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Feeding by heterotrophic protists on the toxic dinoflagellate *Ostreopsis* cf. *ovata*

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ABSTRACT

Ostreopsis cf. *ovata* is a toxic dinoflagellate with a wide distribution, from tropical to temperate waters. This species is primarily an epiphytic benthic dinoflagellate, but it also lives in the water column and sometimes forms harmful red tides. The taxonomy, physiology, and distribution of *O.* cf. *ovata* have been extensively investigated, and toxin production by this species is well documented. However, data regarding potential predators of this toxic dinoflagellate are lacking. In particular, no studies of heterotrophic protistan grazers have been conducted. To investigate feeding by the heterotrophic dinoflagellates (HTDs) and a ciliate on *O.* cf. *ovata*, whether the common HTDs *Gyrodinium dominans*, *Gyrodinium moestrupii*, *Gyrodinium spirale*, *Oxyrrhis marina*, *Pfiesteria piscicida*, *Polykrikos kofoidii*, *Protoperidinium bipes*, and *Stoeckeria algicida* and the naked ciliate *Strobilidium* sp. are able to feed on *O.* cf. *ovata* was tested. In addition, the growth and ingestion rates of *G. moestrupii* and *P. kofoidii* on *O.* cf. *ovata* as a function of prey concentration were measured because this prey supported positive growth of only these two predators. Furthermore, these growth and ingestion rates were compared with those on the other algal prey for exploring comparative nutritional value of this prey. *G. dominans*, *G. moestrupii*, *O. marina*, *P. piscicida*, and *P. kofoidii* were able to feed on *O.* cf. *ovata*; in contrast, *G. spirale*, *P. bipes*, *S. algicida*, and *Strobilidium* sp. were unable to feed on this prey species. The maximum growth rates of *G. moestrupii* and *P. kofoidii* on *O.* cf. *ovata* were 0.86 and 0.73 day^{-1}, respectively, while the maximum ingestion rates were 6.2 and 33.3 ng C predator^{-1} day^{-1}, respectively. The maximum ingestion rates of *G. moestrupii* and *P. kofoidii* on *O.* cf. *ovata* were higher than the previously reported values for these two predators on any other dinoflagellate prey; on the other hand, the maximum specific growth rates of *G. moestrupii* and *P. kofoidii* feeding on *O.* cf. *ovata* were intermediate to the previously reported values for these two predators on any other dinoflagellate prey. With the exception of the small dinoflagellate *Prorocentrum minimum*, the maximum swimming speed of *O.* cf. *ovata* was lower than that of any other dinoflagellate prey. The results of the present study suggest that *O.* cf. *ovata* is an easily edible prey because of its slow swimming speed; however, this species is not a nutritional prey for growth of *G. moestrupii* or *P. kofoidii*.

1. Introduction

Ostreopsis spp. are primarily benthic dinoflagellates with a diverse range of habitats (Faust et al., 1996; Pocsidio and Dimaano, 2004; Penna et al., 2005; Aligizaki and Nikolaidis, 2006; Kim et al., 2011; Laza-Martinez et al., 2011). However, they have frequently been observed in the water column (Nikolaides and Moustaka-Gouni, 1990; MacKenzie, 1991; Faust et al., 1996; Chang et al., 2000, 2003; Gallitelli et al., 2005; Totti et al., 2010; Accoroni et al., 2011; Vila et al., 2012; Gadea et al., 2013; Godrijan et al., 2013; Fani et al., 2014; Reñé et al., 2014). Gallitelli et al. (2005) observed *Ostreopsis* populations blooming with high abundance (>1000 cells ml^{-1}) in the water column off the coast of Bari, southern Italy.

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Ostreopsis ovata is the most commonly found species in the genus *Ostreopsis*. However, there is considerable debate regarding the taxonomy of *O. ovata* for the following reasons: (1) strains have been found in various locations; (2) marked intra-specific variation exists among these strains; and (3) these strains have a very wide size range. In contrast, the shape and plate pattern of these strains show very little variation. Many published literature reports regarding the morphology of *O. ovata* lack supporting genetic characterization. Thus, the name *O.* cf. *ovata* has been proposed for *O. ovata* (e.g., Penna et al., 2010).

Ostreopsis cf. *ovata* (previously *O. ovata*) is a toxic dinoflagellate that is known to produce diverse toxins such as putative palytoxin (pPLTX), ovatoxins (OVTXs), and Ostreol A (Ciminiello et al., 2006, 2008, 2012; Hwang et al., 2013). This species has a wide distribution, from tropical to temperate waters (e.g., Kang et al., 2013). *O.* cf. *ovata* is a primary epiphytic benthic dinoflagellate, but it also lives in the water column and sometimes forms red tides (e.g., Guerrini et al., 2010; Totti et al., 2010). Ciminiello et al. (2006) reported that the abundance of *O. ovata* during a red tide in the coastal waters of Genoa, Italy was 1800 cells ml^{-1}. Totti et al. (2010) subsequently reported the occurrence of *O. ovata* in the water column at all the stations of the Conero Riviera, located in the northern Adriatic Sea; during the period of bloom, the maximum abundance of this species was 25.2 cells ml^{-1} in the water column and 13,500 cells ml^{-1} in the re-suspended mat. *Ostreopsis* spp. is generally known to become abundant when they are re-suspended because of stormy weather (e.g., Totti et al., 2010). This variation in cell abundance of a dinoflagellate species is a function not only of its growth rate, but also of its mortality rate caused by predation. Therefore, the bloom dynamics of this species are affected by its predators. The growth dynamics of *O.* cf. *ovata* have been extensively investigated, but data regarding the potential predators of this toxic dinoflagellate are lacking; in particular, no studies of heterotrophic protistan grazers have been conducted (e.g., Pistocchi et al., 2011; Nascimento et al., 2012; Scalco et al., 2012; Furlan et al., 2013; Tanimoto et al., 2013).

Heterotrophic dinoflagellates (HTDs) and ciliates are major components of marine ecosystems (Porter et al., 1985;Stoecker and Capuzoo, 1990; Jeong, 1999; Jeong et al., 2010b; Yoo et al., 2013a). These organisms are ubiquitous and some genera are cosmopolitan (Lessard, 1984; Rublee et al., 2004; Jeong et al., 2010b). Many HTDs and ciliates are known to be effective grazers on a diverse range of algal prey, and they sometimes control prey populations (Watras et al., 1985; Jeong and Latz, 1994; Tillmann, 2004; Jeong et al., 2010b, 2014, 2015; Yoo et al., 2010, 2013c). Some HTD species are known to feed on toxic algal prey; on the other hand, many HTDs and most ciliates do not feed on toxic algal prey (Hansen, 1989; Matsuyama et al., 1999; Jeong et al., 2001a, 2003, 2007; Yoo et al., 2013b). Thus, the presence and absence of algal prey toxins influences feeding by many HTDs and ciliates. The ability of some species to feed on toxic prey may be an evolutionary trait.

Recently, *Ostreopsis* cf. *ovata* was isolated from the coastal waters of Jeju Island, Korea. A clonal culture of this strain was established and was shown to produce the newly described toxin Ostreol A (Hwang et al., 2013). This toxic dinoflagellate may be a source of differential feeding by HTDs and ciliates. In the present study, the feeding capabilities of the common HTDs *Gyrodinium dominans*, *Gyrodinium moestrupii*, *Gyrodinium spirale*, *Oxyrrhis marina*, *Pfiesteria piscicida*, *Polykrikos kofoidii*, *Protoperidinium bipes*, and *Stoeckeria algicida*, and the naked ciliate *Strobilidium* sp. on *O.* cf. *ovata* were investigated. In addition, the growth and ingestion rates of *G. moestrupii* and *P. kofoidii* as a function of prey concentration were determined. The nutritional value of *O.* cf. *ovata* as prey was also evaluated by comparing these growth and ingestion rates with those previously reported in the literature for *G. moestrupii* and *P. kofoidii* on other algal prey. Finally, the swimming speed of *O.* cf. *ovata* was determined and compared this with the data previously reported in the literature for other dinoflagellate prey.

The findings in the present study provide a basis for understanding the interactions between *Ostreopsis* spp. and heterotrophic protists and the population dynamics of these species in marine food webs.

2. Materials and methods

2.1. Preparation of experimental organisms

For the isolation and culture of *Ostreopsis* cf. *ovata*, macroalgal samples (*Gelidium amansii*) were collected by divers at a depth of ca. 3 m from waters off Chaguido, Jeju Island, Korea. The samples were collected during May 2008, when the water temperature and salinity were 18.6 °C and 31.2, respectively. A clonal culture of *O.* cf. *ovata* was established by using two serial single-cell isolations (GenBank accession number HE793379; Kang et al., 2013). As the concentration of *O.* cf. *ovata* increased, the culture was transferred to 50-ml, 125-ml, and 500-ml polycarbonate (PC) bottles containing fresh f/2-Si seawater medium. The bottles were filled to capacity with freshly filtered seawater, capped, and incubated at 20 °C under an illumination intensity of 20 $\mu E\ m^{-2}\ s^{-1}$ with cool white fluorescent light and a 14-h light:10-h dark cycle. When dense cultures of *O.* cf. *ovata* were obtained, the cells were transferred at approximately 3-week intervals to new 500-ml PC bottles containing fresh f/2-Si seawater media before the feeding experiments were conducted.

For the isolation and culture of the HTD predators *Gyrodinium dominans*, *Gyrodinium moestrupii*, *Gyrodinium spirale*, *Oxyrrhis marina*, *Polykrikos kofoidii*, *Pfiesteria piscicida*, *Stoeckeria algicida*, and *Protoperidinium bipes*, plankton samples were collected by using water samplers, from the coastal waters off Masan, Saemankeum, Keum River Estuary, Incheon or Shiwha, Korea during 2001–2010. A clonal culture of each species was established by using two serial single-cell isolations (Table 1).

For the isolation and culture of the ciliate *Strobilidium* sp. (cell length, 30–40 μm), plankton samples were collected by using a water sampler, from the waters of Shiwha Bay, Korea. The samples were collected during August 2011, when the water temperature and salinity were 27.0 °C and 15.0, respectively (Table 1). A clonal culture of *Strobilidium* sp. was established by using two serial single-cell isolations.

The carbon contents of *Ostreopsis* cf. *ovata* (2.29 ng C per cell, $n = 50$), the investigated HTDs, and the ciliate were estimated from the cell volume, according to the procedure of Menden-Deuer and Lessard (2000). The cell volumes of the predators were estimated using the methods of Kim and Jeong (2004) and Yoon et al. (2012) for *Gyrodinium dominans*, *Gyrodinium moestrupii*, and *Gyrodinium spirale*; Jeong et al. (2008) for *Oxyrrhis marina*; Jeong et al. (2001b) for *Polykrikos kofoidii*; Jeong et al. (2007) for *Pfiesteria piscicida* and *Stoeckeria algicida*; Jeong et al. (2004) for *Protoperidinium bipes*; and Jeong et al. (2011) for *Strobilidium* sp.

2.2. Feeding capability

Experiment 1 was designed to examine the feeding capabilities of *Gyrodinium dominans*, *Gyrodinium moestrupii*, *Gyrodinium spirale*, *Oxyrrhis marina*, *Pfiesteria piscicida*, *Polykrikos kofoidii*; *Protoperidinium bipes*, *Stoeckeria algicida*, and *Strobilidium* sp. on *Ostreopsis* cf. *ovata* (Table 2).

Approximately, 1.6×10^5 *Ostreopsis* cf. *ovata* cells were added to each of two 80-ml PC bottles containing *Gyrodinium spirale* or *Polykrikos kofoidii* (100–150 cells ml^{-1}). Next, each of the other