodinium* cf. *guttula* on *Cafeteria* sp. increased with increasing mean prey concentration before saturating at the mean prey concentration of ca. 100 ng C ml^{-1} (20 000 cells ml^{-1}; Fig. 3). At the given prey concentrations, the observed maximum growth rate of *G.* cf. *guttula* on *Cafeteria* sp. was 0.054 d^{-1}.

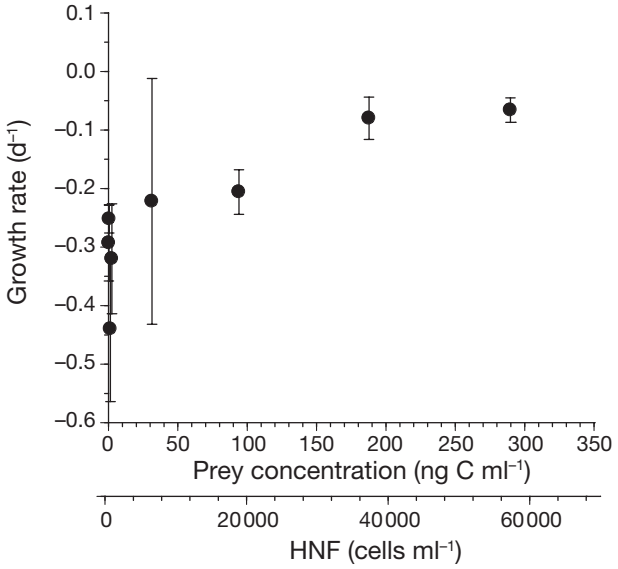

Fig. 4. *Pfiesteria piscicida*. Specific growth rates of *P. piscicida* (HTD) on *Cafeteria* sp. (HNF) as a function of mean prey concentration (x). Symbols represent treatment means (±1 SE)

Specific growth rates of *Pfiesteria piscicida* on *Cafeteria* sp. increased with increasing mean prey concentration. At the given prey concentrations, the observed maximum specific growth rate of *P. piscicida* on *Cafeteria* sp. was –0.08 d^{-1} (Fig. 4).

Ingestion and clearance rates

With increasing mean prey concentration, the ingestion rates of *Oxyrrhis marina*, *Gyrodinium* cf. *guttula*, and *Pfiesteria piscicida* on *Cafeteria* sp. rapidly increased at mean prey concentrations <100 ng ml^{-1} (ca. 20 000 cells ml^{-1}), but slowly increased at higher mean prey concentrations (Figs. 5 to 7). The maximum ingestion rates (cells $predator^{-1}$ d^{-1} in parentheses) of *O. marina*, *G.* cf. *guttula*, and *P. piscicida* on *Cafeteria* sp. were 0.294 (59), 0.034 (7), and 0.028 (6) ng C $predator^{-1}$ d^{-1}, respectively, when the data were fitted to Eq. (3). Gross growth efficiencies, defined as predator biomass produced or lost per prey biomass ingested, where the maximum growth and ingestion rates were obtained, were 23% for *O. marina* and 41% for *G.* cf. *guttula*.

The maximum clearance rates of HTDs on *Cafeteria* sp. were 0.54 µl $predator^{-1}$ h^{-1} for *Oxyrrhis marina*, 0.18 µl $predator^{-1}$ h^{-1} for *Gyrodinium* cf. *guttula*, and 0.39 µl $predator^{-1}$ h^{-1} for *Pfiesteria piscicida*.

Grazing impact

When the abundances of *Oxyrrhis marina* and HNFs (n = 19) in the high-salinity waters (salinity = 36.2 to

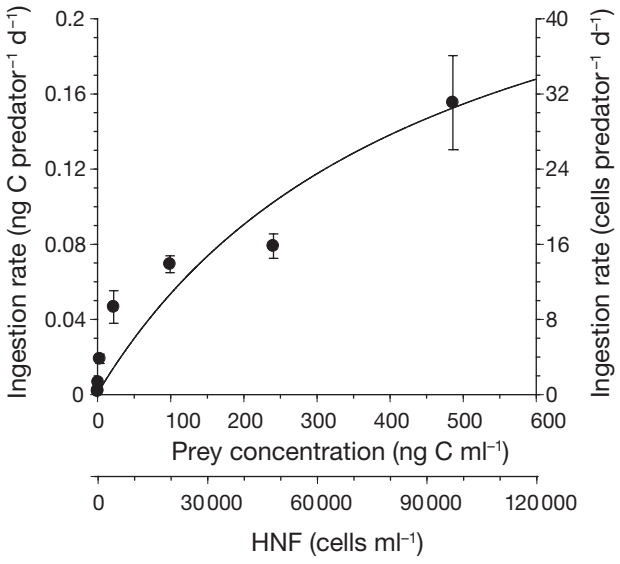

Fig. 5. *Oxyrrhis marina*. Ingestion rates of *O. marina* (HTD) on *Cafeteria* sp. (HNF) as a function of mean prey concentration (x). Symbols represent treatment means (±1 SE). The curve is fitted by a Michaelis-Menten equation (Eq. 3) using all treatments in the experiment. Ingestion rate (IR, ng C grazer^{-1} d^{-1}) = 0.294 [x / (455 + x)], r^2 = 0.874

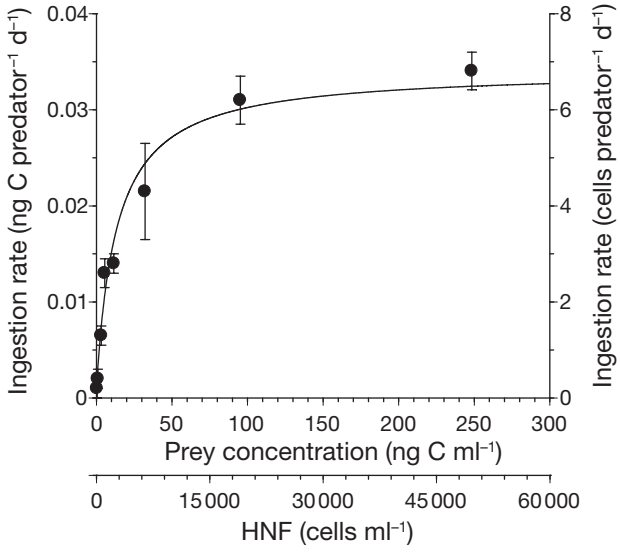

Fig. 6. *Gyrodinium* cf. *guttula*. Ingestion rates of *G.* cf. *guttula* (HTD) on *Cafeteria* sp. (HNF) as a function of mean prey concentration (x). Symbols represent treatment means (±1 SE). The curve is fitted by a Michaelis-Menten equation (Eq. 3) using all treatments in the experiment. Ingestion rate (IR, ng C grazer^{-1} d^{-1}) = 0.034 [x / (13 + x)], r^2 = 0.877

101 psu, mean ± SE = 50.2 ± 3.5 psu) of solar salterns near Shiwha in 2006 were 1 to 6130 cells ml^{-1} (mean ± SE = 2160 ± 420 cells ml^{-1}) and 1900 to 7080 cells ml^{-1} (4570 ± 330 cells ml^{-1}), respectively, the grazing coefficients attributable to *O. marina* on co-occurring HNFs

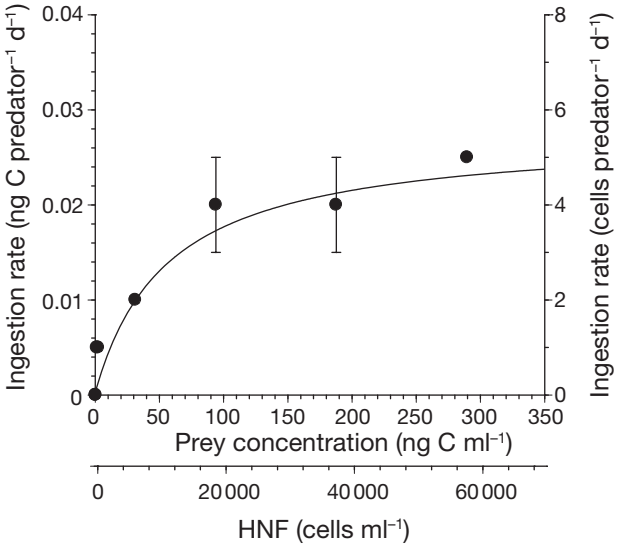

Fig. 7. *Pfiesteria piscicida*. Ingestion rates of *P. piscicida* (HTD) on *Cafeteria* sp. (HNF) as a function of mean prey concentration (x). Symbols represent treatment means (±1 SE). The curve is fitted by a Michaelis-Menten equation (Eq. 3) using all treatments in the experiment. Ingestion rate (IR, ng C grazer^{-1} d^{-1}) = 0.028 [x / (56 + x)], r^2 = 0.886

were 0.06 to 8.25 d^{-1} (mean ± SE = 2.88 ± 0.58 d^{-1}; Fig. 8A). In general, grazing coefficients increased with increasing *O. marina* concentration.

When the abundances of *Gyrodinium* cf. *guttula* (plus *G. dominans*) and HNFs (n = 27) in Masan Bay in 2004 and 2005 were 25 to 750 cells ml^{-1} (mean ± SE = 159 ± 37 cells ml^{-1}) and 66 to 1840 cells ml^{-1} (498 ± 82 cells ml^{-1}), respectively, the grazing coefficients attributable to *G.* cf. *guttula* on co-occurring HNFs were 0.02 to 2.17 d^{-1} (mean ± SE = 0.42 ± 0.11 d^{-1}; Fig. 8B). In general, grazing coefficients also increased with increasing *G.* cf. *guttula* concentration.

DISCUSSION

Predators on HNF

The present study shows that several HTD species (*Oxyrrhis marina*, *Gyrodinium* cf. *guttula*, *Luciella masanensis*, and *Pfiesteria piscicida*) are able to ingest the tested species of HNF. Before the present study, ciliates, rotifers, copepods, and cladocerans had been reported to ingest HNFs in aquatic ecosystems (Turner et al. 1988, Verity 1991, Sanders et al. 1994, Jürgens et al. 1996). Therefore, this is the first study reporting the feeding by HTDs on HNFs in aquatic ecosystems. These predator–prey relationships between HNFs and HTDs may influence our conventional view of energy flow and carbon cycling in the marine planktonic community.

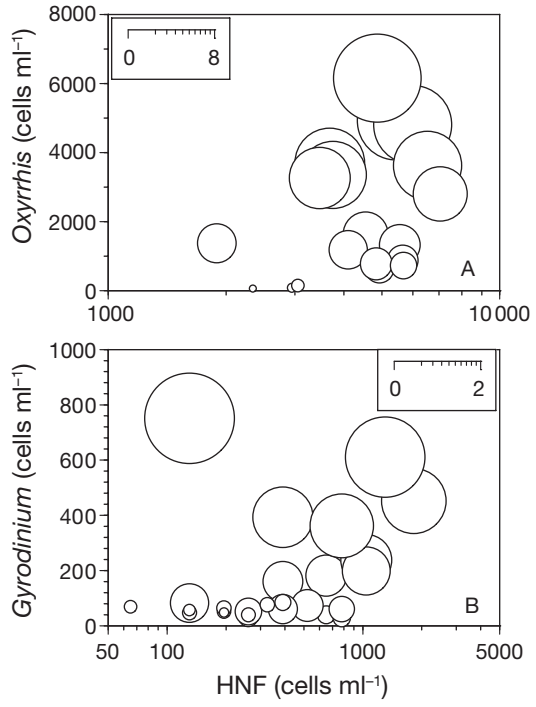

Fig. 8. Calculated grazing coefficients (g, d^{-1}, see scale bars) attributable to *Oxyrrhis marina* (A) and *Gyrodinium* cf. *guttula* (B) on HNFs (see 'Materials and methods' for calculation); n = 19 for *O. marina* and n = 27 for *G.* cf. *guttula*

HNFs and small ciliates are known to be one of the major predators on bacteria (Fenchel 1982, Azam et al. 1983, Sieburth 1984, Vaque et al. 2002). Recently, it has been shown that red-tide dinoflagellates and raphidophytes also feed on marine autotrophic and heterotrophic bacteria and have considerable grazing impact on the prey populations (Jeong et al. 2005c, Seong et al. 2006). Therefore, based on the results of this study and the literature, 4 possible major pathways from bacteria to ciliates in marine planktonic communities can be established: (1) bacteria–red-tide algae–HTDs–large ciliates (Jeong et al. 2001b, 2003, 2004a, 2005c, Seong et al. 2006); (2) bacteria–HNFs–HTDs–large ciliates (Vaque et al. 2002, Jeong et al. 2004a, present study); (3) bacteria–HNFs–ciliates (Verity 1991, Šolić & Krstulović 1994); and (4) bacteria–ciliates (Sherr et al. 1989, Seong et al. 2006). Discovery of the feeding by HTDs on HNFs increases our knowledge of the complexity of marine microbial food webs. HTDs are sometimes likely to compete with some ciliates for HNF prey in marine planktonic communities.

While *Pfiesteria piscicida* fed on *Cafeteria* sp., the *Pfiesteria*-like dinoflagellate (PLD) *Stoeckeria algicida* did not ingest the HNF. *S. algicida* was revealed to only feed on the raphidophyte *Heterosigma akashiwo* and fish blood cells, while *P. piscicida* fed on diverse species of dinoflagellates, raphidophytes, and cryptophytes, as well as on fish blood cells (Jeong et al. 2006). The results of the present study confirm that these 2 HTDs have different responses to prey species, even though their size, shape, and feeding mechanisms are very similar. Therefore, *P. piscicida* and some PLDs may have different ecological niches in marine ecosystems.

Growth and ingestion

Cafeteria sp. supported the positive growth of *Oxyrrhis marina* and *Gyrodinium* cf. *guttula*, while it did not support that of *Pfiesteria piscicida*. The maximum growth rate (MGR) of *O. marina* on *Cafeteria* sp. (0.19 d^{-1}) was higher than that of *G. cf. guttula* (0.05 d^{-1}) or *P. piscicida* (–0.08 d^{-1}). The higher maximum ingestion rate (MIR) of *O. marina* (0.29 ng C predator^{-1} d^{-1}) seemed to be responsible for this higher MGR compared to that of *G.* cf. *guttula* (0.034 ng C predator^{-1} d^{-1}) or of *P. piscicida* (0.028 ng C predator^{-1} d^{-1}). Deploying a tow filament and then engulfing a swimming HNF cell (*O. marina*) may be a more efficient feeding mechanism than directly engulfing the prey cell (*G.* cf. *guttula*) or sucking materials from the prey using a peduncle (*P. piscicida*). In natural environments, *O. marina* is expected to be one of the most abundant HTDs when HNFs are abundant, but alternative prey is rare. However, *G.* cf. *guttula* and *P. piscicida* do not seem to be abundant under these circumstances.

There had been only a single study reporting the growth and/or ingestion rates of marine protistan predators on HNFs before the present study (Verity 1991). MGR of *Oxyrrhis marina* on *Cafeteria* sp. is considerably lower than that of the naked ciliate *Strobilidium spiralis* (0.49 d^{-1}; Table 1). MIR of *S. spiralis* on *Cafeteria* sp. (1.1 ng C predator^{-1} d^{-1}) is also 3 to 4 times higher than that of *O. marina*. The much larger size of *S. spiralis* may be responsible for its higher MIR compared to that of *O. marina*. Based on this comparison of MGRs, the abundance of *O. marina* is expected to be lower than that of *S. spiralis* when *Cafeteria* sp. is abundant and alternative prey is rare.

MGR and MIR of *Oxyrrhis marina* on *Cafeteria* sp. (0.19 d^{-1} and 0.29 ng C predator^{-1} d^{-1}, respectively) are much lower than those of *O. marina* on the raphidophytes *Heterosigma akashiwo* and *Fibrocapsa japonica*, the dinoflagellate *Amphidinium carterae*, or the diatom *Phaeodactylum tricornutum* (0.7 to 1.4 d^{-1} and 1.3 to 7.0 ng C predator^{-1} d^{-1}; Table 2). Therefore, for *O. marina*, the algae may be better prey than HNFs and thus HNFs are likely to be supplementary prey when both the algal prey and HNFs are abundant. However, when the abundance of the algal prey is low, but that of HNFs is high, HNFs may be a major prey item. Data from studies on the feeding of *O. marina* on the HNF and algal prey

listed in Table 2 suggest that either the MGR or MIR of *O. marina* on the 7 different prey species does not correlate significantly with prey size (equipment spherical diameter, ESD) ($p > 0.1$, linear regression ANOVA). This relationship suggests that the sizes of prey may not be a critical factor affecting the growth and ingestion rates of *O. marina*. *Strobilidium spiralis* is known to ingest less *Cafeteria* sp. than *Isochrysis galbana* as well (Verity 1991). Therefore, *Cafeteria* sp. may be a less preferred prey for HTDs and ciliates compared to the algal prey. The C:N ratio of *I. galbana* (6.7) is slightly lower than that of *Cafeteria* sp. (7.1; Verity 1991), and thus their nutritional values may be similar. In our preliminary test, the mean (± SE, n = 30) swimming speed of *Cafeteria* sp. (106 ± 3 µm s^{-1}) measured at 20°C was twice that of *I. galbana* (47 ± 3 µm s^{-1}). *O. marina* may have more difficulty in capturing, handling, and ingesting faster swimming *Cafeteria* cells than slower swimming *I. galbana* cells.

Grazing impact

In the waters of the solar salterns near Shiwha in 2006, 73% (mean) of co-occurring HNF populations were removed by *Oxyrrhis marina* populations in 1 d. Also, in Masan Bay in 2004 and 2005, 34% (mean) of HNF populations was removed by *Gyrodinium* cf. *guttula* (plus *G. dominans*) populations in 1 d. Therefore, the results of the present study suggest that *O. marina* and *G.* cf. *guttula* (plus *G. dominans*) can have a considerable grazing impact on populations of HNFs. Also, the grazing pressure by HTDs on bacterivorous HNFs may affect the population dynamics of marine bacteria, as HNFs occasionally control the populations of marine bacteria (Fenchel 1982, Sieburth 1984). There have been few studies reporting the grazing impact of marine ciliates on HNFs (Šolić & Krstulović 1994); ciliates have been reported to remove 20 to 130% (mean = 57%) of HNF standing stock in a day in the coastal Adriatic Sea. Thus, the grazing impact of HTDs may sometimes be comparable to that by ciliates. However, *Cafeteria* sp. was the less preferred prey for *Strobilidium spiralis* when a mixture of the photosynthetic nanoplankton *Isochrysis galbana* and *Cafeteria* sp. was provided as prey (Verity 1991). Therefore, the grazing coefficients attributable to *O. marina* on co-occurring HNFs and those attributable to *G.* cf. *guttula* might be overestimated if preferred algal prey co-existed.

Table 1. Comparison of growth, ingestion, and clearance rates of heterotrophic protists on the marine heterotrophic nanoflagellates *Cafeteria* sp. (3.5 µm in size) measured at 20°C. PDC: predators' carbon (ng C $cell^{-1}$); μ_{max}: maximum growth rate (d^{-1}); I_{max}: maximum ingestion rate (ng C $predator^{-1}$ d^{-1}); C_{max}: maximum clearance rate (µl $predator^{-1}$ h^{-1}); HTD: heterotrophic dinoflagellate; NC: naked ciliate; TC: tintinnid ciliate

Predator		PDC	μ_{max}	I_{max}	C_{max}	Source
Pfiesteria piscicida	(HTD)	0.05	–0.08	0.028	0.39	Present study
Oxyrrhis marina	(HTD)	0.14	0.19	0.294	0.54	Present study
Gyrodinium cf. *guttula*	(HTD)	0.35	0.05	0.034	0.18	Present study
Strobilidium cf. *spiralis*	(NC)	3.5	0.49[a]	1.1[a]		Verity (1991)
Tintinnopsis dadayi	(TC)	15	0.28[a]			Verity (1991)

[a]Obtained at mean prey concentrations of 250 to 260 ng C ml^{-1}

Ecological importance

The feeding of HTDs on HNFs may be important in marine planktonic communities in the following ways: (1) HTDs are another important protistan predator on HNFs. In general ciliates grow fast when suitable prey is abundant, but die quickly during periods of starvation (Jeong et al. 1999). However, HTDs can survive much longer than ciliates during periods of starvation (Jeong & Latz 1994). Therefore, the pathway from bacteria to HTDs via HNFs may work more frequently in

Table 2. *Oxyrrhis marina*. Comparison of growth, ingestion, and clearance rates of *O. marina* on diverse protistan prey. Rates were corrected to 20°C using $Q_{10} = 2.8$ (Hansen et al. 1997). ESD: equivalent spherical diameter (µm); μ_{max}: maximum growth rate (d^{-1}); I_{max}: maximum ingestion rate (ng C $predator^{-1}$ d^{-1}); C_{max}: maximum clearance rate (µl $predator^{-1}$ h^{-1}); HNF: heterotrophic nanoflagellate; DIA: diatom; PRY: prymnesiophyte; CHL: chlorophyte; MTD: mixotrophic dinoflagellate; RAP: raphidophyte

Prey		ESD	μ_{max}	I_{max}	C_{max}	Source
Cafeteria sp.	(HNF)	3.5	0.19	0.3	0.53	Present study
Phaeodactylum tricornutum	(DIA)	4.2	1.30	2.6	0.002	Goldman et al. (1989)
Isochrysis galbana	(PRY)	4.8	0.79	7.0	0.02	Goldman et al. (1989)
Dunaliella tertiolecta	(CHL)	7.3	0.79	1.4	0.01	Goldman et al. (1989)
Amphidinium carterae	(MTD)	9.7	1.17	2.8	2.35	Jeong et al. (2001a)
Heterosigma akashiwo	(RAP)	11.5	1.43	1.3	0.29	Jeong et al. (2003)
Fibrocapsa japonica	(RAP)	20.3	1.20	2.0		Tillmann & Reckermann (2002)

comparison to a pathway from bacteria to ciliates via HNFs. (2) HNFs support the growth of some HTDs, but do not support that of other HTDs. Therefore, HNFs may play an important role in determining dominant HTD species when HNFs are abundant, and alternative prey is rare. (3) In the present study, large portions of HNFs in Korean waters were removed by HTDs in a single day, and thus some HTDs are sometimes likely to control the populations of HNFs. Therefore, HTDs should be taken into consideration as an important factor affecting the population dynamics of HNFs. To understand interactions and transfer of materials and energy among bacteria, HNFs, HTDs, ciliates, and metazooplankton in marine planktonic food webs better, it would be worthwhile to explore the contribution in terms of predation pressure of co-occurring HTDs, ciliates, and metazooplankton on HNFs.

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