Note

Feeding by the cyclopoid copepod *Oithona similis* on the microplankton assemblage in the Oyashio region during spring

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Abstract: Onboard incubation experiments were conducted to examine the feeding rates and selectivity of the cyclopoid copepod *Oithona similis* on natural assemblages of microplankton during the spring bloom in the Oyashio region. *O. similis* cleared ciliates at higher rates (2.8–11.9 mL copepod^{−1} d^{−1}) than dinoflagellates (1.4 mL copepod^{−1} d^{−1}), with no detectable ingestion of diatoms. Clearance increased with ciliate size, reaching maximum rates at a size of ~35 μm equivalent spherical diameter. The total ingestion rates of protozooplankton (ciliates and dinoflagellates) by *O. similis* ranged between 24.8 and 41.5 ngC copepod^{−1} d^{−1}, which exceeded by about 2–3 times the metabolic expenditure of the copepod. Applying the ingestion rates to abundance data of *O. similis*, we estimated that the copepod population removed each day 0.1–0.4% of the protozooplankton standing stock. These results indicate that protozooplankton, especially aloricate ciliates, are an important food resource available to *O. similis* even under bloom conditions in the Oyashio region, though the predation by the copepod did not have a significant impact on protozooplankton assemblages.

Key words: ciliates, clearance rate, *Oithona similis*, selective feeding

The cyclopoid family Oithonidae is often the most abundant group of pelagic copepods in estuarine, coastal and oceanic waters throughout the world (Paffenhöfer 1993, Gallienne & Robins 2001). Although their numerical importance has been increasingly recognized, our present knowledge on the ecology of oithonid copepods is still rather limited compared to calanoids.

Early laboratory studies using cultured prey have shown that oithonid copepods are omnivorous, ingesting a wide variety of autotrophic and heterotrophic prey items (e.g. Lampitt & Gamble 1982; Drits & Semenova 1984; Uchima & Hirano 1986). Oithonids have also been observed to feed on fecal pellets of other zooplankton (González & Smetacek 1994), though their coprophagous feeding is still controversial (Reigstad et al. 2005; Iversen & Poulsen 2007). Recent experimental studies employing natural prey assemblages have revealed that oithonids feed selectively on microzooplanktonic protists, such as ciliates and heterotrophic dinoflagellates (e.g. Nakamura & Turner 1997; Lonsdale et al. 2000; Castellani et al. 2005a; Atienza et al. 2006). Considering their high abundance, oithonid copepods may serve as an important conduit to trans-

fer microbial production to higher trophic levels in marine pelagic food webs.

Oithona similis Claus, a small oithonid of 0.7–0.9 mm body length, dominates the copepod community in terms of abundance throughout the subarctic Pacific (Mackas & Tsuda 1999), but its trophic ecology and role in this region are not yet well understood. Therefore we examined the feeding rates and selectivity of *O. similis* on natural assemblages of microplankton in the Oyashio region during spring. The emphasis of our study was to assess the importance of protozooplankton (ciliates and dinoflagellates) as a food source for *O. similis* when the diatom bloom occurred, and the predation impact of the copepod population on the protozooplankton assemblage was also estimated.

The present study was conducted at a station (42°00'N, 145°15'E, 4,000 m deep) in the Oyashio region, western subarctic Pacific from 16 to 21 April 2007 during a cruise of the R/V 'Hakuho-Maru'. During the study period, the station was under diatom bloom conditions (chl. *a* concentrations: 2.5–5.8 μg L^{−1}, T Kobari, unpubl. data). Experiments were made on three occasions using female specimens sampled on different dates (Table 1). Copepods were collected by slow vertical hauls from 10 m depth using a ring net (45 cm mouth diameter, 50 μm mesh size) equipped with a large non-filtering cod-end. Adult females of *O. similis* were immediately sorted out in a

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Table 1. Summary of the feeding experiments. Taxonomic group, size and initial abundance of microplankton prey, and clearance and ingestion rates (mean $\pm$ SD) of female *Oithona similis* are included. Significant levels for the difference between prey abundances in control and experimental bottles are also shown (** $p<0.01$, ** $p<0.05$, * $p<0.10$, ns: not significant). ESD: equivalent spherical diameter.

Expt	Date	Temp (°C)	Prey taxon	Size (μ m ESD)	Initial abundance ($\times 10^3$ cells L $^{-1}$)	Clearance rate (mL copepod $^{-1}$ d $^{-1}$)	Ingestion rate (ngC copepod $^{-1}$ d $^{-1}$)
1	16–17 April 2007	5.0	Diatoms	27	70	ns	—
			<i>Myrionecta rubra</i>	22	7.6	3.1 $\pm$ 1.2**	18.8 $\pm$ 5.7
			Aloricate choreotrichs (small)	16	0.86	2.8 $\pm$ 1.3**	0.91 $\pm$ 0.35
			Aloricate choreotrichs (medium)	22	1.3	4.7 $\pm$ 1.7***	4.6 $\pm$ 1.1
			Aloricate choreotrichs (large)	34	0.45	10.7 $\pm$ 3.8***	9.0 $\pm$ 1.3
			Tintinnids	34 ^a	0.13	ns	—
			Athecate dinoflagellates	19	12	ns	—
			Thecate dinoflagellates	28	1.7	ns	—
2	18–19 April 2007	5.4	Diatoms	26	105	ns	—
			<i>Myrionecta rubra</i>	21	2.3	6.6 $\pm$ 0.8**	9.2 $\pm$ 0.7
			Aloricate choreotrichs (small)	16	3.0	4.6 $\pm$ 0.6***	4.5 $\pm$ 0.4
			Aloricate choreotrichs (medium)	24	1.2	5.9 $\pm$ 1.9***	7.0 $\pm$ 1.5
			Aloricate choreotrichs (large)	33	0.62	8.8 $\pm$ 2.3***	11.9 $\pm$ 1.5
			Tintinnids	30 ^a	0.25	4.2 $\pm$ 2.4**	0.88 $\pm$ 0.43
			Athecate dinoflagellates	18	13	1.4 $\pm$ 0.5*	8.1 $\pm$ 2.7
			Thecate dinoflagellates	24	2.8	ns	—
3	20–21 April 2007	5.2	Diatoms	27	62	ns	—
			<i>Myrionecta rubra</i>	18	1.2	3.7 $\pm$ 0.3***	3.0 $\pm$ 0.2
			Aloricate choreotrichs (small)	15	2.1	3.9 $\pm$ 0.4***	3.6 $\pm$ 0.3
			Aloricate choreotrichs (medium)	23	0.76	8.0 $\pm$ 1.5***	5.4 $\pm$ 0.6
			Aloricate choreotrichs (large)	37	0.32	11.9 $\pm$ 4.1***	12.1 $\pm$ 1.2
			Tintinnids	30 ^a	0.29	4.2 $\pm$ 1.4***	0.76 $\pm$ 0.19
			Athecate dinoflagellates	21	23	ns	—
			Thecate dinoflagellates	24	4.1	ns	—

^a Calculated from lorica volume.

constant temperature room ($\sim 4^\circ\text{C}$) using a dissecting microscope and placed into 500 mL glass containers filled with seawater. These specimens were maintained overnight in the room. Seawater for experiments was collected from 5 m depth with 10 L Niskin-X bottles mounted on a rosette multi sampler and screened through an in-line filter (64 μm mesh). This screening procedure may have altered the composition of natural prey assemblages for the experiments by removing large diatom chains or by damaging some fragile ciliates, but was necessary to remove all metazoans. The screened seawater was mixed gently and poured into six 280 mL acid-washed polycarbonate bottles. A 200 mL sample was also taken from the screened seawater and immediately fixed with acid Lugol's solution at a final concentration of 2% to determine the initial prey abundance. A total of 30–40 female specimens were added to each of three bottles, while the other three bottles without copepods served as controls. The copepod concentrations in the bottles were higher than those in the ambient environment, but were chosen to ensure significant reductions in prey density at high food levels (i.e. under bloom conditions). Incubations were carried out for 24 h at *in situ* temperatures in a container on deck with flow-through surface seawater under natural light conditions attenuated to 50% of incident light

using a neutral screen. The temperature was recorded by a temperature logger every 30 min. Except for the ship motion, the bottles in the container were not stirred. At the end of incubation, a 200 mL aliquot from each bottle was fixed with acid Lugol's solution at a final concentration of 2% for enumeration of the remaining prey organisms.

The fixed samples were concentrated to 10 mL by settling in a glass cylinder for at least 24 h. A 0.5–1 mL aliquot of the sample thus concentrated was transferred to a counting chamber. Microplankton cells ($>10 \mu\text{m}$), including ciliates, dinoflagellates and diatoms, were counted under an inverted microscope at a magnification of 200 $\times$. Identification of ciliates was based on Montagnes & Lynn (1991) and Ströder-Kypke et al. (2001). Identified species and morphotypes were pooled into taxonomic categories and size classes (Table 1). Dinoflagellates were assigned as either athecate or thecate forms, since it is difficult to distinguish autotrophic from heterotrophic cells in samples fixed in Lugol's solution. By using epifluorescence microscopy, however, it was found that the dominant taxa of athecate (*Gymnodinium* spp. and *Gyrodinium* spp.) and thecate dinoflagellates (*Oxytoxum* spp.) during the study period were non-pigmented (T Ota, unpubl. data). Cell volume of each prey category was estimated from measurements of linear di-

mensions made with an image analyzer with CCD camera, by assuming simple geometrical shapes.

Clearance and ingestion rates of *O. similis* were calculated for each prey category according to the equations of Frost (1972) only when the difference in prey concentration between control and experimental bottles proved significant (Student's *t*-test, Table 1). The ingestion rates were converted to carbon on the basis of prey cell volumes using a factor of $0.19 \text{ pgC } \mu\text{m}^{-3}$ for ciliates (Putt & Stoecker 1989) and the equation $\log \text{pgC (cell}^{-1}) = -0.119 + 0.819 \times \log \text{volume (}\mu\text{m}^{-3})$ for athecate dinoflagellates (Menden-Deuer & Lessard 2000).

In all three experiments, the microplankton assemblage was dominated by diatoms (Table 1), mainly consisting of *Thalassiosira* spp. and *Chaetoceros* spp. Atecate dinoflagellates were the second most important component of the microplankton, whereas thecate dinoflagellates were less abundant. Ciliates, including *Myrionecta rubra* (Lohmann), aloricate choreotrichs and tintinnids, accounted for 5–11% of the total microplankton abundance. Aloricate choreotrichs were separated into three size classes (<20 , 20 – 30 and $>30 \mu\text{m}$ in maximum cell dimension); dominant genera were *Lohmaniella* for the small group, *Strombidium* and *Leegaardiella* for the medium group, and *Strombidium* and *Strobilidium* for the large group.

Oithona similis fed on all size classes of aloricate choreotrichs and *M. rubra* across the three experiments, with clearance rates of 2.8 – 11.9 and 3.1 – $6.6 \text{ mL copepod}^{-1} \text{ d}^{-1}$, respectively (Table 1). Clearance was detected on tintinnids in two out of the three experiments, with a rate of $4.2 \text{ mL copepod}^{-1} \text{ d}^{-1}$. Atecate dinoflagellates were ingested on only one occasion, with a relatively low clearance rate of $1.4 \text{ mL copepod}^{-1} \text{ d}^{-1}$. No detectable clearance of thecate dinoflagellates and diatoms was measured during any experiment. These results indicate that *O. similis* fed selectively on ciliates over dinoflagellates and diatoms, independent of their relative abundances. Food particle size is an important factor governing prey selection in copepods (Berggreen et al. 1988), but no clear difference was found in cell size, expressed as equivalent spherical diameter (ESD), between the three prey categories examined in the present study (Table 1). Another important factor that potentially influences copepod feeding preference is the motility of prey, especially for ambush feeding copepods such as *O. similis* (Jonsson & Tiselius 1990; Jakobsen et al. 2005). Svensen & Kiørboe (2000) demonstrated that *O. similis* detects its prey remotely from the hydromechanical signals generated by the swimming of prey organisms. Hence, highly motile ciliates may be positively selected for by *O. similis* compared to less motile dinoflagellates and immobile diatoms due to their higher detectability by the copepod. Higher clearance rates on ciliates than on dinoflagellates or diatoms have been reported for *O. similis* (Nakamura & Turner 1997; Castellani et al. 2005a) and other *Oithona* spp. (Lonsdale et al. 2000; Atienza et al. 2006), though the opposite trend has also been observed (Atkinson 1995, 1996).

The highest clearance rates on ciliates by *O. similis* measured in each experiment (8.8 – $11.9 \text{ mL copepod}^{-1} \text{ d}^{-1}$, Table

1) roughly agree with those reported for *Oithona* spp. (mostly *O. similis*) in subpolar and polar waters, where the thermal conditions (0 – 8.8°C) are comparable to the present study (~ 3 – $23.5 \text{ mL copepod}^{-1} \text{ d}^{-1}$, Atkinson 1995, 1996, Castellani et al. 2005a), with the exception of the extremely high value of $\sim 75 \text{ mL copepod}^{-1} \text{ d}^{-1}$ at 0°C in Lonsdale et al. (2000). However, it should be noted that our high clearance rates might be underestimates to some extent due to bottle effects (i.e. incubation with a large number of specimens in a small volume), particularly for the large aloricate choreotrichs, whose abundance could have been greatly reduced before the end of incubation (cf. Table 1).

Clearance rates of *O. similis* were clearly related to size of ciliate prey (Fig. 1); the maximum rates were observed for the large aloricate choreotrichs with a size of $\sim 35 \mu\text{m}$ ESD. This suggests that *O. similis* prefers large ciliates, possibly due to their easier detection and ease of capture of larger prey relative to smaller prey items. Nakamura & Turner (1997) also found such a size-dependent difference in clearance rates for *O. similis* when feeding on aloricate ciliates and dinoflagellates, with the maximum rate at 30 – $35 \mu\text{m}$ ESD. Tintinnids had a relatively large body size of $30 \mu\text{m}$ ESD, but clearance rates on them were similar to or even lower than those on other smaller taxa (Fig. 1). This rather low clearance rate for tintinnid prey may partly be explained by the presence of a lorica around their cells which is considered to reduce successful capture and ingestion by copepods (Stoecker & Sanders 1985).

The total ingestion rates of protozooplankton by *O. similis* ranged from 24.8 – $41.5 \text{ ngC copepod}^{-1} \text{ d}^{-1}$, which is equivalent to 5.1 – 8.7% of its body carbon d^{-1} (Table 2). Large aloricate choreotrichs and *M. rubra* together made up more than half of the total carbon ingested (Table 1). Based on the relationship between respiration rate and temperature (Castellani et al. 2005b), the daily carbon requirement for respiration of *O. similis* was calculated as $12.7 \text{ ngC copepod}^{-1} \text{ d}^{-1}$ or 2.6% of body C d^{-1} , assuming a respiratory quotient of 0.97 (Ikeda et al. 2000). Thus, the ingestion rates measured here were about 2–3

Fig. 1. Clearance rates of female *Oithona similis* on different taxonomic groups of ciliates, as a function of ciliate size. Error bars, which indicate $\pm$ SD, are shown when they exceed the size of a symbol.

Table 2. Carbon weight, total ingestion rate on protozooplankton and daily ration of female *Oithona similis*. Values are mean $\pm$ SD

Expt	Carbon weight (ngC) ^a	Total ingestion rate of protozooplankton (ngC copepod ⁻¹ d ⁻¹)	Daily ration (% body C d ⁻¹)
1	488 $\pm$ 55	33.3 $\pm$ 6.9	6.8 $\pm$ 1.4
2	480 $\pm$ 55	41.5 $\pm$ 2.8	8.7 $\pm$ 0.6
3	485 $\pm$ 83	24.8 $\pm$ 1.7	5.1 $\pm$ 0.3

^a Estimated from prosome length measured for formalin-preserved individuals ($n=30$) using the length-weight regression from Sabatini & Kiørboe (1994).

times higher than the metabolic expenditure of *O. similis*. This indicates that protozooplankton, especially aloricate ciliates, are an important food source for *O. similis* even during the spring diatom bloom, though the contribution of other potential foods including phytoflagellates (Castellani et al. 2005a), copepod nauplii (Nakamura & Turner 1997) and fecal pellets (González & Smetacek 1994) was not assessed in our experiments.

To determine the abundance of *O. similis*, a ring net (36 cm mouth diameter, 63 μ m mesh size) equipped with a flow meter (Rigosha) was hauled vertically at a speed of 0.5 m s⁻¹ from 150 m depth on 16, 18 and 20 April. Samples were preserved immediately on board in a 2% formaldehyde-seawater solution buffered with borax. Adults and copepodites of *O. similis* were enumerated under a stereomicroscope. Abundance of the *O. similis* population (adults and copepodites) ranged between 1,410 and 2,360 indiv. m⁻³ during the study period. Assuming that the weight-specific ingestion rate of *O. similis* was independent among the developmental stages of the copepod (cf. Table 2), the population ingestion rates were estimated as 23.4–55.8 μ gC m⁻³ d⁻¹, which equates to 0.3–1.5% and 0.1–0.4% of the ciliate (3.8–9.8 μ gC L⁻¹, T. Ota, unpubl. data) and total protozooplankton biomass (14.0–35.6 μ gC L⁻¹) in the surface water, respectively. Thus, the predation impact on the protozooplankton assemblage by *O. similis* appeared to be low in the Oyashio region during spring. However, considering that the abundance of *O. similis* reaches as high as $\sim$ 20,000 indiv. m⁻³ in mid summer (Y Nishibe, unpubl. data), the copepod may seasonally be important as a consumer of protozooplankton and as an intermediary in the transfer of microbial production to higher trophic levels in this region.

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