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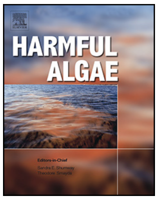

# Interactions between the mixotrophic dinoflagellate *Takayama helix* and common heterotrophic protists

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ABSTRACT

The phototrophic dinoflagellate *Takayama helix* that is known to be harmful to abalone larvae has recently been revealed to be mixotrophic. Although mixotrophy elevates the growth rate of *T. helix* by 79%–185%, its absolute growth rate is still as low as 0.3 $d^{-1}$. Thus, if the mortality rate of *T. helix* due to predation is high, this dinoflagellate may not easily prevail. To investigate potential effective protistan grazers on *T. helix*, feeding by diverse heterotrophic dinoflagellates such as engulfment-feeding *Oxyrrhis marina*, *Gyrodinium dominans, Gyrodinium moestrupii, Polykrikos kofoidii*, and *Noctiluca scintillans*, peduncle-feeding *Aduncodinium glandula, Gyrodiniellum shiwhaense, Luciella masanensis*, and *Pfiesteria piscicida,* pallium-feeding *Oblea rotunda* and *Protoperidinium pellucidum,* and the naked ciliates *Pelagostrobilidium* sp. (ca. 40 μm in cell length) and *Strombidinopsis* sp. (ca. 150 μm in cell length) on *T. helix* was explored. Among the tested heterotrophic protists, *O. marina*, *G. dominans*, *G. moestrupii, A. glandula*, *L. masanensis*, *P. kofoidii*, *P. piscicida*, and *Strombidinopsis* sp. were able to feed on *T. helix*. The growth rates of all these predators except *Strombidinopsis* sp. with *T. helix* prey were lower than those without the prey. The growth rate of *Strombidinopsis* sp. on *T. helix* was almost zero although the growth rate of *Strombidinopsis* sp. with *T. helix* prey was higher than those without the prey. Moreover, *T. helix* fed on *O. marina* and *P. pellucidum* and lysed the cells of *P. kofoidii* and *G. shiwhaense*. With increasing the concentrations of *T. helix*, the growth rates of *O. marina* and *P. kofoidii* decreased, but those of *G. dominans* and *L. masanensis* largely did not change. Therefore, reciprocal predation, lysis, no feeding, and the low ingestion rates of the common protists preying on *T. helix* may result in a low mortality rate due to predation, thereby compensating for this species' low growth rate.

## 1. Introduction

Dinoflagellates are ubiquitous protists in marine environments and occasionally form red tides or harmful algal blooms (Bockstahler and Coats, 1993a, 1993b; Stoecker, 1999; Adolf et al., 2008; Hall et al., 2008; Menden-Deuer and Montalbano, 2015; Wolny et al., 2015; Jeong et al., 2017b). In the last three decades, many dinoflagellates have been revealed to be mixotrophic (i.e., grow through a combination of feeding and photosynthesis) (e.g., Stoecker, 1999; Burkholder et al., 2008; Jeong et al., 2010, 2015). Mixotrophic dinoflagellates play diverse ecological roles in marine planktonic communities (Smalley et al., 1999; Seong et al., 2006; Stoecker et al., 2006; Carvalho et al., 2008; Jeong et al., 2012; Lim et al., 2014). They are predators on diverse prey items (Bockstahler and Coats, 1993a; Burkholder et al., 2008; Yoo et al., 2009; Jeong et al., 2005b, 2008b; Nishitani et al., 2008; Lee et al., 2014c; Johnson, 2015) and are themselves important prey items for other mixotrophic dinoflagellates (Skovgaard, 1996; Jeong et al., 2005a; Lee et al., 2014a, 2016), heterotrophic dinoflagellates (Hansen, 1992; Jacobson and Anderson, 1996; Johnson et al., 2003; Adolf et al., 2007), ciliates (Kamiyama and Matsuyama, 2005; Jeong et al., 2008a), and several varieties of metazooplankton (Stoecker and Sanders, 1985; Turner and Tester, 1997; Turner and Borkman, 2005). Owing to their diverse and important roles in marine ecosystems, studies have suggested that dinoflagellates form one of the major functional groups (e.g., Mitra et al., 2016). Therefore, to understand the roles of mixotrophic dinoflagellates in marine ecosystems, it is important to understand their prey items and predators among co-occurring plankton, and the growth, ingestion, and mortality rates caused by predation of

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the species implied in the predator–prey relationships of the targeted dinoflagellates (e.g., Jeong et al., 2015).

Heterotrophic protists are major microzooplankton in marine ecosystems and play important roles in marine planktonic food webs (Sherr and Sherr, 1994, 2016; Jeong, 1999; Calbet and Landry, 2004; Calbet et al., 2009; Yoo et al., 2013b; Lee et al., 2014b; Turner, 2014; Petitpas et al., 2015; Jang et al., 2016). Heterotrophic protists such as heterotrophic dinoflagellates and ciliates have been revealed as effective grazers on many mixotrophic dinoflagellates (Jeong et al., 2001; Tillmann, 2004; John et al., 2015). Furthermore, the grazing impacts by heterotrophic protists on populations of mixotrophic dinoflagellates are sometimes high enough to control prey populations (e.g., Yoo et al., 2013a). Therefore, mortality due to predation by heterotrophic protists should be studied to understand mixotrophic dinoflagellate dynamics in marine ecosystems.

The species in the genus *Takayama* are phototrophic dinoflagellates belonging to the family Kareniaceae (de Salas et al., 2003). To date, 7 species have been established in this genus (de Salas et al., 2003, 2008; Gu et al., 2013), including *T. acrotrocha* (previously *Gyrodinium acrotrochum*), *T. cladochroma* (*G. cladochroma*), *T. helix*, *T. pulchella* (*Gymnodinium pulchellum*), *T. tasmanica*, *T. tuberculata*, and *T. xiamenensis*. These dinoflagellates form blooms in the waters of many countries, such as Australia, Italy, Japan, New Zealand, Singapore, and USA (Maclean, 1979; Onoue et al., 1985; Larsen, 1994; Steidinger et al., 1998; Zingone et al., 2006; Tang et al., 2012; Leong et al., 2015). Furthermore, their blooms often cause fish kills (Maclean, 1979; Onoue et al., 1985; Larsen, 1994; Steidinger et al., 1998; Mooney et al., 2007; Leong et al., 2015). Thus, the bloom dynamics of *Takayama* spp. are of critical concern to scientists, government officials, and those dependent on the aquaculture and tourism industries. Of these *Takayama* species, *T. helix* has been found in the waters of several countries, such as Australia, Italy, Japan, Korea, New Zealand, South Africa, and Spain (Botes et al., 2003; de Salas et al., 2003, 2005; Zingone et al., 2006; McCarthy, 2013; our unpublished data). This species is known to kill abalone larvae (Botes et al., 2003); 10% of the tested abalone larvae ($LC_{10}$) were killed at a concentration of 840 cells $mL^{-1}$. Recently, *T. helix* has been revealed to be mixotrophic (Jeong et al., 2016). This species is able to feed on diverse algal prey, such as *Alexandrium minutum*, *Alexandrium lusitanicum*, *Alexandrium tamarense*, *Alexandrium pacificum*, *Alexandrium insuetum*, *Cochlodinium polykrikoides*, *Coolia canariensis*, *Coolia malayensis*, *Gambierdiscus caribaeus*, *Gymnodinium aureolum*, *Gymnodinium catenatum*, *Gymnodinium instriatum*, *Heterocapsa triquetra*, *Lingulodinium polyedrum*, and *Scrippsiella trochoidea*. Furthermore, mixotrophy elevates the growth rate of *T. helix* by 79%–185% compared with its autotrophic growth rate (Jeong et al., 2016). However, the absolute maximum growth rate of this species is still as low as 0.3 $d^{-1}$ (Jeong et al., 2016). Therefore, if the mortality rate of *T. helix* due to predation is high, this dinoflagellate may not prevail.

In the present study, to investigate potential effective protistan grazers on *T. helix*, the feeding behaviors of diverse heterotrophic dinoflagellates, such as engulfment-feeding *Oxyrrhis marina*, *Gyrodinium dominans, Gyrodinium moestrupii, Polykrikos kofoidii*, and *Noctiluca scintillans*; peduncle-feeding *Aduncodinium glandula*, *Gyrodiniellum shiwhaense, Luciella masanensis*, and *Pfiesteria piscicida*; pallium-feeding *Oblea rotunda* and *Protoperidinium pellucidum*; and the naked ciliates *Pelagostrobilidium* sp. (ca. 40 μm in cell length) and *Strombidinopsis* sp. (ca. 150 μm in cell length), on *T. helix*, and vice versa, were explored. Most of these heterotrophic protists are known to be present or often abundant in the waters of many countries including Australia, Japan, Korea, New Zealand, South Africa and Spain where *T. helix* has been found (Wood, 1954; Larsen, 1996; Jeong et al., 2001, 2006; de Salas et al., 2003, 2005; Mason et al., 2007; Lowe et al., 2010; Hallegraeff et al., 2010; McCarthy, 2013, our unpublished data). Thus, there is a high possibility that *T. helix* and these heterotrophic protists interact. Furthermore, the growth and ingestion rates of *O. marina*, *G. dominans*, *G. moestrupii, A. glandula*, *L. masanensis*, *P. kofoidii*, *P. piscicida*, and *Strombidinopsis* sp., which were revealed to feed on *T. helix*, were measured at single high prey concentrations. Moreover, the functional and numerical responses by *O. marina*, *G. dominans*, *L. masanensis*, and *P. kofoidii* to prey concentration were examined. The results of the present study provide a basis for understanding the interactions between *T. helix* and common heterotrophic protist species, and their ecological roles in the marine planktonic community.

## 2. Materials and methods

### 2.1. Preparation of experimental organisms

A clonal culture of *Takayama helix* (CCMP3082) was obtained from the National Center for Marine Algae and Microbiota (NCMA),

**Table 1**
Isolation and maintenance conditions for the experimental organisms. Sampling location and time; water temperature (T, °C); salinity (S) for isolation; feeding mechanisms (FM) and cell volume (CV, $\times 10^3$ $\mu m^3$); and prey species and concentrations (PC, cells $mL^{-1}$) for maintenance. HTD, heterotrophic dinoflagellate; EG, engulfment feeder; PE, peduncle feeder; PA, Pallium feeder; CIL, ciliate.

| Organism | Location | Time | T | S | FM | CV | Prey species | PC |
|---|---|---|---|---|---|---|---|---|
| *Aduncodinium glandula* (HTD) | Masan Bay, Korea | Mar. 2013 | 8.1 | 30.3 | PE | 4.8 | *Akashiwo sanguinea* | 1000–2000 |
| *Gyrodinium dominans* (HTD) | Masan Bay, Korea | Apr. 2007 | 15.1 | 33.4 | EG | 4.2 | *Amphidinium carterea* | 30,000–40,000 |
| *Gyrodinium moestrupii* (HTD) | Off Saemankeum, Korea | Oct. 2009 | 21.2 | 31.0 | EG | 3.3 | *Alexandrium minutum* | 3000–5000 |
| *Gyrodiniellum shiwhaense* (HTD) | Geoje, Korea | Aug. 2014 | 26.5 | 33.4 | PE | 0.6 | *Amphidinium carterea* | ~20,000 |
| *Luciella masanensis* (HTD) | Jinhae Bay, Korea | Jul. 2016 | 22.6 | 30.7 | PE | 1.3 | *Apistonema* sp. (CCMP 3085) | ~20,000 |
| *Noctiluca scintillans* (HTD) | Jangheung Bay, Korea | Jul. 2016 | 23.6 | 26.4 | EG | 39,385 | *Lingulodinium polyedrum* | 1500–2000 |
| *Oblea rotunda* (HTD) | Jinhae Bay, Korea | Apr. 2015 | 12.6 | 31.2 | PA | 5.3 | *Amphidinium carterea* | 10,000–20,000 |
| *Oxyrrhis marina* (HTD) | Shiwha Bay, Korea | Nov. 2008 | 16.8 | 27.0 | EG | 2.0 | *Amphidinium carterea* | 8000 |
| *Polykrikos kofoidii* (HTD) | Jangheung Bay, Korea | Jul. 2016 | 23.6 | 26.4 | EG | 43.1 | *Scrippsiella trochoidea* | ~5000 |
| *Pfiesteria piscicida* (CCMP2091) (HTD) | Neuse River, USA | Jan. 1998 | – | – | PE | 1.3 | *Amphidinium carterea* | ~5000 |
| *Protoperidinium pellucidum* (HTD) | Yeosu, Korea | Oct. 2016 | 22.1 | 29.3 | PA | 6.3 | *Prorocentrum triestinum* | ~1000 |
| *Pelagostrobilidium* sp. (CIL) | Jeongok Harbor, Korea | Dec. 2016 | 8.5 | 31.9 | EG | 35.5 | *Heterocapsa rotundata* | 2000–3000 |
| *Strombidinopsis* sp. (CIL) | Off Kunsan, Korea | Jan. 2017 | 4.1 | 29.9 | EG | 1115 | *Scrippsiella trochoidea* | ~5000 |