Mar Biol (2007) 150:893–903
DOI 10.1007/s00227-006-0387-0

RESEARCH ARTICLE

Functional responses of copepod nauplii using a high efficiency gut fluorescence technique

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Received: 31 January 2006 / Accepted: 2 June 2006 / Published online: 4 July 2006

Abstract To investigate copepod nauplii ingestion rates on phytoplankton, we have adapted the traditional gut fluorescence technique as it can be used with lower gut pigment concentrations. With the improved technique, laboratory experiments were performed to estimate functional responses for nauplii of *Calanus helgolandicus* and *Centropages typicus*. Nauplii were raised from eggs to copepodites and the experiments were performed with stages NIV-NV. Gut evacuation rates and ingestion rates were measured on *Isochrysis galbana* at different concentrations. Specific ingestion rates ranged between 0.038–0.244 μg C μg^{-1} nauplii C d^{-1} for *C. typicus* and 0.041–1.412 μg C μg^{-1} nauplii C d^{-1} for *C. helgolandicus*. Both species showed a type III functional response, reaching a saturation concentration at around 600 μgC l^{-1} for *C. typicus* and 800 μgC l^{-1} for *C. helgolandicus*.

Introduction

Copepods are keystone intermediates in the flow of energy and matter through pelagic food webs and copepod nauplii are arguably the most numerous forms of metazoans on the planet (Björnberg 1984). Due to the widespread use of 200 μm nets for sampling mesozooplankton, information about nauplii, and also small copepodites, is relatively scarce. However, when appropriate mesh sizes are used, naupliar abundance can vastly outnumber older copepodite and adult stages (Turner 1982; Chisholm and Roff 1990; Turner and Roff 1993). Despite their high abundance, our knowledge of the ecological role of copepod nauplii is limited. Although important numerically, their contribution to the total community in terms of biomass is usually small and this may be the reason for the lack of research on copepod nauplii. However, recent studies have shown their possible importance. Lonsdale et al. (1996) found weight-specific ingestion rates to be three to four times higher than those of adults, and thus nauplii may play an important role in the food web. Also, as nauplii can ingest pico- and nanophytoplankton (Uye and Kasahara 1983; Berggreen et al. 1988) as well as some forms of bacterioplankton (Turner and Tester 1992; Roff et al. 1995) they may act as a trophic link between the microbial and classical food webs (Turner and Roff 1993).

On the other hand, technical difficulties when working with these small zooplankters may also explain the scarcity of information on their feeding habits. Abundance and distribution can be estimated but effective methods to quantify their *in situ* feeding rates are lacking. Work on laboratory rearing of copepods, fed on unialgal cultures (e.g. Klein Breteler et al. 1982; Fryd et al. 1991; Torres and Escribano 2003), demonstrates that nauplii feed efficiently on cells over a large size range. However, our knowledge of their ingestion rates is derived from incubation experiments, most of

Communicated by S.A. Poulet, Roscoff

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them using phytoplankton cultures as food (e.g. Paffenhöfer 1971; Berggreen et al. 1988; Bonnet and Carlotti 2001; Rey et al. 2001) providing results difficult to extrapolate to natural conditions. In the cases when natural food assemblages have been used, experiments were usually carried with nauplii of the largest species of copepods (e.g. the studies of *Calanus* spp. nauplli by Hansen et al. 2000, Turner et al. 2001 and Irigoien et al. 2003), making these results only representative of the feeding of naupliar communities where *Calanus* spp. is the dominant species. Exceptions are the studies by Tackx et al. (1990), White and Roman (1992) and Uitto (1996) involving incubation experiments with small nauplii and radio labeled phytoplankton.

The gut fluorescence method (Mackas and Bohrer 1976) is one of the most widely used methods when studying ingestion of phytoplankton by adult copepods. The method provides information on *in situ* feeding rates immediately prior to collection. With this technique, one avoids problems associated with lengthy incubations (Roman and Rublee 1980) and studies of diel variations in feeding rates are simple to conduct, as is investigation of the in situ feeding impact of herbivorous zooplankton on a phytoplankton assemblage. Up to now, this technique has not been used with nauplii and small copepodites due to technical problems. Their small size and low gut fluorescence require adaptations of the original methodology.

The first goal of this study was the improvement of the gut fluorescence technique to measure feeding rates of nauplii and small copepodites, using a high efficiency chlorophyll-a fluorometric analysis. Once the new protocol was established, feeding and gut evacuation experiments were performed with nauplii of two species of copepods. The validity of the improved technique was tested by comparing our results with literature data and also indirect calculation based on naupliar metabolism.

Another goal of this study was to determine the functional responses for both species. It is crucial to know zooplankton functional responses to understand the zooplankton-phytoplankton trophic link as a basis for models. In spite of this, experimental studies supporting a particular type of functional response are extremely scarce (reviewed in Gentleman et al. 2003). Two conceptual models (Lam and Frost 1976; Lehman 1976) pointed out that a type III functional response (Holling 1965) is the one that maximizes the net gain of energy. Both of them include a critical food concentration below which the energy expenditure of the feeding process is higher than the gain from the assimilation of the food collected. In this case an animal may reduce its feeding activity to minimize the energy loss or even cease it. Therefore, the most profitable functional response for suspension feeding zooplankters would be a sigmoidal type 3 or one type 2 with a feeding threshold at low food concentrations.

Materials and methods

Adaptation of the gut fluorescence technique

Prior to sample analysis, a series of tests were carried out to examine the influence of various factors on the experimental procedure. A Turner Designs TD700 fluorometer with a detection limit of 0.05 μg l^{-1} of chlorophyll-a (chl-a) and 0.06 μg l^{-1} of phaeophytin-a (pheo-a) in a solution of 90% acetone was used. To increase the sensitivity of pigment analysis an adapter kit for 75–250 μl borosilicate cuvettes was used, enabling a small number of nauplii per sample to be analyzed. The fluorometer was calibrated at high sensitivity, using the Raw Fluorescence Calibration, multi-optional Mode. During the calibration, the instrument range was manually adjusted. The optimal scale for nauplii gut content analysis was the one that assigned a value of 800 fluorescence units (fsu) to a concentration of about 10 μg chl-a l^{-1}. The relationship between chl-a concentration and fluorescence was linear within the range of measured concentrations. The EPA Method 445.0 (Arar and Collins 1997) for chl-a and pheo-a determination was followed.

As the blank fluorescence of individual cuvettes could vary when working at such high sensitivity levels, the fsu values for 30 different empty cuvettes were measured. Each cuvette was measured three times and a one-way ANOVA with a Cochran's *C*-test was carried out to verify the homogeneity of variances. Significant differences in the cuvette fluorescence ($F_{14,45} = 8.81$, $p < 0.05$) were detected. The blank fluorescence variance within cuvettes (std = 3.4 fsu) was much lower than between cuvettes (std = 8.5 fsu). To correct the error due to cuvette variability, a blank solution reading was taken with each cuvette prior to reading the sample. Then, using a Pasteur pipette, the blank solution was removed from the cuvette and the sample was introduced.

To establish the minimum number of nauplii needed to obtain a valid gut fluorescence measurement, samples ($n = 60$) with different numbers of individuals (5, 10, 20, 30) were analyzed. Nauplii were collected at three stations near Cudillero off the north coast of Spain (Central Cantabrian Sea) during January-March 2003. Plankton net tows (27 cm ring diameter and 53 μm mesh) were made from 50 m depth to the

surface. Tows were performed at low speed (0.5 m s^{-1}) and nets were not washed before removing the cod ends to avoid including in the sample, the most stressed and damaged nauplii from the net. The cod end contents were filtered through a 200 μm mesh to remove mesozooplankton and filtered onto a 30 μm mesh. Mesh filters with the nauplii were frozen immediately in liquid nitrogen and kept frozen until analysis. All the filtering process prior to freezing was carried out in a very short period of time to avoid gut evacuation (around 1 min.). For the gut fluorescence analyses, the filters were thawed and washed with filtered seawater to recover the nauplii. Working continually under dim light, nauplii were isolated with a micropipette (0.5–10 μl) and transferred to a petri dish with filtered seawater so that their bodies were washed to remove any adhering phytoplankton cells. Only nauplii without apparent damage were chosen. Afterward, groups of nauplii were picked with the micropipette, without regard to species or developmental stage. Each group was collected in a volume of 4 μl (nauplii and filtered seawater) and placed into cuvettes with 125 μl 90% acetone. The cuvettes were sealed with parafilm to avoid acetone evaporation. Pigments were extracted for 24 h at 4°C in the dark. Samples were not homogenized, as Morales et al. (1990) found that this procedure does not significantly affect copepods gut content measurements. Fluorescence was measured before and after acidification with HCl (Parsons et al. 1984) and gut pigment content was calculated as chl-a equivalent (chl-a eq = chl-a + 1.51 × pheo-a) as suggested by Båmstedt et al. (2000). Data were not corrected for background fluorescence and pigment degradation. During the period January–March 2003 very different values of mean individual gut contents were found, ranging between 0.0062 and 0.0791 ng chl-a eq $nauplii^{-1}$. These values corresponded to 1.3–27.6 fsu $nauplii^{-1}$. Bearing in mind these results and the variability found in the cuvette readings (variance within cuvettes), samples with at least 20 nauplii were picked out to ensure that the fluorometer errors were not significant.

To test the effect of exposure to light during sorting, the fluorescence values obtained for nauplii samples that had been exposed under dim microscope light for different periods of time were compared: immediately after washing (approximately after 2 min under the microscope) and 10 min later. Nauplii of *Calanus helgolandicus* and *Centropages typicus* from laboratory cultures were used to minimize variance between samples. ''Before'' and ''after'' 10 min, measurements were compared using paired-samples *t* test. We did not find any difference between their fluorescence (n = 13, t_{12} = 1.372, p = 0.195), although we observed that longer exposure times did result in decreases in fluorescence, so it is advisable to work as quickly as possible.

Gut fluorescence measurements

Once the new protocol was established, feeding and gut evacuation experiments were performed with nauplii from laboratory cultures.

Rearing of nauplii

Copepods were collected from net tows made off Plymouth (English Channel) between 15 June and 15 August 2004 with a 200 μm mesh net. The cod end contents were transferred to the lab in surface seawater in less than 2 h. Adult female *C. helgolandicus* and *C. typicus* (approximately 200 for each species) were placed in 2 egg-separation tubes in 5 l beakers with filtered seawater. After 24 h, females were removed and eggs were allowed to develop. Nauplii were kept in 5 l beakers at 15°C and with excess *Isochrysis galbana* (approximately 850 μg C l^{-1}). Phytoplankton concentration in the beakers was checked everyday, and it was adjusted by addition or dilution of the cultures. When most of the nauplii reached NIV-NV, the gut evacuation and ingestion experiments were carried out.

Gut evacuation experiments

Approximately 1,500 NIV-NV of each species were selected from the copepod cultures, and fed for several hours with excess *I. galbana* (similar concentration as in the cultures). After this period they were removed with a 53 μm mesh and rinsed into 5 l beakers filled with 0.45 μm filtered seawater. Groups of at least 100 nauplii were removed at 0, 2, 4, 6, 8, 10 and 15 min intervals, and immediately frozen with liquid nitrogen. Gut pigment content was measured for groups of 20 nauplii. For each time point, 8–10 groups of *C. helgolandicus* and 4–8 of *C. typicus* nauplii were analysed, depending on the abundance of nauplii on the filter. To estimate gut evacuation rate we used the following equations:

$$G_t = G_0 \times \exp(-k \times t)$$

assuming that a constant percentage of the gut content is evacuated per unit time (Baars and Oosterhuis 1984; Kiørboe et al. 1985; Christoffersen and Jespersen 1986). In the equation, k is the gut clearance coefficient, and G_o and G_t are the gut contents at times 0 and t.

Ingestion experiments at different concentrations

Groups of approximately 150 NIV-NV from the cultures were incubated in 1 l beakers filled with filtered seawater and an *I. galbana* suspension at different concentrations. They were allowed to feed for 4 h, and then the water in the containers was filtered through 30 μm mesh and the nauplii retained were immediately frozen with liquid nitrogen. Gut pigment contents were measured as above and ingestion rates (I) were calculated with the following equation:

$$I = k \times G$$

Initial concentration and size of phytoplankton cells in the water was measured with a Coulter® Multisizer. Final concentrations were not used to calculate ingestion rates with the "clearance method" as incubation time was too short and concentration of cells too high to get significant differences. To obtain chl-a concentration, 100 ml of water from each bottle was filtered onto GF/F filters and measured with a Turner Designs 10 AU fluorometer. We assumed a C content for *I. galbana* of 7.43 pg cell^{-1} (Rey et al. 2001) and a C/chl-a ratio of 40.5 was obtained for the phytoplankton culture.

Functional responses

The equations for the different types of functional responses (Holling 1965) were fitted by the least-squares criterion to the ingestion data. For the type I fit (rectilinear model) we followed the procedure by Rothhaupt (1990) to calculate where the deflection point should be, and then we obtained the fit for the combination of the two linear regressions:

$$I = a \times C\,,$$

when

$$C \leq Cd$$

$$I = I_{\max}\,,$$

when

$$C > Cd$$

where I is the specific ingestion rate (μg C μg^{-1} nauplii C d^{-1}), a is a constant, C is the phytoplankton concentration (μg C l^{-1}), Cd is the C at the deflection point and $Imax$ is maximum I, calculated as the I average value for $C > Cd$.

For type II we used the Ivlev (1961) equation:

$$I = I_{max} \times [1 - \exp(-a \times C/I_{max})]$$

where I is the specific ingestion rate, $I_{\max}$ is asymptotic maximum I, a is a constant and C is the phytoplankton concentration.

And the logistic equation for type III model:

$$I = I_{max}/(1 + \exp[(Kc - C)/a])$$

where $I_{\max}$ is asymptotic maximum I, C is the phytoplankton concentration, Kc is a constant defined as the food concentration for $I = I_{max}/2$, and a is a constant.

We tested the significance of differences in variances among regressions by a two-tailed F test on the mean-square error (Mullin et al. 1975; Rothhaupt 1990).

Stage duration and growth rates

A sample of at least 25 nauplii was taken from the cultures every 12 h for cohort analysis, and stage durations were estimated using the method of "median development time" (Peterson and Painting 1990).

Gross growth efficiency (GGE) was calculated for NIV-NV to check if the ingestion rates found were adequate to sustain naupliar growth. We used the formula:

$$\mathrm{GGE} = G/I$$

where G is daily growth and I is daily ingestion rate, both expressed in C units.

To calculate GGE, the same nauplii used for the cohort analysis were measured. To calculate dry weight we used the relationship between length and weight found by Klein Breteler et al. for *C. typicus* (1982). Since we could not find a formula for *C. helgolandicus* nauplii, the one by Klein Breteler et al. (1982) for *Pseudocalanus* sp was used. Dry weight was converted to C weight using ratios given by Gorsky et al. (1988). To calculate daily growth we divided the difference in weight between NV and NIV by the duration of stage NIV. The ingestion rates used were those obtained at 862 μg C l^{-1}, as nauplii in the cultures were grown under similar concentrations.

Results

Development times and growth rates

The duration of development stages is presented in Table 1 Development from egg to CI lasted for

Table 1 Duration, in days, of development stages in the cultures at 15°C

Species	*Egg*	*NI*	*NII*	*NIII*	*NIV*	*NV*	*NVI*	*Cumulative duration*
Calanus helgolandicus	1.3	1	1.6	3.8	1.5	1.5	2	12.7
Centropages typicus	1.45	1.25	1.55	2.1	4	1.9	2.05	14.3

12.7 days in *C. helgolandicus* and for 14.3 days in *C. typicus*. Length, weight and growth rate observations for stages NIV and NV reared in our experimental conditions are presented in Table 2.

The gross growth efficiency calculated for NIV-NV *C. helgolandicus* was 0.12 ± 0.05 (mean ± s.d.) and 0.28 ± 0.006 for NIV-NV *C. typicus.*

Gut evacuation rates

Despite the high variance within each group of replicate samples, we observed a trend of gut pigment content decreasing during each experiment and least-squares exponential fits were obtained for both evacuation experiment (Fig. 1.).

Ingestion rates at different concentrations

For both species, gut contents showed an increasing trend with increasing phytoplankton concentration (Figs. 2, 3), with a saturation response at around 600 µgC l^{-1} for *C. typicus*, and at a slightly higher concentration for *C. helgolandicus*. Evacuation rates found in the previous experiments were used to calculate ingestion rates. Specific ingestion rates ranged between 0.038–0.244 µg C $µg^{-1}$ nauplii C d^{-1} for *C. typicus* and 0.041–1.412 µg C $µg^{-1}$ nauplii C d^{-1} for *C. helgolandicus*.

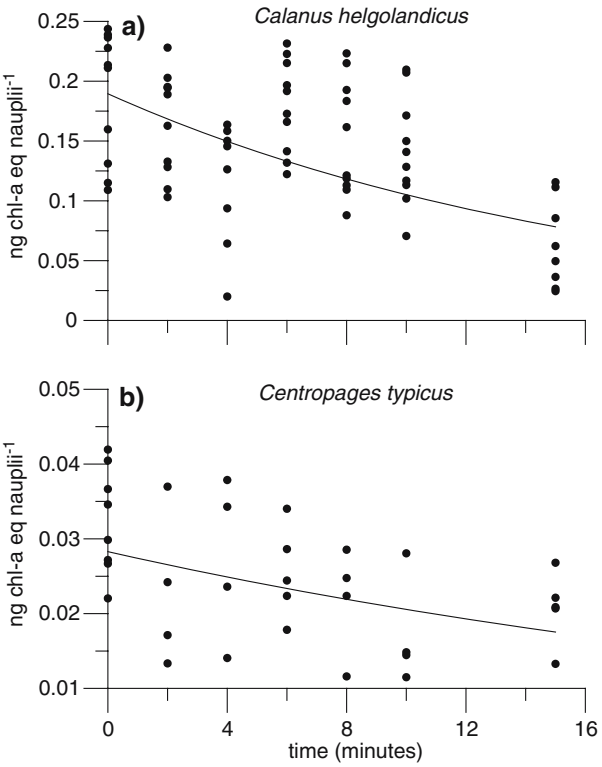

Fig. 1 Gut contents during gut evacuation experiments. *Lines* represent exponential least-squares fits. *C. helgolandicus* ($G_0 = 0.19$, $k = 0.058$, $r^2 = 0.25$, $p < 0.001$). *C. typicus* ($G_0 = 0.03$, $k = 0.032$, $r^2 = 0.19$, $p < 0.01$)

Functional responses

The three types of fits for functional responses were tried. Using minimization of the mean square variance as the criterion for goodness of fit, the type III model was best in both cases (Table 3). However, for *C. typicus,* we did not find significant differences in explained variance between type I, type II and type III models (results not shown). With *C. helgolandicus* it was not possible to obtain a valid outcome for the type II fit, and the type I did not reach the saturation concentration with our data. When comparing the type I and type III responses in *C. helgolandicus*, we did not find significant differences in explained variance either.

Table 2 Body length of nauplii stages NIV and NV (µm, mean ± SD, calculated from 25 values), dry weight (µg) calculated following Klein Breteler et al. (1982) and growth between NIV and NV expressed as µg C day^{-1}. Dry weight was transformed to C weight following Gorsky et al. (1988)

Species	Body length		Dry weight		Growth
	NIV	NV	NIV	NV	
C. helgolandicus	405 ± 16.7	467 ± 12.2	1.414 ± 0.061	1.627 ± 0.044	0.064
C. typicus	166.4 ± 8	206.1 ± 5.2	0.560 ± 0.028	0.689 ± 0.018	0.012

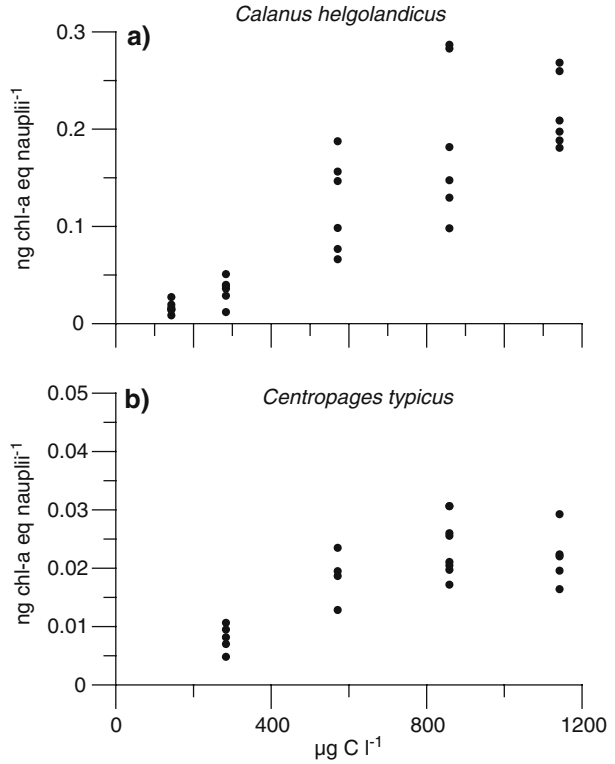

Fig. 2 Gut contents at different *I. galbana* concentrations

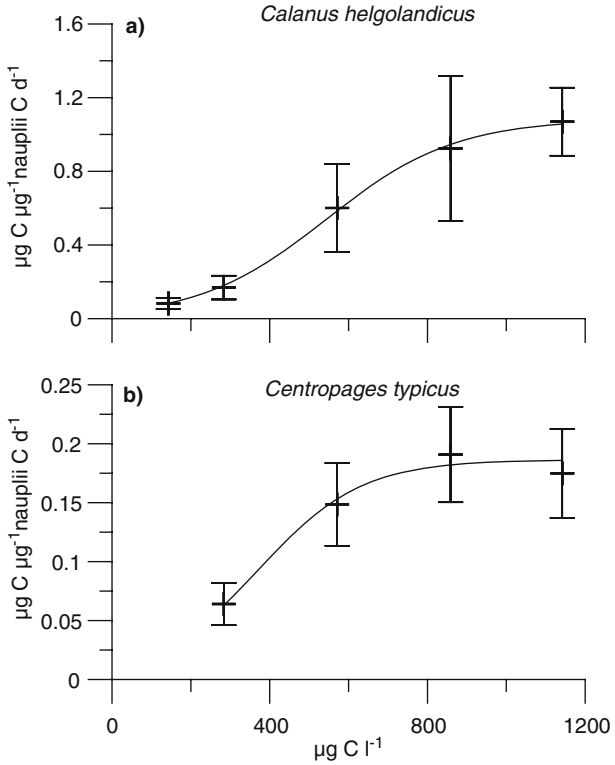

Fig. 3 Specific ingestion rates at different *I. galbana* concentrations. *Lines* represent logistic equation fitted to data by least-squares fits

We assumed a type III functional response because it was the model that better fit the data.

Discussion and conclusions

The exploratory analysis of the improved technique applied to the analysis of gut fluorescence, provides a basis for further investigation of nauplii feeding rates in the laboratory and in the field.

The gut fluorescence technique has some weaknesses: it is limited to ingestion of phytoplankton, there are difficulties in obtaining reliable evacuation rates, and there is a possibility of pigment destruction in copepod guts to non-fluorescent compounds.

The gut evacuation rate calculated in our experiments ($k = 0.058$ min^{-1} for *C. helgolandicus* and $k = 0.032$ min^{-1} for *C. typicus*) is quite similar to that obtained with the equation of Dam and Peterson (1988) that relates gut clearance rate to temperature ($k = 0.038$ min^{-1} at 15°C). This equation has been generally accepted and employed for adult copepods and it seems it could be used for copepod nauplii as well, although more experiments should be done under different conditions and with different species and stages. This would suggest a lack of relation between gut evacuation constant and copepod size (or in this case developmental stage) and is consistent with the results obtained by Morales et al. (1990). In their review of the gut fluorescence method, they found a relationship between the gut evacuation constant and temperature, but no relation with copepod body size. In the estimation of ingestion rates we have assumed that gut evacuation rates obtained would not change with food concentration, although some authors have found that food concentration influences this rate (Dagg and Walser 1987; Pasternak 1994). Irigoien (1998), in a review of literature data, found differences in the gut clearance constant for pre-fed animals and animals collected directly from the environment, indicating that the practice of pre-feeding animals before evacuation experiments could produce biased results due to higher initial gut contents. However, the differences Irigoien (1998) found for both relations were quite low ($k = 0.05$ min^{-1} at 15°C for pre-fed animals and $k = 0.04$ for animals from the environment). Thus, although it seems that k changes with food concentration, a possible error created by using the same constant for all the experiments could not be high, and it would not significantly affect the ingestion rates obtained.

Several authors have pointed out that one of the main weaknesses of the method is the uncertainty

Table 3 Parameters for the model fits and mean-square error (MSE) for the type I, type II and type III models. Cd and Kc (μg C l^{-1}), I_{max} (μg C μg^{-1} nauplii C d^{-1})

Species	Type I				Type II			Type III			
	A	Cd	*I*max	MSE	a	Imax	MSE	a	Kc	*I*max	MSE
C. helgolandicus	9.79×10^{-4}	–	–	0.0487	–	–	–	162	544	1.08	0.0467
C. typicus	2.29×10^{-4}	763	0.175	1.33×10^{-3}	3.7×10^{-4}	0.231	1.45×10^{-3}	130	371	0.186	1.23×10^{-3}

about pigment destruction during digestion to non-fluorescent compounds. Previous studies have reported highly variable degradation rates, ranging from 0 to 100% (reviewed in Dam and Peterson 1988). There is no general agreement about the extent of pigment destruction in copepod guts, as is discussed in Pasternak (1994) and Båmstedt et al. (2000). In any case, studies comparing the gut fluorescence technique with other techniques usually obtain similar results (Dagg and Grill 1980; Kiørboe et al. 1982; Baars and Franz 1984; Baars and Oosterhuis 1984; Kiørboe et al. 1985; Ishii 1990; Peterson et al. 1990), and results presented here are in the same range as those found by other authors using different methodology (see below). This suggests that high degradation rates are not the rule, and it is possible that in the cases when authors have found values as high as 80–100%, results have been influenced by artifacts in the experimental or analytical techniques. Although, further investigation is necessary to understand the processes involved in pigment degradation to know how accurate the gut fluorescence technique is, the method is still very useful to estimate *in situ* copepod ingestion rates on phytoplankton. It could be even more useful with copepod nauplii, as their small size and low ingestion rates make it difficult to perform bottle incubations with them in the field.

The naupliar feeding rates we measured should be considered as approximations as diel feeding periodicities have not been studied. Diel periodicities have been described by many authors for copepods (reviewed in Mauchline 1998) and not having taken them into account could imply a significant error in the extrapolation to daily ingestion, although it is not yet clear whether naupliar feeding also exhibits a diel cycle.

In spite of this, when compared with other data found in the literature (Table 4 and plotted in Fig. 4), our data are in the same range as most of the others. The *C. helgolandicus* ingestion rates on *I. galbana* are not very different from those found by Rey et al. (2001) under similar conditions. The *C. typicus* ingestion rates are lower than the rates found by Bonnet and Carlotti (2001), but differences in the experiments could explain this. Although the culture conditions were quite similar, in Bonnet and Carlotti (2001) both phytoplankton concentration and cell size were higher and there were differences between nauplii too, involving different stages, larger size and faster development in their case (11.55 versus 14.3 d from egg to CI). The main differences are found with the results of Paffenhöfer (1971) which are much higher than ours. Fernández (1979) considered that Paffenhöfer (1971) had underestimated the nauplii carbon content, and this would result in higher specific ingestion rates. Another point is that our results could be influenced by the size of the algal species chosen. Irigoien et al. (2003) found that *C. finmarchicus* nauplii fed on quite large cells from the natural assemblage. *I. galbana* used in our study was probably too small to be efficiently captured. Previous studies have found that *I. galbana* size is near the lower end of the size spectrum available for some species. Harris (1994) reported poor capture efficiency by *C. helgolandicus* feeding on the similarly sized cell, *Emiliania huxleyi*. Fernández (1979) found that the lower size for *C. pacificus* nauplii was between 2 and 4 μm. In his experiments, NV and NVI *C. pacificus* never ingested *I. galbana* in amounts sufficient to support maintenance metabolism. Frost (1972) has suggested that the minimum size on which copepods graze efficiently increases with the size of the copepod. However, in our experiments, although *C. typicus* nauplii are smaller than those of *C. helgolandicus*, they had lower specific ingestion rates, suggesting a less efficient capture of *I. galbana*.

Our feeding experiments supported a type III functional response, although differences with the other types were not significant. The saturation concentrations found are somewhat higher than previously reported in the literature. Berggreen et al. (1988) found a saturation concentration for juvenile stages of *Acartia tonsa* feeding on *Rhodomonas baltica* of around 500 μg C l^{-1}, and Frost (1972) found saturation concentrations, for *Calanus pacificus* females feeding on different species of phytoplankton, ranging between 100–300 μg C l^{-1}. Frost (1972) also observed that the saturation concentration decreased with increasing cell volume, so the small size of the phytoplankton species we used, could explain these differences.

Table 4 Summary of specific ingestion rates of copepod nauplii at different food concentrations found in the literature

Copepod species	Stage	Phytoplankton species	Phytoplankton concentration (μg C l^{-1})	Specific ingestion rate (μg C μg^{-1} nauplii C day^{-1})	Source
Calanus helgolandicus	NIV-NV	*Isochrysis galbana* (4 μm)	143	0.082 ± 0.031	This study
C. helgolandicus	NIV-NV	*I. galbana* (4 μm)	287	0.169 ± 0.064	This study
C. helgolandicus	NIV-NV	*I. galbana* (4 μm)	574	0.600 ± 0.239	This study
C. helgolandicus	NIV-NV	*I. galbana* (4 μm)	862	0.924 ± 0.393	This study
C. helgolandicus	NIV-NV	*I. galbana* (4 μm)	1,149	1.069 ± 0.184	This study
C. helgolandicus	NIII	*Rhodomonas baltica* (7–8 μm)	364	0.674 ± 0.061	Rey et al. (2001) [a]
C. helgolandicus	NIII	*I. galbana* (4–5 μm)	520	0.737 ± 0.358	Rey et al. (2001) [a]
C. helgolandicus	NIII	*Prorocentrum micans* (26–27 μm)	505	2.969 ± 1.079	Rey et al. (2001) [a]
C. helgolandicus	NIII	*Pleurochrysis carterae* (9–10 μm)	768	2.892 ± 0.548	Rey et al. (2001) [a]
C. helgolandicus	NIII	*Thalassiosira weissflogii* (12–14 μm)	429	0.930 ± 0.289	Rey et al. (2001) [a]
C. helgolandicus	NIV	*R. baltica* (7–8 μm)	364	0.759 ± 0.117	Rey et al. (2001) [a]
C. helgolandicus	NIV	*I. galbana* (4–5 μm)	520	0.498 ± 0.014	Rey et al. (2001) [a]
C. helgolandicus	NIV	*P. micans* (26–27 μm)	505	1.704 ± 0.266	Rey et al. (2001) [a]
C. helgolandicus	NIV	*P. carterae* (9–10 μm)	768	1.375 ± 0.612	Rey et al. (2001) [a]
C. helgolandicus	NIV	*T. weissflogii* (12–14 μm)	429	0.907 ± 0.248	Rey et al. (2001) [a]
C. helgolandicus	NV	*R. baltica* (7–8 μm)	364	0.523 ± 0.107	Rey et al. (2001) [a]
C. helgolandicus	NV	*I. galbana* (4–5 μm)	520	0.224 ± 0.008	Rey et al. (2001) [a]
C. helgolandicus	NV	*P. micans* (26–27 μm)	505	1.102 ± 0.148	Rey et al. (2001) [a]
C. helgolandicus	NV	*P. carterae* (9–10 μm)	768	0.988 ± 0.044	Rey et al. (2001) [a]
C. helgolandicus	NV	*T. weissflogii* (12–14 μm)	429	0.836 ± 0.212	Rey et al. (2001) [a]
C. helgolandicus	NVI	*R. baltica* (7–8 μm)	364	0.241 ± 0.025	Rey et al. (2001) [a]
C. helgolandicus	NVI	*I. galbana* (4–5 μm)	520	0.209 ± 0.079	Rey et al. (2001) [a]
C. helgolandicus	NVI	*P. micans* (26–27 μm)	505	1.195 ± 0.196	Rey et al. (2001) [a]
C. helgolandicus	NVI	*P. carterae* (9–10 μm)	768	0.481 ± 0.066	Rey et al. (2001) [a]
C. helgolandicus	NVI	*T. weissflogii* (12–14 μm)	429	0.449 ± 0.096	Rey et al. (2001) [a]
C. helgolandicus	N	Mixture of cultures	120	1.289 ± 0.107	Meyer et al. (2002) [b]
Calanus pacificus	NIV	*Lauderia borealis* (19 μm)	49	0.9	Paffenhöfer (1971) [c]
C. pacificus	NIV	*L. borealis* (19 μm)	101	2.75	Paffenhöfer (1971) [c]
C. pacificus	NIV	*L. borealis* (36 μm)	36	3.55	Paffenhöfer (1971) [c]
C. pacificus	NIV	*Gymnodinium splendens* (60 μm)	95	2.95	Paffenhöfer (1971) [c]
C. pacificus	NV	*L. borealis* (19 μm)	49	2.95	Paffenhöfer (1971) [c]
C. pacificus	NV	*L. borealis* (19 μm)	101	3.45	Paffenhöfer (1971) [c]
C. pacificus	NV	*L. borealis* (36 μm)	36	4.82	Paffenhöfer (1971) [c]
C. pacificus	NV	*G. splendens* (60 μm)	95	3.82	Paffenhöfer (1971) [c]
C. pacificus	NV	*Thalassiosira fluviatilis* (12–17 μm)	177	1.57	Paffenhöfer (1971) [c]
C. pacificus	NVI	*L. borealis* (19 μm)	49	1.55	Paffenhöfer (1971) [c]
C. pacificus	NVI	*L. borealis* (19 μm)	101	2.05	Paffenhöfer (1971) [c]
C. pacificus	NVI	*L. borealis* (36 μm)	36	2.6	Paffenhöfer (1971) [c]
C. pacificus	NVI	*G. splendens* (60 μm)	95	1.9	Paffenhöfer (1971) [c]
C. pacificus	NIII	Different cultures	125	0.1–0.5	Fernández 1979) [e]
C. pacificus	NIV	Different cultures	125	0.06–0.87	Fernández (1979) [e]
C. pacificus	NV	Different cultures	125	0.04–1.3	Fernández (1979) [e]
C. pacificus	NVI	Different cultures	125	0.06–1.25	Fernández (1979) [e]
Calanus finmarchicus	NIII-NV	Natural assemblage	94.08 ± 87.36	0	Hansen et al. (2000) [d]
C. finmarchicus	NIII-NIV	Natural assemblage	177.6 ± 182.04	0.32	Hansen et al. (2000) [d]
C. finmarchicus	NIV-NVI	Natural assemblage	27.6–212	0.11–0.46	Irigoien et al. (2003)[h]
C. finmarchicus	N	Mixture of cultures	120	1.313 ± 0.064	Meyer et al. (2002) [b]
Calanus spp.	N	Natural assemblage	5–20	0.0087–0.012	Turner et al. (2001) [f]
Temora longicornis	NII	*Oxyrrhis marina* (13.2 μm)	755	0.35	Klein Breteler et al. (1990) [g]
T. longicornis	NIII	*O. marina* (13.2 μm)	755	0.35	Klein Breteler et al. (1990) [g]
T. longicornis	NIV	*O. marina* (13.2 μm)	755	0.42	Klein Breteler et al. (1990) [g]
T. longicornis	NV	*O. marina* (13.2 μm)	755	0.48	Klein Breteler et al. (1990) [g]

Table 4 continued

Copepod species	Stage	Phytoplankton species	Phytoplankton concentration (μg C l^{-1})	Specific ingestion rate (μg C μg^{-1} nauplii C day^{-1})	Source
T. longicornis	NVI	*O. marina* (13.2 μm)	755	0.58	Klein Breteler et al. (1990) [g]
Centropages typicus	NIV-NV	*I. galbana* (4 μm)	287	0.064 ± 0.018	This study
C. typicus	NIV-NV	*I. galbana* (4 μm)	574	0.148 ± 0.035	This study
C. typicus	NIV-NV	*I. galbana* (4 μm)	862	0.191 ± 0.040	This study
C. typicus	NIV-NV	*I. galbana* (4 μm)	1,149	0.175 ± 0.038	This study
C. typicus	NVI	*I. galbana* (6 μm)	1,539	0.48 ± 0.25	Bonnet and Carlotti (2001) [h]
Acartia spp.	N	Natural assemblage	300–420	0.28–0.52	Tackx et al. (1990) [i]
Acartia spp.	N	Natural assemblage	180–1,620	0.79–2.8	White and Roman (1992) [j]
Copepod nauplii assemblage	N	Natural assemblage	15–68	0.08–0.29	Uitto (1996) [k]

[a]24 h incubations. Initial and final concentrations measured with Coulter Counter. [b]24 h incubations. Initial and final pigment analysis with HPLC. [c]1–38 h incubations. Initial and final concentrations measured with Coulter Counter. [d]24 h incubations. Chlorophyll clearance method. [e]15–20 h incubations. Initial and final concentrations measured with Coulter Counter. [f]24 h incubations. Microscopic counting and chlorophyll clearance method. [g]24 h incubations. Initial and final concentrations measured with Coulter Counter. [h]24 h incubations. Initial and final concentrations counted under a microscope. [i]Data taken from Uitto (1996).[j]Short incubations with radio labelled natural phytoplankton. [k]An aliquot of radio labelled phytoplankton cultures (*Brachiomonas submarina* and *Pavlova lutheri*) was added to water collected in the study area. Ingestion was calculated with the clearance rates obtained for the before mentioned species during short incubation experiments

The gross growth efficiencies calculated for NIV-NV *C. helgolandicus* and *C. typicus* are consistent with literature data. Rey et al. (2001) found gross growth efficiencies for *C. helgolandicus* nauplii feeding on different diets ranging from 0.12 to 0.59. They observed a strong dependence on the algal diets, implying differential assimilation. Efficiencies determined in experiments with copepods average about 0.33 (Kiørboe et al. 1985; Peterson 1988; Båmstedt et al. 1999). The gross growth efficiencies we observed support the validity of our method for estimating ingestion rates. The much higher ingestion rates found by Paffenhöfer (1971) are likely to be overestimates that would result in low growth efficiencies. Using development times reported by Paffenhöfer (1970), we calculate these efficiencies to be 0.01–0.04 in his experiments.

We consider that the results presented in this paper confirm the value of the gut fluorescence technique for estimating feeding rates of copepod nauplii on phytoplankton. The preliminary tests, as well as data obtained during an annual cycle in the Cantabrian Sea (unpublished), have demonstrated that it can be used with natural assemblages. Thus, it indicates promising approaches for investigating the trophic activity of copepod nauplii on autotrophic components of pelagic ecosystems.

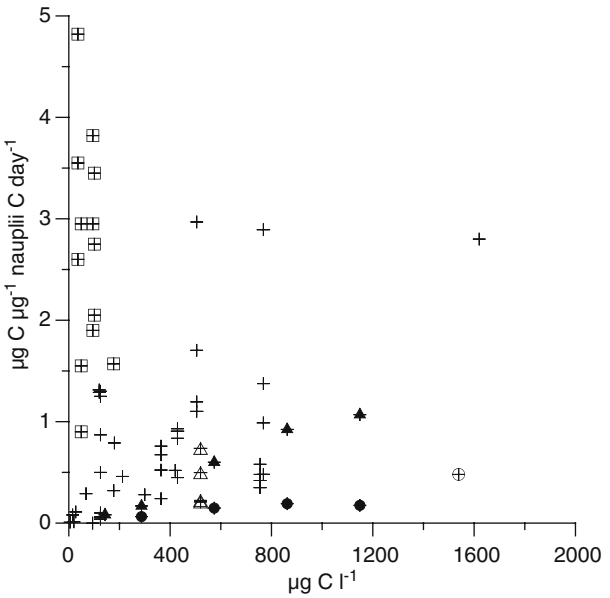

Fig. 4 Specific ingestion rates as a function of phytoplankton concentration. Extreme values have been plotted for the cases that are presented in Table 4 as a range of data. + All data from Table 4, *open square C. pacificus* (Paffenhöfer 1971), *open triangle C. helgolandicus* feeding on *I. galbana* (Rey et al. 2001), *filled triangle C. helgolandicus* feeding on *I. galbana* (this study), *open circle C. typicus* feeding on *I. galbana* (Bonnet and Carlotti 2001), *filled circle C: typicus* feeding on *I. galbana* (this study)

Acknowledgments This work was supported by the collaboration specific agreement between Universidad de Oviedo and Instituto Español de Oceanografia: ''*Control a largo plazo de las condiciones químico-biológicas en la plataforma continental de Asturias*'', the Ministerio de Educacion y Ciencia (MEC) project CARPOS (REN/09532-C03–03) and a FPU grant by MEC to E.L. We are indebted to the crew of the R/V ''Jose Rioja'' and ''Sepia'' for their help to collect zooplankton and seawater. We

thank the ''Group of Biological Oceanography'' of the University of Oviedo for their help with the sampling and their helpful assistance in the laboratory and D. Bonnet, L. Yebra and T. Smith (Plymouth Marine Laboratory) for their technical assistance and constructive criticism. We also want to thank J. L. Acuña for his useful comments on functional responses and A.G. Nicieza and J. Arrontes for their statistical assessment. All the experiments were performed in compliance with the laws of Spain and UK.

References

Arar EJ and Collins GB (1997) Method 445.0: In vitro determination of chlorophyll a and pheophytin a in marine and freshwater algae by fluorescence. http://www.epa.gov/nerlcwww/ordmeth.htm

Baars MA, Franz HG (1984) Grazing pressure of copepods on the phytoplankton stock of the central North Sea. Neth J Sea Res 18:120–142

Baars MA, Oosterhuis SS (1984) Diurnal feeding rhythms in the North Sea copepods measured by gut fluorescence, digestive enzyme activity and grazing on labelled food. Neth J Sea Res 18:97–119

Båmstedt U, Solberg PT, Nejstgaard JC (1999) Utilization of small-sized food algae by *Calanus finmarchicus* (Copepoda: Calanoida) and the significance of feeding history. Sarsia 84:19–38

Båmstedt U, Gifford DJ, Irigoien X, Atkinson A, Roman M, Harris RP, Wiebe PH, Lenz J, Skjoldal HR, Huntley M (2000) Feeding. In: Harris RP, Wiebe PH, Lenz J, Skjoldal HR, Huntley M (eds) ICES Zooplankton Methodology Manual. Academic Press, London, pp 297–380

Berggreen U, Hansen B, Kiørboe T (1988) Food size spectra, ingestion and growth of the copepod *Acartia tonsa* during development: implications for determination of copepod production. Mar Biol 99:341–352

Björnberg TKS (1984) The rejected nauplius, a commentary. In: Schiever G, Schminke HK, Shih CT (eds) Proceedings of the second international conference on copepoda. National Museums of Canada, Ottawa, pp 232–236

Bonnet D, Carlotti F (2001) Development and egg production in *Centropages typicus* (Copepoda: Calanoida) fed different food types: a laboratory study. Mar Ecol Prog Ser 224:133–148

Chisholm LA, Roff JC (1990) Abundances, growth rates, and production of tropical neritic copepods off Kingston, Jamaica. Mar Biol 106:79–89

Christoffersen K, Jespersen AM (1986) Gut evacuation rates and ingestion rates of *Eudiaptomus graciloides* measured by means of the gut fluorescence method. J Plankton Res 8:973–983

Dagg MJ, Grill DW (1980) Natural feeding rates of *Centropages typicus* females in the New York Bight. Limnol Oceanogr 25:597–609

Dagg MJ, Walser WE (1987) Ingestion, gut passage and egestion by the copepod *Neocalanus plumchrus* in the laboratory and in the subarctic Pacific Ocean. Limnol Oceanogr 32:178–188

Dam HG, Peterson WT (1988) The effect of temperature on the gut clearance rate constant of planktonic copepods. J Exp Mar Biol Ecol 123:1–14

Fernández F (1979) Nutrition studies in the nauplius larva of *Calanus pacificus* (Copepoda: Calanoida). Mar Biol 53:131–147

Frost BW (1972) Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. Limnol Oceanogr 17:805–815

Fryd M, Haslund OH, Wohlgemuth O (1991) Development, growth and egg production of the two copepod species *Centropages hamatus* and *Centropages typicus* in the laboratory. J Plankton Res 13:683–689

Gentleman W, Leising A, Frost B, Strom S, Murray J (2003) Functional responses for zooplankton feeding on multiple resources: a review of assumptions and biological dynamics. Deep-Sea Res Part II 50:2847–2875

Gorsky G, Dallot S, Sardou J, Fenaux R, Carré C, Palazzoli I (1988) C and N composition of some northwestern Mediterranean zooplankton and micronekton species. J Exp Mar Biol Ecol 124:133–144

Hansen BW, Hygum BH, Brozek M, Jensen F, Rey C (2000) Food web interactions in a *Calanus finmarchicus* dominated pelagic ecosystem—a mesocosm study. J Plankton Res 22:569–588

Harris RP (1994) Zooplankton grazing on the coccolithophore *Emiliania huxleyi* and its role in inorganic carbon flux. Mar Biol 119:431–439

Holling CS (1965) The functional response of predators to prey density and its role in mimicry and population regulation. Mem Entomol Soc Can 45:60

Irigoien X (1998) Gut clearance rate constant, temperature and initial gut contents: a review. J Plankton Res 20:997–1003

Irigoien X, Titelman J, Harris RP, Harbour D, Castellani C (2003) Feeding behaviour of *Calanus finmarchicus* nauplii in the Irminger Sea. Mar Ecol Prog Ser 262:193–200

Ishii H (1990) In situ feeding rhythms of herbivorous copepods and the effect of starvation. Mar Biol 105:91–98

Ivlev VS (ed) (1961) Experimental ecology of the feeding of fishes. Yale University Press, New Haven, Connecticut, USA

Kiørboe T, Møhlenberg F, Nicolajsen H (1982) Ingestion rate and gut clearance in the planktonic copepod Centropages hamatus (Lilljeborg) in relation to food concentration. Ophelia 21:181–194

Kiørboe T, Møhlenberg F, Riisgard HU (1985) *In situ* feeding rates of planktonic copepods: a comparison of four methods. J Exp Mar Biol Ecol 88:67–81

Klein Breteler WCM, Fransz HG, Gonzalez SR (1982) Growth and development of four calanoid copepod species under experimental and natural conditions. Neth J Sea Res 16:195–207

Klein Breteler WCM, Schogt N, Gonzalez SR (1990) On the role of food quality in grazing and development of life stages, and genetic change of body size during cultivation of pelagic copepods. J Exp Mar Biol Ecol 135:177–189

Lam RK, Frost BW (1976) Model of copepod filtering response to changes in size and concentration of food. Limnol Oceanogr 21:490–500

Lehman JT (1976) The filter-feeder as an optimal forager, and the predicted shapes of feeding curves. Limnol Oceanogr 21:501–516

Lonsdale DJ, Cosper EM, Doall M (1996) Effects of zooplankton grazing on phytoplankton size-structure and biomass in the lower Hudson river estuary. Estuaries 19:874–889

Mackas D, Bohrer R (1976) Fluorescence analysis of zooplankton gut contents and an investigation of diel feeding patterns. J Exp Mar Biol Ecol 25:77–85

Mauchline J (1998) The biology of calanoid copepods. In: Blaxter JHS, Southward AJ, Tyler PA (eds) Adv Mar Biol. Academic Press, London

Meyer B, Irigoien X, Graeve M, Head RN, Harris RP (2002) Feeding rates and selectivity among nauplii, copepodites and adult females of *Calanus finmarchicus* and *Calanus helgolandicus*. Helgol Mar Res 56:169–176

Morales CE, Bautista B, Harris RP (1990) Estimates of ingestion in copepod assemblages: gut fluorescence in relation to body size. In: Barnes M, Gibson RN (eds) Proc 24th Europ Mar Biol Symp. Aberdeen University Press, pp 565–577

Mullin MM, Fuglister-Steward E, Fuglister FJ (1975) Ingestion by planktonic grazers as a function of concentration of food. Limnol Oceanogr 20:259–262

Paffenhöfer GA (1970) Cultivation of *Calanus helgolandicus* under controlled conditions. Helgolander wiss. Meeresunters 20:346–359

Paffenhöfer GA (1971) Grazing and ingestion rates of nauplii, copepodids and adults of the marine planktonic copepod *Calanus helgolandicus*. Mar Biol 11:286–298

Parsons TR, Maita Y, Lalli CM (eds) (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford

Peterson WT (1988) Rates of egg production by the copepod *Calanus marshallae* in the laboratory and in the sea off Oregon, USA. Mar Ecol Prog Ser 47:229–237

Peterson WT, Painting SJ (1990) Developmental rates of the copepods *Calanus australis* and *Calanoides carinatus* in the laboratory, with discussion of methods used for calculation of development time. J Plankton Res 12:283–293

Peterson WT, Painting S, Barlow R (1990) Feeding rates of *Calanoides carinatus*: a comparison of five methods including evaluation of the gut fluorescence method. Mar Ecol Prog Ser 63:85–92

Rey C, Harris RP, Irigoien X, Head R, Carlotti F (2001) Influence of algal diet on growth and ingestion of *Calanus helgolandicus* nauplii. Mar Ecol Prog Ser 216:151–165

Roff JC, Turner JT, Webber MK, Hopcroft RR (1995) Bacterivory by tropical copepod nauplii: extent and possible significance. Aquat Microb Ecol 9:165–175

Roman MR, Rublee PA (1980) Containment effects in copepod grazing experiments: a plea to end the black box approach. Limnol Oceanogr 25:982–990

Rothhaupt KO (1990) Changes of the functional responses of the rotifers *Brachionus rubens* and *Brachionus calyciflorus* with particle sizes. Limnol Oceanogr 35(1):24–32

Tackx MLM, Bakker C, van Rijswijk P (1990) Zooplankton grazing pressure in the Osterschelde (The Netherlands). Neth J Sea Res 25:405–415

Torres CG, Escribano R (2003) Growth and development of *Calanus chilensis* nauplii reared under laboratory conditions: testing the effects of temperature and food resources. J Exp Mar Biol Ecol 294:81–99

Turner JT (1982) The annual cycle of zooplankton in a Long Island estuary. Estuaries 5:261–274

Turner JT, Levinsen H, Nielsen TG, Hansen BW (2001) Zooplankton feeding ecology: grazing on phytoplankton and predation on protozoans by copepod and barnacle nauplii in Disko Bay, West Greenland. Mar Ecol Prog Ser 221:209–219

Turner JT, Roff JC (1993) Trophic levels and trophospecies in marine plankton: lessons from the microbial food web. Mar Microb Food Webs 7:225–248

Turner JT, Tester PA (1992) Zooplankton feeding ecology: bacterivory by metazoan microzooplankton. J Exp Mar Biol Ecol 160:149–167

Uitto A (1996) Summertime herbivory of coastal mesozooplankton and metazoan microplankton in the northern Baltic. Mar Ecol Prog Ser 132:47–56

Uye SI, Kasahara S (1983) Grazing of various developmental stages of *Pseudodiaptomus marinus* (Copepoda: Calanoida) on naturally occurring particles. Bull Plankton Soc Japan 30:147–158

White JR, Roman MR (1992) Seasonal study of grazing by metazoan zooplankton in the mesohaline Chesapeake Bay. Mar Ecol Prog Ser 86:251–261

Mar Biol (2007) 150:1047–1048
DOI 10.1007/s00227-006-0461-7

ERRATUM

Functional responses of copepod nauplii using a high efficiency gut fluorescence technique

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Published online: 5 September 2006

Erratum to: Mar Biol
DOI 10.1007/s00227-006-0387-0

The formula that the author used to calculate copepod dry weight contained an error. This resulted in incorrect values in the "Results" section as well as in Tables 2, 3, and 4. Figures 3 and 4 also contain errors. The corrections are shown below.

Results

Development times and growth rates

Last paragraph should read: "The gross growth efficiency calculated for NIV-NV *C. helgolandicus* was 0.47 ± 0.19 (mean ± s.d.) and 0.35 ± 0.07 for NIV-NV *C. typicus*."

Ingestion rates at different concentrations

Last paragraph should read: "Specific ingestion rates ranged between 0.069–0.443 µg C µg^{-1} nauplii C d^{-1} for *C. typicus* and 0.024–0.812 µg C µg^{-1} nauplii C d^{-1} for *C. helgolandicus*."

The online version of the original article can be found at http://dx.doi.org/10.1007/s00227-006-0387-0.

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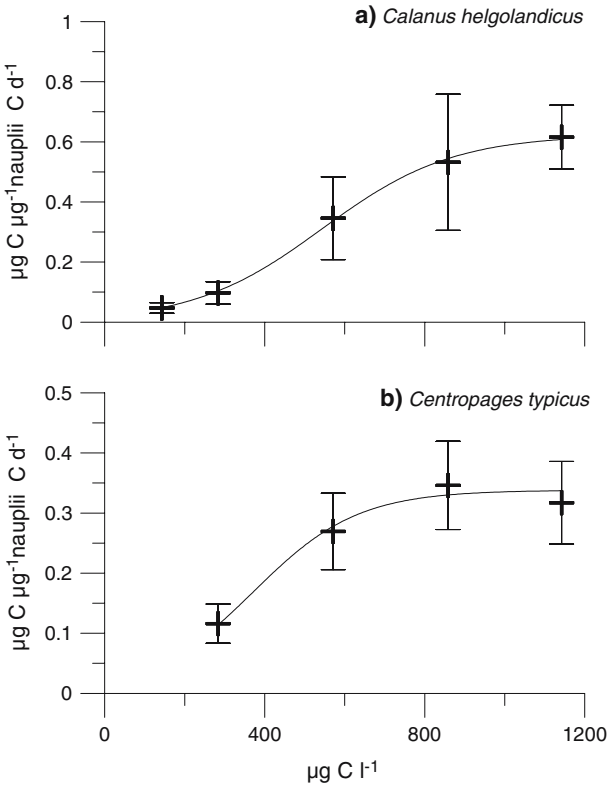
Table 2. Last three columns should read:

Dry weight		Growth
NIV	NV	
2.225 ± 0.219	3.070 ± 0.196	0.255
0.264 ± 0.030	0.425 ± 0.025	0.015

Figure 3. The scale from the *Y*-axis changes in the following way:



Discussion and conclusions

Table 3

Species	Type I				Type II			Type III			
	A	Cd	I_{max}	MSE	a	I_{max}	MSE	a	Kc	I_{max}	MSE
C. helgolandicus	5.63×10^{-4}	–	–	0.0173	–	–	–	162	544	0.623	0.0154
C. typicus	4.57×10^{-4}	732	0.334	4.05×10^{-3}	6.8×10^{-4}	0.418	4.77×10^{-3}	130	371	0.338	4.05×10^{-3}

Table 4. Specific ingestion rates data from this study are incorrect. The correct data are:

Copepod species	Phytoplankton Concentration (µg C l^{-1})	Specific ingestion rate (µg C μg^{-1} nauplii C day^{-1})
C. helgolandicus	143	0.047 ± 0.017
C. helgolandicus	287	0.097 ± 0.037
C. helgolandicus	574	0.345 ± 0.137
C. helgolandicus	862	0.532 ± 0.226
C. helgolandicus	1,149	0.615 ± 0.106
C. typicus	287	0.116 ± 0.032
C. typicus	574	0.269 ± 0.063
C. typicus	862	0.346 ± 0.073
C. typicus	1,149	0.317 ± 0.068

Figure 4. Plotted data from this study would change in the following way: