Feeding activity and swimming patterns of *Acartia grani* and *Oithona davisae* nauplii in the presence of motile and non-motile prey

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ABSTRACT: The feeding behaviour of the nauplii of the copepods *Acartia grani* (calanoid) and *Oithona davisae* (cyclopoid) was investigated in relation to the different motility capabilities of their prey. The prey were the motile dinoflagellate *Heterocapsa* sp. (equivalent spherical diameter, ESD = 12.8 µm) and the non-motile diatom *Thalassiosira weissflogii* (ESD = 14.4 µm). Functional response feeding experiments showed that *A. grani* nauplii exhibited higher ingestion rates on the motile than on the non-motile prey, with maximum daily rations of, respectively, 299 and 185 % body C d^{-1}. *O. davisae* nauplii showed lower ingestion rates (121 % body C d^{-1}) and were unable to feed on the non-motile prey. Video observations showed that *A. grani* nauplii moved with a continuous hopping behaviour, either in a straight or helical pattern; feeding events were observed mainly while swimming in helicoids. *O. davisae* nauplii exhibited a jump and sink behaviour, typical of ambush feeding. Both species showed changes in their behaviour when presented with the motile prey *Heterocapsa* sp. This indicates that the nauplii are capable of adjusting their feeding behaviour to the different prey characteristics, thereby optimising ingestion rates.

KEY WORDS: *Oithona davisae* · *Acartia grani* · Nauplii · Feeding rates · Prey motility · Swimming patterns

INTRODUCTION

Encounters between organisms in the ocean are fundamental for predator–prey interactions. For the copepod–prey interaction, amongst others, the encounter depends on the relative swimming velocity of the predator and the prey (Gerritsen & Strickler 1977). In this sense, some copepods have the capability to create feeding currents, increasing the water flow towards the copepod and, therefore, the encounter rate with prey (Strickler 1985, Van Duren et al. 2003). Encounter rate depends also on the distance at which a prey can be detected. This reaction distance will depend, additionally, on the threshold value of mechano- and chemoreceptors to trigger the copepod response and

the strength of the prey signal (Paffenhöfer & Lewis 1990, Svensen & Kiørboe 2000). Finally, moderate small-scale turbulence augments prey encounter rate in ambush-feeding copepods (Kiørboe & Saiz 1995, Saiz & Kiørboe 1995, Saiz et al. 2003).

Calanoids and cyclopoids are the most important copepod orders in the plankton, and numerous studies have been conducted on the feeding and/or behaviour of the adults of these 2 groups (Uchima & Hirano 1988, Paffenhöfer & Lewis 1990, Tiselius et al. 1997, Svensen & Kiørboe 2000, Paffenhöfer & Mazzocchi 2002, Saiz et al. 2003, Seuront et al. 2004). Only recently some research has been conducted on the feeding behaviour of their first larval stages (nauplii) (Van Duren & Videler 1995, Paffenhöfer et al. 1996, Titelman 2001,

Titelman & Kiørboe 2003a,b, Jiang & Paffenhöfer 2004). This lack of knowledge contrasts with the fact that copepod nauplii are considered the most abundant multicellular zooplankters in the marine environment (Fryer 1986), and an important food source for many fish larvae (Dalpadado et al. 2000, Gaard & Reinert 2002). To be able to better understand the predator–prey interactions in copepod nauplii, quantitative studies on their behaviour and feeding activity are fundamental.

Among the prey characteristics that influence copepod feeding, prey motility has a special relevance because, as mentioned above, it can affect encounter rates by either increasing the relative speed between prey and predator (Gerritsen & Strickler 1977) or by facilitating detection of the prey by the predator because of the hydromechanical signal generated. The effect of prey motility on copepod feeding activity likely will depend on the behavioural characteristics of the copepod species as well. Many cyclopoid nauplii remain virtually motionless in the water while slowly sinking, relocating only occasionally with fast jumps. This has been referred as jump–sink behaviour (Titelman & Kiørboe 2003a, Jiang & Paffenhöfer 2004), and seems to correspond to an ambush strategy. In contrast, nauplii of calanoids seem to exhibit a more diversified behaviour, ranging from nearly 100% cruise moving mode to a more hop-like mode (Van Duren & Videler 1995, Paffenhöfer et al. 1996, Titelman & Kiørboe 2003a, Jiang & Paffenhöfer 2004). From the point of view of prey encounter, the jump–sink behaviour mentioned above suggests a lower encounter, and thus lower feeding, rate in the presence of a non-motile prey than what is expected for a continuous-moving copepod. Therefore, we expect ambush-feeding predators like cyclopoids to be more affected by prey motility, increasing their feeding rate when confronted with a motile prey. In comparison, continuously moving or hopping calanoid nauplii would be expected to exhibit higher encounter and feeding rates due to their increased motility.

This study aims to provide new insights into the feeding behaviour of copepod nauplii by testing the effects of prey motility on encounter and feeding rates of copepods. To do that, we have exposed the nauplii of the copepods *Acartia grani* (Calanoida) and *Oithona davisae* (Cyclopoida) in laboratory incubations to 2 similarly sized, different prey: the non-motile diatom *Thalassiosira weissflogii* and the motile dinoflagellate *Heterocapsa* sp., and the nauplii feeding responses, motion patterns and reaction distances to prey have been determined.

MATERIALS AND METHODS

Acartia grani and *Oithona davisae* specimens came from continuous cultures at the Institut de Ciències del Mar located in Barcelona, Spain. They were kept separated in 20 l transparent plastic tanks, at 20°C and with a 12 h day:12 h night cycle. *A. grani* was fed the Cryptophyceae *Rhodomonas baltica* (equivalent spherical diameter, ESD = 6.6 µm), and *O. davisae* was maintained with the heterotrophic dinoflagellate *Oxyrrhis marina* (ESD = 16.9 µm). To allow comparison between species, special care was taken to use similar-sized nauplii. Due to length differences between newly hatched nauplii of *A. grani* and *O. davisae* different nauplii stages were used for each species (Stages NII and III for *A. grani* and Stages NIII and IV for *O. davisae*).

Typical carbon and nitrogen contents of the nauplii and algal species were determined with a NA2100 Carbon–Nitrogen Analyser (Table 1). Three replicate samples were taken for both nauplii and algae, as well as 3 blank samples. For the nauplii samples, a minimum of 4700 individuals was filtered per replicate. Size-carbon and size-nitrogen conversion factors for both predator and prey were determined and used to calculate weight-specific rates.

Nauplii feeding experiments. For the feeding experiments with *Acartia grani*, nauplii cohorts were obtained by removing adults from the culture with a 250 µm mesh size sieve, and placing them in a new tank. These animals were kept with a concentration of *Rhodomonas baltica* at 8 ppm, and allowed to produce eggs for 15 to 20 h. Thereafter, the eggs were collected and transferred to a new tank to hatch, where the recently hatched nauplii were grown at a 3 ppm *R. baltica* concentration. After 48 h the hatched nauplii had reached Stages NII to NIII and were ready to use for experiments.

Nauplii cohorts for *Oithona davisae* were made similarly. First, egg-bearing females were collected with a 130 µm sieve and transferred into a hatching tank, from which the adults were removed after 15 to 20 h,

Table 1. Typical values of size (either body length [mean ± SE] for nauplii or equivalent spherical diameter for algae), C and N contents (ng ind.$^{-1}$) and C:N weight ratio for *Acartia grani* and *Oithona davisae* nauplii, and for the algae *Thalassiosira weissflogii* and *Heterocapsa* sp. These values have been used to convert body size into carbon and nitrogen contents in the experiments

	A. grani	*O. davisae*	*T. weissflogii*	*Heterocapsa* sp.
Size (µm)	165.5 ± 2.5	120.0 ± 1.5	14.4	12.8
C contents	40.1	32.2	220.5×10^{-3}	188.5×10^{-3}
N contents	10.2	8.0	32.9×10^{-3}	33.8×10^{-3}
C:N	3.95	4.05	6.7	5.6

leaving the newly hatched nauplii in the tank. They were kept for 72 h on a diet of *Oxyrrhis marina* at 3 ppm before initiating the experiments. By then they would have reached Stages NIII to NIV. For both *Acartia grani* and *O. davisae* food concentrations were kept at the desired level in the corresponding tanks by daily adjustments.

The algal species used as prey in the feeding and behavioural experiments were a non-motile species (*Thalassiosira weissflogii*, ESD = 14.4 µm; Culture Collection of Algae and Protozoa, UK) and a motile species (the dinoflagellate *Heterocapsa* sp., ESD = 12.8 µm). They were cultivated in sterile environments in 1 l flat bottom glass flasks with added *f*/2-medium in a 12 h day:12 h night cycle at 100 µmol photon m^{-2} s^{-1}. In the case of the diatom *T. weissflogii*, silicon was also added. At least 3 d before initiation of the experiments, the cultures of *T. weissflogii* were stirred with aeration to facilitate the spine throw off, which otherwise could interfere with the feeding behaviour (Gifford et al. 1981, and authors' pers. obs.).

Feeding experiments were conducted in 72 ml transparent plastic cell culture bottles and covered a range of algal concentrations from 39 to 2500 cells ml^{-1} to allow the determination of functional responses. In the case of *Acartia grani* versus *Heterocapsa* sp. an additional second experiment was conducted with algal cell concentrations from 625 to 4000 cells ml^{-1}. This was done because saturation was not totally achieved in the first experiment. The range of algal concentrations was obtained by successive dilution of stock cultures. The algal suspensions were adjusted by enumerating 4 diluted replicate samples by triplicate counting using a Coulter Multisizer particle counter. Typically, 2 initial, 3 control (only prey) and 3 experimental (prey + nauplii) bottles were filled with prey suspension in a 3-step filling procedure to ensure homogeneity between replicates. Nutrients were added to all bottles (5 ml *f*/2 l^{-1}) to compensate for differentiated algal growth in the experimental bottles due to possible nutrient excretion by nauplii, and to ensure excess of nutrients in all the bottles. The bottles were sealed with a double layer of plastic foil, preventing bubbles from forming, and capped. The bottles were mounted on a plankton-wheel (0.2 rpm), and were incubated at ~20°C (19.6 to 20.8°C), with a 12 h day:12 h night cycle for ca. 24 h. Initial and final samples of the experimental suspension were preserved with acidic Lugol's solution (1% final concentration), and were kept in the dark at 4°C for later microscopic analysis of prey abundance (between 200 and 300 cells counted per replicate).

Clearance and ingestion rates were determined according to Frost (1972). Cell counts were converted into carbon and nitrogen units using conversion factors derived from Table 1 and average cell size from the Coulter Multisizer counts when preparing the suspensions. Carbon- and nitrogen-specific rates were calculated using the average nauplii size determined for each experiment. In the case of *Acartia grani* versus *Heterocapsa* sp., the 2 independent experiments conducted to complete the functional response employed nauplii originating from different cohorts, therefore showing slightly different stage compositions and sizes (average body mass: 31.5 and 33.9 ng C $ind.^{-1}$, respectively). In order to put together the data from these 2 experiments, the respective weight-specific ingestion rates were obtained and the ingestion rates on a per capita basis (cells $ind.^{-1}$ d^{-1}) were computed for an average nauplii (32.7 ng C $ind.^{-1}$).

Video filming of nauplii. Behavioural observations of the nauplii were conducted in 2 different ways. The first one consisted of capturing live 2D video films of the animals under the presence of prey at low magnification. This was done with a CCD video camera fitted with a 100 mm macrophoto-lens and connected to a time–date recorder, a VCR and a monitor, thus providing live images at 50 fields s^{-1}. A 2 l transparent plastic cubic aquarium (13.5 cm side), filled with distilled water, was placed in front of the macro-lens. Then, a 72 ml culture tissue plastic bottle, containing the nauplii and the algal suspension to be observed, was submerged inside the aquarium. It was given a minimum of 1 h to acclimatise before filming was initiated. Concentrations of algal cells and nauplii in the recordings were, respectively, 500 cells ml^{-1} and 2.7 nauplii ml^{-1}. For each nauplii recording, a millimetre scale was attached to the tissue plastic bottle and filmed as well.

In order to better record the details of behavioural events and to be able to register feeding attacks, another set of observations was conducted at higher magnification. The set-up was similar to the first one, however, a horizontally placed dissecting microscope was used instead of the macro-lens (see Saiz & Alcaraz 1991) and images were obtained at 25× magnification. Alga and nauplius concentrations were in the range of 3000 to 4000 cells ml^{-1} and 20 to 25 nauplii ml^{-1}.

For both set-ups, filming was done in a temperature-controlled dark room (19.8 to 21.9°C) and the illumination consisted of backlighting from an infrared light source. Video observations were initiated at 20:00 h, which corresponded to the initiation of the laboratory night cycle, and lasted ~60 min for the swimming observation and ~120 min for the close-up filming.

Video analysis was carried out manually with a Panasonic HS1000-Edit station VCR. The full recording was played, and 20 nauplii that were in focus and provided a minimum of 20 displacements on the screen were randomly chosen for analysis. In the close-up filming, due to a lower presence of animals in focus

and the few observed feeding attacks, the number of events was lower (n = 8 to 11). Nauplius trajectories were traced on a transparent acetate sheet placed on top of the monitor screen; afterwards they were digitalised on a flatbed scanner, and the trajectory coordinates were obtained with the software QuickTrace PPC for Macintosh after calibration. The coordinate data were transferred to Microsoft Excel, where the distance travelled, velocities and time budgets were computed.

Nauplii behaviour definitions. Three main different swimming behaviours were identified. For both *Acartia grani* and *Oithona davisae* nauplii, *sinking* was defined as a downward movement due to gravity solely. *Jumping* was an active movement defined as a rapid displacement of the animal. *Quiet* was defined as a lack of motion, normally after a jump, where the animal was positioned at the same place for a short period. It could have happened that the animal was actually very slowly sinking, but this was not detected because of the short time range and visual limitations.

Video analysis of nauplii feeding attacks. The distance between the nauplius and the prey was measured when an attack was effected, in which case the attack time, the handling time and the pre-attack behaviour was noted. Attack time was defined as the time from the initiation of the attack until the algal cell had disappeared. In most of the cases this was not easy to observe because of shadow from the nauplius itself; hence, only 5 to 7 complete successful attacks were observed in each food scenario. In addition, some attacks leading to eventual rejection of the prey item were recorded. Unless stated otherwise, values are arithmetic means and standard errors (SE).

To evaluate the error in the attack distance estimates based on 2-dimensional pictures, the depth of field of the observations was measured. This was done with a 10 µm interval object micrometer mounted at an angle of 45° to the lens axis. Focus on the object micrometer was set on the first line of the scale, and the following scale line not in focus indicated the maximum distance (300 µm) at which a certain point source would be in focus. The maximum depth of field was calculated using the theorem of Pythagoras. The depth of field was 212 µm, and, in practical terms, this means that the error on the calculated distance values would be ±106 µm.

RESULTS

Feeding experiments

Acartia grani nauplii ingestion rates followed a Holling Type II (on a per cell basis) functional response model on both prey (Fig. 1A, B). Maximum ingestion rates in terms of cells and carbon were, respectively, 50 and 62% higher when feeding on the motile prey (Table 2). The maximum intake of *A. grani* nauplii on the motile prey almost doubled that for the non-motile prey when expressed in terms of nitrogen (Table 2).

Oithona davisae nauplii only exhibited feeding on *Heterocapsa* sp. (Fig. 1C). Several experiments with different nauplii and algal concentrations were conducted to achieve a feeding response for *O. davisae* nauplii on the non-motile prey, always rendering no significant values. Regarding the feeding on *Heterocapsa* sp., *O. davisae* nauplii showed a similar-type response to *Acartia grani*, but overall with much lower maximum ingestion rates (Fig. 1C, Table 2).

For both naupliar species clearance rates decreased with food concentration (Fig. 2), down to values of ca. 0.05 to 0.1 ml ind.$^{-1}$ d^{-1} at the highest food concentrations. At low food availability, high variability on clearance rates was observed (which rendered low r^2 fitted models, Fig. 2). Maximum clearance rates (F_{max}, i.e. clearance rates before any saturating effects became meaningful) were estimated from the Holling Type II fit for ingestion rates (on a per cell basis) as the quotient I_{max}/K_m, where K_m is the half saturation constant for the Holling fit and I_{max} is the maximum ingestion rate. F_{max} for *Oithona davisae* nauplii was 40% lower than for *Acartia grani* nauplii (Table 2).

Prey and predator motility

The mean velocity of *Heterocapsa* sp. was obtained by randomly selecting 27 different cells and following each of them for a minimum of 2 s. The results gave a mean (±SE) transportation velocity of 0.087 ± 0.004 mm s^{-1}.

The *Acartia grani* nauplii exhibited a hop-like motion, with frequent and rapid small jumps (1.4 ± 0.05 bl, body lengths) interrupted by very short periods, when the nauplii were quiet or slowly sinking (Table 3). Overall, the hop-like motility was characterised by either movement in a straight line or in a spiral-like pattern (helical movement, Fig. 3), either downward or upward, in which the nauplii always moved in short hops. It is important to note that cruise swimming, understood as a continuous movement of the animal without stops, was not observed in any case with *A. grani* nauplii. The hop-like behaviour was consistent whether the nauplii were moving in a straight line or in a helical pattern. A Wilcoxon 2-sample test on the percentage time of the total time allocated to the 3 different behaviours (jump, sink and quiet) proved that their time budgets were independent of prey motility (p > 0.24 in all cases).

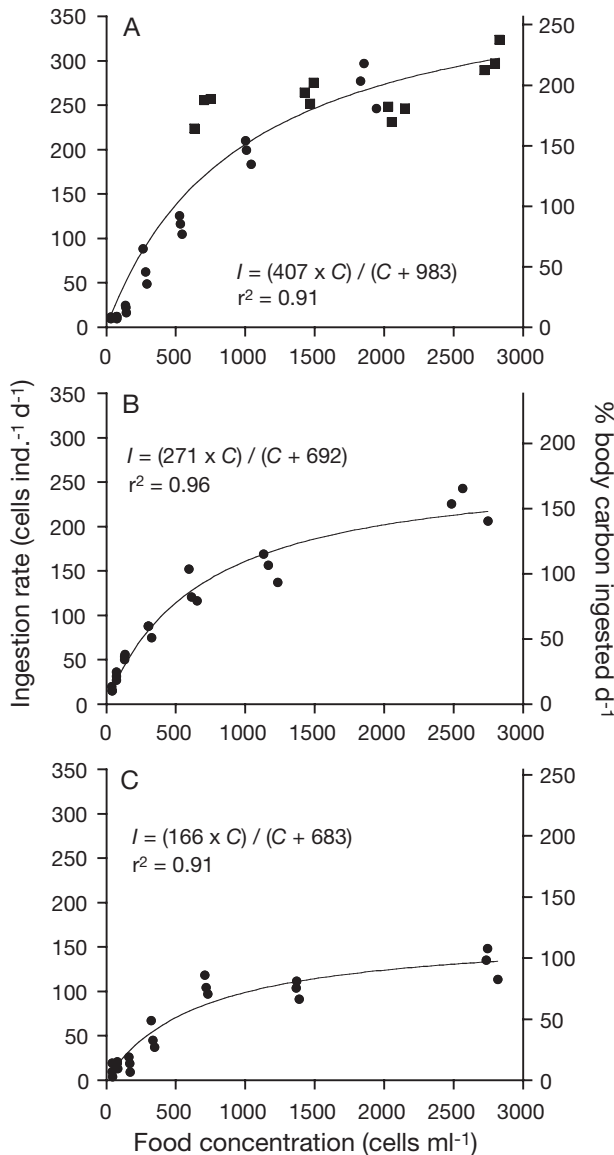There were no significant differences between the lengths of the jumps when feeding on the 2 prey types

Fig. 1. *Acartia grani* and *Oithona davisae*. Ingestion rates (*I*) expressed as either cells ingested ind.$^{-1}$ d^{-1} or percent body carbon ingested daily, as a function of the average food concentration (*C*). Holling Type II equation is fitted to ingestion rates on a cell basis. (A) *A. grani* feeding on *Heterocapsa* sp. (squares: data from a second experiment also used for curve fitting, see 'Materials and methods'). (B) *A. grani* feeding on *Thalassiosira weissflogii*. (C) *O. davisae* feeding on *Heterocapsa* sp. Note that no significant ingestion rates were detected for *O. davisae* feeding on *T. weissflogii*; hence, no graph is presented

Fig. 2. *Acartia grani* and *Oithona davisae*. Clearance rates (*F*) as a function of the average food concentration (*C*). Details as in Fig. 1

(Table 3, Wilcoxon test, p > 0.68). The amount of time spent in helical motion was higher under the presence of non-motile prey (45 ± 0.1 and 34 ± 0.09% for non-motile and motile prey, respectively), although the difference was not statistically significant (Table 3, p > 0.93). The rest of the time, the nauplii were jumping in a non-helical manner. It should be mentioned that due
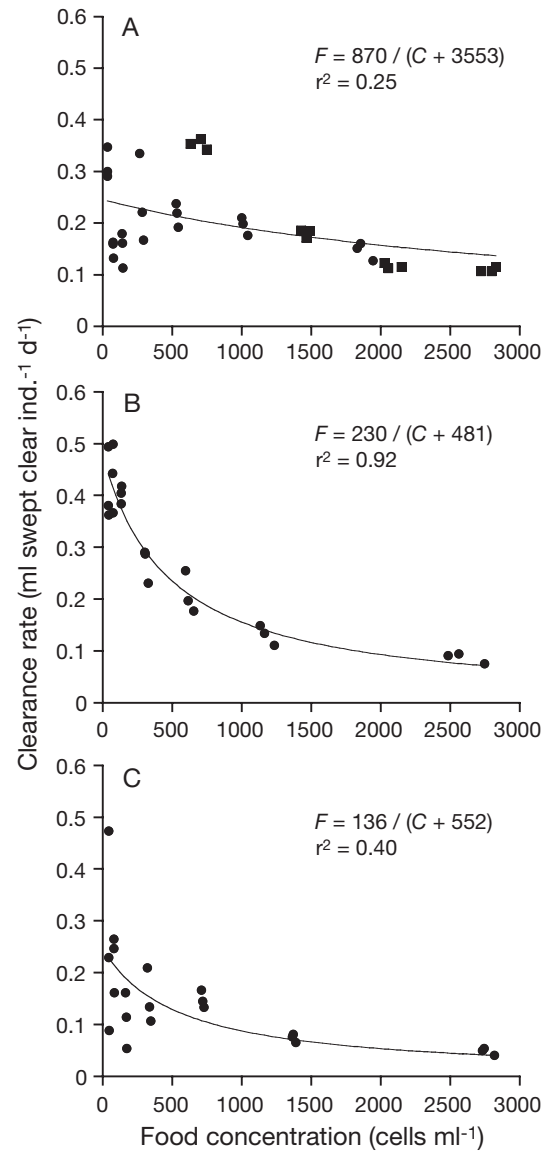
to the relatively short observation time for each individual (2.1 to 14.7 s), nauplii either performed one behaviour or the other, but only in a few cases were both motility behaviours observed in the same individual. Helical movement was performed at a faster speed in the presence of motile prey (1.30 ± 0.18 and 0.69 ± 0.10 mm s^{-1}, respectively, for motile and non-motile prey; Wilcoxon, p > 0.02). This could be a consequence of the higher jump frequency (226 ± 23 jumps min^{-1}) in the presence of the motile prey compared to the non-motile prey (196 ± 14 jumps min^{-1}). On 2 occasions, both with a non-motile diet, the nauplii remained in the same position while spinning frenetically around themselves. This type of behaviour is not presented in

Table 2. *Acartia grani* and *Oithona davisae.* Maximum ingestion and clearance rates of nauplii feeding on *Heterocapsa* sp. (*Het.*) and *Thalassiosira weissflogii* (*T.w.*). Data obtained from the Holling Type II equations of Fig. 1. Body mass of nauplii is also given

Scenario	Body mass (ng C ind.$^{-1}$)	Maximum ingestion rate (I_{max})			Maximum clearance rate (F_{max}) (ml ind.$^{-1}$ d^{-1})
		(cells ind.$^{-1}$ d^{-1})	(% body C d^{-1})	(% body N d^{-1})	
A. grani vs. *Het.*	32.7	407	299	212	0.41
A. grani vs. *T.w.*	32.6	271	185	109	0.39
O. davisae vs. *Het.*	33.3	166	121	88	0.24

Table 3. *Acartia grani* and *Oithona davisae.* Summary of behavioural variables (mean ± SE) analysed to describe the behaviour of the nauplii when offered *Heterocapsa* sp. (*Het.*) and *Thalassiosira weissflogii* (*T.w.*). Values expressed as body lengths or body lengths per second are in parentheses. ⋆: not enough data to calculate SE

	Acartia vs. *Het.*	*Acartia* vs. *T.w.*	*Oithona* vs. *Het.*	*Oithona* vs. *T.w.*
Percentage of time sinking	48.2 ± 4.9	43.4 ± 4.2	98.2 ± 0.15	98.9 ± 0.1
Percentage of time jumping	13.3 ± 1.5	11.5 ± 1.1	1.60 ± 0.15	0.93 ± 0.08
Percentage of time quiet	38.4 ± 3.6	44.0 ± 3.6	0.02⋆	0.08⋆
Sinking event duration (s)	0.76 ± 0.1	0.86 ± 0.09	3.3 ± 0.3	5.1 ± 0.3
Jump frequency (jumps min^{-1})	226 ± 23.0	196 ± 14.0	24 ± 2.0	14 ± 1.1
Jump distance (mm)	0.18 ± 0.006 (1.4 ± 0.05)	0.18 ± 0.008 (1.4 ± 0.06)	0.41 ± 0.02 (3.6 ± 0.2)	0.43 ± 0.03 (3.8 ± 0.3)
Sinking distance (mm)	0.063 ± 0.007 (0.49 ± 0.05)	0.070 ± 0.007 (0.55 ± 0.06)	0.24 ± 0.02 (2.1 ± 0.2)	0.26 ± 0.02 (2.3 ± 0.2)
Jump velocity (mm s^{-1})	4.8 ± 0.17 (37.3 ± 1.3)	5.2 ± 0.15 (40.5 ± 1.2)	9.7 ± 0.5 (85.8 ± 4.6)	10.2 ± 0.5 (89.8 ± 4.1)
Sinking velocity (mm s^{-1})	0.082 ± 0.01 (0.64 ± 0.09)	0.082 ± 0.006 (0.63 ± 0.05)	0.079 ± 0.004 (0.69 ± 0.03)	0.053 ± 0.002 (0.46 ± 0.02)

Table 4. *Acartia grani* and *Oithona davisae.* Behavioural variables (mean ± SE) describing the feeding attacks of nauplii on *Heterocapsa* sp. (*Het.*) and *Thalassiosira weissflogii* (*T.w.*). Values expressed as body lengths are in parentheses. Notice that *O. davisae* did not feed on *T. weissflogii* and consequently no data are presented. n: number of observations

	A. grani vs. *Het.*	*A. grani* vs. *T.w.*	*O. davisae* vs. *Het.*
Attack duration (s)	0.06 ± 0.007 n = 10	0.06 ± 0.003 n = 11	0.08 ± 0.01 n = 8
Handling duration (s)	0.33 ± 0.04 n = 7	0.44 ± 0.2 n = 9	0.51 ± 0.08 n = 6
Attack distance (μm)	91 ± 27 (0.73 ± 0.21) n = 10	96 ± 18 (0.76 ± 0.15) n = 10	113 ± 22 (1.0 ± 0.19) n = 6
Pre-attack behaviour	Helical (n = 10)	Helical (n = 9) Straight (n = 2)	Sinking (n = 8)

Table 3 due to its low frequency (2 observations) and percentage (1.07%) of total time.

Oithona davisae nauplii exhibited swimming patterns different than those of *Acartia grani* nauplii when offered both prey. Their overall motility can be characterised by jump–sink behaviour dominated by long jumps of 3.7 ± 0.18 bl, both upwards and downwards, followed by long sinking periods (Fig. 4). Each sinking sequence was followed by a jump and a new sinking period. *O. davisae* spent ~98% of the total time sinking and ~1% of the time jumping (Table 3). There was no significant difference in jump length between prey types (Wilcoxon, p > 0.9), although they dedicated considerably more time to jumping in the presence of the motile prey (Wilcoxon, p > 0.0008). When *O. davisae* nauplii were on a non-motile diet, they spent significantly more time in each sinking event (5.1 ± 0.3 versus 3.3 ± 0.3 s; p < 0.001). *O. davisae* nauplii jump frequency was significantly higher (Wilcoxon test, p < 0.001) when offered the motile prey (24 ± 2 jumps min^{-1}) compared to the non-motile diet (14 ± 1.1 jumps min^{-1}).

Feeding attacks by nauplii

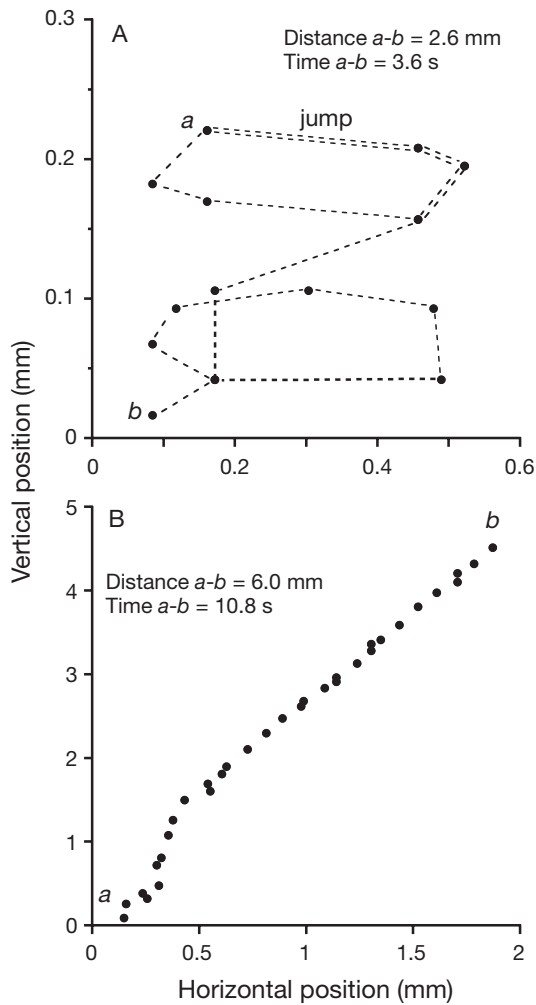Nauplii of *Acartia grani* seemed to be searching for food only when involved in a helical movement pat-

Fig. 3. *Acartia grani.* Representative examples of the 2 swimming behaviours observed in nauplii: (A) helical and (B) straight line motion. Note the different scales used in both panels and between axes

tern. This statement is based on the fact that in 19 out of 21 attack observations the nauplii were performing helical movement prior to the attack. Only in 2 cases were pre-attack nauplii moving in a straight line (Table 4). A rapid continuous movement of the appendages (likely to handle the prey) followed each successful attack, while the animal was moving in short pulses. For *Oithona davisae*, feeding-attack observations were only made with the motile prey, because of no registered feeding when offered the non-motile prey. *O. davisae* nauplii were always in periods of sinking prior to the observed attacks. Each attack was followed by a 'smooth gliding' behaviour, in which the animal gave the impression of handling and ingesting the algal cell.

For *Acartia grani* nauplii, attack duration was consistent regardless of prey type, and averaged 0.06 s;

Oithona davisae nauplii, on the contrary, spent more time in each attack (0.08 s, Table 4). This difference, however, was mostly a result of the longer attack distance completed by *O. davisae* nauplii. *A. grani* attack distances did not differ regardless of whether the animal was offered the motile or the non-motile prey (Table 4, Wilcoxon, p > 0.35). Handling duration was approximately 30% higher with *Thalassiosira weissflogii* than with *Heterocapsa* sp., although the differences were not significant (p > 0.75), likely due to the low number of attacks fully observed. In the case of *T. weissflogii* as prey, some cells were rejected (4 cases out of 11 attacks) and, thus, not ingested. It is worth mentioning that during the microscopic counting of *T. weissflogii* cells from the grazing bottles with *A. grani* nauplii, the algae showed signs of deterioration, which could be an indication of sloppy feeding. This qualitative observation, together with the high number of rejections observed for *A. grani* nauplii when feeding on *T. weissflogii*, gives high credibility to sloppy feeding in this particular feeding scenario.

DISCUSSION

The feeding rates reported here for nauplii of *Acartia grani* and *Oithona davisae* fall within the range of values found in the limited literature on the subject. For instance, field experimentation with *Calanus* nauplii rendered daily rations of up to 40% body C d^{-1} (Irigoien et al. 2003). For smaller nauplii, such as *Oncaea mediterranea*, Paffenhöfer (1993) reported daily rations of 105% in laboratory experiments. Higher ingestion rates have been reported for *Calanus pacificus* (100 to 150%; Fernández 1979), *Calanus helgolandicus* (110 to 297%; Rey et al. 2001) and *Rhin-*
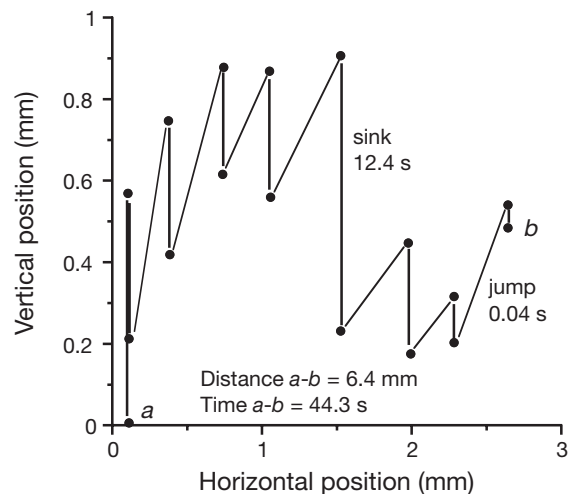

Fig. 4. *Oithona davisae*. Representative example of the jump and sink swimming behaviour observed in nauplii

calanus nasutus (80 to 227%; Mullin & Brooks 1967). The maximum daily rations found in our study (299 and 121% body C d^{-1} for *A. grani* and *O. davisae* nauplii, respectively) are within the higher range of the values found in the literature.

Regarding clearance rates, again it is difficult to find enough data with which to compare. The maximum clearance rates observed in our study varied between 0.2 and 0.4 ml ind.$^{-1}$ d^{-1}, whereas the lowest rates, at high food concentrations, were around 0.05 to 0.1 ml ind.$^{-1}$ d^{-1}. These values were somewhat higher than those in previous reports for small nauplii. For instance, Berggreen et al. (1988) reported clearance rates of ca. 0.044 ml ind.$^{-1}$ d^{-1} for *Acartia tonsa* nauplii feeding on *Thalassiosira weissflogii* at a 0.65 ppm concentration. At a comparable concentration, similarly weighted *Acartia grani* would clear 0.25 ml ind.$^{-1}$ d^{-1}. Other studies report values of up to 3–5 ml ind.$^{-1}$ d^{-1} for much larger calanoid species nauplii (e.g. 0.4 µg C ind.$^{-1}$, Irigoien et al. 2003; 2.8 to 4.8 µg C ind.$^{-1}$, Turner et al. 2001).

The nauplii species studied in this work showed conspicuously different swimming behaviours. *Acartia grani* nauplii swimming was characterised by jerky jumping (referred to as hopping behaviour), as displayed by many other calanoid nauplii species (Titelman & Kiørboe 2003a). Such behaviour is characterised by a high frequency of jumping, e.g. 196 to 226 jumps min^{-1} for *A. grani* nauplii (present study) and 90 to 183 jumps min^{-1} for *Acartia tonsa* nauplii (Titelman & Kiørboe 2003a). *Oithona davisae* nauplii, however, typically displayed low-frequency jump–sink behaviour (14 to 24 jumps min^{-1}), which is a characteristic pattern associated with cyclopoids (Paffenhöfer & Mazzocchi 2002). It is important to note here, however, that the jumps that characterise both behaviours are short (1.4 bl for *A. grani* nauplii, 3.6 to 3.8 bl for *O. davisae* nauplii), far from the displacements reported for escape reactions.

Prior to our study, the effects prey motility could have on the consumption rates of nauplii were not known. Copepod nauplii have simplified feeding appendages, and, accordingly, if they generate any feeding currents at all these should be weak (Paffenhöfer 1998). Copepod nauplii, then, must rely mainly on a raptorial feeding mode, detecting and seizing prey individually. Raptorial feeding should not preclude, in principle, that copepod nauplii could consume motile and non-motile prey indiscriminately. The responses to prey motility exhibited by *Acartia grani* and *Oithona davisae* nauplii in this work were very different. *A. grani* nauplii cleared both prey at similar rates, independent of their motility. One would expect that the motility of *Heterocapsa* sp. would enhance the encounter rate with *A. grani* nauplii. Very likely

Heterocapsa sp. velocity was too small compared to *A. grani* nauplii speed to contribute to the predator–prey velocity difference driving encounter rate (Gerritsen & Strickler 1977). An alternative explanation could be that any enhancement in encounter rate with *Heterocapsa* sp. might have been balanced if *Heterocapsa* sp. had the ability to escape naupliar attacks, resulting in no enhancement of feeding overall. However, this does not seem plausible, firstly, because during video observations no escape responses were registered in *Heterocapsa* sp., and, secondly, because Jakobsen (2001) did not find escape responses in dinoflagellates when exposed to a siphon flow. An interesting conclusion stemming from these results is that, even if *A. grani* nauplii detect prey by mechanoreception, chemoreception must work similarly well for them to detect the non-motile *Thalassiosira weissflogii*.

In terms of ingestion rate, there were meaningful differences in the maximum values observed as a function of prey. Maximum daily rations of *Acartia grani* nauplii on *Heterocapsa* sp. were 62 and 94% higher on a carbon and nitrogen basis, respectively, than those fed on *Thalassiosira weissflogii*. These differences in daily rations between the motile and non-motile prey are not necessarily related to food quality, because the C:N ratio of both algal species was similar. Likely, the feeding saturation at a lower rate in the case of *T. weissflogii* (271 cells ind.$^{-1}$ d^{-1}) was due to the fact that these diatoms fill up the copepod gut volume faster than the naked *Heterocapsa* sp., because of their silicon frustules, allowing for a lower cell packaging in the copepod gut and, therefore, limiting the ingestion rate. In addition, the qualitative observations of broken *T. weissflogii* cells during sample counting and the observations of *T. weissflogii* rejections by *A. grani* nauplii suggest that palatability may play a major issue limiting the maximum ingestion rates. Another nonexclusive explanation might be that the longer handling time observed for *A. grani* nauplii when offered *T. weissflogii* compared to when offered *Heterocapsa* sp. (although not statistically significant) might result in less time allocated to food searching and, therefore, lead to lower effective encounter and clearance rates at high food concentrations.

Our observations on nauplii behaviour agree, in general, with the results from the incubation experiments mentioned above. Thus, the presence or absence of motility in the offered prey also had an effect on the swimming behaviour of the *Acartia grani* nauplii. In the presence of *Heterocapsa* sp., the nauplii of *A. grani* had almost double the transportation velocity when doing helical swimming than in the presence of the non-motile prey. Such changes in predator swimming velocity when in helical mode must result in a combined effect on encounter and

feeding rates. This statement is supported by the fact that 90% of the observed *A. grani* nauplii feeding attacks occurred while in the helical swimming pattern. In fact, from these observations we hypothesise that helical movement would be an optimised search strategy for prey within a food patch, and, therefore, is directly associated with feeding (whereas straight line movement in *A. grani* nauplii could be interpreted as a search strategy for food-rich patches). The mechanisms underlying these changes in behaviour are not clear. Actually, the lower swimming velocity registered in *A. grani* nauplii in the presence of non-motile prey could just as well be interpreted as the result of a lower 'quality' value in *Thalassiosira weissflogii*, as suggested by the sloppy-feeding observations.

Regarding *Oithona davisae* nauplii, several attempts showed no feeding on the non-motile *Thalassiosira weissflogii*. Previous studies have indicated a low capability of adults and nauplii of *Oithona* spp. to feed on non-motile prey, both in the laboratory (Uchima & Hirano 1986) and in natural conditions (Atkinson 1995), although diatoms have also been reported in their diet (Hopkins et al. 1993, Atkinson 1996, Atkinson et al. 1996, Atienza et al. 2006). The low capability of *Oithona* copepodites to feed on non-motile prey has been attributed to the lack of feeding currents (Paffenhöffer 1993), which obliges the copepod to ambush feed. Ambush feeders rely on motile prey to be able to achieve a significant encounter rate with prey (Kiørboe & Saiz 1995). According to our results this seems to be the case for the naupliar stages of *O. davisae*, since they attack their prey in an ambush mode. The fact that both motile and non-motile prey had similar size excludes prey size as an alternative explanation for the dietary preference observed in *O. davisae* nauplii. Although previous work has suggested the role of chemical prey detection in the feeding of *O. similis* (González & Smetacek 1994), Svensen & Kiørboe (2000) showed that such a mechanism would not be an advantage in ambush-feeding copepods, like *Oithona*.

The maximum ingestion rates of *Oithona davisae* nauplii were substantially lower than those exhibited by *Acartia grani* nauplii. This lower intake seems to be a penalty for the ambush-feeding mode, which restricts prey encounter rates. Relying on prey motility to warrant prey encounters and feeding obviously sets a lower limit to the maximum daily ration a copepod can obtain in comparison with cruising or suspension feeders, which can scan a large volume of water for potential prey. The fewer encounters *O. davisae* nauplii exhibited in the presence of either motile or non-motile prey, as compared to *A. grani* nauplii, would only be an ecological success if it meant metabolic sav-

ings in other areas. *Oithona* is assumed to have lower metabolic demands than calanoids, providing an energetic advantage that allows it to thrive in environments at a low nutrient threshold (Paffenhöfer 1993, Castellani et al. 2005). This adaptation may be due to the lack of 2 energy-demanding characters: a feeding current and an active motility pattern. However, other adaptive morphological and sensorial traits, through evolutionary selection, may also help *O. davisae* nauplii to survive in such environments (Paffenhöfer 1998). *O. davisae* nauplii perceived approaching *Heterocapsa* sp. from further away than *A. grani* nauplii. The perceptive reaction distance is the single factor having the greatest effect on the encounter rate between a predator and its prey, because reaction distance scales squared with encounter rate (Kiørboe & Saiz 1995).

The behavioural response of *Oithona davisae* nauplii to prey motility was different than that exhibited by *Acartia grani*. As mentioned above, *O. davisae* nauplii are ambush feeders and detect their prey while slowly sinking. The present results showed that *O. davisae* nauplii increased their average duration of sinking events by 55% in the presence of the non-motile prey as compared to the motile one (Table 3). Furthermore, their jump frequency was as low as 14 jumps min^{-1} when presented with the non-motile prey and rose to 24 jumps min^{-1} in the presence of the motile prey (Table 3). As the motile and non-motile prey concentrations were the same, such changes in behaviour are likely triggered by the swimming capability of *Heterocapsa* sp. and by the ability of *O. davisae* nauplii to detect it, as evidenced by the feeding incubations.

It is interesting to note the behavioural changes in *Oithona davisae* nauplii in the presence of the non-motile prey (longer sinking events, lower transportation velocity and lower jump frequency) agree well with the reported effects of filtered seawater (no food) on *Acartia clausi* adults (Takahashi & Tiselius 2005). Such behavioural changes could indicate a strategy to save energy in an environment in which they are not able to sustain their normal energy demands through accessible food.

Finally, swimming behaviour has implications, not only for feeding, but also for the exposure to predation risks. *Acartia grani* nauplii showed a maximum of 226 jumps min^{-1} with the motile prey and 196 jumps min^{-1} when offered the non-motile prey (Table 3). Sinking nauplii are hydrodynamically almost undetectable, since the distance at which they can be detected by a predator tends towards zero (Titelman & Kiørboe 2003b). Low-frequency jump–sink nauplii (e.g. *Oithona davisae*) generate a small hydrodynamic signal, whereas high-frequency jumpers (e.g. *A. grani* nauplii) would be at the highest risk (Titelman & Kiørboe 2003b). By spending more time in a low-velocity type

of behaviour, such as sinking, a prey, in this case *O. davisae* nauplii, may reduce the hydrodynamic signal that can be perceived by a predator, and thus limit the time exposed to predators (Titelman 2001).

CONCLUSIONS

Acartia grani and *Oithona davisae* nauplii clearly show different behaviours and feeding performances. The swimming of *A. grani* nauplii can be described as a typical high-frequency hopping behaviour. Feeding seems to be associated with helical paths, as a strategy to optimise water clearing. In contrast, *O. davisae* nauplii are typical ambush feeders, which exhibit low-frequency jump–sink behaviour and should be less conspicuous to potential predators and prey. These different strategies, which commit *O. davisae* to feeding on motile prey, result, overall, in lower daily rations in comparison to those of *A. grani* nauplii. Prey motility can enhance encounter and feeding rates, but other factors like cell-packaging restrictions in the gut and lower palatability of prey can set the limits to actual feeding rates.

Finally, copepod nauplii showed plasticity in their behaviour as a function of the presence or absence of motility in potential prey. The fact that not only prey but predators are subject to behavioural changes has important implications for encounter- and individual-based models, which must take into account such variability to improve their predictive power.

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