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Feeding interactions between planktonic copepods and red-tide flagellates from Japanese coastal waters

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ABSTRACT: Feeding interactions between inshore marine copepods *Pseudodiaptomus marinus* and *Acartia omorii*, and 15 red-tide flagellates were studied by examining egestion rate, mortality and egg production rate of the copepods offered a suspension of each phytoplankton species. Among several species of poor quality as food, *Olisthodiscus luteus* (Raphidophyceae) was nearly completely rejected by *P. marinus*, and *Gymnodinium nagasakiense* (Dinophyceae), *Heterosigma akashiwo* (Raphidophyceae, Nagasaki University strain), *Chattonella marina* (Raphidophyceae) and *Fibrocapsa japonica* (Raphidophyceae) were almost entirely rejected by *A. omorii*. Since these flagellates are of preferred cell size and have no hard undigestible cell walls, the rejective feeding by copepods was suspected to be chemically mediated. Effects of chemical stimuli from *O. luteus* (to *P. marinus*) and *G. nagasakiense* (to *A. omorii*) were examined in detail by conducting comparative feeding experiments in a suspension of *Heterocapsa triquetra* (Dinophyceae), a normally edible species. Addition of filtrate from the cell-homogenate of exponentially growing *O. luteus* or *G. nagasakiense* to the suspension reduced copepod filtering rate on *H. triquetra*, indicating deterrent chemical compounds are intracellularly present. These compounds were ephemeral, being deactivated within 12 h at 20°C. Chemically-mediated rejection by copepods is an important factor in the development of monospecific red tides.

INTRODUCTION

A considerable amount of data has been compiled on feeding behavior of copepods, since they are the major constituents of herbivorous zooplankton. Suspension-feeding copepods were thought to be passive or mechanical feeders: their particle selection was size-dependent based on the morphology of the filtering mesh of the second maxillae (Boyd 1976, Nival & Nival 1976, Frost 1977). However, recent studies show copepods are discriminating feeders. Donaghay & Small (1979) reported that a copepod, *Acartia clausi*, selected phytoplankton cells over plastic beads of similar size and equal availability. Poulet & Marsot (1978) first demonstrated chemically-mediated selective feeding of copepods, which ingested microcapsules enriched with phytoplankton homogenate at much higher rates than those without enrichment. The importance of chemical properties of particles was suggested by Friedman & Strickler (1975) who found chemoreceptor-like organs in copepod feeding appendages. Using high-speed cinematography, Alcaraz et al. (1980) and Koehl & Strickler (1981) showed real selection behavior of copepods: they captured, handled (probably tasted) and ingested (or rejected) particles according to their quality.

Probably for similar chemical reasons, several bloom-forming flagellate species are rejected or fed upon at lower rates by copepods. Tomas & Deason (1981) reported *Olisthodiscus luteus* (this is suspected to be *Heterosigma akashiwo*; Hara et al. 1985, Hara & Chihara 1987) which chronically forms red tides in Narragansett Bay, Rhode Island, USA, was rejected by *Acartia hudsonica* and *A. tonsa*. Huntley (1982) also found that *Gymnodinium flavum*, a causative species of red tides in the waters off La Jolla, California, USA, was ingested at extremely low rates by *Calanus pacificus*. Recent studies (Turner & Anderson 1983, Huntley et al. 1986, Van Alstyne 1986, Gill & Harris 1987, Ives 1987, Sykes & Huntley 1987) show further rejected species, including *Gonyaulax tamarensis*, *Gyrodinium aureolum*, *Protoceratium reticulatum* (= *Gonyaulax grindleyi*), *Ptychodiscus brevis* and *Scrippsiella trochoidea*.

Growth of phytoplankton populations in the field is controlled not only by various environmental factors

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(e.g. temperature, salinity, light intensity, nutrients, growth-promoting substances, water turbulence, etc.), but also by zooplankton grazing pressure (Steele 1974, Frost 1980). The importance of zooplankton grazing pressure has been demonstrated in the course of development to red tides (Turner & Anderson 1983, Watras et al. 1985, Uye 1986). If bloom-causing phytoplankton species have adapted to employ chemical defences against being grazed (Huntley et al. 1986), the effect of zooplankton grazing pressure will be unimportant. There are 62 bloom-forming phytoplankton species in Japanese coastal waters including species causing mass kills of finfish and shellfish (Nishio 1982, Okaichi 1987), but none of these species have been examined to determine whether they might be rejected by planktonic copepods.

In this study, we offered 15 red-tide flagellates, which had been originally isolated from Japanese coastal waters, to 2 inshore marine copepods, *Pseudodiaptomus marinus* and *Acartia omorii,* in order to examine their feeding interaction. We found that a dinoflagellate *(Gymnodinium nagasakiense)* and 4 raphidophycean species (*Olisthodiscus luteus, Heterosigma akashiwo* [NU], *Chattonella marina* and *Fibrocapsa japonica*) were rejected by copepods. The effects of grazing pressure by zooplankton in the course of development to red tides are discussed.

MATERIALS AND METHODS

Phytoplankton. Fifteen species of phytoplankton (8 species of Dinophyceae, 4 species – one of them represented by 2 different strains – of Raphidophyceae, 2 species of Prasinophyceae and 1 species of Euglenophyceae) were obtained from the culture collections of Nagasaki University or the National Institute of Environmental Studies (Table 1). They ranged in maximum cell width from 12.5 to 90 μm (Table 1), a preferred particle size range for suspension-feeding copepods (Richman et al. 1977, Uye & Kasahara 1983). Phytoplankton were cultured in f/2 medium (Guillard & Ryther 1962) at 20°C (± 1°C) under a 12-12 h light-dark cycle. Only cells in the logarithmic phase of growth were suspended in glassfiber (Whatman GF/C) filtered seawater and offered as food for copepods. The egestion rate, survivorship and egg production rate of copepods were examined under bloom conditions, with concentrations ranging from 2×10^3 to 2×10^4 cells ml^{-1}. Although concentrations for each diet were not equivalent, each diet was presented in excess, with carbon concentration (calculated from Mullin et al. 1966) ranging from 600 to 2400 $\mu g\ l^{-1}$.

Copepods. Zooplankton samples were collected by a 0.45 m plankton net (200 μm mesh opening) with 1 l volume cod-end in Fukuyama Harbor, Hiroshima Pre-

Table 1. Species of red-tide flagellates used and their source and size

Species	Source or strain number	Size (μm)
Dinophyceae		
Heterocapsa triquetra	NIES-235[a]	30 × 22.5 × 22.5
Gymnodinium sanguineum	NIES-11	55 × 35 × 25
Gymnodinium nagasakiense	NU[b]	25 × 20 × 10
Gonyaulax spinifera	NIES-292	30 × 20 × 20
Protoceratium reticulatum	NIES-319	30 × 30 × 30
Prorocentrum triestinum	NIES-219	30 × 10 × 10
Prorocentrum micans	NIES-12	50 × 30 × 15
Pyrophacus steinii	NIES-222	90 × 80 × 25
Raphidophyceae		
Olisthodiscus luteus	NIES-15	25 × 15 × 5
Heterosigma akashiwo	NU	17.5 × 10 × 7.5
Heterosigma akashiwo	NIES-145	20 × 10 × 10
Chattonella marina	NU	50 × 25 × 25
Fibrocapsa japonica	NIES-136	25 × 20 × 15
Prasinophyceae		
Pterosperma cristatum	NIES-221	12.5 × 7.5 × 7.5
Pyramimonas aff. *amylifera*	NIES-251	15 × 10 × 10
Euglenophyceae		
Eutreptiella sp.	NU	25 × 12.5 × 12.5

[a] NIES: National Institute for Environmental Studies
[b] NU: Nagasaki University

fecture, Japan, and brought back to our laboratory within 30 min. Adult females of 2 copepod species, *Pseudodiaptomus marinus* (collected mainly in early summer and fall 1986 and 1987) and *Acartia omorii* (collected in fall 1986 and 1987 and spring 1987), were sorted from the plankton samples and used as grazers. These copepods were starved for 24 h in glassfiber-filtered seawater before each experiment. Subsequent experiments were conducted at 20 °C and under a 12-12 h light-dark cycle, unless otherwise noted.

Egestion rate. Copepods were individually transferred into test tubes containing 30 ml of a suspension and incubated for 24 h. Five to 10 tubes were prepared for each phytoplankton species. At the end of the experiment, survival of copepods was checked and fecal pellets deposited on the bottom of tubes were pipetted into a counting tray. Pellets were counted and their length and width measured for up to 10 randomly selected pellets. When fecal pellets were broken, the ends of pellets were counted, and half of this number was assumed to be the number of intact pellets.

Mortality. Fifty copepods were transferred into each beaker containing 500 ml of a suspension and reared for 20 d for *Pseudodiaptomus marinus* and 15 d for *Acartia omorii*. The same number of copepods were also kept in beakers containing only filtered seawater. Dead individuals were counted and removed daily. At the same time, fecal pellets on the bottom of the beakers were pipetted out, and about a quarter of the content was replaced by newly prepared medium or filtered seawater.

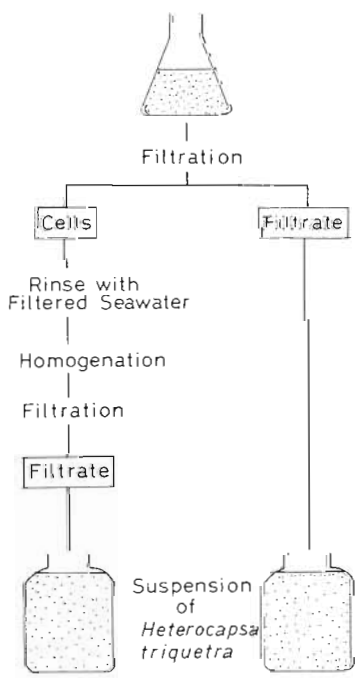

Fig. 1 Schematic presentation for the preparation of filtrate from culture and filtrate from cell homogenate of *Olisthodiscus luteus* or *Gymnodinium nagasakiense*

Egg production. A preliminary experiment revealed that egg sac production by *Pseudodiaptomus marinus* ceased after 4 d starvation. Hence, *P. marinus* were first kept in filtered seawater for 4 d to expend their stored energy and then 10 copepods were transferred into a beaker containing 500 ml of a suspension. Production of a new egg sac was monitored for 7 d.

A preliminary experiment indicated that spawning of *Acartia omorii* ceased after 3 d starvation but recovered to the previous level after 3 d of feeding on normally edible phytoplankton. Hence, *A. omorii* were first starved for 3 d, fed with respective phytoplankton species for 3 d, and then individually incubated in each test tube containing 30 ml of a suspension. Eggs produced over 24 h were counted.

Chemical compounds which inhibit copepod feeding. The experiments mentioned above (see 'Results') revealed that *Olisthodiscus luteus* was almost completely rejected by *Pseudodiaptomus marinus* and *Gymnodinium nagasakiense* was one of 4 species rejected by *Acartia omorii*, while *Heterocapsa triquetra* was a preferable phytoplankton for both copepod species. Since differences in shape, cell size and cell wall hardness did not explain the difference in food value, we suspected the rejective feeding was chemically mediated, as reported by Huntley et al. (1986) and Van Alstyne (1986).

In order to elucidate the origin of the inhibitory compounds, whether intracellular or extracellular, feeding experiments were conducted following the procedures shown in Fig. 1. A culture of *Olisthodiscus luteus* or *Gymnodinium nagasakiense* was filtered through glassfiber filters to separate filtrate and cells. Cells on a filter were rinsed with ca 50 ml of filtered seawater, transferred to a homogenation tube which was externally cooled with ice, and homogenized in 5 to 10 ml of filtered seawater. This homogenate was filtered through a glassfiber filter to obtain the filtrate of cell homogenate. Either the filtrate of culture or the filtrate of cell homogenate was sufficiently diluted on a suspension of *Heterocapsa triquetra*, which had been placed in a dark room for 24 h to prevent its growth, to contain the algal compounds derived from ca 2000 cells per ml of the suspension. Bottles containing a suspension of only *H. triquetra* were also prepared. Twenty *Pseudodiaptomus marinus* or 10 *Acartia omorii* were transferred to each grazing bottle (450 ml for *P. marinus*, 250 ml for *A. omorii*). Four experimental bottles and 2 control bottles (containing no copepods) were prepared at each cell concentration. These bottles were placed on a grazing wheel (1 rpm) for 6 h in darkness. The concentration of *H. triquetra* cells before and after the experiment was determined with a Coulter Counter

(Model ZB). Filtering rate of copepods was calculated after Frost (1972).

To test heat resistance of the inhibitory compounds, the filtrate of cell homogenate was heated at 40, 60 and 80 °C (but only 80 °C for *Gymnodinium nagasakiense*) for 10 min, cooled to 20 °C and added to a suspension of *Heterocapsa triquetra*. Grazing experiments were conducted as above. Durability of the inhibitory effect was also tested by adding the filtrate of cell homogenate which had been kept at 20 °C for 2, 3, 4, 6, 12 and 24 h (but only for 12 and 24 h in the case of *G. nagasakiense*) before grazing experiments.

RESULTS

Egestion rate

Pseudodiaptomus marinus and *Acartia omorii* produced cylindrical fecal pellets with mean lengths of 196 and 144 μm and mean widths of 35 and 38 μm, respectively. The number and size of fecal pellets varied depending on the phytoplankton species provided as food. Hence, egestion rates of copepods, in terms of the volume of fecal pellets produced per individual per day, differed markedly with phytoplankton species (Fig. 2).

Pseudodiaptomus marinus egested (Fig. 2A) more than 8.95×10^6 μm^3 copepod^{-1} d^{-1} in suspensions of *Heterocapsa triquetra, Gymnodinium sanguineum, Prorocentrum triestinum, P. micans, Pyrophacus steinii* and *Eutreptiella* sp. On the other hand, copepods fed with *Gymnodinium nagasakiense, Gonyaulax spinifera, Protoceratium reticulatum, Olisthodiscus luteus, Fibrocapsa japonica* and *Pyramimonas* aff. *amylifera* egested less than 2.68×10^6 μm^3 copepod^{-1} d^{-1}. The egestion rates were intermediate, ranging from 5.34×10^6 to 7.32×10^6 μm^3 copepod^{-1} d^{-1}, for copepods with *Heterosigma akashiwo (NU), H. akashiwo* (NIES-145), *Chattonella marina* and *Pterosperma cristatum*.

The egestion rates of *Acartia omorii* (Fig. 2B) were higher than 3.82×10^6 μm^3 copepod^{-1} d^{-1} when fed with *Heterocapsa triquetra, Gymnodinium sanguineum, Gonyaulax spinifera, Prorocentrum triestinum, P. micans, Olisthodiscus luteus, Heterosigma akashiwo* (NIES-145) and *Eutreptiella* sp. But less than 2.26×10^6 μm^3 copepod^{-1} d^{-1} were egested by copepods in suspensions of the remaining flagellate species. There was almost no defecation (mean: 0.5 pellets copepod^{-1} d^{-1}) in a suspension of *Gymnodinium nagasakiense*.

Survival

In filtered seawater, mortality of *Pseudodiaptomus marinus* was low during the first 8 d, but this was followed by high mortality, and all individuals died by the 13th day (Fig. 3A). Median survival time was 9 d. As shown in Fig. 3A, more than 66 % of *P. marinus* survived to the end of the experiment in suspensions of *Heterocapsa triquetra, Gymnodinium sanguineum, Gonyaulax spinifera, Protoceratium reticulatum, Prorocentrum triestinum, P. micans, Pyrophacus steinii, Heterosigma akashiwo* (NU), *H. akashiwo* (NIES-145), *Chattonella marina, Pterosperma cristatum, Pyramimonas* aff. *amylifera* and *Eutreptiella* sp. High mortality (52 to 100 %) was observed for *P. marinus* fed with *Gymnodinium nagasakiense, Fibrocapsa japonica* and *Olisthodiscus luteus* with median survival times of 20, 15 and 11 d, respectively. In an experimental suspen-

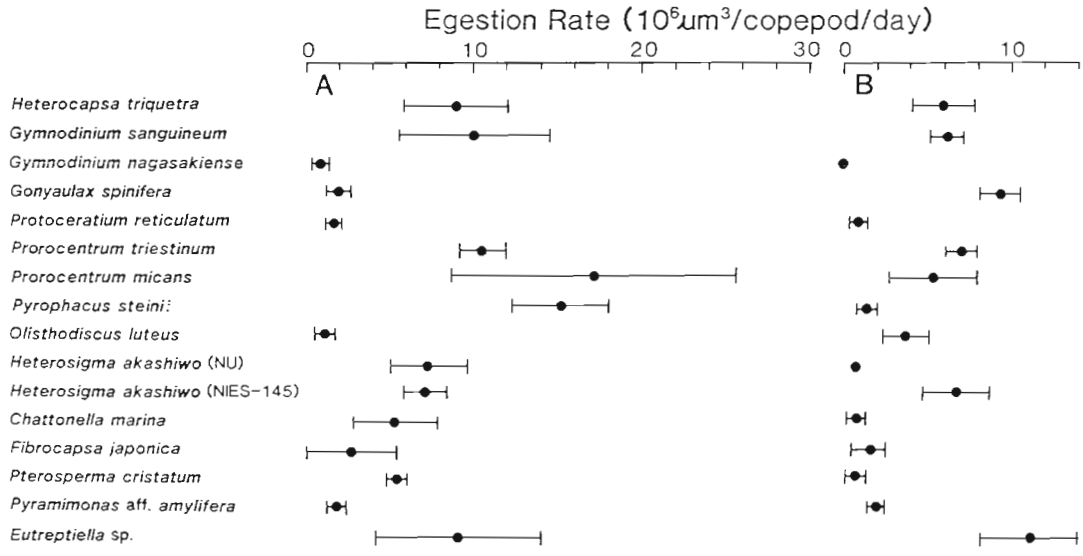

Fig. 2. (A) *Pseudodiaptomus marinus* and (B) *Acartia omorii*. Egestion rates of adult females fed on various red-tide flagellate species. Bars indicate standard deviation of the mean

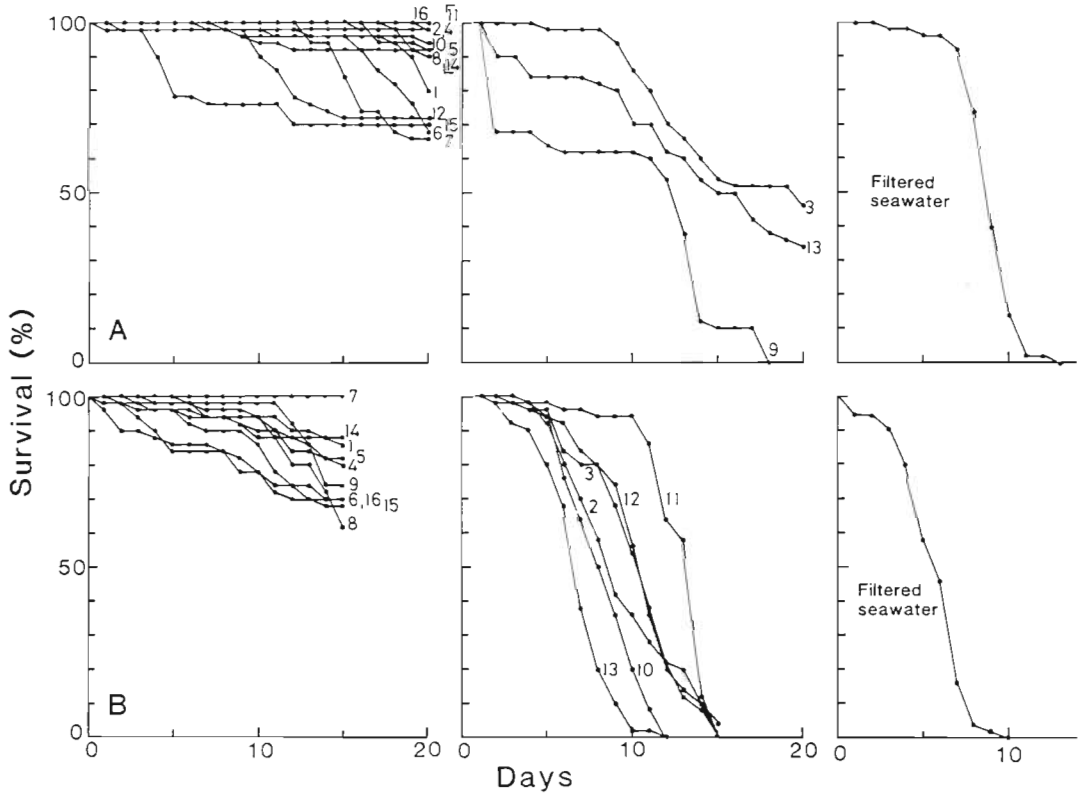

Fig. 3. (A) *Pseudodiaptomus marinus* and (B) *Acartia omorii*. Survival curves of adult females fed on various red-tide flagellate species. (1) *Heterocapsa triquetra*, (2) *Gymnodinium sanguineum*, (3) *Gymnodinium nagasakiense*, (4) *Gonyaulax spinifera*, (5) *Protoceratium reticulatum*, (6) *Prorocentrum triestinum*, (7) *Prorocentrum micans*, (8) *Pyrophacus steinii*, (9) *Olisthodiscus luteus*, (10) *Heterosigma akashiwo*, NU, (11) *Heterosigma akashiwo*, NIES-145, (12) *Chattonella marina*, (13) *Fibrocapsa japonica*, (14) *Pterosperma cristatum*, (15) *Pyramimonas* aff. *amylifera*, and (16) *Eutreptiella* sp.

sion of *O. luteus*, 32 % died on the 2nd day (Fig. 3A), a much higher mortality than that in filtered seawater.

Acartia omorii could survive up to 10 d under starved conditions (Fig. 3B) with a median survival time of 6 d. Mortality was relatively low (0 to 38 %) when given *Heterocapsa triquetra, Gonyaulax spinifera, Protoceratium reticulatum, Prorocentrum triestinum, P. micans, Pyrophacus steinii, Olisthodiscus luteus, Pterosperma cristatum, Pryramimonas* aff. *amylifera* and *Eutreptiella* sp. (Fig. 3B). However, almost all individuals died by the end of the experiments with *Gymnodinium nagasakiense, G. sanguineum, Heterosigma akashiwo* (NU), *H. akashiwo* (NIES-145), *Chattonella marina* and *Fibrocapsa japonica* with median survival times of 9, 11, 8, 14, 11 and 7 d, respectively (Fig. 3B).

Egg production

As shown in Fig. 4, 100 % of *Pseudodiaptomus marinus* produced a new egg sac, containing more than 20 eggs, 3 to 6 d after being fed with *Heterocapsa triquetra, Gymnodinium sanguineum, Gonyaulax spinifera, Protoceratium reticulatum, Prorocentrum triestinum, P. micans, Pterosperma cristatum, Pyramimonas* aff. *amylifera* and *Eutreptiella* sp. Although *Gymnodinium nagasakiense* and *Fibrocapsa japonica* supported egg production for 90 and 80 % of females, respectively, their newly produced egg sac was much smaller, containing ca 10 eggs. Similarly, a small egg sac was produced by 20 to 50 % of *P. marinus* fed with

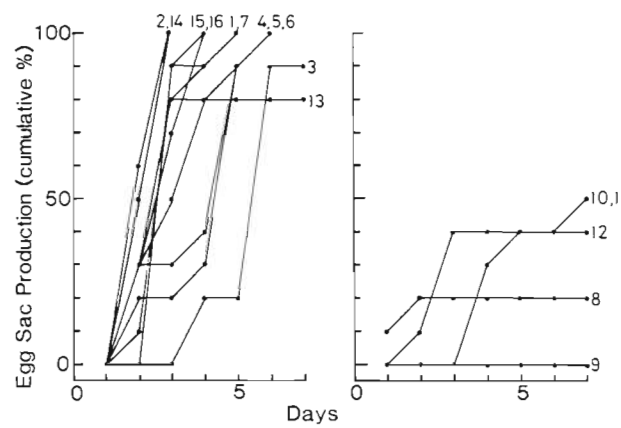

Fig. 4. *Pseudodiaptomus marinus*. Cumulative percentage of females carrying an egg sac, when fed on various red-tide flagellate species (see Fig. 3 for species name)

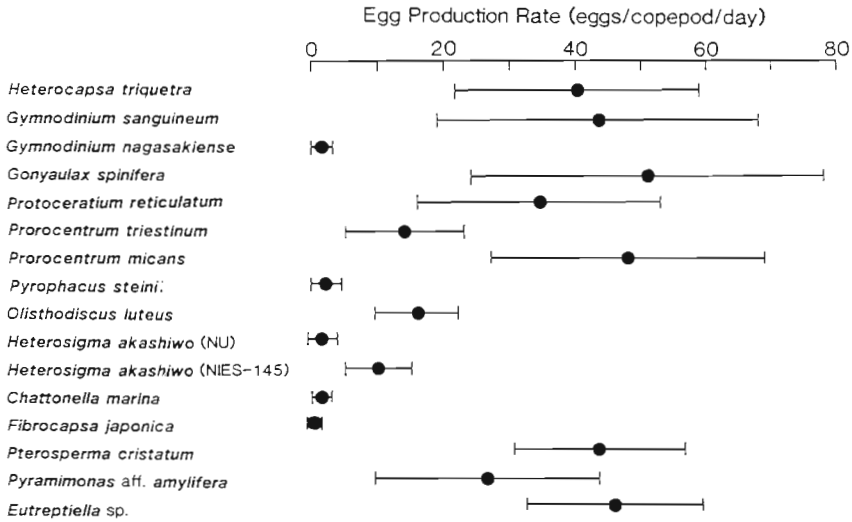

Fig. 5. *Acartia omorii*. Egg production rates when fed on various red-tide flagellate species. Bars indicate standard deviation of the mean

Pyrophacus steinii, *Chattonella marina*, *Heterosigma akashiwo* (NU), and *H. akashiwo* (NIES-145) (Fig. 4). No eggs were produced by *P. marinus* in a suspension of *Olisthodiscus luteus* (Fig. 4).

Acartia omorii showed relatively high egg production rates, 26.5 to 51.2 eggs copepod^{-1} d^{-1}, when given *Heterocapsa triquetra*, *Gymnodinium sanguineum*, *Gonyaulax spinifera*, *Protoceratium reticulatum*, *Prorocentrum micans*, *Pterosperma cristatum*, *Pyramimonas* aff. *amylifera* and *Eutreptiella* sp. (Fig. 5). On the other hand, egg production rates were extremely low, 0.4 to 1.7 eggs copepod^{-1} d^{-1}, for copepods with *Gymnodinium nagasakiense*, *Pryrophacus steinii*, *Heterosigma akashiwo* (NU), *Chattonella marina* and *Fibrocapsa japonica* (Fig. 5). *Prorocentrum triestinum*, *Olisthodiscus luteus* and *H. akashiwo* (NIES-145) supported intermediate rates of egg production, 10.1 to 15.9 eggs copepod^{-1} d^{-1} (Fig. 5).

Evaluation of food quality

Table 2 summarizes results of experiments to evaluate the food quality of phytoplankton species for

Table 2. Evaluation of food quality (+: good, −: bad, ±: intermediate) of each flagellate species for *Pseudodiaptomus marinus* (P.m.) and *Acartia omorii* (A.o.) based on the results of egestion, survival and egg production rates

Species	Egestion		Survival		Egg production	
	P.m.	A.o.	P.m.	A.o.	P.m.	A.o.
Dinophyceae						
Heterocapsa triquetra	+	+	+	+	+	+
Gymnodinium sanguineum	±	+	+	−	+	+
Gymnodinium nagasakiense	−	−	±	−	±	−
Gonyaulax spinifera	−	+	±	+	+	+
Protoceratium reticulatum	−	−	+	+	+	+
Prorocentrum triestinum	+	+	±	+	+	±
Prorocentrum micans	+	±	±	+	+	+
Pyrophacus steinii	+	±	+	+	±	−
Raphidophyceae						
Olisthodiscus luteus	−	+	−	+	−	±
Heterosigma akashiwo (NU)	±	−	+	±	±	−
Heterosigma akashiwo (NIES-145)	±	+	±	−	±	±
Chattonella marina	±	−	+	−	±	−
Fibrocapsa japonica	−	−	±	−	±	−
Prasinophyceae						
Pterosperma cristatum	±	−	+	+	+	+
Pyramimonas aff. *amilifera*	−	±	+	+	+	+
Euglenophyceae						
Eutreptiella sp.	+	+	+	+	+	+

Pseudodiaptomus marinus and *Acartia omorii*. We simply judged 'good', 'intermediate' and 'poor' quality arbitrarily based on the results of each experiment.

We concluded that half the flagellates examined were of 'good' quality, i.e. *Heterocapsa triquetra, Gonyaulax spinifera, Protoceratium reticulatum, Prorocentrum triestinum, P. micans, Pterosperma cristatum, Pyraminomas* aff. *amylifera* and *Eutreptiella* sp. Although egestion rates were not always high, these species supported high survival and egg production rates in both copepod species. *Gymnodinium sanguineum* and *Pyrophacus steinii* were 'good quality' food for *Pseudodiaptomus marinus*, but for *Acartia omorii*, the former did not support high survival rates and the latter did not provide high egg production rates. The remaining phytoplankton species were evaluated as more or less 'poor quality' food. Remarkably poor results, i.e. extremely low egestion, survival and egg production rates, were observed for *Olisthodiscus luteus* fed to *P. marinus*, and for *Gymnodinium nagasakiense, Heterosigma akashiwo* (NU), *Chattonella marina* and *Fibrocapsa japonica* fed to *A. omorii*.

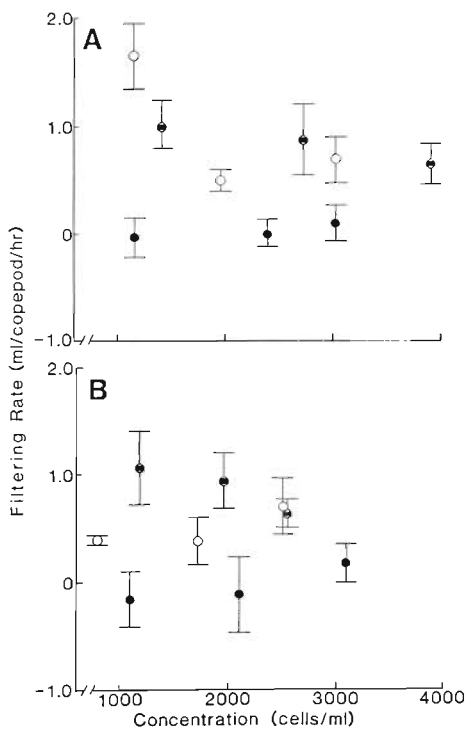

Fig. 6. (A) *Pseudodiaptomus marinus* and (B) *Acartia omorii*. Filtering rates of adult females on *Heterocapsa triquetra*. (○) Control cells (*Heterocapsa triquetra*) only; (⊖) control cells + filtrate of *Olisthodiscus luteus* culture (A) or *Gymnodinium nagasakiensa* culture (B); (●) control cells + filtrate of the homogenate of *Olisthodiscus luteus* cells (A) or *Gymnodinium nagasakiense* cells (B). Bars indicate standard deviation of the mean

The differences in food quality are probably related to differences in nutritional chemical compounds (e.g. carbon, nitrogen, protein, lipid) at least for those species which were ingested (evidenced by fecal pellet production). It was apparent, for most species judged as 'poor quality' that non-feeding was a reason for the high mortality and low egg production rates of copepods. However, this non-feeding behavior could not be explained by differences in shape, cell size or hardness of cell walls. Hence, we suspected some chemical compounds were involved, as was reported by Huntley et al. (1986) and Van Alstyne (1986). In the subsequent grazing experiments, responses of *Pseudodiaptomus marinus* to *Olisthodiscus luteus* and of *Acartia omorii* to *Gymnodinium nagasakiense* were investigated in detail.

Occurrence of inhibitory compounds: intra- or extracellular?

Filtering rates of *Pseudodiaptomus marinus* on *Heterocapsa triquetra*, a normally edible species, were determined at 3 different cell concentrations in 3 different treatments: *H. triquetra* was suspended in (1) filtered seawater, (2) filtered seawater containing filtrate of *Olisthodiscus luteus* culture, and (3) filtered seawater containing filtrate from the homogenate of *O. luteus* cells (Fig. 6A). Although *P. marinus* filtered at relatively high rates in Treatments (1) and (2) (overall means 0.95 and 0.85 ml copepod^{-1} h^{-1} respectively), the copepods did not filter appreciably (0.02 ml copepod^{-1} h^{-1}) in Treatment (3). At concentrations of 1156 to 1412 cells ml^{-1}, the mean filtering rate (1.65 ml copepod^{-1} h^{-1}) in Treatment (1) was significantly (t-test, $p < 0.05$) higher than the mean filtering rate (1.03 ml copepod^{-1} h^{-1}) in Treatment (2), which was in turn significantly ($p < 0.01$) higher than the mean value (0.035 ml copepod^{-1} h^{-1}) in Treatment (3). Although, at concentrations of 2737 to 3037 cells ml^{-1}, there was no significant difference in mean filtering rates between Treatments (1) and (2) (0.69 and 0.88 ml copepod^{-1} h^{-1}, respectively), these values were significantly ($p < 0.01$) higher than the filtering rate (0.1 ml copepod^{-1} h^{-1}) in Treatment (3). From these results, it was concluded that the filtrate of *O. luteus* culture did not inhibit feeding of *P. marinus* on *H. triquetra*, but the filtrate from the cell homogenate did inhibit feeding, indicating the inhibitory compounds were present intracellularly.

Fig. 6B shows the filtering rates of *Acartia omorii* in each treatment: *Heterocapsa triquetra* was suspended in (1) filtered seawater, (2) filtered seawater containing filtrate of *Gymnodinium nagasakiense* culture, and (3) filtered seawater containing filtrate from the homo-

genate of *G. nagasakiense* cells. Overall means were 0.49, 0.88 and −0.03 ml copepod^{-1} h^{-1}, respectively. At concentrations of 795 to 1196 and 1730 to 2107 cells ml^{-1}, the mean filtering rates (1.07 and 0.94 ml copepod^{-1} h^{-1}, respectively) in Treatment (2) were significantly ($p < 0.05$) higher than the mean rates (0.39 and 0.38 ml copepod^{-1} h^{-1}) in Treatment (1), but this difference was not significant at concentrations of 2517 to 2556 cells ml^{-1}. At concentrations of 795 to 1196 cells ml^{-1}, the mean filtering rate (−0.16 ml copepod^{-1} h^{-1}) in Treatment (3) was significantly ($p < 0.05$) lower than the mean rate in Treatment (1), but at 1730 to 2107 cells ml^{-1}, this difference (mean in Treatment [3]: −0.11 ml copepod^{-1} h^{-1}) was not significant. These results indicate the addition of homogenate filtrate of *G. nagasakiense* cells may reduce the filtering rate of *A. omorii,* but the addition of culture filtrate may increase the filtering rate. Hence, it was concluded the inhibitory compounds were also present within *G. nagasakiense* cells.

Physico-chemical properties of inhibitory compounds

Fig. 7 shows the effect of heated filtrate from *Olisthodiscus luteus* cell homogenate on the filtering rate of *Pseudodiaptomus marinus* on *Heterocapsa triquetra*. Filtering rate tended to increase with the temperature at which the cell homogenate was treated. However, the mean filtering rate even in experimental suspension with 80°C-heated filtrate (0.33 ml copepod^{-1} h^{-1}) was not significantly different from that in control suspension (treated at 20°C, 0.01 ml copepod^{-1} h^{-1}).

Similarly the filtering rate of *Acartia omorii* increased to 0.59 ml copepod^{-1} h^{-1} in experimental suspension with *Gymnodinium nagasakiense* cell homogenate heated to 80°C (Fig. 7). However, this was again not significantly different from the mean filtering rate in control suspension (−0.11 ml copepod^{-1} h^{-1}).

Fig. 8 shows the effect of aged filtrate from *Olisthodiscus luteus* cell homogenate on the filtering rate of *Pseudodiaptomus marinus*. There was a clear tendency for filtering rate to increase as the filtrate became older. Mean filtering rates in experimental suspension with 2 to 6 h old filtrate (0.01 to 0.27 ml copepod^{-1} h^{-1}) were not different from the filtering rate in control suspension, which contained 0 h old filtrate (0.01 ml copepod^{-1} h^{-1}). Mean filtering rates with 12 and 24 h old filtrate (0.63, 0.84 ml copepod^{-1} h^{-1}, respectively) were significantly ($p < 0.05$) higher than the mean value in control suspension.

Acartia omorii filtered at significantly higher rates in experimental suspension with 12 h old (0.53 ml copepod^{-1} h^{-1}) and 24 h old (0.52 ml copepod^{-1} h^{-1}) filtrate from *Gymnodinium nagasakiense* cell homogenate than in control suspension (Fig. 8). These results indicate that the inhibitory compounds produced by both *Olisthodiscus luteus* and *G. nagasakiense* were labile.

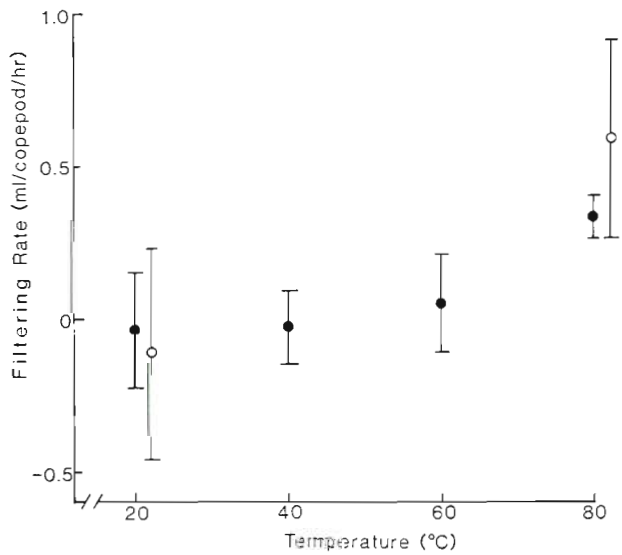

Fig. 7. *Pseudodiaptomus marinus* and *Acartia omorii*. Effect of heated filtrate from the homogenate of *Olisthodicus luteus* or *Gymnodinium nagasakiense* on filtering rate of copepods on *Heterocapsa triquetra*. (●) Filtering rate of adult female *P. marinus*; (○) filtering rate of adult female *A. omorii*. Bars indicate standard deviation of the mean

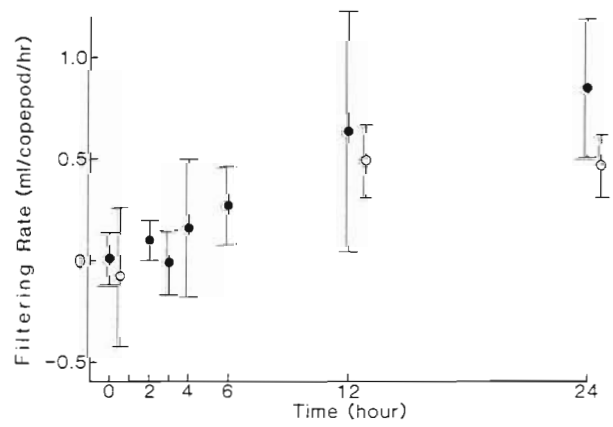

Fig. 8. *Pseudodiaptomus marinus* and *Acartia omorii*. Effect of aged filtrate from the homogenate of *Olisthodicus luteus* or *Gymnodinium nagasakiense* on filtering rate of copepods on *Heterocapsa triquetra*. (●) Filtering rate of adult female *P. marinus*; (○) filtering rate of adult female *A. omorii*. Bars indicate standard deviation of the mean

DISCUSSION

Since *Pseudodiaptomus marinus* and *Acartia omorii*, the latter being much more abundant than the former (Uye et al. 1983, Ueda 1986, 1987), occur in inlet waters of Japan, where red tides often take place, both copepods are likely to encounter blooms of various phytoplankton species including the 15 flagellates used in the present study. Since these flagellates are of preferred cell size for adult female copepods, they should be ingested and assimilated, unless they lack some nutritional requirements for copepods or have hard undigestible cell walls. Some flagellate species were readily eaten by copepods and supported high survival and egg production rates, but others were eaten at low rates or almost completely rejected. Among the latter, *Olisthodiscus luteus* was almost completely rejected by *P. marinus*, and *Gymnodinium nagasakiense*, *Heterosigma akashiwo* (NU), *Chattonella marina* and *Fibrocapsa japonica* were totally rejected by *A. omorii*. We found that the rejective feeding was due to the effect of deterrent chemical compounds in the intracellular product, and this was confirmed for 2 representative species, *O. luteus* and *G. nagasakiense*.

Feeding interaction between flagellates and copepods

The present study shows raphidophycean algae are, in general, undesirable food to copepods. However, the food value of these flagellates differed from one copepod species to another. For example, *Olisthodiscus luteus* was readily eaten by *Acartia omorii*, and *Heterosigma akashiwo* (NU), *Chattonella marina* and *Fibrocapsa japonica* were efficiently utilized by *Pseudodiaptomus marinus* (Table 2). Owing to the similar morphologies of *O. luteus* and *H. akashiwo*, the latter has often been misidentified as *O. luteus* (Hara et al. 1985, Hara & Chihara 1987). *O. luteus* (it is likely to have been *H. akashiwo*) was reported as an unsatisfactory food for copepods (Tomas & Deason 1981) and tinntinnids (Verity & Stoecker 1982). Chotiyaputta & Hirayama (1978) reported that *H. akashiwo* (NU) (the same strain as that used in the present experiment) was also an unsatisfactory food for a rotifer, *Brachionus plicatilis*. Although *C. marina* was inferior food in the present study, Ito & Imai (1986) and Uye (1986) reported *Chattonella* (*antiqua* and *marina*) were readily ingested by copepods. However, they did not examine whether *Chattonella* spp. were utilized by these copepods to support high survival and egg production rates.

Among 8 dinoflagellate species examined, only *Gymnodinium nagasakiense* was an unsatisfactory food for both copepod species. This was more marked for *Acartia omorii* than for *Pseudodiaptomus marinus*; the latter could to some extent utilize it for survival and egg production (Table 2). *Gyrodinium aureolum*, which is morphologically very similar and hence suspected as a synonym of *G. nagasakiense* (Tangen 1977, Partensky et al. 1988), was an inferior food for *Calanus helgolandicus* and *Temora longicornis* (Gill & Harris 1987). Although Huntley et al. (1986) demonstrated *Protoceratium reticulatum* (subsequently referred to by its synonym, *Gonyaulax grindleyi*, by Sykes & Huntley 1987 and Huntley et al. 1987) was rejected by *Calanus pacificus* and *Paracalanus parvus*, in our study this species supported high survival and egg production rates of both copepods in spite to low egestion rates. These results suggest food value (or rejection or non-rejection) of a given phytoplankton species may differ with strain or culture condition of phytoplankton, as well as with grazer species.

If many herbivores respond similarly to *Pseudodiaptomus marinus* and *Acartia omorii* in these experiments, then reduction of their feeding pressure may be a significant factor in the development of flagellate populations to red tide conditions. Avoidance of a bloom area by zooplankton (Fiedler 1982) may also favor red-tide formation. When monospecific blooms of these flagellates occur over a wide area, our results suggest disastrous effects to herbivore populations. Under such conditions, they could not acquire the energy to meet minimum requirements for respiration and metabolism. Then a significant number of grazers would disappear from the bloom area, further contributing to maintenance of the bloom. Such deleterious conditions for copepods may be alleviated if a considerable amount of good quality phytoplankers coexist.

In contrast, most dinoflagellate species (but see exceptionally low results for *Gymnodinium sanquineum* and *Pyrophacus steinii* with *Acartia omorii*; Table 2), prasinophytes and a euglenophyte used in the present experiments were satisfactory food to copepods and no harmful effects were found even under bloom concentrations. Hence, the development and persistence of blooms of these species are expected to be influenced by grazing pressure of zooplankton.

Production of inhibitory chemical compounds

A variety of dissolved organic compounds are produced by phytoplankton (Fogg 1966). These compounds surrounding cells may be detected by copepods through chemoreceptors to enable selection of food particles (Koel & Strickler 1981, Andrews 1983, Poulet et al. 1986). In particular, free amino acids are important feeding stimuli (Poulet & Marsot 1980, Poulet & Quellet 1982, Gill & Poulet 1988). On the other hand, some phytoplankton

species produce metabolites which are deterrent or toxic toward phytoplankton (i.e. allelopathy; cf. Pratt 1966, Lewis 1986), zooplankton (Huntley et al. 1986) or fish (White 1980, 1981). The production of toxic compounds, especially toward zooplankton, is considered a chemical defense against grazers. However, except for a report by Abe & Hirayama (1979), who found a lethal effect of *Gymnodinium nagasakiense* on a rotifer *(Brachionus plicatilis)*, production of toxins causing acute mortality of grazers has not to our knowledge been demonstrated in marine phytoplankton.

In the present study, 5 (1 dinoflagellate and 4 raphidophytes) out of 15 flagellate species were presumed to produce compounds which deter feeding by copepods. These compounds, however, did not cause acute mortality of copepods. Since 2 flagellates, *Olisthodiscus luteus* and *Gymnodinium nagasakiense*, were found to contain the inhibitory compounds within their cells, their toxic chemical properties may not be detected by copepods before ingestion, but can be detected after the cells are ingested and broken. However, Huntley et al. (1986) found that compounds which inhibited feeding of *Calanus pacificus* were derived from the extracellular products of *Protoceratium reticulatum*. In addition, Van Alstyne (1986) reported that feeding of *Centropages hamatus* was inhibited by both intra- and extracellular products of *O. luteus* and intracellular compounds of *Scrippsiella trochoidea*. Sykes & Huntley (1987) observed, through a video system, the behavior of *C. pacificus* presented with *P. reticulatum* and found the copepods actually ingested the cells but 40 % of the copepods regurgitated after 45 to 120 min. These findings suggest that chemical properties of extracellular products might not be successfully detected by copepods before cells are ingested.

Responses to cell exudates (smell) are more ecologically relevant than responses to cell extracts (taste), because copepods would not necessarily have to kill cells to detect their chemical properties. As demonstrated by Huntley et al. (1986) for *Protoceratium reticulatum*, the active compounds derived from *Olisthodiscus luteus* and *Gymnodinium nagasakiense* were also ephemeral, being deactivated within 12 h at 20 °C. The importance of plant defensive mechanisms, especially the role of secondary metabolites as chemical defenses against herbivores, has been recognized (Whittaker & Feeny 1971). It has been argued that the evolution of defense mechanisms in terrestrial plants may be responsive to the plant's cost/benefit relationships. In this sense, production of defensive chemical compounds is costly for phytoplankton. This was explicitly examined by Huntley et al. (1986) who found growth rates of dinoflagellates exuding toxic compounds were significantly lower than those which do not contain such compounds. If ephemeral bioactive compounds reside extracellularly, the production of such compounds is energetically expensive because they must be continuously produced and released. If the compounds reside intracellularly, their production is energetically more economical, although individual cells are exposed to the risk of being killed by grazers in this case. We speculate grazers are capable of discriminating toxic cells through trial-and-error experiences in ingesting bad-tasting species, although such a learning effect has not been explicitly demonstrated in marine zooplankton. Ingested cells are a sacrifice for the remaining population. Being bad-tasting is then a selective advantage for the population, but not for a single cell.

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