

Note

Cell lysis of a phagotrophic dinoflagellate, *Polykrikos kofoidii* feeding on *Alexandrium tamarense*

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In many cases, phytoplankton blooms terminate suddenly within a few days. For bloom-forming phytoplankton, grazing is one of the major factors in the decline of blooms as is sexual reproduction to produce non-dividing gametes and planozygotes (Anderson et al. 1983; Frost 1991). Matsuoka et al. (2000) reported growth rates of a phagotrophic dinoflagellate, *Polykrikos kofoidii* Chatton, using several dinoflagellate species as food organisms. They noted that *P. kofoidii* showed various feeding and growth responses to strains of *Alexandrium* and *Prorocentrum*. This fact suggests that for ecological control of phytoplankton blooms, we should collect information on the food preferences of predator organisms. Phagotrophic dinoflagellates include both thecate species, for example, *Diplopsalis lenticula* Bergh (Naustvoll 1998), *Oblea rotunda* (Lebour) Balech ex Sournia (Strom & Buskey 1993), *Oxyphysis oxytoxoides* Kofoid (Inoue et al. 1993), *Protoperdinium conicum* (Gran) Balech and *P. depressum* (Bailey) Balech (Gaines & Taylor 1984), *P. cf. divergens* (Jeong 1994), *P. hirobis* Abé (Jacobson & Anderson 1993) and *Protoperdinium* spp. (Jacobson & Anderson 1986), and naked species, for example, *Gymnodinium fungiforme* Anissinova (Spero & Morée 1981) and *Polykrikos kofoidii* (Matsuoka et al. 2000). Food items used in the previous papers varied from diatoms, dinoflagellates, even to ciliated protozoa and copepod eggs (Table 1).

So far three main feeding mechanisms have been discovered in phagotrophic dinoflagellates: engulfment of an intact prey item, sucking out the contents of a prey item through a peduncle (feeding tube), or enveloping prey with a pallium which is sometimes called a feeding veil or pseudopod (Jacobson & Anderson 1986; Hansen 1992; Naustvoll 1998). A phagotrophic dinoflagellate, *P. kofoidii* can capture intact prey organisms with a nematocyst and ingest them whole by direct engulfment (Matsuoka et al. 2000). However, not all feeding allows *P. kofoidii* to grow well. In this paper, we describe cell lysis of *P. kofoidii* when feeding on the autotrophic and toxic

dinoflagellate, *Alexandrium tamarense* (Lebour) Balech.

P. kofoidii was collected from Isahaya Bay in western Kyushu, Japan, on 3 November, 1998. We isolated actively swimming *P. kofoidii* using a capillary pipette and individually transferred them into multi-well tissue culture plates containing a dense suspension of the autotrophic dinoflagellate, *Gymnodinium catenatum* Graham (approximately 700 cells ml⁻¹) isolated from a bloom near Amakusa Island, western Japan, 1997. The *P. kofoidii* were cultured at 20°C with constant lighting to a density of 60 indiv. ml⁻¹, and then starved until no *G. catenatum* food particles remained in the body. Finally, 5 ml of culture medium containing *P. kofoidii* was injected into 35 ml of an *A. tamarense* suspension (approximately 2000 cells ml⁻¹) that was cultured in a 50 ml plastic flask at 20°C with constant lighting. The strain of *A. tamarense* (ATHS-92) used in this experiment was established from Hiroshima Bay in the Seto Inland Sea by Dr H. Takayama at Hiroshima Fisheries Experimental Station, in 1992. Observations were carried out under an inverted microscope equipped with a camera at 200 and 400 times magnification.

P. kofoidii started to hunt as soon as they were transferred into the *A. tamarense* culture flask. Nematocysts were used to capture *A. tamarense* as previously observed when engulfing *G. catenatum* (Matsuoka et al. 2000). *A. tamarense* was ingested by *P. kofoidii* through a sulcus of the posterior zooid. The predator swam around for a while after engulfing a whole *A. tamarense* cell before sinking to the bottom of the culture flask where it intermittently rotated in place (Fig. 1A). A few minutes later, the *A. tamarense* cell that had been engulfed by the predator, had moved towards the posterior end of the predator. Finally it was egested from the posterior sulcus through which the engulfment had occurred. The egested *A. tamarense* cell was unable to move and appeared to be covered with mucilaginous material which had adhered to the cell while inside the predator. The prey remained attached to the predator for several minutes probably due to the mucilage (Fig. 1B). Whether the cell recovered its vitality after becoming detached from the predator was unable to be observed. *P. kofoidii* continued rotating and started to change to a spherical shape,

Table 1. Phagotrophic dinoflagellate species, their optimal food items and feeding mechanisms observed in previous papers.

Predator Species	Food Items	Feeding Mechanism*	References
Thecate species			
<i>Diplopsalis lenticula</i>	<i>Ditylum brightwellii</i> (diatom)	pallium	Naustvoll 1998
<i>Oblea rotunda</i>	<i>Ditylum brightwellii</i> (diatom)	pallium	Strom & Buskey 1993
	various to detrital particle	pallium	Jacobson & Anderson 1986
<i>Oxyphysis oxytoxoides</i>	loricated ciliates	feeding tube	Inoue et al. 1993
<i>Protoperidinium</i> spp.	almost diatoms	pallium	Jacobson & Anderson 1986
<i>P. conicum</i>	chain-forming diatoms	feeding veil	Gaines & Taylor 1984
<i>P. crassipes</i>	<i>Gonyaulax polyedra</i> (dinoflagellate)	pallium	Jeong & Latz 1994
<i>P. depressum</i>	chain-forming diatoms	feeding veil	Gaines & Taylor 1984
<i>P. cf. divergens</i>	copepod eggs and early naupliar stages	pallium	Jeong 1994
	<i>Gonyaulax polyedra</i> (dinoflagellate)	pallium	Jeong & Latz 1994
<i>P. hirobis</i>	<i>Leptocylindrus danicus</i> (diatom)	pallium	Jacobson & Anderson 1993
<i>P. pellucidum</i>	<i>Skeletonema costatum</i> (diatom)	pallium	Hansen 1992
<i>Zygabikodinium lenticulatum</i>	various to detrital particle	pallium	Jacobson & Anderson 1986
Naked species			
<i>Amphidinium crassum</i>	<i>Rhodomonas baltica</i> (cryptophyte)	peduncle	Hansen 1992
<i>Gymnodinium fungiforme</i>	<i>Condylostoma magnum</i> (protozoa)	peduncle	Spero & Morée 1981
	<i>Dunaliella salina</i> (chlorophyte)		
<i>Gyrodinium</i> sp.	<i>Rhodomonas baltica</i> (cryptophyte)	engulfment	Hansen 1992
<i>G. dominans</i>	<i>Heterocapsa triquetra</i> (dinoflagellate)	engulfment	Hansen 1992
<i>G. spirale</i>	<i>Heterocapsa triquetra</i> (dinoflagellate)	engulfment	Hansen 1992
<i>Polykrikos kofoidii</i>	<i>Gymnodinium catenatum</i> (dinoflagellate)	engulfment	Matsuoka et al. 2000

* In feeding mechanism column, pallium is a synonym for feeding veil and peduncle is for feeding tube. The expression used follows that in the original paper.

the cingulum and sulcus became deformed, and their grooves disappeared without leaving a trace behind (Fig. 1C–E). The originally ellipsoidal *P. kofoidii*, eventually, became spherical (Fig. 1F). Subsequently the cell burst, and several nematocysts were left in its place (Fig. 1G). The whole process took about 10 min. All the *P. kofoidii* in the flask disappeared within one hour due to cell lysis after feeding on *A. tamarense*.

According to Matsuoka et al. (2000), the best food for *P. kofoidii* was *G. catenatum*, with *Alexandrium affine* (Inoue & Fukuyo) Balech, *A. tamarense*, *Gyrodinium aureolum* Hulburt, *Lingulodinium polyedrum* (Stein) Dodge and *Scrippsiella* cf. *trochoidea* also giving good growth rates, while *A. fundyense* Balech, *A. lusitanicum* Balech, *A. monilatum* Howell, *Heterocapsa triquetra* (Ehrenberg) Stein and *Heterocapsa* sp. caused *P. kofoidii* to die. Although the strains of *A. tamarense* (CCMP115, CCMP116 and CCMP1312) supported good growth of *P. kofoidii* in Matsuoka et al. (2000), the *A. tamarense* strain (ATHS-92) used in this study was egested from the predator. This may be due to different toxin contents between strains of *A. tamarense*, as suggested by Oshima et al. (1990) where the total toxin content in *A. tamarense* varies significantly depending on culture conditions. According to Asakawa et al. (1995), the toxin profile of *A. tamarense* (ATHS-92) featured a large amount of PX2. However no hard

evidence is presently available that can explain the different responses of *P. kofoidii* to *A. tamarense*. The egestion is not, it would seem, because of the rigid thecate plates of *A. tamarense*, because good growth rates of *P. kofoidii* are observed with other armored dinoflagellate species.

We observed the egestion of an intact cell of *A. tamarense* by *P. kofoidii* before lysis. *P. kofoidii* preyed on *A. tamarense* actively, without any observable preference either for or against it, before actual ingestion. However, *A. tamarense* caused *P. kofoidii* to die. This paper contributes to our understanding of the mechanism by which *P. kofoidii* disappears suddenly after feeding on *A. tamarense* in the field. From these observations it is clear that all grazing activity is not necessarily helpful in the survival of a predator, and this predator, *P. kofoidii*, appears not to possess the ability to choose suitable foods for itself, as previously shown by Matsuoka et al. (2000).

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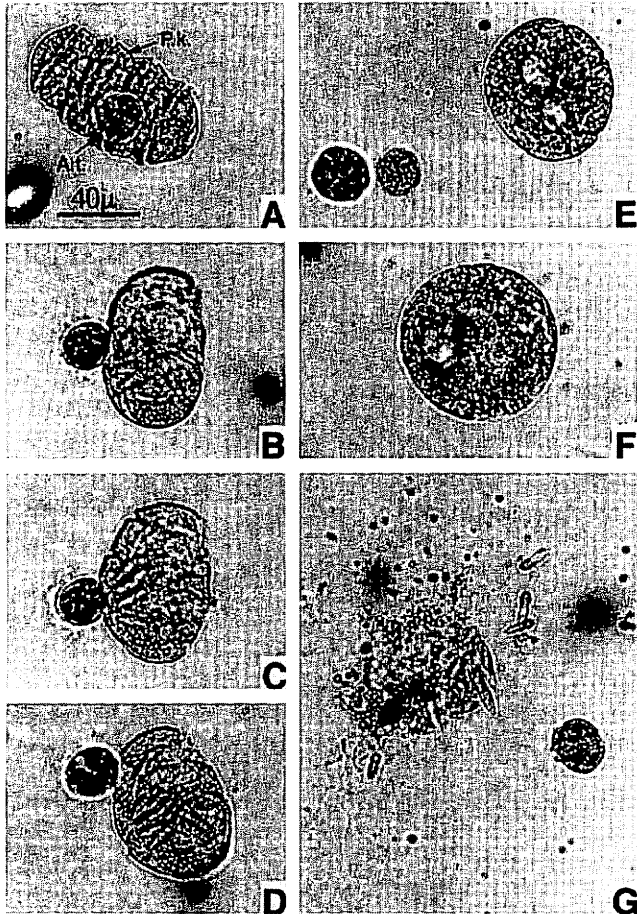


Fig. 1. Cell lysis process of *Polykrikos kofoidii* feeding on *Alexandrium tamarensis*. The scale bar is 40 μ m. **A.** *P. kofoidii* (P.k.) has fed on *A. tamarensis* (A.t.). **B.** After repeated rotation, *P. kofoidii* egests *A. tamarensis* from a sulcus of the posterior zooid. **C–F.** The shape of *P. kofoidii* which is originally ellipsoidal, starts to change. It becomes spherical; the cingulum and sulcus deform, and the grooves disappear. **G.** Finally, the spherical cell bursts and several nematocysts are left in its place.

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