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# Interspecific differences among meiobenthic copepods in the use of microalgal food resources

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ABSTRACT: We examined the potential for interspecific differences among meiobenthic copepods in their exploitation of microalgal food resources in a natural benthic community. The feeding behaviors of *Coullana* sp., *Cletocamptus deitersi*, *Microarthridion littorale*, and *Pseudostenhelia wellsi* were examined using $^{14}C$-radiotracer grazing experiments and gut-pigment analyses. In one grazing experiment, laboratory-cultured microalgae were labeled using $NaH^{14}CO_3$ and injected into intact sediment cores to determine whether copepods were grazing on algae from the water column and/or at the sediment-water interface. In another grazing experiment, $NaH^{14}CO_3$ was injected directly into sediment cores and grazing on $^{14}C$-labeled natural algae was measured. Fluorometric analyses of gut-pigments were used to determine the recent feeding histories of copepods. Functional responses of copepod feeding to variation in sedimentary chlorophyll (chl) *a* concentrations were also used to discern interspecific differences in feeding. *Coullana* sp. grazed on microalgae from the water-column and at the sediment-water interface. *C. deitersi* grazed predominantly on microalgae from the sediment-water interface. Grazing on laboratory-cultured algae was minimal in *M. littorale* and *P. wellsi*, but grazing experiments with $^{14}C$-labeled natural algae and gut-pigment analyses indicated that these copepods grazed on microalgae in the field. However, a positive functional response to chl *a* concentrations by *M. littorale* and a lack of a functional response by *P. wellsi* suggest that these 2 species exploit algal resources differently. Collectively, our observations indicate that each copepod species examined exploits microalgal resources differently.

KEY WORDS: Meiofauna · Grazing · Microalgae · Harpacticoid copepods · *Cletocamptus deitersi* · *Pseudostenhelia wellsi* · *Microarthridion littorale* · *Coullana* sp.

## INTRODUCTION

Allocation of available food resources affects the type and number of organisms that may coexist in a given system (Begon et al. 1996). Understanding how food is used by organisms in aquatic systems is thus essential to the study of marine food webs. Harpacticoid copepods constitute an important component of marine food webs, both as consumers (Montagna 1995) and producers (Gee 1989). They are often the most abundant or second most abundant meiofaunal group present and serve as an important food source for macrofauna, as well as many larval and juvenile fish species (McCall & Fleeger 1995). As consumers, copepods feed on diatoms, phytoflagellates, cyanobacteria, bacteria, fungi, and yeasts (Hicks & Coull 1983). Evidence of interspecific feeding differences among harpacticoid copepods has been found in laboratory studies (Reiper 1978, 1982, Vanden Berghe & Bergmans 1981, Chandler 1986) and field studies (Carman & Thistle 1985, Decho & Castenholtz 1986, Montagna et al. 1995), suggesting that meiobenthic copepods have different nutritional requirements and utilize available food resources differently. Further, food resources used by copepod species may change ontogenetically (Decho & Fleeger 1988a), seasonally (Lee et al. 1976), or over shorter time scales such as a tidal cycle (Decho 1988, Souza-Santos et al. 1995). Exploitation of different microbial food sources by copepods has been suggested as an explanation for their spatially and temporally heterogeneous distributions (Lee et al. 1977, Reiper 1984, Carman & Thistle 1985, Decho & Castenholz 1986, Decho & Fleeger 1988b).

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Few studies have addressed how microbial food resources are exploited by natural harpacticoid copepod communities. Field studies (Lee et al. 1977, Carman & Thistle 1985, Decho & Castenholz 1986) have provided evidence of interspecific feeding differences among harpacticoid copepods as well as positive spatial correlations between the distribution of copepods and their potential microbial food resources (Decho & Fleeger 1988b, Blanchard 1991, Montagna et al. 1995). Other field studies (Montagna et al. 1983, Alongi 1988), however, have detected no correlation between harpacticoid copepods and their microbial food resources.

Here, we present the results of research designed to gain insight into the mechanisms of *in situ* copepod feeding behavior. We tested the hypothesis that the co-occurring meiobenthic harpacticoid copepods *Coullana* sp., *Cletocamptus deitersi* Richard, *Microarthridion littorale* Poppe and *Pseudostenhelia wellsi* Coull & Fleeger exploit different microalgal resources. With the exception of Decho (1988), previous studies addressing feeding in these copepod species have been limited to laboratory studies (see 'Materials and Methods'). In order to gain a better understanding of feeding behaviors of copepods in the field, in the present study we examined feeding on benthic and planktonic microalgae by copepods using intact sediments with natural faunal and microalgal assemblages. Several techniques were employed to determine whether copepods were grazing on planktonic or benthic microalgae. Through collective consideration of data obtained by using a variety of techniques, a better understanding of harpacticoid feeding behavior was realized.

## MATERIALS AND METHODS

The study site, located near Cocodrie, Louisiana, USA (30° 15' N, 91° 21' W), is a mudflat surrounded by stands of *Spartina alterniflora* Loisel. Salinity at the site varies between 2 and 26 ppt. Tidal fluctuations are small (0 to 0.3 m) and water movement is predominately wind driven (Phillips & Fleeger 1985).

Grazing experiments were performed using minimally disturbed, intact cores of sediment. Core liners were constructed from clear butyrate tubing with a cross-sectional area of 9.6 $cm^2$. Cores were collected at low tide in areas of exposed sediment in May and June 1995. *Coullana* sp., *Cletocamptus deitersi*, *Microarthridion littorale*, and *Pseudostenhelia wellsi* were the dominant harpacticoid copepod species present at the study site, and these species were chosen to examine natural copepod feeding behavior

*Coullana* sp. is a semi-sessile burrow dweller and has been described as a suspension feeder (Decho 1986, Chandler & Fleeger 1987, Decho & Fleeger 1988b). It feeds by creating a current using its oral appendages, drawing in planktonic algae, detritus, and benthic microalgae that may be near the opening of its burrow (Decho 1986). *Cletocamptus deitersi* is an epibenthic copepod (Fleeger 1980) that has been shown to graze on benthic diatoms (Decho 1986, 1988). *Microarthridion littorale* is a mud-dwelling, epibenthic copepod (Palmer & Coull 1980, Morris & Coull 1992) that can consume benthic as well as planktonic diatoms (Decho 1986). *Pseudostenhelia wellsi* is a mucus-tube dweller whose feeding biology is unknown. It has not been seen foraging outside of its tube and no feeding current has been observed (Chandler & Fleeger 1984). It has been hypothesized that *P. wellsi* 'gardens' microbial organisms on the walls of its mucus tube and feeds on these microbes (Chandler & Fleeger 1984, 1987).

**Labeled microalgal grazing experiments.** Copepod feeding on benthic versus planktonic microalgae was examined by adding $^{14}C$-cultured microalgae ($^{14}C$-CM) to the overlying water or sediment of cores. For all grazing experiments, cores were collected from the study site and overlying water was adjusted to 2 cm above the sediment surface. Cores were placed under artificial light for the duration of the 4 h incubation. A 500 µl syringe (Hamilton) was used to administer $^{14}C$-CM to the sediment surface or overlying water of intact sediment cores. Algae added to the sediment surface were administered through a silicon-sealed port located in the side of each core. Algae added to the overlying water of cores were added from the top of the core. Two diatom species, *Thalassiosira weissflogii*, a planktonic diatom common to Louisiana salt marshes, *Amphora coffeaeformis*, a benthic diatom similar in size to *T. weissflogii*, and a dinoflagellate, *Isochrysis galbana*, were used as food.

An experiment was performed to determine if microalgae injected into the overlying water of cores remained in suspension and, thus, were appropriate for use as indicators of planktonic feeding. $^{14}C$-labeled *Thalassiosira weissflogii*, *Amphora coffeaeformis*, and *Isochrysis galbana* in 1 ml of artificial sea water, were injected into the overlying water of cores (5 cores per algal species). At 0, 1, 2, 3, and 4 h, a 1 ml aliquot of overlying water was collected from each core and assayed for radioactivity to determine the proportion of algal cells that had settled from the water during the experimental period.

For grazing experiments, sediment cores were collected from the study site and returned to the laboratory. After a 1 h settling period, 1 ml $^{14}C$-labeled *Thalassiosira weissflogii* ($2.5 \times 10^6$ cells, 1.8 dpm $cell^{-1}$) were injected into the overlying water of 5 cores (*T. weissflogii* water experiment). In an additional 5 cores, 500 µl of $^{14}C$-labeled *Amphora coffeaeformis* ($2.5 \times 10^6$ cells, 0.38 dpm $cell^{-1}$) was injected onto the sedi-

ment surface (*A. coffeaeformis* sediment experiment). Killed controls were injected with labeled cells followed by an injection of 500 µl 37% formaldehyde. Five replicates for each algal species were performed. Controls for position of food (water or sediment) and algal species (*T. weissflogii* or *A. coffeaeformis*) were performed in an additional set of grazing experiments. Treatments were as described above, except that *T. weissflogii*, the planktonic diatom, was injected onto the sediment surface of 5 cores (*T. weissflogii* sediment experiment) and *A. coffeaeformis*, the benthic diatom, was injected into the overlying water of 5 cores (*A. coffeaeformis* water experiment). Killed controls were repeated as above. Data were expressed as algal cells consumed / µg copepod dry weight / 4 h.

Grazing experiments using the dinoflagellate *Isochrysis galbana* (*I. galbana* water experiment) were also performed. Live *I. galbana* are motile and do not remain at the sediment surface. This alga was injected only into the overlying water of cores and served as an indicator of planktonic grazing. Sediment cores were collected as described above and 1 ml $^{14}$C-labeled *I. galbana* ($1.26 \times 10^7$ cells, 0.37 dpm cell$^{-1}$) was injected into the overlying water of 5 cores. Killed controls were performed as above. Cores were incubated for 4 h under artificial light and then harvested.

Cores from all grazing experiments were harvested by collecting the top 2 cm of sediment and preserving it in 4% (final concentration) formaldehyde. Samples were stained with rose bengal, and copepod species were separated and enumerated. Ten adult female copepods of each species, when available, were collected from cores and placed into scintillation vials. No fewer than 4 individuals of a species were used in each assay. Copepods were solubilized in 200 µl of TS-2 tissue solubilizer (Research Products International, Mount Prospect, IL, USA) and incubated overnight at 60°C on a slide warmer, after which 100 µl of 1.2 N HCl was added to neutralize the tissue solubilizer. BioSafe II liquid scintillation cocktail (Research Products International) was added and samples were assayed for radioactivity using a Beckman LS 6000IC liquid scintillation counter (Beckman Instruments, Fullerton, CA, USA). Quenching was corrected for using external standards.

**Grazing on $^{14}$C-labeled natural microalgae.** Copepod grazing on $^{14}$C-natural microalgae ($^{14}$C-NM) was measured in sediment cores collected from the study site in May and June 1995. Cores were injected with 50 µCi $NaH^{14}CO_3$ following the injection technique described by Carman et al. (1989), incubated for 5 h, and harvested as described above. Five cores were incubated in the dark to account for label uptake due to biotic, nonphotosynthetic processes (Montagna 1983, Carman & Thistle 1985, Carman 1990). Radioactivity in dark controls was subtracted from radioactivity in light treatments to calculate $^{14}$C uptake from grazing on photoautotrophs. Data were expressed as dpm / µg copepod dry weight / 5 h.

The 5 h incubation period for labeled algae and natural algae grazing experiments is longer than the recorded gut-passage time for these copepods (Decho 1988), and thus actual grazing rates were underestimated. We assumed, however, that copepod grazing rates did not change over the experimental period and, thus, that statistical comparisons of grazing rates were appropriate.

**Gut-pigment analyses.** Copepods used for gut-pigment analyses were obtained by collecting the upper 3 to 5 mm of sediment from exposed areas of the mudflat. Sediment was poured through a 125 µm sieve and rinsed with marsh water to remove small sediment particles and concentrate copepods. The concentrate was transferred to a beaker and live copepods were aggregated using a fiber-optic light source. Copepods were removed by aspirating water and a portion of those extracted was immediately frozen in liquid nitrogen. Remaining live copepods were collected, rinsed with filter-sterilized (0.45 µm) marsh water (FMW), and transferred to 40 ml FMW. Animals were starved for 36 h, examined microscopically to insure gut clearance, and then frozen in liquid nitrogen.

To determine total gut-pigments in copepod guts [chlorophyll (chl) *a* and phaeopigment], animals were thawed and sorted into species. For each replicate, 30 adult female copepods were transferred, under subdued lighting, to 6 ml DMSO:acetone (1:1). Samples were sonicated with a Branson Model 450 sonifier for 1 min at 90 W and refrigerated overnight. Samples were then centrifuged for 5 min at 650 × *g*. Fluorescence was determined using a Turner Model 10 fluorometer before and after acidification with 1.2 N HCl. Five replicates were performed for each copepod species. The average pigment content of starved copepods was subtracted from values recorded for copepods that were immediately frozen.

**Functional responses.** In a separate experiment designed to test the influence of hydrocarbons on benthic food webs, grazing by meiofauna was measured using the $^{14}$C-NM technique (K. R. Carman, J. W. Fleeger & S. M. Pomarico unpubl.). Microcosms of sediment (15 cm i.d., see Carman et al. 1995) were collected at the study site and transported to the Louisiana Universities Marine Consortium facility in Cocodrie, where they were maintained on wet tables. Microcosms were irrigated with 5 µm-filtered marsh water via a drip system, and thus very little phytoplankton was available for consumption (Carman et al. unpubl.). Microcosms were maintained in the laboratory for 28 d, during which time the effects of hydrocarbons were examined. Algal biomass and copepod grazing

rates were determined at 0, 7, 14, and 28 d. Here, we report data from control (uncontaminated) microcosms.

**Statistical analyses.** Data from each experiment were analyzed using a 1-way analysis of variance (ANOVA). In addition, a 2-way ANOVA was performed on data from the gut-pigment analyses and $^{14}$C-NM grazing experiments to test for temporal effects of time × species interactions. Where significant differences were found, pairwise multiple comparisons were performed using Bonferroni's method (alpha = 0.05). Functional responses were examined by linear regression, with grazing rate as the dependent variable and algal biomass as the independent variable. When necessary, data were $\log_{10}$ transformed to meet assumptions of normality and homogeneity of variance.

**Dry weights.** Copepods were extracted from sediment and frozen in liquid nitrogen. For each of 5 replicates, 40 adult, female *Coullana* sp., *Cletocamptus deitersi*, *Microarthridion littorale*, or *Pseudostenhelia wellsi* were rinsed in deionized water and transferred to a tared, aluminum weighing dish. Copepods were dried at 55°C for 4 d. After drying, copepods were transferred to a desiccator, cooled to room temperature and weighed using a Cahn C-31 microbalance. Average dry weights/copepod for each harpacticoid species were as follows: 3.15 µg for *Coullana* sp., 1.37 µg for *M. littorale*, 1.15 µg for *C. deitersi*, and 0.87 µg for *P. wellsi*. Dry weights were used to standardize data from grazing experiments.

## RESULTS

### Grazing on prelabeled laboratory-cultured microalgae

The purpose of the $^{14}$C-CM grazing experiments was to examine differences in benthic and planktonic feeding among copepod species. Planktonic algae, *Thalassiosira weissflogii* and *Isochrysis galbana*, added to the overlying water of cores remained in the water column for the duration of the experiment (Fig. 1). Most (87%) of the benthic alga *Amphora coffeaeformis* settled to the sediment surface after 1 h. Because *T. weissflogii* and *I. galbana* (planktonic microalgae) remained in suspension, feeding on these 2 algae was assumed to be via planktonic feeding. Copepod feeding on *A. coffeaeformis*, the benthic alga which quickly settled out of the water column (Fig. 1), was via benthic grazing. Consumption of *T. weissflogii* and *A. coffeaeformis* added to the sediment was assumed to be from benthic feeding at the sediment-water interface.

Grazing rates differed significantly among copepod species in the $^{14}$C-CM experiments (Table 1). Benthic and planktonic feeding by *Coullana* sp. was greater than other copepod species tested in both the *Thalassiosira weissflogii* (Fig. 2) and *Isochrysis galbana* (Fig. 3) water experiments. *Coullana* sp. fed on *Amphora coffeaeformis* at significantly higher rates than all other copepod species in the sediment experiment (Fig. 2). *Coullana* sp. and *Cletocamptus deitersi* fed on *A. coffeaeformis* at equivalent rates in the water experiment, and both rates were significantly higher than those of *Microarthridion littorale* and *Pseudostenhelia wellsi*.

Benthic grazing by *Cletocamptus deitersi* was also significantly higher than grazing rates of *Microarthridion littorale* and *Pseudostenhelia wellsi* in the *Thalassiosira weissflogii* sediment and *Amphora coffeaeformis* water experiments (Fig. 2). Benthic grazing rates were not significantly different among these 3 species in the *A. coffeaeformis* sediment experiment.

Grazing experiments using $^{14}$C-CM indicated that *Microarthridion littorale* and *Pseudostenhelia wellsi* fed relatively little from the water column. Benthic and planktonic grazing rates of *M. littorale* and *P. wellsi* were not significantly different (Figs. 2 & 3). In the *Thalassiosira weissflogii* sediment and *Amphora coffeaeformis* water experiments, grazing rates of *Coullana* sp. and *Cletocamptus deitersi* were significantly higher than *M. littorale*. Lowest overall grazing rates were seen in *P. wellsi* (Figs. 2 & 3) and its feeding was predominately benthic. Planktonic grazing by *P. wellsi* in the *T. weissflogii* water experiment was not significantly different from killed controls.

### Grazing on $^{14}$C-labeled natural microalgae

Although our initial goal was to analyze all 4 species using $^{14}$C-CM, $^{14}$C-NM, and gut pigments, variable

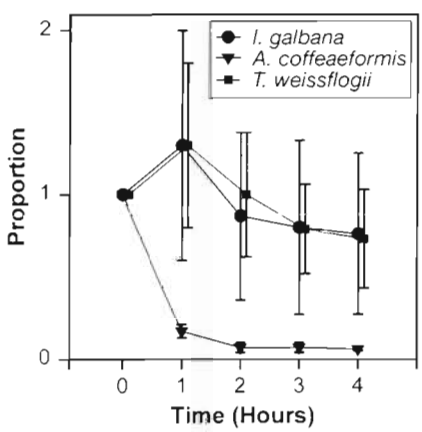


Fig. 1. *Isochrysis galbana*, *Amphora coffeaeformis*, *Thalassiosira weissflogii*. Settlement of algal cells over the experimental period. Error bars are ± 1 SD (n = 4). Points are proportion of cells remaining in the water column at each sampling time in relation to time 0

Table 1 Results of the 1-way ANOVA for copepod feeding behavior experiments. Abbreviations are as follows: $^{14}$C-CM = copepod grazing on laboratory-cultured microalgae; $^{14}$C-NM = copepod grazing on natural microalgae; gut-content analyses = analysis of copepod total gut-pigments and % chl *a*; sed = algae added to the sediment surface of cores; water = algae added to the overlying water of cores; BIC = $NaH^{14}CO_3$ labeled natural microalgae; total pigment = total amount of chl *a* plus phaeopigment in copepod guts; % chl *a* = [chlorophyll *a*/(chl *a* + phaeopigment)] × 100%

| Source | df | *F*-value | p |
|---|---|---|---|
| $^{14}$C-CM | | | |
| *T. weissflogii* sed | 3 | 20.4 | <0.0001 |
| *T. weissflogii* water | 3 | 22.4 | <0.0001 |
| *A. coffeaeformis* sed | 3 | 5.84 | 0.017 |
| *A. coffeaeformis* water | 3 | 16.4 | <0.0001 |
| *I. galbana* water | 2 | 14.3 | 0.0009 |
| $^{14}$C-NM | | | |
| BIC (May) | 3 | 11.3 | 0.0021 |
| BIC (June) | 2 | 1.85 | 0.22 |
| Gut-content analyses | | | |
| Total pigment (May) | 2 | 3.34 | 0.02 |
| Total pigment (June) | 2 | 2.13 | 0.15 |
| % chl *a* (May) | 2 | 5.99 | 0.04 |
| % chl *a* (June) | 2 | 2.17 | 0.16 |

abundances of *Cletocamptus deitersi* and *Microarthridion littorale* prevented us from being able to do so. In both May and June, we were unable to collect a sufficient number of *M. littorale* for pigment analysis. In June, *C. deitersi* were not present in cores used for $^{14}$C-CM.

The purpose of $^{14}$C-NM grazing experiments was to measure copepod grazing on the natural microalgal assemblage. In the May grazing experiment (Fig. 4A), dark controls accounted for an average of 112% of the label in *Coullana* sp., and 0.83, 29 and 16% of label in *Cletocamptus deitersi*, *Pseudostenhelia wellsi*, and *Microarthridion littorale*, respectively. Dark controls in the June experiment (Fig. 4B) accounted for 45, 9, 3, and 32% of the label in *Coullana* sp., *C. deitersi*, *M. littorale*, and *P. wellsi*, respectively.

Grazing rates on $^{14}$C-NM were significantly different among copepod species in May but not June (Table 1). Grazing rates of *Cletocamptus deitersi* collected in May were significantly higher than those of *Coullana* sp. (Fig. 4A). Grazing rates of *Microarthridion littorale* and *Pseudostenhelia wellsi* in May were not significantly different from grazing rates of *Coullana* sp. or *C. deitersi*. In May, *C. deitersi* grazed at a rate more than double that of *M. littorale* and *P. wellsi*, though the difference was not significant, probably due to low sample size. In contrast to the May experiment, grazing in June by *Coullana* sp. was higher than that of *M. littorale* and *P. wellsi*, though the 1-way ANOVA detected no significant differences in grazing rates among species (Fig. 4B, Table 1). A 2-way ANOVA revealed that grazing rates by *Coullana* sp. in May and June were significantly different (Table 2). Grazing rates in the other copepod species were not significantly different between May and June, and there were no significant time × species interactions.

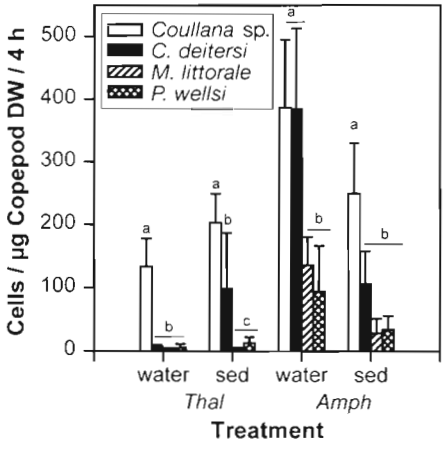


Fig. 2. *Coullana* sp., *Cletocamptus deitersi*, *Microarthridion littorale*, *Pseudostenhelia wellsi*. Harpacticoid grazing on $^{14}$C-labeled *Thalassiosira weissflogii* (*Thal*) and *Amphora coffeaeformis* (*Amph*) added to the water (water) or sediment (sed) ($^{14}$C-CM). Different letters indicate significant differences among species. Horizontal bars are mean cells/µg copepod dry weight (DW). Error bars are + 1 SD (n = 5)

### Gut-pigment analyses

Total gut-pigment levels were significantly higher in *Pseudostenhelia wellsi* than in *Cletocamptus deitersi*

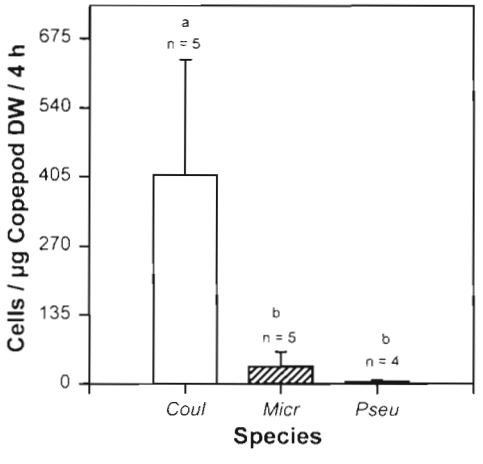


Fig. 3. Harpacticoid grazing on $^{14}$C-labeled *Isochrysis galbana* ($^{14}$C-CM). Species designations are as follows: *Coul* = *Coullana* sp., *Micr* = *Microarthridion littorale*, *Pseu* = *Pseudostenhelia wellsi*. Different letters indicate significant differences among species. Error bars are + 1 SD

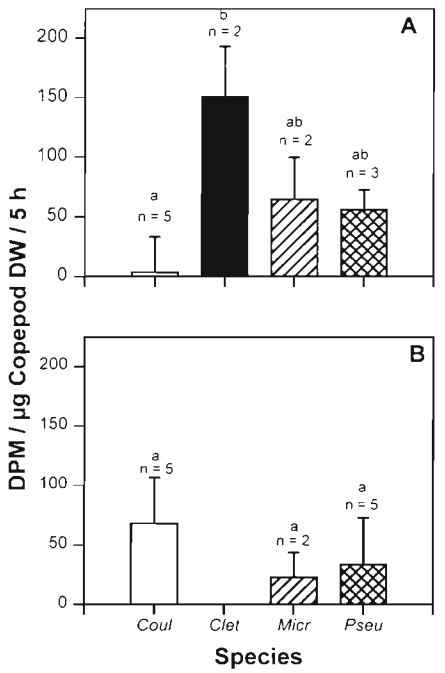


Fig. 4. (A) May and (B) June 1995 $^{14}$C-natural microalgae ($^{14}$C-NM) grazing experiment results. Abbreviations are as follows: *Coul* = *Coullana* sp., *Clet* = *Cletocamptus deitersi*, *Micr* = *Microarthridion littorale*, *Pseu* = *Pseudostenhelia wellsi*. Different letters indicate significant differences among species. Error bars are + 1 SD

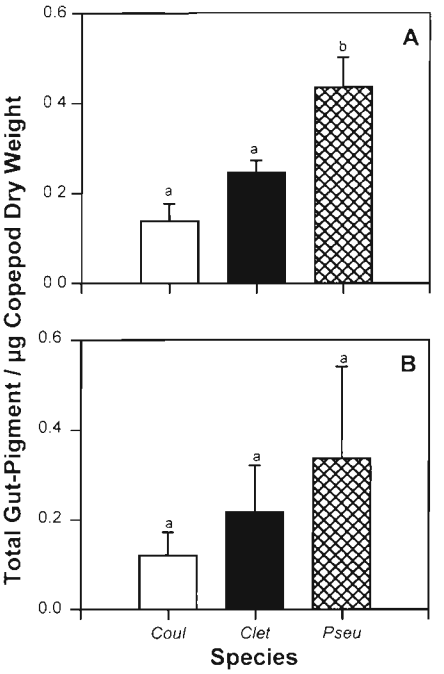


Fig. 5. Total gut-pigment / µg copepod dry weight for animals collected in (A) May and (B) June 1995. Total gut-pigment is defined as the sum of chl *a* and phaeopigment. Abbreviations are as follows: *Coul* = *Coullana* sp., *Clet* = *Cletocamptus deitersi*, *Pseu* = *Pseudostenhelia wellsi*. Different letters indicate significant differences among species. Error bars are + 1 SD (n = 5)

and *Coullana* sp. for May samples (Fig. 5A, Table 1). Total gut-pigment levels were not significantly different in *Coullana* sp., *C. deitersi*, and *P. wellsi* collected in June (Fig. 5B, Table 1). A 2-way ANOVA revealed that over May and June total pigment in *P. wellsi* was significantly higher than in *C. deitersi* and *Coullana* sp. (Table 2).

Percent chl *a* detected in the guts of *Coullana* sp., *Cletocamptus deitersi*, and *Pseudostenhelia wellsi* collected in May were significantly different; although the pattern of interspecific differences was similar in June, % chl *a* did not differ significantly among species (Table 1, Fig. 6). A 2-way ANOVA revealed that over May and June % chl *a* in *P. wellsi* was significantly lower than in *C. deitersi* and *Coullana* sp. (Table 2).

### Functional responses

Benthic microalgal biomass in control microcosms varied from 1.02 to 7.25 µg chl *a* cm$^{-2}$ over the 28 d experiment. Relationships between microalgal biomass and grazing rates of the 4 copepod species con-

Table 2. Results of a 2-way ANOVA analyzing copepod grazing experiments in May and June 1995. Abbreviations are as follows: gut-pigments = total gut-pigments; % chl *a* = [chl *a* / (chl *a* + phaeopigment)] × 100; $^{14}$C-NM = copepod grazing on natural microalgae

| Assay | Factor | p | *A posteriori* |
|---|---|---|---|
| Gut-pigments | Species | < 0.001 | *P. wellsi* > *C. deitersi* and *Coullana* sp. |
| | Month | 0.078 | |
| | Species × Month | 0.350 | |
| % chl *a* | Species | 0.005 | *P. wellsi* < *C. deitersi* and *Coullana* sp. |
| | Month | 0.087 | |
| | Species × Month | 0.719 | |
| $^{14}$C-NM | Species | 0.810 | June *Coullana* sp. > May *Coullana* sp. |
| | Month | 0.774 | |
| | Species × Month | 0.021 | |

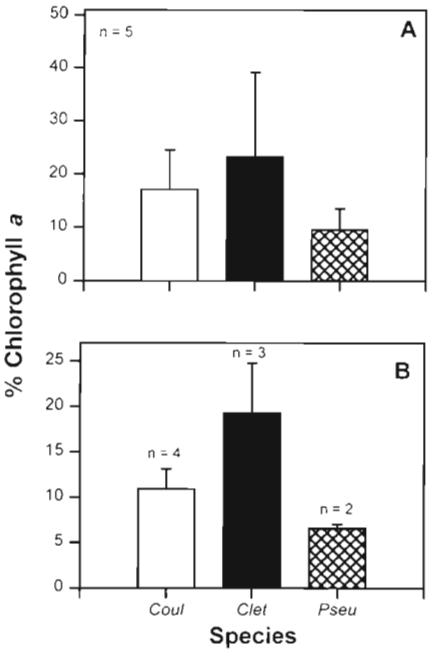


Fig. 6. Chl *a* as a percent of total gut-pigment (chl *a* and phaeopigment) in harpacticoid species collected in (A) May and (B) June 1995. Abbreviations are as follows: *Coul* = *Coullana* sp., *Clet* = *Cletocamptus deitersi*, *Pseu* = *Pseudostenhelia wellsi*. Error bars are + 1 SD

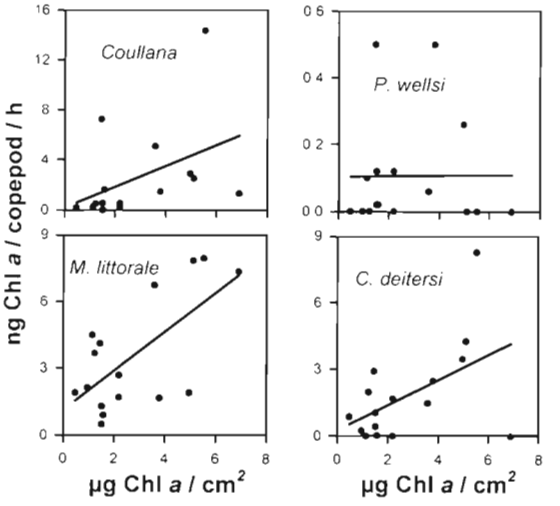


Fig. 7. Functional responses of copepod grazing to variability in benthic chl *a*. Lines are best fit linear regressions. Regression statistics, where $y$ = µg chl *a* consumed, are as follows: *Coullana* sp.: $y = 0.0009 + (0.0042 \times \mu g\ chl\ a\ cm^{-2})$, $r^2 = 0.181$, $p = 0.1134$; *Pseudostenhelia wellsi*: $y = 0.0005 + (0.000005 \times \mu g\ chl\ a\ cm^{-2})$, $r^2 = 0.0001$, $p = 0.9650$; *Microarthridion littorale*: $y = 0.0055 + (0.00443 \times \mu g\ chl\ a\ cm^{-2})$, $r^2 = 0.442$, $p = 0.0049$; *Cletocamptus deitersi*: $y = 0.00128 + (0.00283 \times \mu g\ chl\ a\ cm^{-2})$, $r^2 = 0.256$, $p = 0.0455$

sidered here are presented in Fig. 7. Significant positive functional responses to algal abundance were detected for *Microarthridion littorale* and *Cletocamptus deitersi*, but not *Pseudostenhelia wellsi* or *Coullana* sp.

## DISCUSSION

The purpose of this study was to determine whether interspecific differences in the use of microalgal food resources existed among co-occurring harpacticoid copepods in saltmarsh sediments. Our data suggest that although all 4 copepod species studied grazed on microalgae, feeding patterns differed among species. Different assays of grazing yielded different types of information, and sometimes qualitatively different results.

$^{14}$C-CM grazing experiments illustrated potential interspecific differences in benthic versus planktonic feeding, and suggested that overall microalgal grazing by *Microarthridion littorale* and *Pseudostenhelia wellsi* was relatively low (Figs. 2 & 3). *Coullana* sp. grazed effectively on planktonic and benthic algae and was especially effective at consuming algae at the sediment-water interface. *Cletocamptus deitersi* grazed predominantly at the sediment surface (Fig. 2) and its benthic grazing rates were comparable to *Coullana* sp.; it was not, however, an effective plankton feeder. *M. littorale* and *P. wellsi* were almost completely ineffective at consuming planktonic algae and consumed benthic microalgae at lower rates than did *C. deitersi* or *Coullana* sp. (Figs. 2 & 3).

Laboratory studies by Decho (1986) examined benthic and planktonic feeding by *Coullana* sp., *Cletocamptus deitersi*, and *Microarthridion littorale* on diatoms (*Thalassiosira weissflogii* and *Amphora tenerrina*) in azoic sediments, and feeding experiments were conducted separately for each species. Thus, copepods in Decho's (1986) experiments did not interact with other meiofaunal species and fed only on introduced algal food. Our experiments, involving the addition of $^{14}$C-labeled benthic and planktonic algae, were similar to those of Decho (1986), except they were conducted using natural sediments with their resident meiofaunal and microalgal communities intact. Data presented here are consistent with Decho's (1986) conclusions that *Coullana* sp. is an effective plankton feeder and *C. deitersi* feeds at the sediment-water interface, but our observation that *Coullana* sp. also feeds effectively on benthic microalgae differs from that of Decho (1986). It is possible that grazing by *Coullana* sp. in natural sediments (this study) differs from its grazing behavior in azoic sediment (Decho 1986).

Our observations of *Microarthridion littorale* grazing also differed from the results of Decho (1986) who found *M. littorale* to graze on planktonic and benthic algae at rates similar to or higher than *Coullana* sp. Other studies involving *M. littorale* have strongly implicated it as a consumer of benthic microalgae (Decho 1988, Souza-Santos et al. 1995). Further, $^{14}$C-NM assays of feeding behavior (discussed below) indicated consumption of benthic microalgae by *M. littorale*.

In this study, natural microalgae as well as $^{14}$C-CM were present in intact sediment cores and *Microarthridion littorale* could have fed on a natural algal source and not on the laboratory-cultured algae. One limitation of the $^{14}$C-CM technique is that only grazing on the introduced $^{14}$C-labeled microalgae can be measured while grazing on unlabeled species is not accounted for; the offered $^{14}$C-labeled algae may be preferentially consumed or avoided by copepods, leading to over- or underestimation of actual copepod grazing rates.

Gut-pigment analyses as well as grazing experiments using $^{14}$C-NM indicated that copepods grazed on algae in the field, but the 2 methods yielded qualitatively different results. The $^{14}$C-NM method indicated that grazing by *Coullana* sp. (but not other species) was affected by tidal exposure, but gut pigments did not vary with tidal exposure for any of the copepods analyzed. Previous studies indicate that tidal cycle and presence or absence of water overlying the sediment surface may affect copepod grazing rates (Decho 1986, 1988, Souza-Santos et al. 1995). In both May (late low water) and June (early low water), *Coullana* sp. gut-pigments were lower than in *Cletocamptus deitersi* and *Pseudostenhelia wellsi* (Fig. 5). The $^{14}$C-NM method, however, showed that in May grazing by *Coullana* sp. was greatly reduced compared to other species (Fig. 4A), and that the grazing rate of *C. deitersi* was more than double that of the other copepod species; in June, however, *Coullana* sp. grazing was higher than in other species (Fig 4B). Further, *P. wellsi* gut-pigments were highest in both May and June samplings, but grazing determined by $^{14}$C-NM was intermediate at both times. Thus, interspecific and temporal variation in gut-pigments and grazing rates determined from $^{14}$C-NM seem to be largely independent of each other. Nevertheless, the dramatic difference in $^{14}$C-NM grazing rates observed for *Coullana* sp. in May and June is intriguing. *Coullana* sp. tends to occupy a subtidal habitat and is not often exposed at low tide, while the other copepod species are intertidal and exposed more frequently. A field study by Decho (1988) examined grazing of *Coullana* sp. (and other species) at high water, early low water, and late low water using gut-pigment analysis. Decho (1988) observed that grazing by *Coullana* sp. was highest at early low water and lowest at late low water. Decho (1988) suggested that inability to feed on plankton at late low water caused the reduced feeding by *Coullana* sp., and our $^{14}$C-NM observations would appear to support that conclusion. However, Decho's (1988) grazing rates were calculated based on gut-pigment measurements, which did not vary with sediment exposure in our study.

There are no previous laboratory or field grazing studies for *Pseudostenhelia wellsi* against which we can compare our observations. It has been suggested, however, that *P. wellsi* grazes on bacteria gardened on the inner walls of its mucus tube (Chandler & Fleeger 1984). Morphological and physiological similarities between *P. wellsi* and *Diarthrodes nobilis*, a phytal copepod presumed to garden bacteria on its mucus capsule (Hicks & Grahame 1979), are also consistent with the hypothesis that *P. wellsi* gardens its food (Williams-Howze et al. 1987). Our data indicate, however, that *P. wellsi* feeds on nonplanktonic algae to at least some degree as relatively high amounts of gut pigments and moderate $^{14}$C-NM grazing rates were detected. In principle, *P. wellsi* could feed on sedimentary algae or algae growing in its tube. If it does not leave its burrow or create a feeding current, grazing on labeled algae in the $^{14}$C-NM experiments by *P. wellsi* would have been restricted to algal cells that settled into its mucus tube, which could explain the low grazing rates by *P. wellsi* observed in the $^{14}$C-CM grazing experiments. $^{14}$C label consumed by *P. wellsi* in the $^{14}$C-NM grazing experiments may have resulted from the labeling of algae growing on the inner walls of its mucus tube. In contrast to *Coullana* sp., grazing rates of *P. wellsi* were seemingly unaffected by the extreme low tide observed in May. If this copepod were grazing on organisms growing on the inner walls of its mucus tube, grazing rates during an extreme low tide would presumably be unaffected. While the presence of pigment in the guts of *P. wellsi* indicated that it was consuming algal material, the small fraction of *P. wellsi* gut-pigment that was in the form of chl *a* suggested that *P. wellsi* was ingesting predominantly detrital plant material or that digestion efficiency in *P. wellsi* was high relative to other copepod species.

Another means of examining feeding is to determine the relationships of grazers with their putative food source. If grazers are limited by food availability, grazing rates should increase with increasing food concentration (Karrh & Miller 1994). Significant functional responses by *Microarthridion littorale* and *Cletocamptus deitersi*, but not *Coullana* sp. or *Pseudostenhelia wellsi* provided further evidence of dietary differentiation among copepod species. Specifically, they indicate that *M. littorale* and *C. deitersi* feeding rates are

closely linked to the availability of benthic microalgae, but that *P. wellsi* and *Coullana* sp. are more dependent on other food sources such as planktonic algae and/or detritus.

Blanchard (1991) reported that meiofaunal grazing on microphytobenthos exceeded production, and suggested that this relationship could lead to food limitation for meiofauna. Montagna et al. (1995) observed that increases in meiofaunal grazing rates (microalgal biomass consumed per individual) were correlated with high biomass of microphytobenthos. Both studies (Blanchard 1991, Montagna et al. 1995) considered meiofaunal grazing at the level of taxon (e.g. harpacticoids, nematodes, and ostracods). Our observations concerning functional relationships between meiofauna and microalgae are consistent with those of Montagna et al. (1995), but demonstrate that the functional response of individual species varies considerably. Thus, consideration of species composition may be necessary to accurately characterize and predict meiofaunal-microphytobenthos interactions.

Overall, various lines of evidence indicate that interspecific feeding differences exist among the harpacticoid copepods examined in this study. While *Coullana* sp. grazed on both benthic and planktonic diatoms, its reduced feeding activity in response to prolonged exposure of the sediment, as well as its weak functional response to benthic algal biomass, suggest that its diet is derived primarily from planktonic material, which is generally consistent with the observations of Decho (1986, 1988). *Cletocamptus deitersi* grazed predominantly on benthic diatoms and fed at high rates even after prolonged exposure of the sediment. These observations, in conjunction with a significant functional response to benthic algal biomass, strongly implicate benthic algae as its primary food source (Decho 1988). While field data show that *Pseudostenhelia wellsi* consumes algae (high gut-pigment content and moderate $^{14}$C-NM grazing rates), the inability of *P. wellsi* to consume labeled ($^{14}$C-CM) planktonic algae, and the fact that prolonged tidal exposure did not influence feeding (in contrast to *Coullana* sp.), suggest that it does not feed on plankton. The very low consumption of added benthic prelabeled ($^{14}$C-CM) algae, combined with the lack of a functional response to benthic algal biomass, is consistent with the hypothesis that *P. wellsi* feed on algal material growing in their tubes. Clearly, further work is needed to determine the factors influencing the feeding biology of *P. wellsi*. Data for *Microarthridion littorale* are incomplete and equivocal, especially given that previous studies have strongly implicated *M. littorale* as a feeder on benthic and planktonic algae. The lack of feeding on algae in the $^{14}$C-CM experiment may have occurred because of dietary selectivity; at a minimum, however, the $^{14}$C-CM experiment illustrates that *M. littorale* feeding preferences differ from those of *Coullana* sp. and *C. deitersi*. Field data ($^{14}$C-NM) indicate that *M. littorale* does feed on microalgae, and that feeding is relatively unaffected by prolonged exposure of the sediment. The significant functional response of *M. littorale* to benthic microalgal biomass implicates benthic algae as an important resource, and differentiates its feeding from that of *P. wellsi*.

Interspecific differences in the use of microalgal resources suggests that food-resource partitioning may occur among these 4 copepod species. By definition, resource partitioning implies that the partitioned resource is limiting (Begon et al. 1996), and we did not examine food limitation in this study. We suggest, however, that consideration of food limitation among meiofaunal species would be an important topic for future studies.

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