Vol. 17: 99–103, 1999 | AQUATIC MICROBIAL ECOLOGY Aquat Microb Ecol | Published April 30

NOTE

# Feeding by the small calanoid copepod *Paracalanus* sp. on heterotrophic dinoflagellates and ciliates

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ABSTRACT: Feeding of the calanoid copepod *Paracalanus* sp. on natural populations of heterotrophic (h-) dinoflagellates and ciliates was examined during the summer in the Seto Inland Sea, Japan. *Paracalanus* sp. ingested h-dinoflagellates with clearance rates of 35 to 146 ml ind.$^{-1}$ d$^{-1}$, comparable to those for autotrophic (a-) dinoflagellates. Feeding rates of the copepod on h-dinoflagellates (0.35 to 0.93 µg C ind.$^{-1}$ d$^{-1}$) were close to, or in some cases exceeded, the basic metabolic demands (0.5 µg C ind.$^{-1}$ d$^{-1}$) of the copepod. Ciliates were also ingested by *Paracalanus* sp., but the corresponding clearance rates (23 to 60 ml ind.$^{-1}$ d$^{-1}$) were lower than those for h-dinoflagellates. These results, together with data on the abundance/biomass of *Paracalanus* sp. and h-dinoflagellates in the study area, indicate that the population of *Paracalanus* sp. can have a significant feeding impact on h-dinoflagellates.

KEY WORDS: Copepod · Heterotrophic dinoflagellate · Ciliate · Predation *Paracalanus*

Ciliates and heterotrophic (h-) dinoflagellates are now recognized as important consumers of phytoplankton in marine ecosystems (e.g. Sherr & Sherr 1992, Burkil et al. 1993, Buck & Newton 1995, Nakamura et al. 1995, 1996). Furthermore, ciliates are also regarded as a link between pico-/nano-sized particles (bacteria, picophytoplankton and nanoflagellates) and metazoans such as copepods (e.g. Sherr & Sherr 1988, Stoecker & Capuzzo 1990). From the standpoint of carbon flow through planktonic food webs, it thus becomes inevitable to assess the fate of ciliates and h-dinoflagellates. In this context, studies on the ciliate-copepod trophic link have been conducted over the past 15 yr. They indicated that copepods including 'herbivorous' species ingest ciliates actively and have a significant impact on ciliate populations (e.g. Stoecker & Capuzzo 1990, Dolan 1991, Sanders & Wickham 1993, Fessendenn & Cowells 1994, Froneman et al. 1996, Merrell & Stoecker 1998). On the other hand, studies on the interactions between h-dinoflagellates and copepods are rather restricted (Jeong 1994, Froneman et al. 1996, Verity & Paffenhöfer 1996, Nakamura & Turner 1997).

A calanoid copepod, *Paracalanus* sp., which has been identified as *P. parvus* (Hirota 1979), but is morphologically more like *P. quasimodo* (J. Hiromi unpubl.), is one of the most abundant copepods in Japanese coastal waters (Liang & Uye 1996). In the present study, feeding of *Paracalanus* sp. on ciliates, h-dinoflagellates and phytoplankton species in natural seawater was examined to assess the fate of ciliates and h-dinoflagellates in the Seto Inland Sea, where microprotozoans play important roles in the collapse of phytoplankton blooms (Nakamura et al. 1995, 1996).

**Materials and methods.** ***Field monitoring:*** In summer 1997 (17 July to 11 August), a field survey was conducted at Stn B (34° 35' N, 134° 30' E, 21 m depth; see Nakamura et al. 1993) around the Ie-shima Islands, in the Seto Inland Sea, Japan. Monitoring and routine sampling were conducted daily except on 25 and 26 July due to a typhoon. Temporal and vertical changes in water temperature, salinity, nutrients, chlorophyll *a*, and abundance of pico/nanoplankton and netzooplankton are reported elsewhere (Nakamura 1999). Samples for enumeration of ciliates/phytoplankton (100 ml) were taken at 0 and 10 m, fixed with acid Lugol's solution (final conc. = 2%), stored at 5°C and counted by the Utermöhl method (10 ml) within 1 mo after completion of the survey. Samples for h-dinoflag-

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ellates (80 ml) were also taken from 0 and 10 m, fixed with glutaraldehyde (final conc. = 1%), and stained with DAPI (final conc. = 1 µg $ml^{-1}$). The stained samples (20 ml) were concentrated onto black Nuclepore filters (pore size = 0.8 µm), and h-dinoflagellates were enumerated by observing the filters using an epifluorescence microscope. Detailed conditions for the enumerations have been summarized in a previous paper (Nakamura et al. 1995). Ciliates and h-dinoflagellates for field monitoring were enumerated for cells with length >15 and >20 µm, respectively.

Samples for enumeration of copepods (1000 ml) were taken at 0, 5, 10, 15 and 19 m, concentrated through a 20 µm mesh sieve to a volume of ~80 ml, fixed with neutralized formalin (final conc. = 5%) and observed under a stereo microscope within 5 mo after collection. Copepods (adults and copepodites) were identified and counted, and their prosome lengths (PL) were measured to the nearest 10 µm for all individuals in the samples. PL was converted into carbon weight from the species specific equation given by Uye (1982, 1991). Since the sampling volume in the present study was low (1000 ml for each depth) in comparison with other studies, only the values averaged over the water column were reported for abundance and biomass.

***Feeding experiments:*** Individuals of *Paracalanus* sp. for feeding experiments were obtained by gentle vertical tows from 15 m at Stn B. Adult females of *Paracalanus* sp. were isolated and preconditioned overnight in filtered seawater (<100 µm, 1000 ml) at the temperature of 15 m depth (±1°C). On the following day, filtered surface seawater from Stn B (<100 µm; ~20 l) was prepared on board and brought back to the field laboratory within 10 min. The filtrate ('initial assemblage') was mixed gently and siphoned into 6 bottles (polycarbonate, 1100 ml). 100 and 80 ml aliquots of the initial assemblage were also fixed with acid Lugol's solution and glutaraldehyde for enumeration of ciliates/phytoplankton and h-dinoflagellates, respectively. Then individuals of *Paracalanus* sp. were introduced to 3 bottles (15 ind. $bottle^{-1}$), while the other bottles served as controls. The bottles were rendered free of air space or bubbles by the use of parafilm, wrapped with black sheeting to suppress algal growth, and suspended at 3 m depth from the pontoon of the field laboratory for 22.5 h. Following completion of the incubation, the bottles were retrieved and the survival of *Paracalanus* sp. was confirmed. Then 100 and 80 ml aliquots were taken from each bottle and used for the enumeration of ciliates/phytoplankton and h-dinoflagellates, respectively. These experiments were conducted 4 times.

Each sample for enumeration of h-dinoflagellates (80 ml with 4 ml of glutaraldehyde solution) was stained with DAPI (final volume = 92 ml) and filtered through 3 Nuclepore filters (30 ml × 3; pore size = 0.8 µm) for epifluorescence microscopic enumeration. By observing 3 filters per sample (~100 fields $filter^{-1}$) at a magnification of ×250, h-dinoflagellates on the filters were counted for size categories of 15 to 20, 20 to 40 and >40 µm. The countings were conducted within 3 d after sampling.

Preserved ciliate/phytoplankton samples were concentrated by settling 30 ml of the sample for at least 24 h. The entire settling chamber was enumerated for all aloricate ciliates with size categories of 15 to 20, 20 to 40 and >40 µm using an inverted microscope at ×200 magnification. Phytoplankton was enumerated only for dominant species by observation at ×200.

The sizes of ciliates and the dominant phytoplankton species were measured and converted to cell volumes assuming simple geometric cell shapes. Cell volumes of h-dinoflagellates were assumed to be 1760, 4800 and 12000 $µm^3$ for cells of 15 to 20, 20 to 40 and >40 µm (Nakamura et al. 1995), respectively. Phytoplankton cell volume was converted to carbon using Strathmann's (1967) equation, and a conversion factor of 0.15 pg C $µm^{-3}$ was used for the estimation of the C-contents of ciliates and h-dinoflagellates (cf. Putt & Stoecker 1989, Lessard 1991).

Clearance rates (CR) of *Paracalanus* sp. were calculated using Frost's (1972) equation for prey items whose number in the initial sample exceeded 60 cells, only when the difference in the prey abundance between control and experimental bottles proved significant (*t*-test, $p < 0.05$).

**Results and discussion.** During the survey period, 4 peaks of chlorophyll *a* (18 and 27 July, 1 and 11 August) were observed (Fig. 1A); the second peak was due to a surface bloom of an autotrophic (a-) dinoflagellate, *Gymnodinium mikimotoi*, and the other peaks were due to diatom blooms (cf. Nakamura 1998). Ciliates were dominated by naked oligotrichs throughout the survey period; tintinnids were found only occasionally (<5% of total abundance). The abundance of ciliates (Fig. 1A) was relatively constant at ~10 cells $ml^{-1}$ during the first half of the survey, then showed a prominent peak on 2 to 3 August, after which it declined rapidly.

Based on the observations of live samples, h-dinoflagellates were dominated by naked gymnodinials such as *Gyrodinium dominans* (<40 µm) and *Gyrodinium spirale* (>40 µm) throughout the survey period. Thecate cells were observed only occasionally (<10% of the total abundance). The abundance of h-dinoflagellates was around ~15 cells $ml^{-1}$ during the first week of the survey, then showed a prominent peak on 28 July just after the bloom of *Gymnodinium mikimotoi* (Nakamura 1998; cf. Nakamura et al. 1995). In the second half of the survey, the abundance of h-dinoflagellates decreased to ~5 cells $ml^{-1}$ (Fig. 1A).

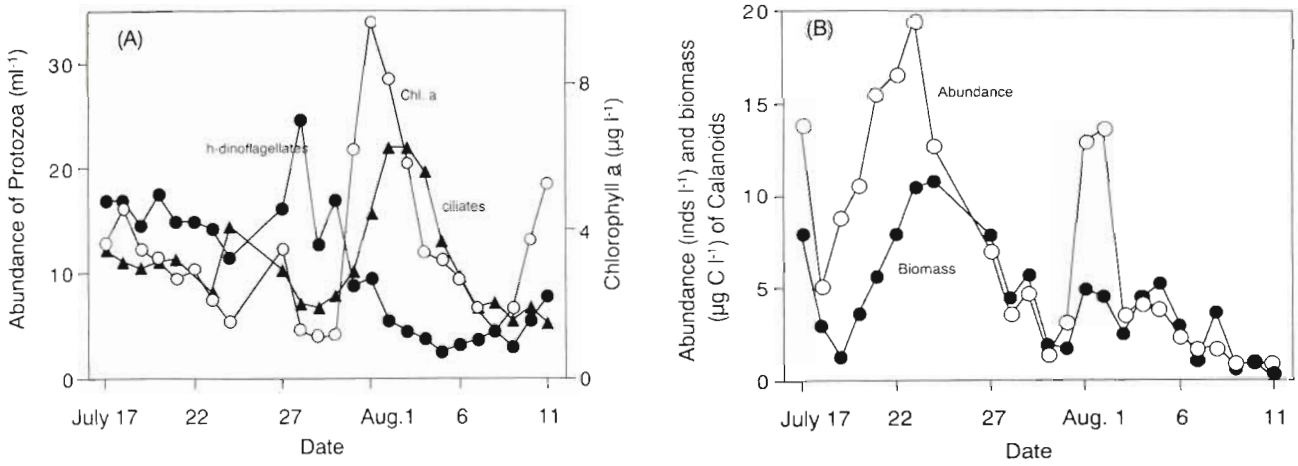


Fig. 1. Field observations. (A) Temporal changes in chlorophyll *a* (○) averaged over the water column; abundance of heterotrophic dinoflagellates (●) averaged between 0 and 10 m depth; and abundance of ciliates (▲) averaged between 0 and 10 m depth. (B) Temporal changes in abundance (○) and biomass (●) of calanoid copepods (nauplii not included)

Calanoid copepods were composed almost exclusively of *Paracalanus* sp. throughout the survey period. The abundance and biomass of calanoids averaged over the water column showed several peaks and ranged from 0.7 to 19.5 ind. $l^{-1}$ and 0.3 to 10.8 (temporal average = 4.4) µg C $l^{-1}$, respectively (Fig. 1B).

Results of the feeding experiments are summarized in Table 1. *Paracalanus* sp. ingested h-dinoflagellates of all size categories with a CR of 35 to 146 ml ind.$^{-1}$ d$^{-1}$ (n = 9, average = 76 ml ind.$^{-1}$ d$^{-1}$); no trends were apparent with a CR as a function of prey cell volume (Fig. 2A). *Paracalanus* sp. also ingested ciliates with a

Table 1. *Paracalanus* sp. Feeding experiments conducted in summer 1997. $N_{int}$: initial prey abundance, <P>: average of prey biomass in experimental bottles; CR: clearance rate; F: feeding rate; h-dinos: heterotrophic dinoflagellates. ns: not significant

| Expt (Date and conditions) | Prey item | $N_{int}$ (cells ml$^{-1}$) | <P> (µg C l$^{-1}$) | CR (ml ind.$^{-1}$ d$^{-1}$) | F (µg C ind.$^{-1}$ d$^{-1}$) |
|---|---|---|---|---|---|
| Expt 1 | H-dinos (15–20 µm) | 10.5 | 3.0 | 63 ± 8 | 0.19 |
| 19–20 Jul (23.7°C, 31.8‰) | (20–40 µm) | 10.2 | 6.4 | 35 ± 4 | 0.22 |
| | Ciliates (15–20 µm) | 14.3 | 1.8 | 35 ± 9 | 0.06 |
| | *Gymnodinium mikimotoi* | 22.0 | 3.8 | 47 ± 21 | 0.18 |
| | *Scripsiella* sp. | 69.8 | 5.3 | 45 ± 13 | 0.23 |
| Expt 2 | H-dinos (15–20 µm) | 20.1 | 4.0 | 45 ± 7 | 0.18 |
| 23–24 Jul (24.8°C, 31.9‰) | (20–40 µm) | 10.9 | 5.4 | 76 ± 10 | 0.41 |
| | (>40 µm) | 5.4 | 5.0 | 68 ± 7 | 0.34 |
| | Ciliates (15–20 µm) | 10.1 | 1.3 | 42 ± 14 | 0.05 |
| | (20–40 µm) | 3.1 | 1.3 | 30 ± 16 | 0.04 |
| | (>40 µm) | 2.1 | 4.2 | 60 ± 20 | 0.25 |
| | *Gymnodinium mikimotoi* | 21.3 | 3.3 | 50 ± 12 | 0.17 |
| Expt 3 | H-dinos (15–20 µm) | 8.3 | 1.6 | 61 ± 4 | 0.10 |
| 31 Jul–1 Aug (24.5°C, 29.5‰) | (20–40 µm) | 9.9 | 4.3 | 93 ± 32 | 0.40 |
| | Ciliates (15–20 µm) | 6.0 | 0.8 | ns | 0.00 |
| | *Ceratium fusus* | 9.2 | 7.5 | ns | 0.00 |
| Expt 4 | H-dinos (15–20 µm) | 9.4 | 1.8 | 85 ± 3 | 0.15 |
| 8–9 Aug (25.2°C, 31.1‰) | (20–40 µm) | 6.7 | 1.4 | 146 ± 17 | 0.20 |
| | Ciliates (15–20 µm) | 8.7 | 1.2 | 23 ± 18 | 0.03 |
| | *Scripsiella* sp. | 6.7 | 0.4 | 98 ± 72 | 0.04 |
| | *Gymnodinium mikimotoi* | 2.4 | 0.5 | 55 ± 40 | 0.03 |
| | *Ceratium fusus* | 2.9 | 1.7 | 55 ± 45 | 0.09 |

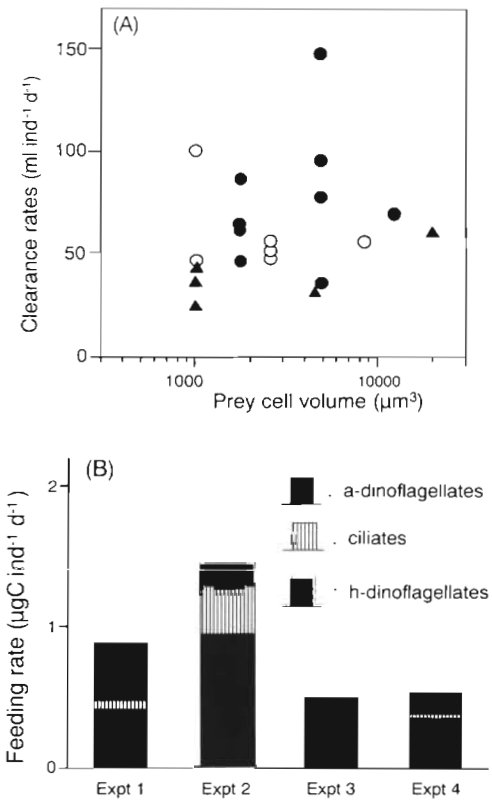


Fig. 2. *Paracalanus* sp. Feeding experiments. (A) Clearance rates for heterotrophic dinoflagellates (●), autotrophic dinoflagellates (○) and ciliates (▲) as a function of prey cell volume. (B) Feeding rates on motile prey

CR of 23 to 60 ml ind.$^{-1}$ d$^{-1}$ (n = 5, average = 38 ml ind.$^{-1}$ d$^{-1}$), except for Expt 3, where feeding was insignificant; CR values for ciliates (not including the results in Expt 3) were significantly less than for h-dinoflagellates (*t*-test, $p < 0.05$). Although not conclusive, the lower CR values for ciliates may be due to the difference in the swimming mode between h-dinoflagellates and ciliates: in contrast to the smooth and steady motion of h-dinoflagellates, ciliates (dominated by oligotrichs during the survey period) show a jerky motion with a much higher maximum swimming speed. The quick motion of the ciliates might enable them to escape much more easily from the feeding currents created by *Paracalanus* sp. (cf. Jonsson & Tiselius 1990), which probably cannot change its feeding mode to 'ambush' predation like *Acartia tonsa* (Bartram 1980, Kiørboe et al. 1996).

CR values for a-dinoflagellates were in the range 45 to 98 ml ind.$^{-1}$ d$^{-1}$ (n = 6, average = 58 ml ind.$^{-1}$ d$^{-1}$) except for a large thecate species, *Ceratium fusus*, in Expt 3; the difference in the CR values between h- and a-dinoflagellates (except the data for *C. fusus* in Expt 3) was not significant (*t*-test; $p > 0.1$). Since there are no differences in the swimming mode between a- and h-dinoflagellates, the comparable values of CR for both groups suggest that *Paracalanus* sp. does not discriminate prey items based on the presence of the chloroplast.

CR values obtained in the present study are higher than those for *Paracalanus* sp. fed the raphidophicean flagellate *Chattonella antiqua* (equivalent spherical diameter = 35 µm) (20 ml ind.$^{-1}$ d$^{-1}$; Uye 1986). The carbon specific clearance rates of *Paracalanus* sp. for h-dinoflagellates and ciliates were 33 and 17 ml µg C$^{-1}$ d$^{-1}$, respectively (C-content of the copepod [2.3 µg C ind.$^{-1}$] was estimated by the prosome length-body carbon relationship proposed by Uye [1991]). Furthermore, these values were converted to volume specific clearance rates of $2 \times 10^5$ and $1 \times 10^5$ h$^{-1}$ for h-dinoflagellates and ciliates, respectively (carbon:volume conversion factor of 0.16 pg C µm$^{-3}$ was assumed; Durbin & Durbin 1978); these values are comparable to those reported for other copepods (Hansen et al. 1997).

Diatoms such as *Chaetoceros lorenzianum* and *Skeletonema costatum* were also grazed by *Paracalanus* sp. with apparent clearance rates of 24 to 40 ml ind.$^{-1}$ d$^{-1}$ in some experiments, but not at all in others (data not shown). Since the bottles for feeding experiments were suspended from the pontoon, the obtained values might have been somewhat (but not correctable) underestimated due to sedimentation of the prey. Thus, feeding of *Paracalanus* sp. on diatoms is not discussed further in the present communication.

During the survey period, the biomass of calanoid copepods (composed almost exclusively of *Paracalanus* sp.) averaged over the water column was in the range 0.3 to 10.8 µg C l$^{-1}$ (temporal average = 4.4 µg C l$^{-1}$). Using the assumption that carbon specific clearance rates of *Paracalanus* sp. for h-dinoflagellates and ciliates are independent of the development stage of the copepod, the population of *Paracalanus* sp. cleared h-dinoflagellates and ciliates with rates of 10 to 360 (temporal average = 150) and 5 to 180 (temporal average = 75) ml l$^{-1}$ d$^{-1}$, respectively. Thus, when calanoids were abundant in the study area, their feeding impact on microprotozoans, especially h-dinoflagellates, was substantial.

Feeding rates of *Paracalanus* sp. for h-dinoflagellates were 0.35 to 0.93 µg C ind.$^{-1}$ d$^{-1}$; those for motile prey items (a-/h-dinoflagellates plus ciliates) were in the range of 0.50 to 1.44 µg C ind.$^{-1}$ d$^{-1}$ (Table 1, Fig. 2B). Since the minimum carbon requirement of *Paracalanus* sp. is calculated to be 0.5 µg C ind.$^{-1}$ d$^{-1}$ (cf. Ikeda 1985, Uye 1991; respiratory quotient of 0.8 is assumed), the feeding rates of *Paracalanus* sp. for h-dinoflagellates were close to or higher than the carbon requirement to maintain basic metabolic activities. This, together with the fact that *Paracalanus* sp. could have a

significant impact on the population of h-dinoflagellates (see above), indicates the importance of the trophic link between h-dinoflagellates and copepods for the carbon flow in the study area.

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*Editorial responsibility: John Dolan, Villefranche-sur-Mer, France*

*Submitted: October 12, 1998; Accepted: January 24, 1999*
*Proofs received from author(s): April 9, 1999*