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Article *in* Marine Biology · April 2010

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DOI 10.1007/s00227-009-1353-4

ORIGINAL PAPER

Trophic role of small cyclopoid copepod nauplii in the microbial food web: a case study in the coastal upwelling system off central Chile

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Received: 15 May 2009 / Accepted: 18 November 2009 / Published online: 4 December 2009

Abstract Copepod grazing impact on planktonic communities has commonly been underestimated due to the lack of information on naupliar feeding behaviour and ingestion rates. That is particularly true for small cyclopoid copepods, whose nauplii are mainly in the microzooplankton size range (<200 μm). The trophic role of *Oithona* spp. nauplii was investigated off Concepción (central Chile, ~36°S) during the highly productive upwelling season, when maximum abundances of these nauplii were expected. Diet composition, ingestion rates, and food-type preferences were assessed through grazing experiments with different size fractions of natural planktonic assemblages (<3, <20, <100, and <125 μm) and cultures of the nanoflagellate *Isochrysis galbana*. When the *Oithona* spp. nauplii were offered a wide range of size fractions as food (pico- to microplankton), they mostly ingested small (2–5 μm) nanoflagellates (5–63 × 10^3 cells nauplius^{-1} day^{-1}). No ingestion on microplankton was detected, and picoplankton was mainly ingested when it was the only food available. Daily carbon (C) uptake by the nauplii ranged between 28 and 775 ng C nauplius^{-1}, representing an overall mean of 378% of their body C. Our relatively high ingestion rate estimates can be explained by methodological constraints in previous studies on naupliar feeding, including those dealing with "over-crowding" and "edge" effects. Overall, the grazing impact of the *Oithona* spp. nauplii on the prey C standing stocks amounts up to 21% (average = 13%) for picoplankton and 54% (average = 28%) for nanoplankton. These estimates imply that the nauplii of the most dominant cyclopoid copepods exert a significant control on the abundances of nanoplankton assemblages and, thereby, represent an important trophic link between the classical and microbial food webs in this coastal upwelling system.

Communicated by X. Irigoien.

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Introduction

Small (<1 mm total length) cyclopoid copepods of the genus *Oithona* are ubiquitous and abundant in the world's ocean (Gallienne and Robins 2001) but knowledge of their trophic, ecological, and biogeochemical role in pelagic systems is minimal compared to what is known for larger calanoids (reviewed in Turner 2004). Most research on the feeding of *Oithona* species has been dedicated to adults (e.g. Lampitt and Gamble 1982; Atienza et al. 2006; Castellani et al. 2008), and extrapolation to younger stages is extremely difficult when considering the differences in feeding behaviour due to changes in the morphology of the mouthparts, combined with differences in body size and bioenergetic requirements (Fernandez 1979; Brucet et al.

2008). If, as a first approximation, the optimal food size for planktonic predators varies according to the predator/prey size ratios (Hansen et al. 1994), the size range of food ingested by nauplii would, as a consequence, be much smaller than that of adults.

Feeding studies with adult *Oithona* using a limited range of cultured organisms or mixed natural assemblages revealed that they have an omnivorous diet of autotrophic and heterotrophic cells including nanoflagellates, dinoflagellates, ciliates, diatoms, and small calanoid nauplii (e.g. Lampitt and Gamble 1982; Nakamura and Turner 1997; Vargas and Gonzalez 2004a). So far, only two feeding studies have been reported for *Oithona* nauplii. Lonsdale et al. (2000) incubated *Oithona* spp. with natural microzooplankton assemblages (<64 μm) and found higher ingestion of ciliates compared to dinoflagellates. The study of Henriksen et al. (2007) showed that nauplii of *Oithona davisae* fed on motile prey (*Heterocapsa* sp.) but not on non-motile prey (*Thalassiosira weissflogii*). Adults and nauplii of *Oithona* as well as other small calanoid copepods are, in turn, prey for higher trophic levels such as fish larvae and other planktivores (Turner 2004) and, thus, can act as a trophic link between the microbial and classical food webs.

In the coastal zone of the highly productive upwelling system off Concepción, central Chile, the microbial and classical food webs coexist and small cyclopoid as well as calanoid copepods are highly abundant (Escribano et al. 2007; Vargas et al. 2007). Among the cyclopoids, two cosmopolitan species of *Oithona* co-occur, *O. similis* and *O. nana*. They display several peaks in abundance throughout the annual cycle (Escribano et al. 2007) and continuously reproduce throughout the year (Torres 2006), which implies that their populations never experience food limitation. According to our observations, most of the naupliar stages of *Oithona* in this area are smaller than 200 μm in body length (stages NI-NVI in *O. nana*: <160 μm and stages NI-NV in *O. similis*: <193 μm) and, therefore, constitute part of the microzooplankton size fraction. Given an optimal ratio of 18:1 of predator/prey sizes (Hansen et al. 1994), their potential food supply should be composed of picoplankton and small (<10 μm) nanoplankton assemblages. Since *Oithona* spp. nauplii are a relatively stable component in this coastal area (Torres 2006), together with picoplankton (Alarcon 2008) and nanoplankton (Böttjer and Morales 2007), we hypothesize that they exert a relatively high grazing pressure upon the abundance and biomass of microbial assemblages. To test this, naupliar grazing experiments using natural microbial assemblages as food were performed during the productive upwelling period, when maximum in naupliar abundances were expected.

Materials and methods

Field collection and initial preparation of the grazing experiments

Plankton samples were taken at the mouth of Coliumo Bay (36°32′S, 72°56′W), central Chile, during the austral spring-summers of 2004, 2005, 2006, and 2007. Copepods were sampled by slow horizontal hauls in the surface layer (0–10 m) using a WP-2 plankton net (mesh size 200 μm) fitted with a non-filtering cod-end (0.5 L). These samples were immediately poured into a thermo-box (20 L), previously filled with surface seawater, and transported within 1 h after collection to the laboratory of the Marine Biological Station in Dichato. After zooplankton collection, seawater for the incubation of *Oithona* adult females was obtained from 10 m depth using a Niskin bottle (General Oceanic® Model 1010, equipped with interior rubber-coated springs, 10 L volume), which was gently transferred by silicone tubing into clean, acid-washed polyethylene carboys (10 L). Once in the laboratory, all samples were maintained in a cold room at the appropriate in situ water temperature (Table 1) for a couple of hours until the completion of sample processing.

Undamaged adult females of *O. nana* and/or *O. similis* carrying ovigerous sacs were sorted from the zooplankton collections by examining sub-samples under a stereomicroscope (Zeiss Stemi® 2000-C). Between 100 and 150 of these females were transferred to several 1-L glass beakers (~30 females per beaker) containing natural food (collected seawater screened through 100-μm mesh) and incubated at a temperature similar to in situ conditions (11.3–12.2°C), at the irradiance level expected at approximately 10 m depth (110 μmole photon m^{-2} s^{-1}) on a 12:12 h light/dark cycle. Every day, the water and food contained in the beakers was changed. For this purpose, the water was very gently poured through a 20-μm mesh fitted to a filtering funnel, so that the females and freshly hatched nauplii were retained and examined under a stereomicroscope. Dead females were removed, and the hatched nauplii were transferred to separate glass bowls filled with ~300 mL filtered seawater (0.2 μm) and the food type corresponding to the subsequent experiment. The remaining females that still carried ovigerous sacs were relocated in the beakers with new filtered seawater (0.2 μM) and food supply. These procedures were maintained until a sufficient number (21–60) of *O. nana* NV (~150 μm) and/or *O. similis* NIV-NV (size range: 155–180 μm) stages was obtained to start the grazing experiments. The naupliar stages of *O. nana* and *O. similis* were distinguished and selected according to their length, as described by Haq (1965; *Oithonina nana* = *Oithona nana*).

Table 1 Experimental conditions during the *Oithona* spp. nauplii incubations with natural food assemblages and cultures of *Isochrysis galbana*

Exp.	Date	*T*	Food offered	Predator density	Initial food concentration		
	(D/M/Y)	(°C)	(μm)	(nauplii 600 mL^{-1})	μg Chl-*a* L^{-1}	10^6 cells L^{-1}	μg C L^{-1}
1	22/11/2004	11.5	Natural <125	20[a]	6.8	3.3*	143*
2	20/01/2005	11.5	Natural <125	12[a]	3.1	2.3*	69*
3	02/02/2005	11.5	Natural <100	9[a]	3.2	1.7*	70*
4	30/03/2005	12.0	Natural <100	9[a]	0.4	0.8*	50*
5	26/09/2005	12.0	Natural <100	7[a, b]	6.5	5.3*	587*
6	27/10/2005	11.5	Natural <100	13[a, b]	6.1	6.5*	306*
7	04/10/2006	11.3	Natural <20	11[a]	–	1,346	34
8	05/10/2006	11.3	Natural <20	12[a]	–	1,330	34
9	28/02/2007	12.2	Natural <20	13[a, b]	0.8	2,089	54
10	01/03/2007	12.0	Natural <20	10[a, b]	0.9	2,964	75
11	04/10/2006	11.3	Natural <3	11[a]	–	591	14
12	05/10/2006	11.3	Natural <3	12[a]	–	736	18
13	28/02/2007	12.2	Natural <3	11[a, b]	0.02	1,138	28
14	01/03/2007	12.0	Natural <3	11[a, b]	0.02	1,289	31
15	23/11/2004	11.5	*I. galbana*	15[a]	6.4	6.0	79
16	19/01/2005	11.5	*I. galbana*	10[a]	11.7	10.5	167

Initial prey concentrations are expressed as chlorophyll-*a* concentration, abundance or biomass

T in situ temperature; – not determined

[a] Presence of *Oithona nana*

[b] Presence of *Oithona nana* and/or *O. similis*

* Picoplankton size-fraction not included

Natural food assemblages were offered in 14 experiments (Table 1) with water collected (Niskin bottles) on the same day (at ~9 AM) before the start of the grazing experiments and from the same location and depth where the adult copepods had been previously collected. Seawater was transferred gently by silicone tubing into clean, acid-washed polyethylene carboys (10 L), transported to the laboratory and maintained in the same cold room as the copepods. Subsequently, it was screened through the appropriate mesh size, according to the selected food type treatment (<125, <100, <20 or <3 μm; Table 1).

Two supplementary experiments were carried out with the cultured nanoflagellate *Isochrysis galbana* (~5 μm cell length), that was directly obtained from a hatchery system. *I. galbana* was used in its exponential growth phase, and 3 mL of its stock culture were added to each of the incubation bottle containing filtered seawater <0.2 μm.

Grazing experiments

Grazing experiments were carried out according to the food removal technique (Gifford 1993), using bottle incubations of nauplii and food for a period of time. Nine bottles (600 mL) were used in each experiment: 6 controls (3 for t_1 and 3 for t_2) containing only the food and 3 including the food and a known number of nauplii (Table 1). These bottles were completely filled and sealed with Parafilm® to exclude or prevent the formation of air bubbles. They were then placed on a rotator wheel at ~0.5 rpm to keep the food and nauplii in suspension, and incubated for ~24 h under the light and temperature conditions described in the previous section. Initial samples for cell abundance, biomass, and total Chl-*a* were taken from the control bottles after 1 h incubation, following the recommendation of Gifford (1993). For natural microplankton and *Isochrysis galbana* samples, 100 mL sub-sample were preserved with Lugol's solution (5% final concentration) and stored cold (4°C) in the dark. Samples for nano- and picoplankton (20 and 10 mL, respectively) were preserved with glutaraldehyde (2% final concentration) and stored in the same way as the microplankton samples. For Chl-*a* analysis, triplicate 100 mL samples were filtered onto fiberglass GF/F filters and frozen; these filters were subsequently extracted in 10 mL 90% acetone for ~20 h, and the fluorescence was measured using a Turner Designs® TD-700 Fluorometer. At the end of the incubations, samples from the control and grazing bottles (t_2) were collected and treated as described above. Finally, the remaining water in each bottle was gently poured through a 20-μm mesh fitted to a filtering funnel in order to recover the nauplii and check their condition.

In experiments 1–6 (Table 1), nano- to microplankton size food was offered to the *Oithona* spp. nauplii. These samples

were analyzed using an inverted microscope (Nikon® TE2000S equipped with a digital camera Nikon® Coolpix 4500) after settling 50 mL sub-samples in sedimentation chambers for 24 h (Utermöhl 1958). Depending on cell size, cells were enumerated and measured at 400× or 1,000× magnification and separated into the main functional groups (nanoflagellates, diatoms, dinoflagellates, and ciliates). At least 150 cells in each taxon were counted (counts of chain-forming diatoms refer to the number of cells not chains). Nanoflagellates and dinoflagellates were distinguished according to size (nanoflagellates: 2–5, 5–10, and 10–15 μm; dinoflagellates: 8–19, 20–39, and >40 μm). The picoplankton in these experiments was not analyzed, since we initially assumed that it was too small to be food for the nauplii. This assumption, however, was tested in an additional set of experiments (7–14; Table 1), in which pico- and/or nanoplankton size food was offered to the *Oithona* spp. nauplii. Those samples were analyzed by epifluorescence microscopy (Porter and Feig 1980) using the same microscope as above equipped with an epifluorescence unit and using UV, blue, or multiple excitations (Nikon® Filter Blocks DAPI UV-2E/C, NB-2A and DAPI/FITC/TRITC). Sub-samples of 3 mL (picoplankton) and 20 mL (nanoplankton) were stained with DAPI (4′, 6-diamidino-2-phenylindole) at a final concentration of 0.01% and filtered onto black polycarbonate filters (0.2 μm for picoplankton and 0.8 μm for nanoplankton) supported by 0.45-μm membrane filters. The polycarbonate filters were mounted on slides and stored at −20°C in the dark until examination. Cells were enumerated at 1,000× magnification; picoplankton was distinguished as bacterioplankton (heterotrophs) and cyanobacteria (autotrophs) and nanoplankton as autotrophs/mixotrophs and heterotrophs, according to the type of fluorescence emitted by the cells. Mean cell size in each group (30 cells per group) was estimated using the software Image Pro Plus® (Version 4.5). The samples obtained from the experiments with cultured nanoflagellates as food were processed as already described for the nano- to microplankton size food of experiments 1–6, except that 10 mL sub-samples were settled in sedimentation chambers for 12 h.

Oithona spp. naupliar clearance and ingestion rates, as well as prey growth, were assessed from changes in total Chl-*a* and in cell abundance per prey type between the start and end of the incubations, using the equations provided by Frost (1972). Clearance and ingestion rate estimates were only considered in further analysis when (1) the difference in prey abundances between the control and grazing treatments at the end of the incubation proved to be significantly higher in the control and (2) prey net growth rates were significantly higher in control than in experimental bottles (Student's *t*-test; Zar 1999). Cases in which prey net growth in control bottles was significantly lower than in experimental bottles suggest a trophic cascading effect (Tang et al. 2001). In incubation experiments dealing with natural assemblages, several trophic relationships are co-occurring. In this study, the growth of prey, which are potentially consumed by the nauplii (pico- and nanoplankton), could be higher in the experimental bottles if the nauplii also exert a predation on protistan heterotrophs (e.g. larger nanoflagellates, dinoflagellates, or ciliates) feeding on the same food items. On the other hand, if the nauplii do not consume that type of protistan predators but they both consume the same type of prey (pico- and smaller nanoplankton), their ingestion rates would be overestimated. In order to correct for this potential bias, prey and protistan predator net growth rates in the final control and grazing bottles plus their grazing rates were examined.

Carbon ingestion rates on the different prey types were estimated from the carbon content of the prey. For this purpose, cell dimensions and an appropriate geometric formula (Sun and Liu 2003) for each food type were used to calculate cell volume (V, in μm^3), and the carbon content per cell (C) was obtained from the following conversion factors:

Bacterioplankton: $C\ (fg) = 90.06 \times V^{0.59}$ (Simon and Azam 1989)
Cyanobacteria: $C\ (fg) = 82\ cell^{-1}$ (Worden et al. 2004)
Flagellates: $C\ (fg) = 220 \times V$ (Børsheim and Bratbak 1987)
Diatoms: $\log_{10} C\ (pg) = -0.541 + 0.811 \times \log_{10} V$ (Menden-Deuer and Lessard 2000)
Dinoflagellates: $\log_{10} C\ (pg) = -0.353 + 0.864 \times \log_{10} V$ (Menden-Deuer and Lessard 2000)
Ciliates: $\log_{10} C\ (pg) = -0.639 + 0.984 \times \log_{10} V$ (Menden-Deuer and Lessard 2000)

Daily carbon rations for the *Oithona* spp. nauplii were estimated from the proportion of the carbon ingestion rates and the mean carbon content of the nauplii. To estimate the latter, a mean naupliar length of 165 μm was applied in the equation of Berggreen et al. (1988):

$$C(ng) = 3.18 \times 10^{-6} L^{3.31}$$

where L represents total length of the copepod (in μm); this resulted in an estimate of 69.55 ng C $nauplius^{-1}$.

Selective feeding by the nauplii was assessed by using an electivity index (E^*) (Vanderploeg and Scavia 1979):

$$E_i* = \frac{W_i - (1/n)}{W_i + (1/n)}$$

with n as the total number of kinds of prey in a given experiment, and the selectivity coefficient W_i is defined by:

$$W_i = \frac{F_i}{\sum F_i}$$

where F_i is the clearance rate of the ith food type, and ΣF_i is the sum of clearance rates on all food types. The

electivity index E^* ranges between −1 and +1; negative values correspond to avoidance; zero values represent neutrality, and positive values represent selectivity. The use of this index has been especially recommended in cases where the different food types are not equally abundant.

Results

Initial prey characteristics

The initial food concentrations in the first six experiments, with natural assemblages in the <100 or <125-μm fractions (excluding the picoplankton), were distributed over a wide range in terms of carbon (50–587 μg C L^{-1}; Table 1). The relative contribution to total cell abundance in these experiments (Fig. 1a) was dominated by nanoflagellates (31–82%) and/or chain-forming diatoms (7–60%). The nanoflagellates were for the most part composed of the 2–5 μm size range and the chain-forming diatoms usually by *Skeletonema* spp. Ciliates, dinoflagellates, and other diatoms, either in solitary or chain forms, were less abundant in these experiments (Table 2) and contributed a small amount (<8%) to total cell abundance (Fig. 1a). In terms of initial carbon biomass (Table 2), chain-forming diatoms were, during most of the experiments, the largest contributors to the total (11–70%, Fig. 1b), but occasionally nanoflagellates and dinoflagellates constituted an important component (up to 41%). During grazing experiments 7–14, with pico- to nanoplankton (<20 μm) or only picoplankton (<3 μm) as food for the nauplii (Table 3), the cyanobacteria and the nanoflagellates were a minor component, whereas the bacterioplankton represented >90% of the total initial abundance and biomass. Detailed prey compositions during

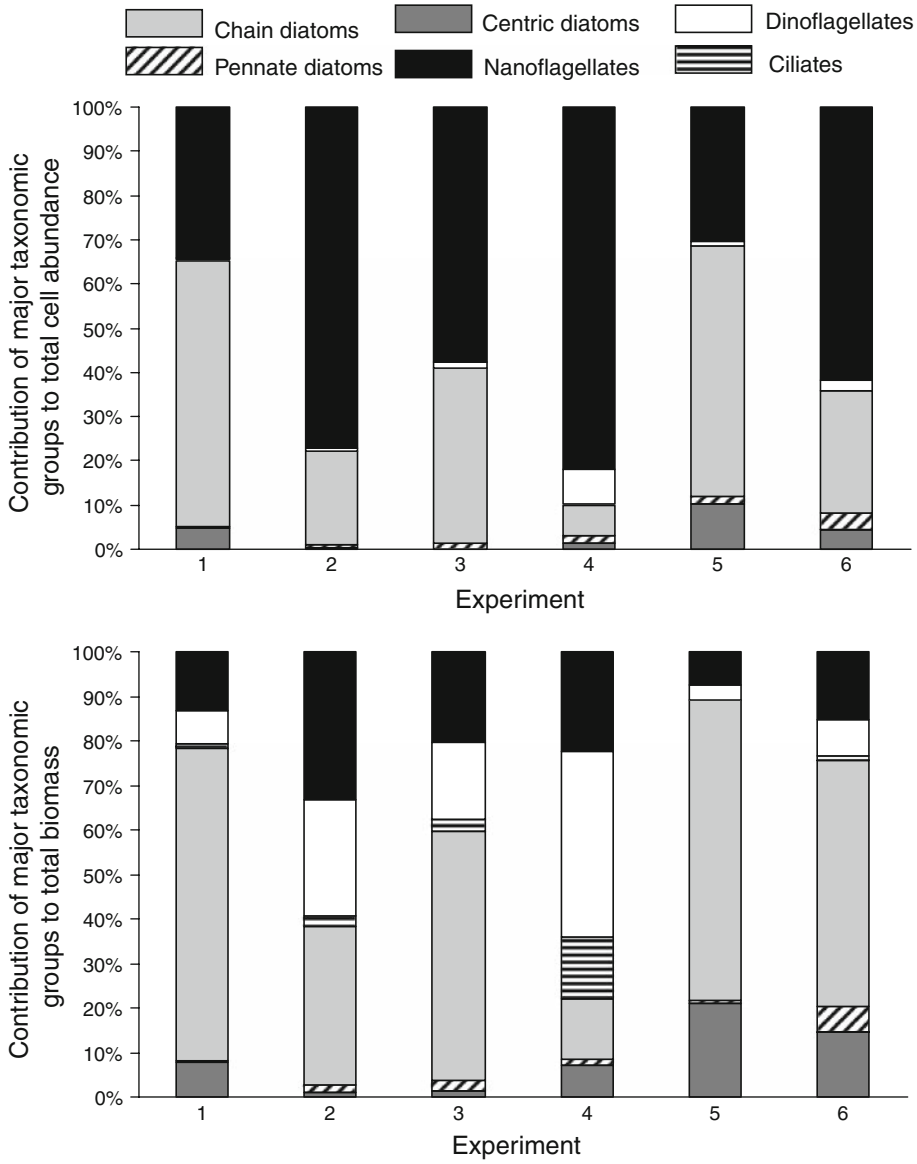

Fig. 1 Relative contribution of main taxonomic groups to total abundance (*upper panel*; cells mL^{-1}) and biomass (*lower panel*; μg C L^{-1}) of the natural nano- to microplanktonic assemblages at the beginning of the grazing experiments with *Oithona* spp. nauplii (experiments 1–6)

Table 2 *Oithona* spp. nauplii grazing experiments 1–6

Experiment	1	2	3	4	5	6
Nanoflagellates						
2–5 μm	908 ± 117 (5.1 ± 0.7)	1,525 ± 204 (8.6 ± 1.2)	809 ± 57 (4.6 ± 0.3)	423 ± 31 (2.4 ± 0.2)	1,010 ± 100 (5.7 ± 0.6)	3,519 ± 303 (20 ± 1.7)
5–10 μm	213 ± 14 (11 ± 0.7)	281 ± 50 (14 ± 2.6)	183 ± 14 (9.4 ± 0.7)	154 ± 43 (7.8 ± 2.2)	461 ± 55 (24 ± 2.8)	413 ± 112 (21 ± 5.7)
10–15 μm	29 ± 8.2 (2.8 ± 0.8)	2.1 ± 0.4 (0.2 ± 0.04)	3.5 ± 0.8 (0.3 ± 0.1)	111 ± 20 (11 ± 2.0)	146 ± 19 (14 ± 1.9)	58 ± 7.1 (5.7 ± 0.7)
Dinoflagellates						
8–19 μm	3.6 ± 1.2 (0.3 ± 0.1)	3.5 ± 0.6 (0.3 ± 0.04)	8.3 ± 0.2 (0.7 ± 0.08)	52.6 ± 2.9 (3.8 ± 0.2)	32.2 ± 0.7 (2.7 ± 0.2)	151 ± 17.6 (11.8 ± 0.9)
20–39 μm	8.7 ± 0.7 (3.6 ± 0.3)	7.1 ± 2.4 (3.7 ± 0.8)	11.2 ± 1.3 (5.9 ± 0.5)	12.6 ± 0.4 (7.2 ± 0.5)	14.0 ± 1.0 (6.4 ± 0.5)	8.7 ± 2.0 (5.9 ± 1.2)
> 40 μm	4.2 ± 0.3 (6.8 ± 0.2)	3.9 ± 0.6 (14.1 ± 2.0)	1.6 ± 0.5 (5.3 ± 2.4)	2.6 ± 1.1 (5.6 ± 3.1)	5.4 ± 1.6 (33 ± 8.7)	4.4 ± 3.7 (7.2 ± 5.2)
Ciliates (40 μm)	0.9 ± 0.3 (1.5 ± 0.4)	0.7 ± 0.2 (1.6 ± 0.7)	0.8 ± 0.1 (2.0 ± 0.4)	1.3 ± 0.1 (5.5 ± 0.6)	0.1 ± 0.03 (0.4 ± 0.2)	1.3 ± 0.1 (3.2 ± 0.5)
Pennate diatoms						
Asterionellopsis (s; 27 μm)	0	0	22 ± 4.4 (1.4 ± 0.3)	5.3 ± 3.7 (0.3 ± 0.2)	0	0
Asterionellopsis (ch)	9.4 ± 2.3 (0.6 ± 0.1)	0	0	0	34 ± 12 (2.1 ± 0.7)	36 ± 12 (2.2 ± 0.7)
Cylindrotheca (s; 50 μm)	6.3 ± 1.4 (0.5 ± 0.1)	1.2 ± 0.4 (0.1 ± 0.03)	1.1 ± 0.5 (0.1 ± 0.04)	2.9 ± 1.7 (0.2 ± 0.1)	53 ± 11 (4.0 ± 0.8)	213 ± 7.2 (16 ± 0.5)
Navicula (s; 24 μm)	9.4 ± 2.4 (0.5 ± 0.1)	2.2 ± 0.9 (0.1 ± 0.04)	0.5 ± 0.4 (0.03 ± 0.03)	3.5 ± 0.9 (0.2 ± 0.04)	27 ± 7.2 (1.3 ± 0.4)	31 ± 5.4 (1.5 ± 0.3)
Pseudonitzschia (s; 45 μm)	0	17 ± 2.8 (1.0 ± 0.2)	0	0.7 ± 0.1 (0.04 ± 0.1)	0	0
Pseudonitzschia (ch)	0	24 ± 4.8 (1.4 0.3)	0	2.6 ± 0.2 (0.1 ± 0.1)	0	17 ± 7.2 (1.0 ± 0.4)
Centric diatoms						
Chaetoceros (s; 14 μm)	0	0	0.4 ± 0.6 (0.1 ± 0.1)	4.8 ± 0.5 (0.6 ± 0.1)	113 ± 19 (14 ± 2.4)	20 ± 19 (2.6 ± 2.4)
Chaetoceros (ch)	0	0	69 ± 4.6 (8.7 ± 0.6)	25 ± 4.8 (3.2 ± 0.6)	1,365 ± 177 (173 ± 22)	133 ± 80 (17 ± 10)
Coscinodiscus (s, 25 μm)	7.0 ± 2.4 (3.3 ± 1.1)	0.8 ± 0.7 (0.4 ± 0.4)	2.1 ± 0.3 (1.0 ± 0.1)	2.2 ± 0.7 (1.0 ± 0.3)	139 ± 9.8 (65 ± 4.6)	17 ± 2.7 (8.0 ± 1.3)
Leptocylindrus (ch; 35 μm)	0	0	0	0	0	33 ± 9 (3.7 ± 3.3)
Skeletonema (s; 12 μm)	154 ± 48 (7.7 ± 2.4)	6.2 ± 2.2 (0.3 ± 0.1)	0	0	0	0
Skeletonema (ch)	2,015 ± 312 (100 ± 16)	465 ± 82 (23 ± 4.1)	610 ± 98 (30 ± 4.8)	27 ± 3.7 (1.4 ± 0.2)	113 ± 19 (5.7 ± 0.9)	839 ± 80 (42 ± 4.0)
Thalassiosira (s; 17 μm)	0	0	0	3.5 ± 1.2 (0.5 ± 0.2)	294 ± 31 (41 ± 4.3)	246 ± 109 (34 ± 15)
Thalassiosira (ch)	0	0.9 ± 1.6 (0.1 ± 0.2)	0.8 ± 0.8 (0.1 ± 0.1)	1.7 ± 1.8 (0.2 ± 0.2)	1,490 ± 77 (207 ± 11)	742 ± 341 (103 ± 47)

Initial mean ($n = 3$) abundance (cells mL^{-1}) and biomass (μg C L^{-1}, in parenthesis) ± standard deviation of nano- and microplankton food. Diatoms (s = single cell with size indication, ch = chain-forming), ciliates (mostly oligotrichous), dinoflagellates (mostly gymnodinoids), and nanoflagellates

Table 3 *Oithona* spp. nauplii grazing experiments 7–14

Experiment	Bacterioplankton	Cyanobacteria	Nanoflagellates
7	1,346 ± 25 (33 ± 0.6)	377 ± 27 (0.03 ± 0.00)	171 ± 26 (1.0 ± 0.1)
8	1,330 ± 98 (32 ± 2.4)	456 ± 33 (0.04 ± 0.00)	267 ± 55 (1.5 ± 0.3)
9	2,086 ± 86 (51 ± 2.1)	3,142 ± 486 (0.3 ± 0.04)	623 ± 63 (3.5 ± 0.4)
10	2,962 ± 199 (72 ± 4.8)	1,562 ± 322 (0.1 ± 0.03)	636 ± 55 (3.6 ± 0.3)
11	591 ± 30 (14 ± 2.0)	263 ± 23 (0.02 ± 0.00)	–
12	736 ± 107 (18 ± 2.6)	193 ± 33 (0.02 ± 0.00)	–
13	1,138 ± 38 (28 ± 0.9)	658 ± 219 (0.05 ± 0.02)	–
14	1,289 ± 20 (31 ± 0.5)	731 ± 127 (0.06 ± 0.01)	–

Initial mean ($n = 3$) abundance (cells mL^{-1}) and biomass (µg C L^{-1}, in parenthesis) ± standard deviation of pico- and nanoplankton food. Bacterioplankton abundance in 10^3 cells mL^{-1}; – = not included

Table 4 *Oithona* spp. nauplii grazing experiments 1 to 6

	Estimate	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 6
Nanoflagellate 2–5 µm	Difference in abundance	***	**	*	*	*	**
	Difference in prey growth	***	**	*	*	*	**
	Prey growth control	0.27	0.28	0.50	0.38	0.53	0.32
	Prey growth grazing	−0.33	−0.13	0.23	0.23	0.18	0.00
	Cell ingestion	14 ± 1	30 ± 4	16 ± 6	5 ± 0.4	33 ± 6	51 ± 3
	Carbon ingestion	78 ± 6	167 ± 23	93 ± 31	28 ± 2	184 ± 35	290 ± 19
Nanoflagellates 5–10 µm	Difference in abundance	*	ns	**	ns	ns	***
	Difference in prey growth	*	#	**	#	#	**
	Prey growth control	0.84	–	0.47	–	–	1.00
	Prey growth grazing	0.30	–	−0.21	–	–	−0.34
	Cell ingestion	4 ± 1	–	5 ± 1	–	–	12 ± 1
	Carbon ingestion	107 ± 26	–	196 ± 37	–	–	486 ± 22
Nanoflagellates 10–15 µm	Difference in abundance	ns	ns	ns	ns	**	*
	Difference in prey growth	#	#	#	#	**	ns
	Prey growth control	–	–	–	–	0.35	–
	Prey growth grazing	–	–	–	–	−0.28	–
	Cell ingestion	–	–	–	–	4 ± 1	–
	Carbon ingestion	–	–	–	–	490 ± 56	–
Chlorophyll-*a*	Difference in abundance	***	***	***	***	ns	**
	Chl-*a* ingestion	54	18	21	4	–	19

Prey net growth rates (day^{-1}), mean ingestion rates (±SD; $n = 3$), and the comparison of changes in prey net growth rates and abundances (Student's *t*-test). Ingestion rates are expressed in cells (10^3 cells $nauplius^{-1}$ day^{-1}) and carbon (ng C $nauplius^{-1}$ day^{-1})

*** $P < 0.001$, ** <0.01, * <0.05, *ns* non-significant; # no test applied after the first test resulted in ns

all above described experiments are listed in Tables 2 (experiments 1–6) and 3 (experiments 7–14). In the experiments with *Isochrysis galbana* as food (15 and 16), the initial abundances and biomasses were higher than those of nanoflagellates in the natural assemblages (Table 2).

Diet and ingestion rates of the nauplii incubated with natural and cultured food

Among the diverse prey types in the nano- and microplanktonic size fractions offered in experiments 1–6, *Oithona* spp. nauplii displayed significant feeding on nanoflagellates in all 6 experiments (Table 4) but mostly on the smallest size fraction (2–5 µm). Total ingestion rates on the three nanoflagellate sizes ranged between 5 and 63 × 10^3 cells $nauplius^{-1}$ day^{-1}, equivalent to 28 and 775 ng C $nauplius^{-1}$ day^{-1} (Fig. 2). On the contrary, no explicit feeding on diatoms, dinoflagellates, or ciliates was detected. Even though in few cases these prey concentrations (e.g. solitary diatoms) were significantly different between the final control and the grazing treatments, suggesting ingestion, the values were very low and, therefore not considered

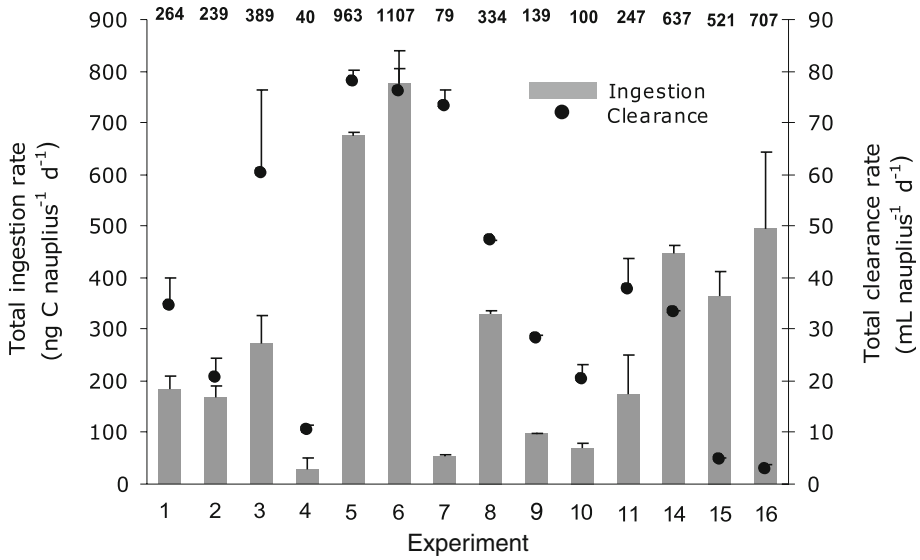

Fig. 2 Total carbon ingestion and clearance rates of *Oithona* spp. nauplii during the grazing experiments carried out with natural food assemblages (experiments 1–14) and *Isochrysis galbana* (experiments 15 and 16). Values on top of each bar represent daily carbon ratios (% body C day^{-1})

Table 5 *Oithona* spp. nauplii grazing experiments 7–14

	Estimate	Exp. 7	Exp. 8	Exp. 9	Exp. 10	Exp. 11	Exp. 12	Exp. 13	Exp. 14
Bacterioplankton	Difference in abundance	ns	*	ns	ns	*	ns	ns	***
	Difference in prey growth	#	*	#	#	*	#	#	***
	Prey growth control	–	0.12	–	–	0.10	–	–	0.69
	Prey growth grazing	–	−0.06	–	–	−0.14	–	–	0.49
	Cell ingestion	–	9,734 ± 3,362	–	–	7,079 ± 3,078	–	–	18,238 ± 723
	Carbon ingestion	–	237 ± 82	–		173 ± 75	–	–	444 ± 18
Cyanobacteria	Difference in abundance	***	ns	ns	ns	*	ns	ns	*
	Difference in prey growth	***	#	#	#	*	#	#	*
	Prey growth control	0.33	–	–	–	0.53	–	–	0.59
	Prey growth grazing	−0.34	–	–	–	0.02	–	–	0.18
	Cell ingestion	11.2 ± 0.9	–	–	–	8.8 ± 2.9	–	–	20.5 ± 4.9
	Carbon ingestion	0.9 ± 0.1	–	–	–	0.7 ± 0.2	–	–	1.7 ± 0.4
Nanoflagellates	Difference in abundance	***	***	***	*	nd	nd	nd	nd
	Difference in prey growth	***	**	***	*	nd	nd	nd	nd
	Prey growth control	0.79	0.23	0.57	0.28	nd	nd	nd	nd
	Prey growth grazing	−0.55	−0.53	−0.04	−0.06	nd	nd	nd	nd
	Cell ingestion	9.6 ± 0.3	7.9 ± 0	17.2 ± 0.3	12.4 ± 1.4	nd	nd	nd	nd
	Carbon ingestion	36.2 ± 1.5	44.6 ± 0	96.9 ± 1.7	70.2 ± 8.1	nd	nd	nd	nd

Prey net growth rates (day^{-1}), mean ingestion rates (±SD; $n = 3$) as well as the comparison of changes in prey net growth rates and abundances (Student's *t*-test). Ingestion rates are expressed in cells (10^3 cells nauplius^{-1} day^{-1}) and carbon (ng C nauplius^{-1} day^{-1}). Other symbols as in Table 4

in further analyses. Chlorophyll-*a* ingestion was in most of the cases significant but the total was not proportional to that of C ingestion implying that the nauplii feed omnivorously.

In the experiments with pico- and nanoplankton as food (7–10), the *Oithona* spp. nauplii displayed significant feeding on nanoplankton (mainly nanoflagellates in the 2–5 μm

size range), whereas it was mostly non-significant for picoplankton (Table 5). Total C ingestion in this case ranged between 55 and 329 ng C nauplius^{-1} day^{-1} (Fig. 2); nanoflagellate ingestion was in the lower range (8–17 $\times$ 10^3 cells nauplius^{-1} day^{-1}) of those reported for the experiments 1–6 (Tables 4, 5). In the experiments in which picoplankton was the only available food (11–14), the nauplii did not feed on two occasions but ingested bacterioplankton and cyanobacteria in the other two. Carbon ingestion amounted to 174–446 ng C nauplius^{-1} day^{-1} in this case; however, cyanobacteria were consumed at a very low level (<1% of the total C ingestion; Table 5). Total carbon ingestion by the nauplii including all 14 experiments with natural food ranged between 28 and 775 ng C nauplius^{-1} day^{-1}, equivalent to 40 and 1,107% body carbon ingested day^{-1} (Fig. 2), and can be derived in similar amounts either from nanoplankton or picoplankton prey.

The ingestion estimates for the *Oithona* spp. nauplii detailed above are not masked by the grazing of other small predators (e.g. dinoflagellates, ciliates) in the incubations bottles. First, prey net growth rates (pico- and nanoplankton) were significantly higher in the final control compared to the experimental bottles (Tables 4, 5). That is, there was no cascading effect involved in the estimations, which is supported by the fact that there was no significant ingestion of protistan predators by the nauplii (Table 4). Most likely, however, the nauplii and the protistan predators were preying on the same food type in these experiments and therefore, both can account for the decrease in prey abundance. Since there was no significant loss of the protistan predators (ciliates or dinoflagellates) in the first six experiments (Table 4), their contribution to the decrease in prey abundance should be similar in both, final control and experimental bottles. Therefore, the difference in prey abundances between the two types of bottles can be attributed exclusively to the *Oithona* spp. nauplii. In experiments 7 and 8, the nauplii and the nanoflagellates were feeding on picoplankton but, at the same time, the nauplii were feeding on nanoflagellates. Since the nauplii largely reduced nanoflagellate abundance in those experimental compared to the final control bottles (63–74%; Table 6), it is highly likely that most of the decrease in picoplankton abundance during these two experiments is a result of consumption by the nauplii.

The *Oithona* nauplii also ingested the nanoflagellate *I. galbana* when offered as the only food (experiments 15 and 16). Ingestion rates in terms of cells, Chl-*a*, and carbon (Table 7) were the following: (1) similar in both experiments even though there was a twofold difference in food concentration (Table 1) and (2) in the same range as those obtained from the incubations with natural nanoflagellates (Tables 4, 5; Fig. 2). In this case, protistan predators were not part of the incubations and, therefore, no interferences with the estimates of ingestion rates in the *Oithona* spp. nauplii is expected.

Table 6 Extent of decrease in nanoflagellate abundance due to *Oithona* spp. grazing during incubation experiments 7–10

Experiment	Nanoflagellates (NF)	
	Abundance (cells mL^{-1})	NF decrease in the presence of nauplii (%)
7		
CoT_1	171 ± 26	74
CoT_2	377 ± 27	
Graz T_2	98 ± 10	
8		
CoT_1	267 ± 55	63
CoT_2	338 ± 27	
Graz T_2	145 ± 23	
9		
CoT_1	623 ± 63	47
CoT_2	1,101 ± 99	
Graz T_2	579 ± 35	
10		
CoT_1	636 ± 55	33
CoT_2	838 ± 92	
Graz T_2	566 ± 60	

Co = control bottles at the beginning (T_1) and end (T_2) containing only the prey. Graz T_2 = bottles including the prey and a known number of nauplii

Table 7 *Oithona* spp. nauplii grazing experiments (15–16) with *Isochrysis galbana*

Experiment	Estimate	15	16
I. galbana	Difference in abundance	**	*
	Difference in prey growth	**	*
	Prey growth control	0.13	0.14
	Prey growth grazing	0.01	0.06
	Cell ingestion	28 ± 3.5	31 ± 9.3
	Carbon ingestion	365 ± 46	495 ± 149
	Difference in Chl-*a*	***	***
	Chl-*a* ingestion	18 ± 3.7	20 ± 5.0

Mean ingestion rates (±SD; $n = 3$) expressed as cells (10^3 cells nauplius^{-1} day^{-1}), carbon (ng C nauplius^{-1} day^{-1}), and chlorophyll-a concentration (ng Chl-*a* nauplius^{-1}day^{-1}). Other symbols as in Table 4

Feeding behaviour of the *Oithona* spp. nauplii

An analysis of the functional relationships between the clearance and feeding rates of the *Oithona* spp. nauplii and the food concentrations (in carbon) revealed a trend of decrease in clearance rate and increase in ingestion rate with increasing food concentration (Fig. 3a, b). In addition,

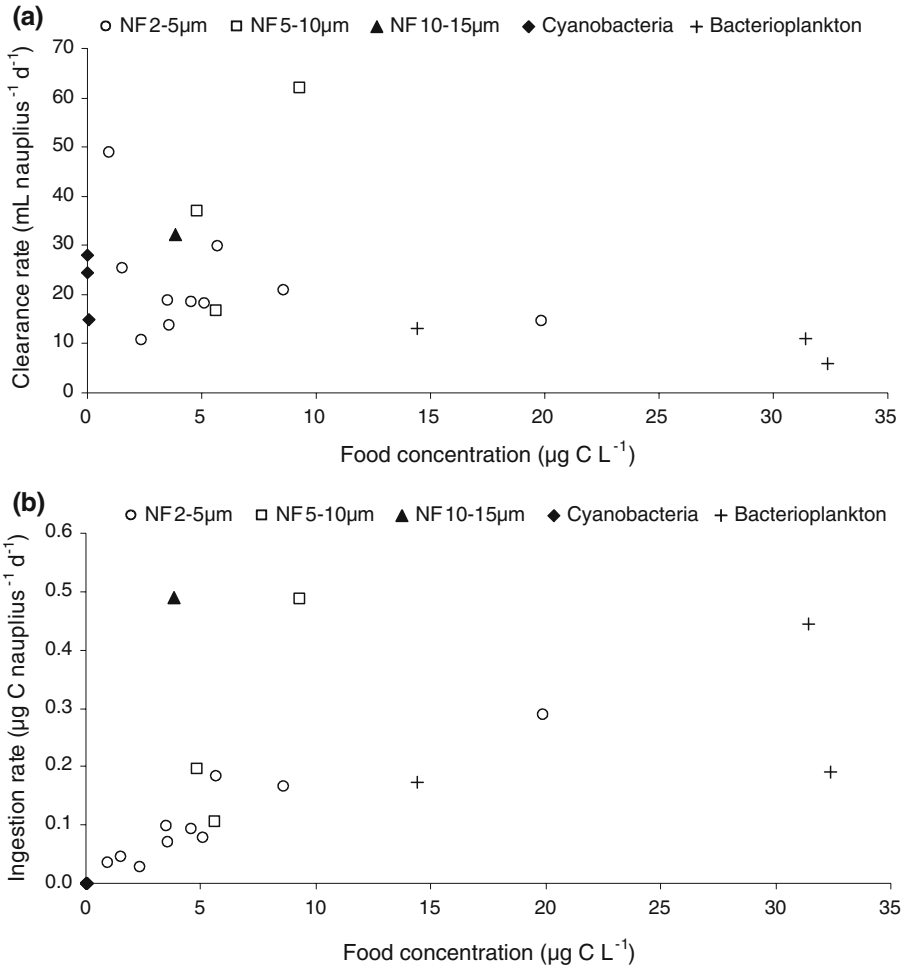

Fig. 3 Functional response of *Oithona* spp. nauplii to food type and concentration: **a** clearance and **b** ingestion rates

no saturation response was detected in the data despite the wide range of initial food concentration in the experiments and, therefore, ingestion rate can be described as a linear function of it ($r^2 = 0.54$; $P < 0.05$, $n = 18$; Fig. 3b). In terms of food preferences, the values of the electivity index (E_i*) for the different food types were predominantly in the −0.25 to +0.25 range, suggesting non-selective feeding by the nauplii (Fig. 4).

Discussion

Food spectrum and ingestion rates by Oithonid nauplii and other small metazoans

To date, there have only been few examinations of *Oithona* spp. feeding (adults and developmental stages) on mixed diets of natural plankton despite their pivotal role in pelagic food webs (Turner 2004). A wide variety of natural prey assemblages in terms of size, type, and motility were offered to nauplii of *Oithona* spp. in the present study as to understand their role in the trophic transfer in an upwelling system. Our results indicate that the size of the nauplii prey is mostly <15 μm, which includes nanoflagellates, bacterioplankton, and, to a minor extent, cyanobacteria. According to the size ratio relationship between planktonic predators and their prey (Hansen et al. 1994), the optimum prey size for nauplii of 165 μm in length (as in this study) is 9.2 μm, a figure consistent with our results. In contrast, diatoms, which are a dominant component of the plankton during the upwelling period in this system (Vargas et al. 2007), were not consumed by the nauplii probably because they are too big and heavily armored. In addition, nauplii of *O. davisae* have been shown to have a preference for motile prey (Uchima and Hirano 1986; Henriksen et al. 2007), and this might be the same case in other *Oithona* species. Dinoflagellates and ciliates were much less abundant than other prey items in this study (Table 2), therefore less likely to have been ingested, and the larger sizes (>20 μm) might have been too wide to be ingested (Paffenhöfer 1998).

Bacterioplankton has been generally considered too small to be efficiently ingested by most adult copepods but a few

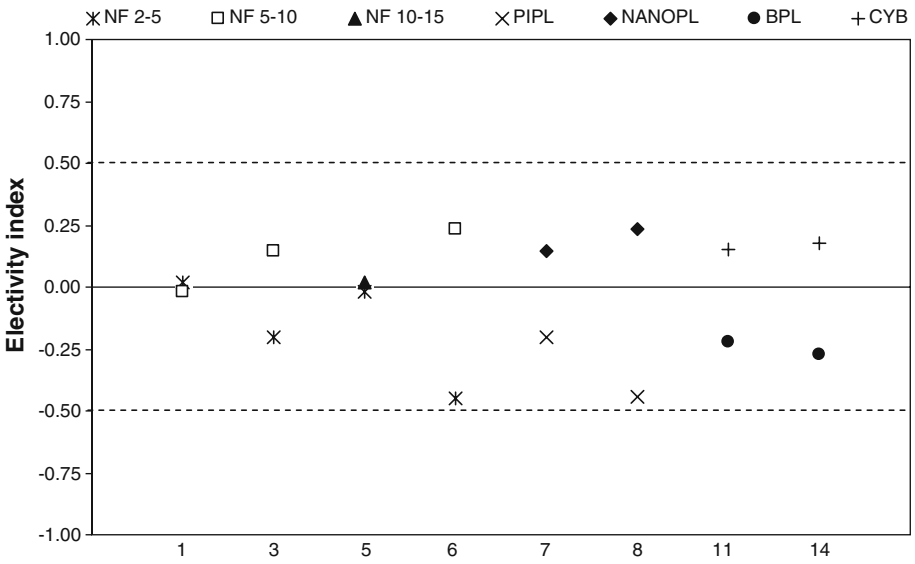

Fig. 4 Electivity index for the different food types ingested by *Oithona* spp. nauplii during the experiments with natural assemblages of nano- and microplankton (experiments 1–6), pico- and nanoplankton (experiments 7 and 8) or only picoplankton (experiments 11 and 14). NF = nanoflagellates, PIPL = picoplankton, NANOPL = nanoplankton, BPL = bacterioplankton, CYB = cyanobacteria

studies have shown that their nauplii do ingest them. Roff et al. (1995) indicated that NII-NIII stages of *Oithona* spp. consumed fluorescently labeled bacteria (FLB) but no ingestion rates were reported. Turner and Tester (1992) estimated nauplii bacterivory using the FLB technique in NI-NIII stages of *Acartia tonsa*. Their mean estimate (5.7×10^6 FLB nauplius^{-1} day^{-1}) is about half that in our study for NIII-NV stages of *Oithona* spp. This difference might be explained by the larger average cell volume of the FLB (0.7 μm^3) compared with the natural bacterioplankton cells in our study (0.15 μm^3) and by the smaller size of the *A. tonsa* nauplii (75–132 μm) compared with the *Oithona* spp. nauplii in this study (mean: 165 μm). Unfortunately, there are no other studies dealing with copepod nauplii feeding on picoplankton to compare with the present results; also, our results are mostly exploratory and remain to be assessed in further detail using fluorescently marked particles.

The apparent lack of food selection by the *Oithona* spp. nauplii in this study suggest that they act as opportunistic feeders. Certainly, this strategy might favor their persistence throughout the year in the upwelling system off Concepción (Torres 2006). Our rate estimates of total carbon ingestion (range: 0.03–0.8 μg C nauplius^{-1} day^{-1}) are higher than those few reported for other oithonid nauplii and in the higher part of the range of those reported for *Oithona* spp. adults and small calanoid nauplii (Table 8). The latter contrasts with the common assumption that cyclopoids have lower ingestion and metabolic rates compared to similarly sized calanoids (Saiz and Calbet 2007), although a few studies have shown comparable estimates between them in terms of ingestion, growth, and/or development (Sabatini and Kiørboe 1994; Calbet et al. 2000).

Methodological differences or artifacts between studies can, at least in part, explain the higher ingestion rates obtained in our study when compared with those published so far. Most of the differences can be attributed to the so-called "edge" effect linked to the size of the incubation containers used. O'Brien (1988) and Köster et al. (2008) reported rising ingestion and metabolic rates with increasing size of the container; they attributed this to the fact that a copepod tends to veer away from the side of a container when coming within 1–2 cm of the walls, thereby reducing the area of feeding. For example, the ingestion rates of *O. davisae* nauplii obtained by Henriksen et al. (2007) are very low compared to ours (Table 8); their incubation containers were small (72 mL). The authors did not report the naupliar density per container but we suspect that it was very high. To be comparable to our rate estimates, they should have incubated 1–3 nauplii in that type of container. In the same way, some of the low carbon ingestion rates reported for *O. nana* females (Lampitt and Gamble 1982) and *O. similis* adults + CV copepodites (Nakamura and Turner 1997; Castellani et al. 2005) might be the result of potential food limitation during the incubations, since the densities per container ranged between 15 to 50 copepods in 100 to 200 mL bottles, compared with the much higher ingestion rates obtained for *O. similis* adults when using 10 copepods in 1 L containers (Vargas and Gonzalez 2004a) (Table 8).

Other potential explanations for the wide variation in the reported ingestion rates of oithonid nauplii can be attributed to differences in the experimental conditions related to the factors that affect these rates, such as temperature, food quantity, and food quality (Mauchline 1998). Even though metabolic processes of marine copepods, such as respiration, excretion, or growth, have been clearly shown to increase with a rise in temperature (Ikeda et al. 2001), the relationship between temperature and ingestion appears to

Table 8 Comparison of clearance (CL) and carbon ingestion rates (I; as total) of small cyclopoid and calanoid metazoans in coastal areas

Species	*V*(L)	*T* (°)	*D* (N° L^{-1})	Food types offered	Food concentration			CL	I	DR (%)
					Chl-*a*	Abundance	Biomass			
Oithona nana/O. similis nauplii[1]	0.6	11–12	12–33	Natural assemblages < 125 and 100 μm	0.4–6.8	0.8–6.5*	51–306*	7–62	0.03–0.8	40–1,107
Oithona nana/O. similis nauplii[1]	0.6	11–12	12–33	Natural assemblages < 2 μm	0.02	591–1,289	14–31	11–38	0.07–0.4	53–637
O. davisae nauplii[2]	0.07	20	–	*Heterokapsa* sp.	–	0.04–2.5	–	0.05–0.5	0–0.03	Maximum 121
Oithona spp. nauplii[3]	2	0	22–25	Microprotozooplankton < 64 μm	0.8–5.8	–	–	0.03–12	–	–
O. nana adults[4]	0.6 + 1.2	22–27	60–200	Natural assemblages <100 μm	0.2–1.1	293–1,783	–	0–36	–	–
O. nana C5 females[5]	0.16	10	480–720	Algal cultures + nauplii	–	–	1–350	0.1–10	0.1–0.3	20–60
O. similis late copepodites and adults[6]	0.5	12	10	Natural assemblages <200 μm	–	–	400–480	0–280	2.6–2.9	85–352
O. similis C5 and adult females[7]	0.24	19–21	120–200	Natural assemblages <8, 20 & 73 μm + nauplii	–	0.001–8	–	2–9	0.01–0.1	1–27
O. similis adult females[8]	0.2	1–12	75–125	Natural assemblages (Nano + microplankton)	–	–	29–206	0–39	0.001–0.5	Average 0.5–10
Oithona spp. mixed[9]	0.6	18–23	6–22	Natural assemblages <100 μm	–	–	10–140	0–26	0.04–0.7	7–99
O. davisae adult females[10]	0.5	12 + 22	80	Natural assemblages unsieved	1–3	2.5–5	215–315	7–46	0.02–0.3	2–23
Calanus finmarchicus nauplii[11]	0.2	1–12	75–125	Natural assemblages (Nano + microplankton)	–	–	29–206	2–27	0.04–0.2	8–40
C. helgolandicus/C. finmarchicus nauplii[12]	1	10 + 15	70	Cultured algae (mixed)	–	–	120	3–5	0.4–0.7	117–137
C. helgolandicus nauplii[13]	8	15	35–45	*Lauderia boreales* *Gymnodinium splendens*	–	–	36–101	4–21	0.2–0.8	292–481
C. glaciales/C. finmarchicus nauplii[14]	0.6	5	100	Natural assemblages <200 μm	1.5–6	–	–	0–30	0.02–0.07	0.5–2
Acartia tonsa nauplii[15]	0.07	22	285	*Aureococcus anophagefferens* *Isochrysis galbana*	–	70–500	–	0.1–31	0.03–0.6	25–500

Table 8 continued

Species	*V*(L)	*T* (°)	*D* (N° L^{-1})	Food types offered	Food concentration			CL	I	DR (%)
					Chl-*a*	Abundance	Biomass			
A. tonsa nauplii[16]	0.25	20	40	Cultured ciliates (mixture)	0.002–0.003	–	–	0–49	0.01–0.3	17–500
A. tonsa nauplii[17]	0.018	–	166	Fluorescently labeled bacteria	–	1,440–2,380	–	1–8	0.1–1.2	194–1,643
A. tonsa nauplii[18]	0.2	20	100	*Euplotes*	–	–	75–600	–	0.3–1.1	400–1,600
A. grani nauplii[2]	0.07	20	–	*Thalassiosira weissflogii* *Heterokapsa* sp.	–	0.04 – 4	–	0.1–0.5	0.004–0.1	Maximum 185–299
A. tonsa/ *Paracalanus parvus* copepodites[19]	0.5	11–17	6–10	Natural assemblages <200 μm	5–30	–	40–1,200	–	2.7–7.4	–

V volume of incubation container; *T* temperature; *D* density. Food density is expressed as chlorophyll-*a* concentration (μg Chl-*a* L^{-1}), abundance ($\times 10^6$ cells L^{-1}), and biomass (μg C L^{-1}), CL = clearance rate (mL Ind^{-1} day^{-1}), I = ingestion rate (μg C Ind^{-1} day^{-1}), DR = daily carbon rations (% body carbon ingested $naplius^{-1}$ day^{-1}); – = no data

[1] = present study, [2] = Henriksen et al. (2007), [3] = Lonsdale et al. (2000), [4] = Atienza et al. (2006), [5] = Lampitt and Gamble (1982), [6] = Vargas et al. (2004a), [7] = Nakamura and Turner (1997), [8] = Castellani et al. (2005), [9] = Broglio et al. (2004), [10] = Gifford et al. (2007), [11] = Irigoien et al. (2003), [12] = Meyer et al. (2002), [13] = Paffenhöfer (1971), [14] = Turner et al. (2001), [15] = Smith et al. (2008), [16] = Stoecker and Egloff (1987), [17] = Turner and Tester (1992), [18] = Isma et al. (2008), [19] = Vargas et al. (2007)

be less defined. In contrast, it has been fairly well demonstrated that ingestion rates are positively correlated to food concentrations ($r^2 = 0.52$, $P < 0.001$; Saiz and Calbet 2007). In oithonids, Castellani et al. (2005) reported a much lower range of adult female ingestion rates compared to ours but their incubation temperature was much lower (1.2°C), whereas the initial food concentrations in both cases were similar (Table 8). Also, Nakamura and Turner (1997) obtained lower ingestion rates than the ones of the present study; they incubated adults at higher temperatures (19–21.2°C) but they used a much lower range of initial food abundance (Table 8). In terms of clearance rates, the maximum value obtained in the present study (62 mL nauplius^{-1} day^{-1}; Fig. 3a) is substantially higher than the one previously reported for cyclopoid nauplii (Lonsdale et al. 2000) but it is similar to that described for calanoid nauplii (Table 8). This is not too surprising, since a wide range of clearance rate values has been reported for copepodite and adult stages of copepods. For example, maximum clearance rates of *Oithona similis* can be one order of magnitude different, from values <15 mL copepod day^{-1} (Castellani et al. 2008) up to 280 mL copepod day^{-1} (Vargas and Gonzalez 2004a).

The daily carbon rations (DCR) of the *Oithona* spp. nauplii during our study ranged from 40 to 1,107% (mean: 500 ± 431%) when feeding on nanoflagellates (Table 4), and the highest were achieved in experiments (5 and 6) when the initial food concentrations were highest (Table 1). Under a nanoflagellate-picoplankton diet (Table 5), the DCR values were lower (from 79 to 637%; mean: 256 ± 211%) and the overall mean of the experiments was 378% (Fig. 2). That is, a nauplii daily consumed nearly four times its own body carbon, in contrast with values obtained for adult cyclopoids that are around 100% and less (Saiz and Calbet 2007). Adult stages usually contain large amounts of lipids (wax esters) that are mainly used as energy reserves to sustain reproduction (Lee et al. 1972; Kattner et al. 2003), after growth has stopped or becomes very slow. In contrast, energy reserves in naupliar stages are lacking (Finlay and Roff 2006) but their metabolic expenditures are expected to be quite high because of frequent molting and high, nearly exponential, growth rates (0.2 day^{-1}) within a relatively short period (Sabatini and Kiøboe 1994). For example, the nauplii of *Acartia hudsonica* were found to ingest more food per unit respired compared to copepodite stages (Finlay and Roff 2006). We suspect that copepod nauplii might not exhibit a high percentage of assimilation of the food ingested, because their gut system is not fully developed in the earlier stages but studies on this aspect remain unknown.

A few other DCR values have been documented for other small copepod nauplii: a maximum of 121% in *O. davisae* (Henriksen et al. 2007) and 299% in *A. grani* (Henriksen et al. 2007), and a mean of 1,110% in *A. tonsa* (Campbell 1993). In the present study, the DCR showed a large increase only at the highest food concentration (366 ± 263% at <50 μg C L^{-1}, $n = 7$; 216 ± 129% at 50–100 μg C L^{-1}, $n = 5$; 778 ± 451% at >100 μg C L^{-1}, $n = 3$) in contrast to a smooth trend as described by Saiz and Calbet (2007) for small calanoid copepods. This increase supports the dependence of their growth on food concentration, as it has been reported for other naupliar stages (Lonsdale and Jónasdóttir 1990; Torres and Escribano 2003).

Grazing impact by *Oithona* nauplii in the upwelling area off Concepción

In the highly productive, coastal upwelling area off Concepción, significant proportions of primary and bacterial productions are channeled through microheterotrophs, thus making the microbial food web a fundamental trophic pathway in the water column (Böttjer and Morales 2005; Vargas et al. 2007). Nevertheless, Böttjer and Morales (2005) lumped together all protistan and metazoan heterotrophs <200 μm, whereas Vargas et al. (2007) excluded small metazoans by size-fractionation through a 115-μm mesh. Therefore, the specific grazing impact of metazoans <200 μm has so far not been evaluated in this area. The same is true for many other coastal or oceanic systems, except for the studies of White and Roman (1992) and Lonsdale et al. (1996), which have stated that the grazing impact of micrometazoans can be remarkable.

Oithona spp. nauplii are most likely competing with other microheterotrophs (<200 μm) for similar food types (pico- and nanoplankton). In order to explore their grazing impact, we first compare their ingestion rates (Table 9). The minimum carbon ingestion rate of picoplanktonic prokaryotes by the *Oithona* spp. nauplii can be of similar magnitude to that of heterotrophic nanoflagellates or ciliates, but their maximum value can be 3–5 orders of magnitude higher than the latter. Rates of prokaryotic cell ingestion are as well orders of magnitude higher for the nauplii compared to other protistan predators (heterotrophic dinoflagellates and ciliates). With nanoflagellates as food, the naupliar ingestion rates are also significantly higher than those of other large protists but overall, these differences might mostly be due to predator size differences.

Since the abundances of copepod nauplii are typically orders of magnitude lower than those of heterotrophic protists, naupliar carbon consumption rates are likely to be similar or even lower than those of protistan grazers, as shown by Verity et al. (1993); unfortunately, comparisons of this kind are almost non-existent. In an attempt to evaluate this, we calculated naupliar carbon consumption

Table 9 Comparison of ingestion rates (IR) by different microheterotrophs feeding on the same prey types as *Oithona* spp. nauplii. These rates have been obtained either directly (grazing experiments) or indirectly (using Peters 1994). *PIP* prokaryotic picoplankton; *FLB* fluorescent labelled bacteria; *BAC* heterotrophic bacteria; *SYN Synechococcus*; *PRO Prochlorococcus*; *CRYP* cryptophytes; *NF* natural nanoflagellates; *COC* coccolithophorids; – no data; IRa = ($\times 10^3$ cells nauplius^{-1} day^{-1}); IRb = (ng C nauplius^{-1} day^{-1})

Predator type	Food spectra	IRa	IRb	Method	Reference
Copepod nauplii					
Oithona spp.	NF	5–64	28–775	Direct	This study
Oithona spp.	PIP	5–18,238	0.9–444	Direct	This study
Ciliates					
Strombidium sulcatum	SYN, PRO	22	–	Direct	Christaki et al. (1999)
Mixed ciliates	NF	0.02–0.5	–	Indirect	Vargas and González (2004b)
Mixed ciliates	PIP	0.17–1.8	–	Direct	Ichinotsuka et al. (2006)
Oligotrichous	PIP	15–31	0.4–0.7	Indirect	This study
Oligotrichous	NF	0.01–0.2	0.3–2.7	Indirect	This study
Dinoflagellates					
Oxyrrhis marina	NF, COC	0–0.1	–	Direct	Hansen et al. (1996)
Gyrodinium galatheanum	CRYP	0–0.0002	–	Direct	Li et al. (2001)
Mostly Gymnodinoids	NF	0.003–0.05	0.1–0.3	Indirect	This study
Heterotrophic nanoflagellates					
Mixed	FLB	0.1–0.7	–	Direct	Sherr et al. (1988)
Mixed	SYN,PRO	0.13	–	Direct	Christaki et al. (2005)
Mixed	BAC	0.4–1.8	–	Indirect	Vargas and Gonzalez (2004b)
Mixed	PIP	0.1–1.3	0.04–0.2	Indirect	Böttjer and Morales (2007)
Mixed	PIP	0.2–0.3	0.005–0.007	Indirect	This study

* Estimated maximum value

Table 10 Consumption rate estimates (μg C L^{-1} day^{-1}) of *Oithona* spp. nauplii and protistan heterotrophs using the data from the grazing experiments in this study

Experiment	Predator	Prey	Consumption rate
1–6	*Oithona* spp. nauplii	Nanoflagellates	0.4–17.1
	Dinoflagellates		0.6–6.3
	Ciliates		0.7–1.6
7–10	*Oithona* spp. nauplii	Prokaryotes	2.6–6.7
	Nanoflagellates		0.8–2.8
	Ciliates		0.3–0.5

rates using their density (Table 1) and carbon ingestion rates from the experiments (Tables 4, 5), whereas those of the protists (heterotrophic nanoflagellates, dinoflagellates, and ciliates) were obtained from their abundances in the experiments (Tables 2, 3) and their carbon ingestion (derived from a generic model on protistan grazing rates; Peters 1994). These results show that carbon consumption by *Oithona* spp. nauplii on nano- and picoplankton can be up to twofold to one order of magnitude higher than those of heterotrophic protists (Table 10). Furthermore, the grazing pressure exerted by the *Oithona* spp. nauplii on the carbon standing stocks of the different prey categories was calculated using their carbon consumption rates and the carbon biomass of the prey (Tables 2, 3). These results show that the nauplii can exert a significant control on the carbon standing stock of nanoflagellates (2–54%), whereas their impact is lower on picoplankton (1–21%).

In conclusion, the present study illustrates that *Oithona* spp. nauplii are important in controlling nanoplanktonic assemblages in the highly productive upwelling system off central Chile. Given that these prey assemblages display a relatively low seasonal variation in this otherwise strongly seasonal environment (Böttjer and Morales 2007), the nauplii most likely experience adequate food conditions throughout the year, explaining their year-round persistence. At the same time, our study shows that these nauplii are not capable of controlling the abundances of diatoms, one of the dominant components of the system during upwelling conditions. Overall, metazoan microzooplankton can act as a link between the microbial and the classical food web in this system by transferring pico- and nanoplankton biomass to large omnivorous copepods and small pelagic fishes.

Acknowledgments We thank the crew of the RV *Kay Kay* (U. de Concepción, Chile), especially J. Caamaño, for assistance during field

trips and sampling. C. Torres and Dr. P. Hidalgo (COPAS Center, U. de Concepción, Chile) helped in planning the experiments and picking up adult oithonids. Dr. C. Vargas (EULA, U. of Concepción, Chile) advised on the calculations of ingestion rates and trophic cascading. Dr. A. G. Davies (MBA, UK) is acknowledged for language corrections. Two anonymous reviewers made very useful comments on an earlier version of this MS. D. B. was supported by a doctoral scholarship from the DAAD (German Academic Exchange Service) during this study. This research was financed by the FONDAP Program (CONICYT, Chile) awarded to the COPAS Centre (Project #150100007).

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