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The Predation Impact by the Heterotrophic Dinoflagellate *Protoperidinium* cf. *divergens* on Copepod eggs in the Presence of Co-occurring Phytoplankton prey

HAE JIN JEONG
Marine Life Research Group 0218, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093-0218

I investigated the predation impact by the heterotrophic dinoflagellate *Protoperidinium* cf. *divergens* on copepod eggs in the presence of co-occurring phytoplankton prey (a preferred red-tide dinoflagellate *Gonyaulax polyedra*) and the selective feeding on mixtures of both prey. The ingestion rates of *P.* cf. *divergens* on Egg N (unidentified round copepod eggs with a smooth surface, about 80 in diameter) decreased by only 1.7-2 times when mean *G. polyedra* concentration increased by 57-115 times. In mixed prey experiments, *P.* cf. *divergens* preferred Egg N over *G. polyedra* even at 1.1 μgC ml^{-1} or 470 cells ml^{-1} of the latter. A strong preference of *P.* cf. *divergens* for Egg N over *G. polyedra* can be responsible for this relatively small effect. *Protoperidinium* may sometimes have a considerable predation impact on the populations of Egg N even during phytoplankton blooms or red-tide periods.

INTRODUCTION

Species in the genus *Protoperidinium* are ubiquitous heterotrophic dinoflagellates in the world ocean (Lessard and Rivikin, 1986; Ochoa and G-mez, 1987; Stoecker *et al.* 1993). They often dominate the biomass of heterotrophic dinoflagellates (20 - 200 μm in size) in coastal (Jacobson, 1987) and oceanic waters (Lessard, 1984). They are present all year in the coastal waters off southern California and possibly most areas (Allen, 1949; Lessard and Rivikin, 1986, Hallegraeff and Reid, 1986) and are often particularly abundant during red tides of autotrophic dinoflagellates (Allen, 1949; Jeong, 1995) or during diatom blooms (Jacobson 1987). Several studies (Allen, 1949; Paasche and Kristiansen, 1982; Dale and Dahl, 1987; Jacobson, 1987) reported abundanczes$\geq$20 *Protoperidinium* ml^{-1} in phytoplankton blooms or red-tide periods.

Protoperidinium is believed to play important roles in food webs in plankton community because it has a very broad range of prey species and can be an important prey for macrozooplankton (Jeong, 1994a). *Protoperidinium* has been observed to prey on diatoms (Hansen, 1992; Jacobson and Anderson; 1993, Buskey *et al.*, 1995), autotrophic dinoflagellates, con-specific cells (Hansen, 1991, Jeong and Latz, 1994, Latz and Jeong, 1996), copepod eggs and early naupliar stages (Jeong, 1994b), and detritus (Jeong, personal observation).

My previous studies (Jeong, 1994a, 1994b) showed that a free-living *Protoperidinium*, which is prey for adult copepods, is also an important potential predator on copepod eggs and early naupliar stages. In the latter study (Jeong, 1994b) I suggested that *Protoperidinium* may sometimes have a considerable predation impact on the populations of copepod eggs, based on estimated daily consumption of eggs by *Protoperidinium* when eggs were the only prey.

When a very broad range of prey species is considered, *Protoperidinium* seems to be a voracious

Present address:
[1]Department of Oceanography, College of Natural Sciences, Kunsan National University, Kunsan 573-701, South Korea

and non-selective feeder. An important further question therefore is whether *Protoperidinium* has a considerable predation impact on the populations of copepod eggs even when the concentrations of co-occurring algal prey are high, i.e. in phytoplankton blooms or red-tide periods. The answer depends on whether predation by *Protoperidinium* on eggs is significantly affected by the presence of co-occurring algal prey, and whether *Protoperidinium* can select eggs over co-occurring algal prey. The precapture behavior of *Protoperidinium*, which involves spinning around a target prey cell (Jacobson and Anderson, 1986), might permit prey selection, and Jeong and Latz (1994) found a strong selective feeding of *Protoperidinium* between two red-tide dinoflagellates.

The objectives of this study were to test the following hypotheses;

$H_0 1$: The predation rate of *Protoperidinium* on copepod eggs is independent of the presence of co-occurring algal prey.

$H_0 2$: *Protoperidinium* do not distinguish between (i.e. do not have a preference for) copepod eggs and co-occurring algal prey.

MATERIALS AND METHODS

Preparation of experimental organisms

Gonyaulax polyedra Stein, known as the best red-tide dinoflagellate prey for *Protoperidinium* cf. *divergens* (Ehrenberg) Balech (Jeong and Latz, 1994), was grown in enriched f/4 seawater media (Guillard and Ryther, 1962) without silicate, at room temperature (20-23°C) with continuous illumination of 100 $\mu E\ m^{-2}s^{-1}$ of cool white fluorescent lights. Cultures in exponential growth phase were used for feeding experiments.

A dense population of cultured *Protoperidinium* cf. *divergens*, originally collected from the Scripps pier (La Jolla, California, USA) during October, 1994, was used for these experiments. Details of culturing this species are described by Jeong and Latz (1994).

Copepods were collected from the coastal waters off La Jolla Bay, CA using a 303 μm mesh net. The copepods (several species) were placed 2 four liter jars with mixtures of *Scrippsiella trochoidea* and *Gymnodinium sanguineum*. Eggs were also collected from jars every day and sieved by 70 and 90 μm mesh nets. Unidentified round eggs (about 80 μm in diameter, hereafter Egg N) with a smooth surface and very thin-yellowish contents (no empty space between the outer surface layer and contents) were collected with a Pasteur micropipette in a multiwell chamber under a dissecting microscope and also kept at 0°C in the dark.

Egg N had been kept at 0°C in the dark for 3 days before used for Experiments 1 and 2 (see Table 1), to prevent them from hatching to nauplii during incubation (Jeong, 1994b). In the present entire experiments, no egg hatched to a nauplius.

Experimental designs

The initial densities of the predator and prey are given in Table 1. Experiments 1 and 2, where the initial concentration of Egg N was fixed, while that of *Gonyaulax polyedra* varied in each experiment, were designed to test the hypotheses ($H_0 1$ and $H_0 2$) stated previously.

Dense cultures of *Protoperidinium* cf. *divergens*

Table 1. Design of experiments. The numbers in prey and predator columns are the initial densities of prey and predator

Experiment No	Prey		Predator
	Species in mixtures	Initial density (inds. ml^{-1})	*Protoperidinium* cf. *divergens* (inds. ml^{-1})
1	#Egg N	0.38	10, 14.5
	Gonyaulax polyedra	6.8, 12.1, 29.0, 98.3, 517.1	
2	Egg N	0.75	10
	G. polyedra	8.4, 13.0, 29.5, 96.9, 475.6	

#Egg N: Unidentified round copepod eggs (about 80 μm in diameter, hereafter Egg N) with a smooth surface.

were sieved through 54 μm mesh; the large cells retained were transferred to a multiwell chamber. Most *P.* cf. *divergens sieved* recovered their normal swimming ability within 30 minutes. In all experiments, the initial concentrations of *P.* cf. *divergens* and eggs were obtained by individual transfer with a Pasteur micropipette into 32 ml polycarbonate (PC) bottles under a dissecting microscope. In experiment 1, 320 *P.* cf. *divergens* (10 *Protoperidinium* ml^{-1}) for 6.8 *Gonyaulax polyedra* ml^{-1} and about 460 (14.5 Protoperidinium ml^{-1}) for other prey concentrations were added to the 32 ml PC bottles. In experiments 1 and 2, three 1 ml aliquots of a *G. polyedra* culture were counted to determine concentration. *G. polyedra* concentrations was obtained by volume conversion with an autopipette. Duplicate experiment bottles in experiments 1 and 2 were set up. In experiments 1 and 2, triplicate control bottles contained only *G. polyedra* and Egg N at all prey concentration combinations. To determine actual initial *G. polyedra* concentrations, the concentration of one control bottle at each prey concentration combination was measured by counting all cells for two initial concentrations of 6.8-13.0 *G. polyedra* ml^{-1} and more than 200 cells for the other concentrations in multiwell chambers by removal of individual cells with a Pasteur micropipette.

Experimental and control bottles were placed on rotating wheels at 0.9 RPM under dim light at 19°C for 14-17 (in experiment 1) or 14-22 (experiment 2) hours.

Ingestion rates, and mean prey and predator concentrations were calculated using Frost's (1972) and/or Heinbokel's (1978) equations. Following incubation, the final concentrations of *Protoperidinium* cf. *divergens* were measured by counting cells in a 8 ml aliquot from bottles by removal of individual cells with a Pasteur micropipette. The final concentrations of eggs were measured by counting all eggs in multiwell chambers. In experiments 1 and 2, the final concentrations of *G. polyedra* were measured by counting all cells for the initial concentrations of 6.8-13 *G. polyedra* ml^{-1} and more than 200 cells for the other concentrations.

Carbon contents for *Gonyaulax polyedra* (2.3 ngC per cell) were estimated from cell volume according to Strathmann (1967) and for Egg N (45 ngC per egg) were obtained from Kiøboe *et al.* (1985).

Test of hypotheses

In experiments 1 and 2, the initial concentration of Egg N was fixed, while that of *Gonyaulax polyedra* varied in each experiment (Table 1). If ingestion rates of Egg N by *Protoperidinium* cf. *divergens* on at one *G. polyedra* concentration are significantly different from those at other *G. polyedra* concentrations, $H_0 1$ can be rejected. The Analysis of Variance (ANOVA, Zar, 1984) was used for the statistical test.

$H_0 2$ can be rejected if there are values consistently below or above the line of unity (means no preference) in a plot of the ratios of ingestion rates of *Protoperidinium* cf. *divergens* on each prey (*G. polyedra*: Egg N) versus ratios of prey availability.

RESULTS

Test of $H_0 1$ (the predation rate of Protoperidinium on copepod eggs is independent of the presence of co-occurring algal prey)

With increasing mean G. polyedra concentration by 115 (experiment 1) or 57 (experiment 2) times, the ingestion rates of Egg N by *Protoperidinium* cf. *divergens* decreased by only 1.7-2 times (Fig. 1A), while ingestion of *G. polyedra* generally increased (Fig. 1B). *P.* cf. *divergens* still prey on Egg N even at 1.1 μgC ml^{-1} or 470 cells ml^{-1}, the highest mean *G. polyedra* concentration tested in this study. Ingestion rate by *P.* cf. *divergens* on Egg N at *G. polyedra* concentrations of 400-500 cells ml^{-1} and mean Egg N concentration of 0.1-0.4 eggs ml^{-1} was 0.03-0.04 eggs $Protoperidinium^{-1}$ d^{-1}.

Ingestion rates of Egg N by *Protoperidinium* cf. *divergens* at one *Gonyaulax polyedra* concentration were significantly different from those at other *G. polyedra* concentrations (ANOVA, $p < 0.05$ in both experiments). Therefore, $H_0 1$ can be rejected. These

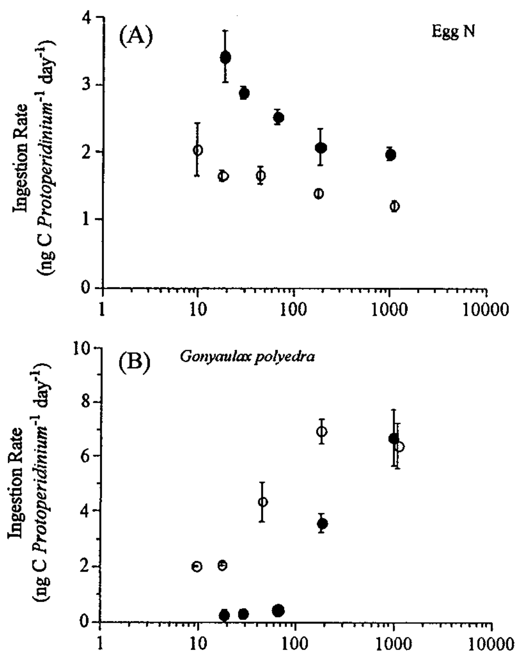

Fig. 1. Ingestion rates of *Protoperidinium* cf. *divergens* on mixed diets of *Gonyaulax polyedra* and Egg N (unidentified round copepod eggs with smooth surface), the latter at 0.38 and 0.75 eggs ml^{-1}, as a function of mean *G. polyedra* concentration. Symbols represent treatment means±1 S.E. (A) Ingestion of Egg N. Open circles: Experiment 1 (refer to Table 1). Solid circles: Experiment 2. (B) Ingestion of *G. polyedra* prey. Symbols as in (A).

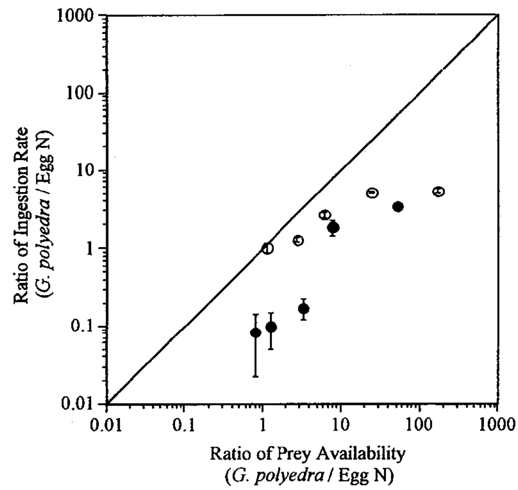

Fig. 2. Prey selection of *Protoperidinium* cf. *divergens* on mixed diets of *Gonyaulax polyedra* and Egg N. Ingestion rate on *G. polyedra*, relative to ingestion rate on Egg N, compared to relative availability of *G. polyedra*. The ratio of prey availability is the mean *G. polyedra* concentration divided by the mean Egg N concentration. Symbols represent treatment means±1 S.E. Solid and open circles as in Fig. 1.

results show that the presence of *G. polyedra* significantly affects, but did not reduce to zero ($p < 0.01$, 1-tailed t-test; Zar, 1984), the ingestion rates of *P.* cf. *divergens* on Egg N.

Test of $H_0$2 (Protoperidinium do not distinguish between copepod eggs and co-occurring algal prey)

The ratio of ingestion rates of *Protoperidinium* cf. *divergens* on each prey as a function of ratios of prey availability indicated a strong preference for Egg N over *Gonyaulax polyedra* (Fig. 2). The ratio of prey availability is the mean *G. polyedra* concentration divided by the mean Egg N concentration. The line of unity means no preference (Murdoch, 1969). $H_0$2 can be rejected because there were values consistently below the line of unity at all ratios of prey availability in experiment 2 and all but one (at the lowest mean *G. polyedra* concentration) in experiment 1.

DISCUSSION

The results of these experiments reject $H_0$1 (the predation rate of *Protoperidinium* on copepod eggs is independent of the presence of co-occurring algal prey) and $H_0$2 (*Protoperidinium* do not distinguish between copepod eggs and co-occurring algal prey).

The ingestion rates of *P.* cf. *divergens* on Egg N decreased by only 1.7-2 times when mean *G. polyedra* concentration increased by 57-115 times. A strong preference of *P.* cf. *divergens* for Egg N over *G. polyedra*, known as the optimal phytoplankton prey for *P.* cf. *divergens* (Jeong and Latz, 1994), can be responsible for this relatively small effect.

At a *Protoperidinium* cf. *divergens* density of 2 cells ml^{-1}, at Egg N concentrations of 0.1-0.4 ml^{-1} and at *G. polyedra* concentrations of 400-500 cells ml^{-1}, 20-40% of the Egg N population could be consumed in a day. This result suggests that predation by *Protoperidinium* on Egg N would significantly affect the populations of copepod eggs even in phytoplankton blooms or red-tide periods. Because *Protoperidinium* is itself prey for adult copepods, there may be a severe battle between the populations of *Protoperidinium* and copepods after a phytoplankton bloom or red-tide period when both groups are abundant.

Although *Protoperidinium* can ingest a very broad range of prey species, my previous (Jeong and Latz, 1994) and present studies consistently show that *Protoperidinium* has an ability to select among various prey species. Chemosensory detection may be a major mechanism of prey selection of *Protoperidinium* rather than mechanosensory detection. Strom and Buskey (1993) found that the heterotrophic dinoflagellate *Oblea rotunda* (Lebour) Balech, another pallium-feeding dinoflagellate, responds to chemosensory stimulation but not to mechanosensory. If a chemosensory detection is a major mechanism for the pallium feeding dinoflagellates, the smell of Egg N may be more attractive to *P.* cf. *divergens* than that of *Gonyaulax polyedra*. Otherwise, larger Egg N may be more easily detected by *P.* cf. *divergens* than smaller *G. polyedra* or the motionless egg caught and handled than the swimming dinoflagellate. To understand more fully the prey selection of *Protoperidinium*, it is worthwhile to explore the prey detection mechanisms of *Protoperidinium* in details.

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