

Is diatom size selection by harpacticoid copepods related to grazer body size?

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Abstract

Copepods are known as important consumers of primary production and are eaten by larger animals. They therefore form a main link to higher trophic levels. While feeding pathways and specificity of planktonic copepods have been well studied, the selectivity of the benthic harpacticoid copepods is far less documented. A better knowledge of the functional ecology of harpacticoids as important grazers on primary producers may have consequences for the re-evaluation of basic energy flow in benthic ecosystems.

We tested whether size selectivity for diatoms exists in harpacticoid copepods. We hypothesized that size selectivity of harpacticoid copepod species is strongly related to body size. Because of morphological constraints, we expected smaller copepods to prefer smaller diatoms while larger copepods should be able to consume both small and large diatoms. We tested this hypothesis in four harpacticoid copepod species of varied body size: *Tigriopus brevicornis*, *Harpacticus obscurus*, *Amphiascus minutus* and *Paramphiascella fulvofasciata*. As food source we used two ¹³C labelled strains of the benthic diatom *Seminavis robusta* with a four-fold difference in cell biovolume.

Three out of four harpacticoid species showed size selectivity: *H. obscurus* and *A. minutus* preferred the larger *Seminavis* cells, while *P. fulvofasciata* selected the smaller *Seminavis* cells. Based on monoclonal treatments, there was no clear preference found for *T. brevicornis* although there was a small preference for large cells in the mixed treatments. Except for *P. fulvofasciata*, all species showed a lower uptake when offered the mixed diet (both small and large cells). Although most species showed a size selectivity, our results suggest that this selectivity was not related to their body size. However, the only species that ate significantly more of small diatoms was characterised by comparatively small mouthparts in relation to its body size.

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1. Introduction

Harpacticoid copepods are known to consume a wide spectrum of food sources that includes microalgae

(Carman and Thistle, 1985; Rieper, 1985; Decho, 1988), protozoans (Rieper and Flotow, 1981), bacteria (Rieper, 1978, 1982), organic detritus (Rudnick, 1989; Rieper-Kirchner et al., 1991) and even flesh (Lopez, 1982; Guidi, 1984; Seifried and Durbaum, 2000) and play a significant role in the transfer of energy from primary producers to top trophic levels in aquatic food

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webs (e.g. Sogard, 1984; De Troch et al., 1998; Buffan-Dubau and Carman, 2000). Copepods are either directly consumed by top predators (e.g. Gee, 1989; De Troch et al., 1998; Turner, 2004; Gorokhova et al., 2005) or linked to them through the food-web (e.g. Haro-Garay, 2003). From such a diverse resource pool, one can wonder if consumers indiscriminately gather particles as they encounter food or selectively ingest according to specific nutritional values. The results of studies of pelagic copepods (mainly Calanoida) illustrate they are able to select the ingested food particles both by sizes (e.g. Sommer et al., 2000) and chemical properties ('taste', DeMott, 1988).

In general, the size selection is also related to handling time and energy costs involved in searching for food. Classical optimal foraging theory (OFT; Stephens and Krebs, 1986) predicts that individuals should feed exclusively from the most profitable resource as long as that resource is in sufficient supply. From this one would expect that species adopt feeding modes that generate the highest energy intake rate, i.e. that prey selection changes according to the relative concentrations of alternative prey (e.g. Kiorboe et al., 1996; Cruz-Rivera and Hay, 2000). For example, the planktonic copepod *Acartia tonsa* spends disproportionately more time in the feeding mode (suspension feeding or ambush feeding) that provides the greatest reward (Kiorboe et al., 1996). However, the possibility to consume certain food items (e.g. larger ones) may not only depend on prey availability but also upon morphological constraints such as the size and functionality of mouthparts of the grazer. Furthermore, the degree of digestion and the survival of gut passage by some food particles are factors that can also influence food-grazer interactions, although this aspect of assimilation has been relatively neglected (Porter, 1973; Moore, 1975; Underwood and Thomas, 1990). In nature, however, the efficiency with which an organism utilizes a certain food source does not only depend on the ability of the consumer to digest the material but also on the specific food available and its abundance (Hicks and Coull, 1983; unpubl. data).

At present, most of our knowledge on food selectivity of copepods results from pelagic systems (e.g. DeMott, 1989; Irigoien et al., 2000; Sommer et al., 2000; Tackx et al., 2003) while the benthic counterpart, mainly represented by harpacticoid copepods, remains less documented. Although a better understanding of food selectivity of harpacticoids as important grazers on primary producers may have consequences for the re-evaluation of basic energy flow in benthic ecosystems.

Vanden Berghe and Bergmans (1981) tested three *Tisbe* species for differential food utilization and found a clear preference for bacteria by *Tisbe furcata* while two sibling species showed a generalised or indiscriminate selectivity. Rieper (1982) showed that *Tisbe holothuriae* could even discriminate for specific strains of Gram-negative bacteria. Carman and Thistle (1985) found that three co-occurring benthic copepod species fed on different microbial food sources in their natural environment. Decho and Castenholz (1986) found that *Mesochra lilljeborgi* fed on phototrophic purple bacteria and selectively assimilated these bacteria. *Canuella perplexa*, however, preferred microalgae and cyanobacteria to phototrophic purple bacteria (Buffan-Dubau et al., 1996). The results of later studies have supported these findings showing a great variability in food selection by harpacticoid copepods (e.g. Pace and Carman, 1996; Buffan-Dubau and Carman, 2000). More recently, Fechter et al. (2004) inferred that two interstitial harpacticoids were able to locate food by sensing water-borne cues.

Here, we specifically addressed selectivity of four harpacticoid copepods in terms of food particle size. Feeding experiments were conducted with ¹³C labelled strains of a single benthic diatom species of different cell size. The advantage of using only one species as food is that confounding factors of taste, toxic chemicals, or other structural or chemical properties are removed in the design. *Tigriopus brevicornis*, *Paramphiascella fulvofasciata*, *Harpacticus obscurus* and *Amphiascus minutus* were selected as grazers because of their different body sizes. Parallel to this experiment, we also studied the mouthparts of these copepods as little or no information on functional morphology is available so far.

2. Materials and methods

2.1. Laboratory conditions and labelling technique

Four intertidal harpacticoid copepod species were isolated from natural samples: *T. brevicornis* (family Harpacticidae, southwest coast of Iceland), *H. obscurus* (family Harpacticidae, north-coast of France, brown alga *Fucus serratus*), *A. minutus* (family Diosaccidae, north-coast of France, brown alga *F. serratus*) and *P. fulvofasciata* (family Diosaccidae, Germany, intertidal mudflat). In the laboratory, the copepods were maintained in 1 L glass beakers, with artificial seawater (c. 32 psu, Instant Ocean® salt, Aquarium Systems, France) and regularly provided with a mixture of benthic diatom

cultures (*Navicula phyllepta*, *Seminavis robusta* and *Cylindrotheca closterium*).

Two strains of the marine pennate diatom *S. robusta* were used in the present investigation (Fig. 1). Previously, a series of principal biological attributes of this species (life cycle, sexual reproduction and mating system) has been studied in culture (Chepurnov et al., 2002) underpinning its usage as an appropriate diatom ‘model’ for various experiments. Both of the clones were obtained experimentally, and they represented a single lineage derived from a cross (*S. robusta* is heterothallic) of two natural clones (68 and 88 in Chepurnov et al., 2002). The clones were started from single initial cells (each c. 65 μm in length), which resulted from sexual reproduction. As typical for most diatoms, vegetative multiplication of *Seminavis* cells was accompanied by gradual cells size reduction, according to the MacDonald–Pfitzer rule (e.g. Chepurnov et al., 2004). One of the clones (referred to as ‘small’ elsewhere in the text; voucher designation Sem-F1-6 in our diatom culture collection) was established on November 12, 2001 and by the time the present study began (October 22, 2004), the cells decreased in size to 22.2 μm on average (S.D.=1.22; range=20.1–24.5 μm ; $N=15$). The other clone was isolated 3.5 years later (referred to as ‘large’; voucher Sem-F2-35A) and the cells were measured to be 59.7 μm long (S.D.=1.13; range=58.4–61.8 μm ; $N=15$) just before the experiment was set. The clones were reported to be of the same mating type and hence no auxosporeulation and cell enlargement occurred when they were grown together in a mixed culture. Biovolume of the cells was calculated as recommended for *Seminavis* in Hillebrand et al. (1999): average values were 1980 (small clone) and 8280 (large clone) μm^3 . F2 culture medium (Guillard, 1975) based on filtered and sterilized seawater (c. 30 psu) from the North Sea was used to maintain the diatom cultures.



Fig. 1. *Seminavis robusta*: mixed culture of two different-sized clones which were used in the present study.

For labelling the diatom cells with ^{13}C , 5 ml of a solution which contained $\text{NaH}^{13}\text{CO}_3$ (336 mg in 100 ml milliQ H_2O) was added per 100 ml of the culture medium. Diatoms underwent several subsequent cell divisions in order to reach high densities of cells and high levels of labelling. This labelling technique resulted in an increase in $\delta^{13}\text{C}$ from -5‰ to 85,000 ‰ for the *small* cells and from -2‰ to 50,000 ‰ for the *large* cells (see further standardisation of the data). Prior to the experiment, the labelled medium was replaced by artificial seawater.

Single specimens of copepods were picked up by micropipette from the original stock cultures and placed into Petri dishes containing the artificial seawater where they were starved of food. After 24 h, the copepods of single species were transferred into polystyrene 50-mm Petri dishes, with artificial seawater (15 ml) and labelled diatoms for 96 h. To detect $^{13}\text{C}/^{12}\text{C}$ ratios in the tissue of the harpacticoids, a minimum of 15 μg C per species was analysed corresponding to 8, 20, 20 and 40 adults of *T. brevicornis*, *H. obscurus*, *P. fulvofasciata* and *A. minutus* in a Petri-plate, respectively. This resulted into 4 petri-plates per replicate of a treatment, i.e. one dish for each copepod species.

The cultures were kept and the experiments were performed at 17 ± 1 $^\circ\text{C}$ and with 12:12 h light–dark cycle.

2.2. Experimental design

To test whether the copepods can graze the diatoms selectively making a choice among the cells of different sizes, four treatments were performed:

- (1) S*: a total of 4×10^6 cells of small diatom clone, labelled
- (2) L*: a total of 1×10^6 cells of large clone, labelled
- (3) S*L: a total of 4×10^6 cells of small clone (labelled) and 1×10^6 of large clone (unlabelled)
- (4) SL*: a total of 4×10^6 cells of small clone (unlabelled) and 1×10^6 cells of large clone (labelled).

To estimate the density of diatom cells in cultures, the cells were homogeneously suspended by shaking and then 50 μl of the cell suspension was transferred into a well of a multidish (96 wells). In a few hours, after all the cells confidently settled to the bottom of the well, their number was counted under a Zeiss Axiovert 135 inverted microscope (Zeiss Gruppe, Jena, Germany); the values obtained have allowed to estimate the densities in the experimental vessels.

Control treatments consisted of the natural ^{13}C signature of copepods that came from the original stock culture and were starved for 24 h. They had not been feeding on the labelled diatoms but on a mixture of unlabelled food and as such they are typically depleted in the ^{13}C stable isotope.

Since the biovolume of cells in the large diatom strain was about 4 times higher compared to the small clone (see above), proportionally less number of large cells was used in the treatments to equalize biomasses of the different diatom clones. The mixture of diatom strains (S*L and SL*) contained twice as much cell biomass if compared with the monoclonal cultures (S* and L*). Diatoms were offered in excess densities (i.e. more than what the copepods were likely to consume, see De Troch et al., 2005). Diatom growth in the experimental containers was negligible as the feeding experiments were conducted in artificial seawater (without silica) and growth rate was very low compared to the growth noted when kept in f2 medium (De Troch, pers. observ.).

Altogether 46 experimental units were used permitting the four treatments, each in triplicate, except treatments L* and SL* for *H. obscurus* where only 2 replicates were included due to limited number of copepods available. All experimental units (Petriplates) were placed randomly on a tray in a climate room. The uptake of labelled diatoms by copepods was analysed after 96 h of grazing.

2.3. Analytic techniques and data treatment

At the end of the experiment (after 96 h), faecal pellets were collected from both monoclonal treatments and from the biclonal treatment with a micro-needle. They were transferred to glass slides, were covered by a cover slip and thus slightly squashed. Observations were made with a standard light microscope (Leitz Diaplan, Wetzlar, Germany) connected to a digital camera (Olympus DP10, Melville, USA).

Subsequently, experimental units containing the copepods were frozen and stored for further analysis. Afterwards, the specimens were sorted manually, washed several times in deionised water and transferred by a micro-needle into tin capsules (8 × 5 mm, Elemental Microanalysis Limited). The samples were then desiccated in an oven at 60 °C for 12 h. Measurements of $\delta^{13}\text{C}$ values and copepod biomass (total carbon) were made using an isotope ratio mass spectrometer (type Europa Integra) at the UC Davis Stable Isotope Facility (University of California, USA).

The values of $\delta^{13}\text{C}$ were standardised per individual copepod (total carbon) and per unit carbon of copepod as recommended by Middelburg et al. (2000): incorporation of ^{13}C is reflected as excess (above background) ^{13}C , expressed as total uptake (I) in milligrams of ^{13}C per individual and calculated as the product of excess ^{13}C (E) and individual biomass (organic carbon). Excess ^{13}C is the difference between the fraction ^{13}C of the control (F_{control} , i.e. based on the natural signature of copepods that did not feed on labelled diatoms) and the sample (F_{sample}), where $F = ^{13}\text{C}/(^{13}\text{C} + ^{12}\text{C}) = R/(R + 1)$. The carbon isotope ratio (R) was derived from the measured $\delta^{13}\text{C}$ values as $R = (\delta^{13}\text{C}/1000 + 1) \times R_{\text{VPDB}}$ with $R_{\text{VPDB}} = 0.0112372$ as $\delta^{13}\text{C}$ is expressed relative to Vienna Pee Dee Belemnite (VPDB).

Since the labeled diatoms had different initial $\delta^{13}\text{C}$ signatures (see before), the uptake per individual and uptake per unit carbon of copepod were further standardized taking into account the proportion of ^{13}C in each diatom food source. The amount of diatom carbon that was taken up by copepods and expressed both per individual and per unit carbon of copepod was calculated by multiplying with a factor derived from the atomic percentage ^{13}C of the diatom food source.

Differences in diatom uptake by copepods among the various treatments were tested with one-way and two-way analyses of variance (ANOVA) with Statistica 6.0 software (StatSoft Inc., 2001). A posteriori comparisons were carried out with the Tukey test using 95% confidence limits. Prior to the ANOVA, the Cochran's *C*-test was used to check the assumption of homoscedasticity.

Mouthparts' morphology of the copepods used in the experiments was studied by detailed morphological observations. For each copepod species, some individuals from the stock culture were dissected with two dissecting needles (tungsten wire, 2.2 mm diameter, projecting about 2–3 cm from a holder) under a Wild M5 binocular. Observations and drawings of dissected mouthparts mounted in glycerine were made using a light microscope (Leica DM LS) equipped with a drawing tube.

3. Results

3.1. Stable isotope signatures

Three of the four harpacticoid species (*H. obscurus*, *A. minutus*, *P. fulvofasciata*) exhibited a significant difference in $\delta^{13}\text{C}$ enrichment (Fig. 2A) while maintained in monoclonal diatom cultures (S* or L*). Except for *H. obscurus* (see *t*-test) ANOVA tests were allowed based

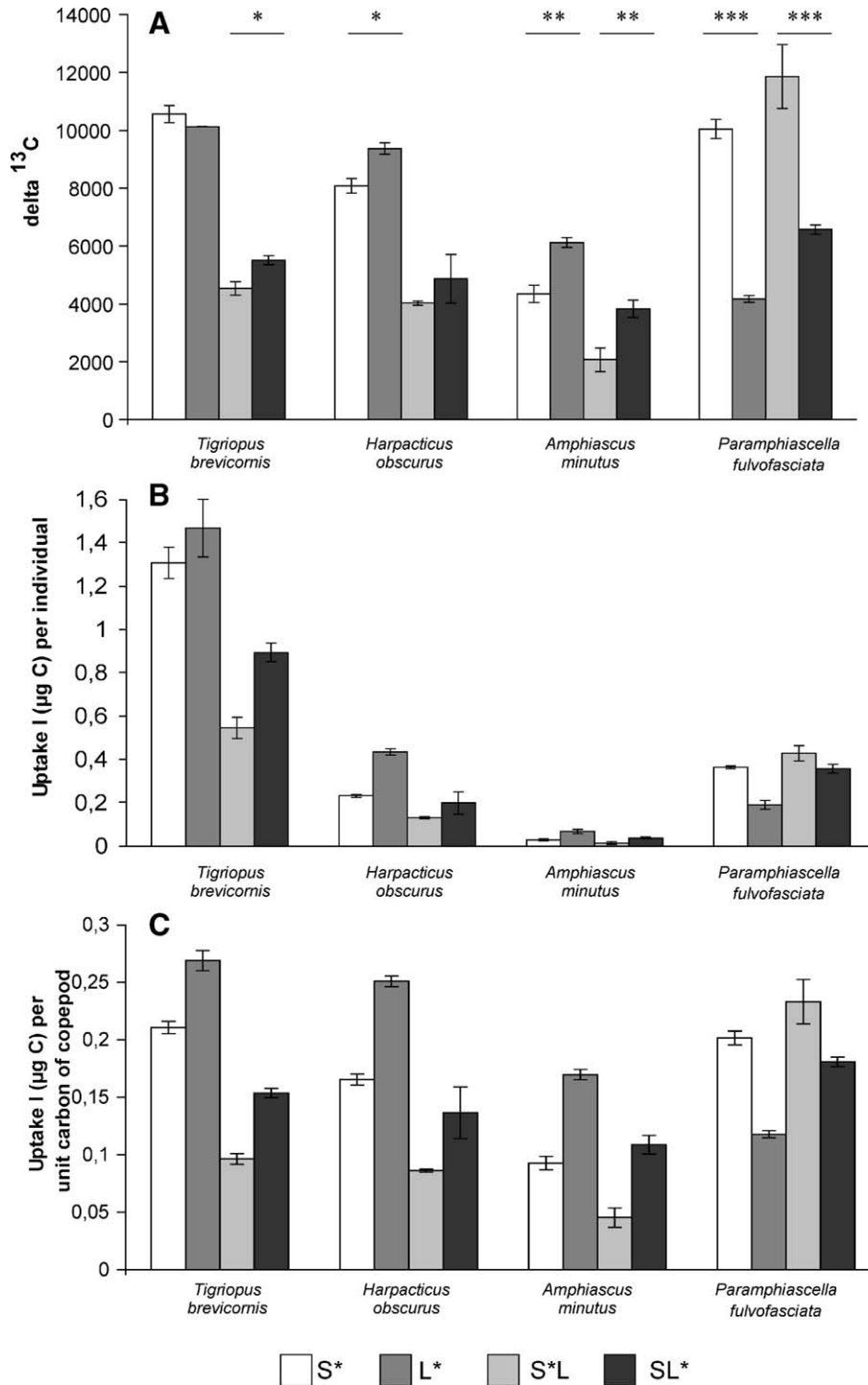


Fig. 2. Diatom uptake (mean \pm standard error) by four harpacticoid copepod species for the different treatments (S=small, L=large diatom cells) expressed as (A) delta ^{13}C , (B) total uptake per individual relative to individual biomass, and (C) total uptake per unit carbon of copepod. (significant differences within monoclonal treatments and within the mixed diet indicated by $*=p<0.05$, $**=p<0.01$, $***=p<0.001$).

on the outcome of the Cochran's *C*-test. A small but statistically significant preference for large diatom cells was found to occur in *H. obscurus* (*t*-test, $p<0.1$) and

more significantly in *A. minutus* (one-way ANOVA, $p<0.01$). In contrast, *P. fulvofasciata* took up significantly more small cells since both the monoclonal dia-

tom treatments were significantly differed in $\delta^{13}\text{C}$ enrichment (one-way ANOVA, $p < 0.001$, Fig. 2A).

In the mixed diet (S*L and SL*), *T. brevicornis* (one-way ANOVA, $p < 0.05$) and *A. minutus* (one-way ANOVA, $p < 0.05$) incorporated more ^{13}C from the large cells. *P. fulvofasciata* was the only species which showed a clear preference for the small cells in the combined treatments: $\delta^{13}\text{C}$ in the SL* treatment was only half the signal from the S*L treatment (one-way ANOVA, $p < 0.01$).

In terms of biomass uptake of the labelled diatoms (Fig. 2B), in all the treatments, the largest copepod (i.e. *T. brevicornis*) took the highest amount while the smallest copepod (i.e. *A. minutus*) took the smallest amount. In the mixed diet, the biomass dropped to about half that of

the monoclonal diet for all species except *P. fulvofasciata*. Both in monoclonal and mixed diet, a similar biomass of small *Seminavis* cells was taken by *P. fulvofasciata* irrespective of the presence of large cells.

The values after standardisation per unit carbon of copepod (Fig. 2C) showed the same trend as the $\delta^{13}\text{C}$ values (Fig. 2A). The relatively lower enrichment of the larger cells (see Materials and methods) was reflected in a relatively higher carbon uptake by copepods.

3.2. Faecal pellets

In all the treatments, faecal pellets of copepods represented packed remains of diatoms' siliceous exoskeleton, the frustule (Fig. 3). In mixed diets, cells of

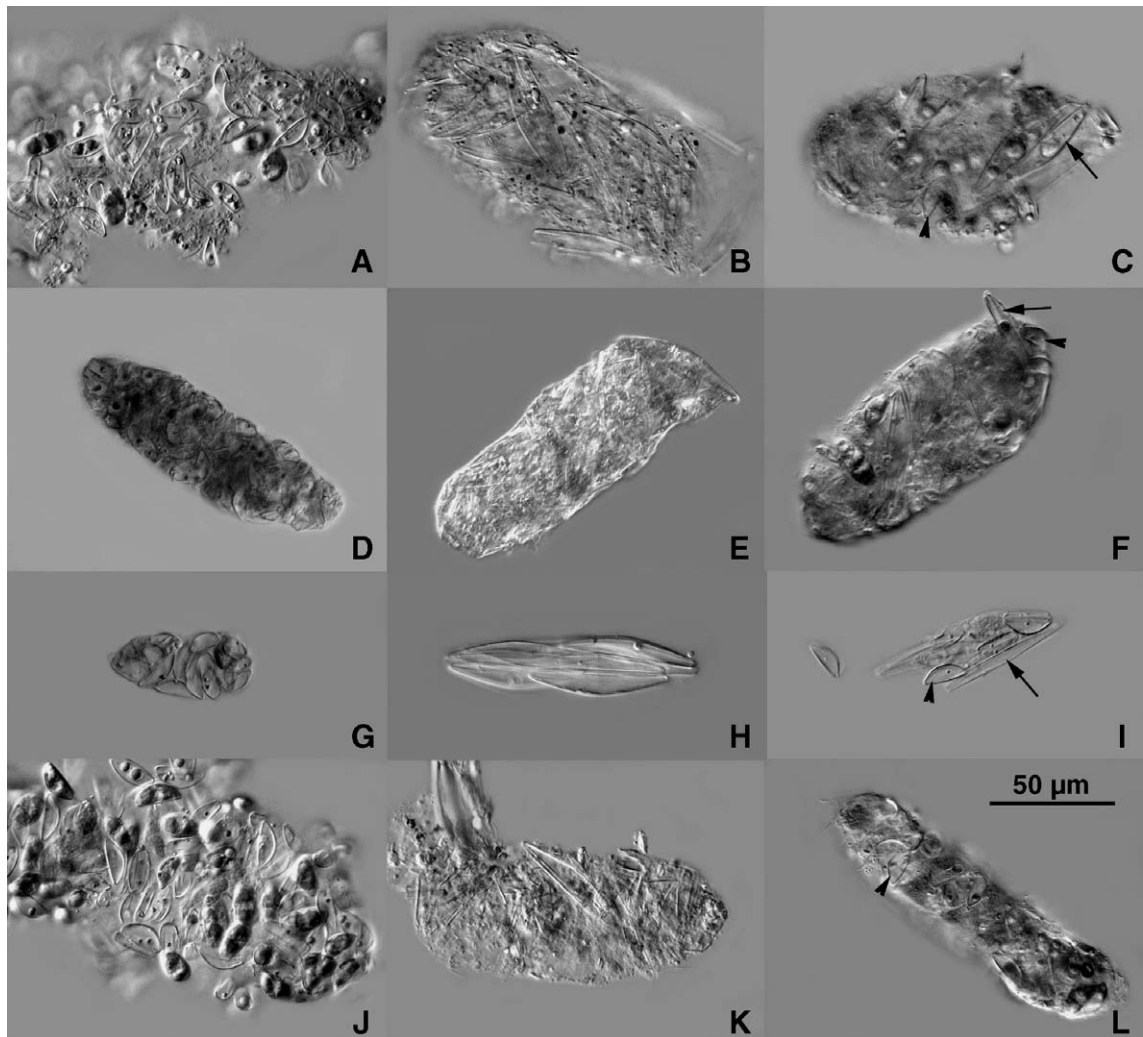


Fig. 3. Faecal pellets of *Tigriopus brevicornis* (A–C), *Harpacticus obscurus* (D–F), *Amphiastus minutus* (G–I) and *Paramphiascella fulvofasciata* (J–L) fed in cultures of *Seminavis robusta*: clone 'small' (A, D, G, J), clone 'large' (B, E, H, K) and a mixture of the two clones (C, F, I, L) with indication of small (arrowhead) and large diatom cells (arrow).

both size classes could be identified in the mass excreted (see arrows and arrowheads in Fig. 3C, F, I), except for *P. fulvofasciata* (Fig. 3L, see further). Other more-or-less structured fragments observed in the faecal pellets were spherical particles, colourless or brownish-pigmented, which most likely represented non-digested protoplast of diatom cells.

The frustules of the small clone were always intact in the faecal pellets of all the copepod species.

Intact and broken frustules of the large diatom clone were also found in the faeces of all copepods, but in the faecal pellets of *A. minutus* the frustules were always intact (Fig. 3H, I). In contrast, large diatom cells in faecal pellets of *H. obscurus* (Fig. 3E) and *P. fulvofasciata* (Fig. 3K) were found predominantly crushed, sometimes into small fragments. Moreover, no large cells could be recognised as such in the faecal pellets of *P. fulvofasciata* from the mixed diet

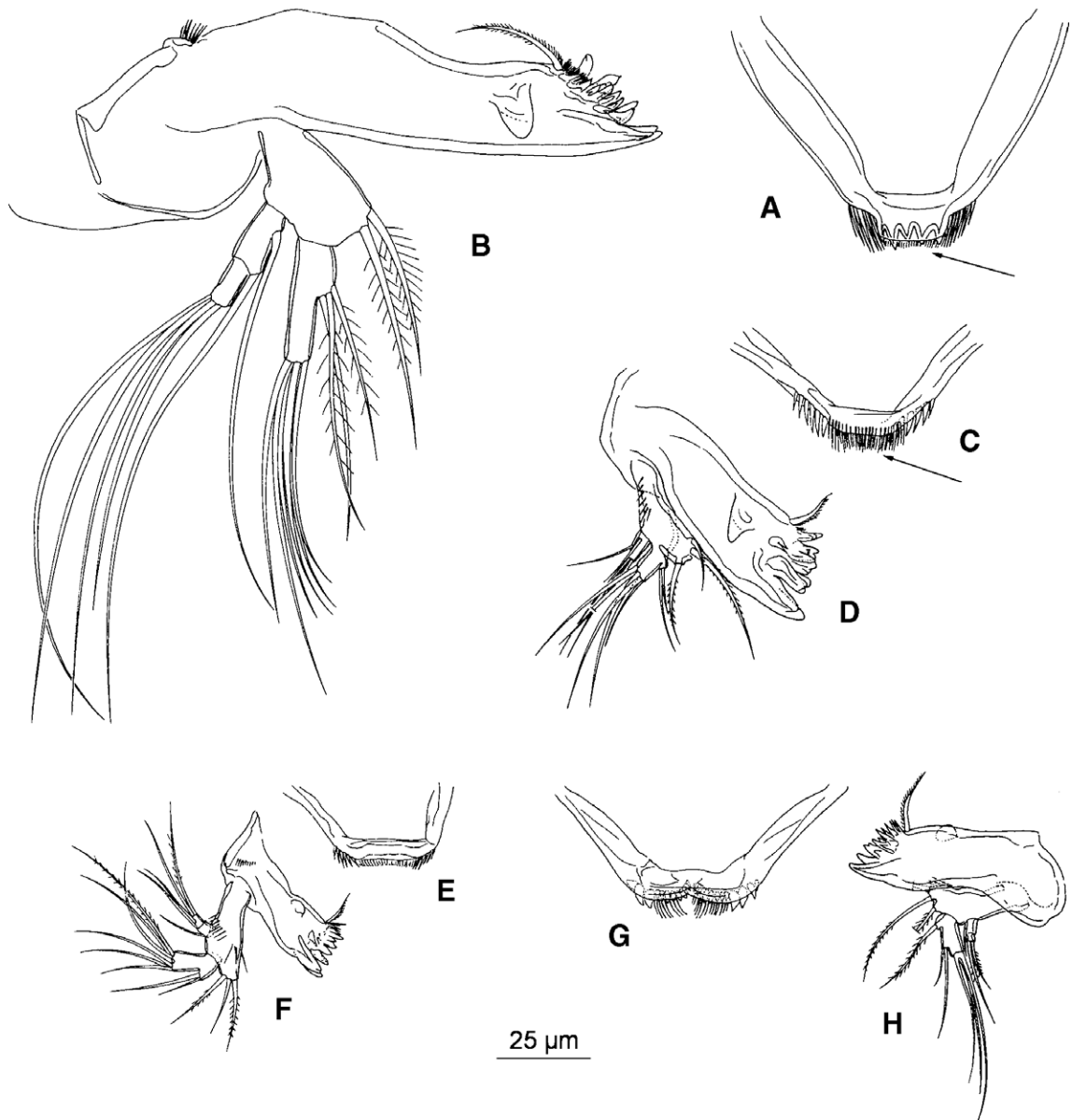


Fig. 4. Labrum and right mandible of *Tigriopus brevicornis* (A–B), *Harpacticus obscurus* (C–D), *Amphiascus minutus* (E–F) and *Paramphiascella fulvofasciata* (G–H: posterior view). Arrows indicate specific structure of the labrum (see text). Drawings by H. Gheerardyn.

treatments (see Fig. 3L). Finally, most of the large diatom cells consumed by *T. brevicornis* seemed to be swallowed as a whole since only a minority of frustules were broken (Fig. 3B).

A. minutus exhibited a great extent of digestion of diatom cell contents at all the diets provided (Fig. 3G–I). In the other three harpacticoid species, our visual impression was that the contents of small cells was utilized less effectively than that of large cells, especially in *T. brevicornis* and *P. fulvofasciata* fed in the monoclonal cultures: essential number of small diatoms, after getting through the alimentary tract, looked almost or even completely undigested (Fig. 3A, J).

3.3. Mouth parts' morphology

In general, the overall body size [*T. brevicornis* (c. 850 μm) > *P. fulvofasciata* (c. 780 μm) > *H. obscurus* (c. 550 μm) > *A. minutus* (c. 350 μm)] correlates with the dimensions of the mouthparts of the harpacticoid species. *T. brevicornis* was characterised by the largest mouthparts, whereas the mouthparts of *A. minutus* were substantially smaller. *H. obscurus* was of intermediate size and so were its mouthparts. Remarkably, *P. fulvofasciata* has much smaller mouthparts than one would expect from its overall body size. For example, the maximum basis length (from attachment point to gnathobasis) of the mandible is 131 μm in *T. brevicornis* while it measures only 48 μm in *P. fulvofasciata*. The ratio of the mandible basis length to the total body size is 1:6 in *T. brevicornis*, 1:9 in *A. minutus* and *H. obscurus*, 1:16 in *P. fulvofasciata*. The ratio of the mandible basis lengths to the maximum width of the cephalothorax is 1:3 in *T. brevicornis* and *A. minutus* and 1:4 in *H. obscurus* and *P. fulvofasciata*.

Detailed observation of the mandibles of the 4 species (Fig. 4) showed rather short and compact teeth on the mandibular gnathobases (Fig. 4B, D, F, H). The labrum of *T. brevicornis* (arrowed in Fig. 4A) bears strong teeth where there are several stout spinules and hairs bordering the labrum of *H. obscurus* (arrowed in Fig. 4C) (both species belonging to the family Harpacticidae). The labrum of *A. minutus* (Fig. 4E) bears rather slender spinules and hairs, while it is broader in *P. fulvofasciata* (Fig. 4G), with stout spinules and several long hairs (both species belonging to the family Diosaccidae).

4. Discussion

In the present study, two of the four harpacticoid species (*H. obscurus*, *A. minutus*) showed a selectivity

for the larger *Seminavis* cells, while *P. fulvofasciata* clearly preferred the smaller *Seminavis* cells. Based on monoclonal treatments, no clear preference was found for the largest copepod species *T. brevicornis* although there was a slight preference for large cells in the mixed treatments.

For all harpacticoid copepods, except *P. fulvofasciata*, the enrichment level of copepods grazing on the mixed diet dropped to half of the ^{13}C level found at monoclonal diet, suggesting that both diatom sizes contribute equally to the diet. Although *P. fulvofasciata* was the second largest species used in our experiments, it showed the highest uptake of small cells even in the mixed diet.

Consequently, our data suggest that the selected diatom size was not related to the body size of the grazer. The fact that body size seems not to be determinant for food selection is rather surprising as recent studies stress the important effect of body size (e.g. in combination with temperature) on individual metabolic rate and global C cycle (e.g. Allen et al., 2005). Moreover, the results from our case study question whether body size alone provides a useful surrogate measure of the niche of each species, and, by extension, of entire ecological networks, as was recently put forward by Woodward et al. (2005).

In general, one can distinguish between active selectivity (active choice of food components based on prey morphology or size) and passive feeding preferences (depending on differential food uptake, assimilation or digestibility) (Aberle et al., 2005). Investigations on food resource utilization by meiobenthic organisms have indicated both ingestion selectivity (e.g. Brown and Sibert, 1977; Rieper, 1978; Vanden Berghe and Bergmans, 1981; Carman and Thistle, 1985) and digestion selectivity (e.g. Decho and Castenholz, 1986) (in Decho and Fleeger, 1988). In the present study, these passive and active ways of selectivity may be linked to (1) constraints of mouthparts' morphology (passive preferences or ingestion selectivity) or (2) differences of chemical nature of the food (e.g. the extracellular polymeric substances covering the diatom frustule, associated bacteria; cfr. 'taste'; DeMott, 1988) (more active selectivity or digestion selectivity). Both points will be discussed in more detail for harpacticoid copepods.

In terms of functional morphology, Marcotte (1977) found that *T. furcata* uses its second antenna and second maxilla for initial food manipulation, the first maxilla for food movement within the oral frustum, and the mandible for crushing the particles captured. However, it is quite unlikely that the functional attributes of mouthparts as specified for *T. furcata* are universal for all harpacticoid

coids. The relative importance of particular oral appendages might be different between various species and also when manipulating different food items. For instance, in planktonic Calanoida, maxillae and associated filtering setae are correlated to the size of food particles (Anraku and Omori, 1963) while raptorial feeders mainly grasp particles with the maxillules (Marcotte, 1977).

Michels and Schnack-Schiel (2005) studied the importance of mandibular gnathobases for the diet of planktonic Antarctic copepod species (Calanoida). They concluded that calanoids with comparatively short and compact teeth mainly feed on diatoms as these teeth assist to crack the siliceous diatom frustules by directed pressure. From our observations, there are no major differences in mandible morphology *per se* between the four harpacticoid species examined. *P. fulvofasciata* had relatively smaller mandibles than one would expect from its overall body length. These morphological constraints are the primary reasons for its preference for small *Seminavis* cells.

In addition to its preference for smaller cells, *P. fulvofasciata* showed different assimilation abilities as several small *Seminavis* cells looked intact in its faecal pellets while large cells were empty. The recovery of intact small cells from faecal pellets suggests that small diatoms have a relatively short gut passage time and concomitant less time for enzymatic breakdown. Moreover, breaking of cells (as seen for larger cells) favours a more effective digestion of the cells. On the other hand, handling and uptake of larger diatom cells should be more energy-consuming than for smaller diatom cells.

Taking this inefficient assimilation of small cells into account, there must be a functional explanation for consuming a lot of small diatoms but leaving a large portion of them intact. Previous experiments indicated that the uptake of diatom cells by *P. fulvofasciata* was clearly promoted by the presence of faecal pellets while the diatom uptake by *T. brevicornis* and *H. obscurus* was not affected (De Troch et al., 2005). The re-use of faecal pellets seems to be advantageous for *P. fulvofasciata* as they consist of several intact diatom cells and probably bacteria because of microbial gardening (De Troch et al., 2005). However, these data are not entirely comparable since we used different diatom species in these experiments. The importance of bacteria for *P. fulvofasciata* was supported by the results of Rieper (1978) who reported that another *Paramphiascella* species, *P. vararensis*, grew well on a diet of solely bacteria.

In addition to associated bacteria, the extracellular polymeric substances (EPS) excreted by diatoms may

be an additional food source. It is partly because of this EPS that epipelagic microalgae form a microphytobenthic biofilm which is thought to play a key role in sediment biostabilisation (e.g. Underwood and Paterson, 1993; Orvain et al., 2003). Moreover, in natural environments, microalgae are associated with adherent bacteria, particularly diatoms producing an extracellular mucus rich in colourless bacteria (Buffan-Dubau et al., 1996).

From our study, we conclude that *T. brevicornis* was not size-selective as diatom grazer, while *H. obscurus* and *A. minutus* had a preference for large diatom cells. *P. fulvofasciata* showed a clear preference for small cells and probably this selectivity is closely linked to its microbial gardening strategy. The size selectivity of these grazers was not related to overall body size, but the maximum width of the cephalothorax seems to be a good estimator for the size of the mouthparts and related size selectivity. The cephalothorax width gives a good indication of the basis length of the mandibles, which are essential to manipulate food particles. As dissection of the mouthparts is not always evident, the width of the cephalothorax might be a good surrogate. As we demonstrated in this case study a species-specific food size selection even within one copepod family, a further generalisation towards all harpacticoid species is not appropriate. Here, we show for the first time that harpacticoid copepods are able to select between different sizes of the same diatom species.

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