

## Diel feeding behavior of meiofauna and their relationships with microalgal resources

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### Abstract

Diel variation of feeding by ostracods and harpacticoid copepod species on microalgae was examined in a mudflat of a *Spartina alterniflora* saltmarsh. Feeding (gut pigments and consumption of <sup>14</sup>C-labeled algae) and benthic microalgal abundance and composition (HPLC analysis of photopigments) were measured every 6 h over a 48-h period. Midday feeding peaks were detected in *Coullana* sp. juveniles and adult females (but not males), *Microarthridion littorale*, *Pseudostenhelia wellsi*, and ostracods and were coincident with peaks in microalgal biomass. This pattern of midday feeding peaks by meiofauna differs from classical models of zooplankton feeding, which typically peak at night. Midday feeding peaks were apparently influenced by functional responses to food availability (ostracods and *Coullana* sp. females and juveniles), entrained diel rhythms (*M. littorale*), influence of light—positive (*M. littorale*) or negative (*Coullana* sp.), and possibly by vertical migration of diatoms within sediment. The negative influence of light on grazing by *Coullana* sp. may have implications for its feeding activity during midday low tides. Diatoms dominated the benthic microalgae, but cyanobacteria and chlorophytes contributed significantly to the planktonic community. *Coullana* sp. gut pigments indicate that at least part of its diet is planktonic, but it selectively avoids planktonic cyanobacteria. Diel variation of microalgal biomass appears to be influenced by meiofaunal grazing, which implies that meiofauna represent an important link between microalgal primary production and higher trophic levels.

Microphytobenthos and their consumers are essential components of coastal ecosystems (MacIntyre et al. 1996); they influence sediment biogeochemistry via uptake and release of nutrients (Rizzo et al. 1992), and sediment erosion via their production of exopolymers (Grant et al. 1986). Microphytobenthos represent up to 40% of macrophyte production in saltmarshes (e.g., Pinckney and Zingmark 1993), and likely contribute a much larger fraction to foodwebs because they are more nutritious and labile than *Spartina* spp. and other vascular plants (Miller et al. 1996). Indeed, several stable-isotope studies have concluded that microphytobenthos are a major source of nutrition that fuels secondary production (Peterson and Howarth 1987; Sullivan and Moncreiff 1990; Currin et al. 1995). Stable-isotope analyses, however, reveal little about specific interactions between primary producers and consumers. In particular, we know very little about whether benthic grazers are limited by the regeneration of microphytobenthos, and in turn, if microphytobenthic biomass is controlled by grazing (Miller et al. 1996). Further, little is known concerning the trophic pathways by which microphytobenthic production reaches higher trophic levels. To address these questions, more information concerning grazer-microalgae interactions are needed.

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Benthic meiofauna constitute an important component of marine-benthic food webs, both as producers (Gee 1989) and consumers (Montagna 1995). Among the meiofauna, harpacticoid copepods and ostracods are particularly important consumers of microphytobenthos (Montagna 1995; Carman et al. 1997), and serve as a food source for macrofauna, as well as larval and juvenile fish (McCall and Fleeger 1995). Limited information indicates that meiofaunal grazing on microphytobenthos may equal or exceed primary production, which may in turn lead to food limitation (Blanchard 1991). The hypothesis of food-limitation is consistent with field observations that meiofauna can adjust their feeding rate in response to changes in microphytobenthos biomass (Montagna et al. 1995), and that functional responses vary among species due to interspecific differences in exploitation of food sources (Pace and Carman 1996). Thus, meiofauna may represent an important link between primary producers and higher trophic levels, and it is therefore important to develop a better understanding of how meiofauna exploit microalgal resources.

Diel patterns of phytoplankton consumption by zooplankton are well documented in marine habitats, and typically characterized by feeding peaks at night when zooplankton migrate from deeper water up to phytoplankton-rich surface water (Durbin et al. 1990). However, virtually nothing is known about diel variation in trophic relationships between microphytobenthos and their consumers. Limited studies indicate that meiofaunal-grazing rates may change over short time scales such as a tidal cycle (Decho 1988). In the only study of meiofaunal diel-feeding patterns that we are aware of, Souza-Santos et al. (1995) determined in laboratory experiments that feeding by the harpacticoid copepod *Harpacticus flexus* peaked approximately every 6 h. To our knowledge, however, no previous studies have examined diel-feeding patterns of meiofauna (or macrofauna) in the

field. A better understanding of diel variation of feeding on microalgae would contribute significantly toward the development of more general models of foodweb structure in coastal ecosystems. Accordingly, the purpose of this study was to investigate in situ diel-feeding behavior of meio-benthic copepods and ostracods, and their relationships with benthic and planktonic algal biomass.

## 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. Tidal fluctuations are small (0–0.3 m) and predominately wind driven (Phillips and Fleeger 1985). During the sampling period, temperature of the sediment/water interface varied from 29.4 to 32.8°C and salinity from 5 to 9‰, respectively. Sampling was carried out at neap tide to limit the influence of variable water cover. Water depth was approximately 0.4 m throughout the study period.

Samples were collected at dawn (Da), noon (No), dusk (Du), and midnight (Mi) (every 6 h) over a 48-h period in June 1997. Da and Mi samplings occurred during darkness whereas No and Du samplings occurred during daylight. For phytoplankton pigment analysis, ambient marsh water was sampled using three replicate 1-liter plastic bottles and 250-mL subsamples were filtered through 50-mm diameter GF/F filters to concentrate microalgae. Filters were immediately frozen in liquid nitrogen and stored in the dark at –80°C for subsequent HPLC pigment analysis. For <sup>14</sup>C-grazing experiments and HPLC pigment analyses of benthic microalgae, sediment cores were collected using 3.5-cm i.d. clear butyrate tubing and were returned to the laboratory for treatment with minimal disturbance. Sediment for copepod pigment analysis was collected from the sediment surface using a vacuum pump and washed through a 125- $\mu$ m sieve to concentrate copepods. Sediment subsamples for HPLC analyses of copepod pigments were stored in the dark at –80°C.

**<sup>14</sup>C-Prelabelled microalgal grazing experiment**—Diel variation of copepod and ostracod grazing rates were examined using the protocol described by Pace and Carman (1996). Overlying water of sediment cores was adjusted to 2 cm above the sediment surface. A 1-ml aliquot of <sup>14</sup>C-labeled microalgae (*Isochrysis galbana*,  $17 \times 10^6$  cells mL<sup>-1</sup>, 0.3 dpm cell<sup>-1</sup>) was injected into the overlying water using a 1-mL pipette. <sup>14</sup>C-labeled *I. galbana* was stored at –80°C, then thawed immediately prior to use in experiments. At each of the eight sampling times, four replicate cores were incubated under artificial (fluorescent) light (100  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) and four were incubated in the dark for 3 h; two killed controls (37% formaldehyde injection) were also performed at each time. The parallel light and dark incubations were performed to determine if exposure to light per se influenced feeding activity. Cores were harvested by collecting the top 2 cm of sediment. Samples were preserved in 4% formaldehyde (final concentration) and stained with Rose Bengal. Ostracods, copepod species, and *Coullana* sp., females, males, and juveniles were sorted, and placed into scintillation vials. Animals were assayed for radioactivity as described by Pace and Carman (1996).

**HPLC analyses of pigments**—Frozen filters containing phytoplankton were cut into small pieces and sonicated for 30 s in 4 ml 100% cold acetone (Mallinckrodt, HPLC quality) using a Branson Sonifer 450 (3-mm-diameter probe, output setting on 4, duty cycle on 80%). Acetonic extracts were filtered (Corning, 0.2  $\mu$ m) and 100- $\mu$ l aliquots were injected through the HPLC system within a few hours. Three replicates were analyzed for each sampling time.

Four replicate sediment cores were vertically partitioned into 0–4, 4–8, 8–12, and 12–20 mm layers to examine the vertical distribution of microalgae. Slices were frozen and stored in the dark within 2 h following sampling, and freeze-dried prior to pigment extraction. Pigments were extracted by sonicating each sample for 30 s in 100% cold acetone [0.3–0.8 g dry weight (gdw) sediment in 5 mL acetone or 1.3 to 2 gdw in 10 ml acetone] and incubating overnight in the dark at –20°C. Acetonic extracts were then separated from sediment by centrifugation, filtered (Corning 0.2  $\mu$ m), and stored in the dark at –80°C. Immediately before HPLC analysis, extracts were diluted with water (Mallinckrodt, HPLC quality) to 66% acetone to improve the sharpness of peaks (Wright et al. 1991). One hundred  $\mu$ l of each diluted extract was analyzed by HPLC within 48 h following extraction.

For pigment analysis of copepods (ostracods were not assayed for pigments), aliquots of frozen sediment were gently washed under tap water through 250- $\mu$ m (*Coullana* sp.) and 125- $\mu$ m (*M. littorale* [Poppe] and *P. wellsi* [Coull and Fleeger]) sieves. Copepods were stained with Rose Bengal overnight (*Coullana* sp.) at 4°C to facilitate sorting of copepod species. Sorting was conducted under minimum light exposure and sorted individuals were kept on ice to prevent pigment degradation. Copepods were washed twice in cold distilled water to remove sediment particles. Forty to 250 copepods were sonicated (Branson Sonifer 450, 3-mm-diameter probe, output setting on 1.5, duty cycle on 80%) in 1 ml of 100% cold acetone for 30 s and incubated in darkness overnight at 4°C. The acetonic extracts were separated from copepod debris by filtration (Corning, 0.2  $\mu$ m). Because Rose Bengal is not soluble in pure acetone the stain was concentrated on copepod debris only and filtration completely removed the stain from the extracts (Buffan-Dubau unpubl. obs.). Extracts were concentrated to 180  $\mu$ l under nitrogen gas, and stored in the dark at –80°C. Immediately before injection, extracts were diluted with water to 66% acetone and 100  $\mu$ l of each diluted extract were injected through the HPLC system within 24 h following extraction. Because <sup>14</sup>C-grazing experiments indicated feeding peaks at noon, HPLC analysis focused on noon and midnight copepod samples. When sufficient numbers of copepods were available, three replicate analyses of adult female copepods were performed. Copepod individual dry weights were used to standardize pigment concentrations: 3.15  $\mu$ g for female *Coullana* sp., 1.37  $\mu$ g for *M. littorale*, and 0.87  $\mu$ g for *P. wellsi* (Pace and Carman 1996).

Pigment contents of copepods starved for 36–48 h were subtracted from values of field-collected copepods. Chl *a* and pheophytin *a* content of starved copepods was 11–30%, and 3–10% of values from field-collected copepods, respectively. The carotenoid pigments zeaxanthin, diatoxanthin,

and diadinoxanthin were at least as concentrated in starved copepod samples as in field-collected copepods, suggesting that these carotenoids originated from copepod tissue and/or epibiotic microorganisms.  $\beta$ -Carotene in starved copepods was 38% of concentrations in field-collected animals. Pheophorbide *a*, Chl *b*, fucoxanthin, and lutein were not detected in starved copepod extracts.

**High-performance liquid chromatography**—Pigment extracts were analyzed using a Hewlett Packard 1100 liquid chromatograph consisting of a 100- $\mu$ l loop autosampler, a quaternary solvent delivery system coupled to a diode array spectrophotometer, and a Hewlett Packard 1046A fluorescence detector. The diode array detector was set at 436 nm for detection of carotenoid and chlorophyll pigments, and at 405 nm for detection of pheopigments (Wright et al. 1991). Chlorophylls and pheopigments were detected without interferences from carotenoids using the fluorescence detector set at 407 nm (excitation)/672 nm (emission) and at 430 nm (excitation)/665 nm (emission). When Chl *b* was present in low concentrations, it was quantified using a fluorescence detector set at 453 nm (excitation)/652 nm (emission) (Jeffrey et al. 1997). Separation of pigments was performed by reverse-phase liquid chromatography using a C18, 5- $\mu$ m column (250 mm  $\times$  4.6 mm i.d.) coupled to a guard column. Data analysis was performed using Hewlett Packard HPChemStation software.

The mobile phase was prepared and programmed according to the analytical gradient protocol described by Wright et al. (1991). All organic solvents and water were HPLC grade (Mallinckrodt). Pigments were identified by comparing their retention time and absorption spectra with those of authentic standards ([Sigma] Chl *a*, Chl *b* and  $\beta$ -carotene), or standards prepared from algal cultures. Peridinin and diatoxanthin were obtained from *Amphidinium carterae* (dinoflagellate), zeaxanthin from *Oscillatoria* sp. (cyanobacteria), and diadinoxanthin from *I. galbana* (prymnesiophyceae). Dr. Thomas Bianchi (Tulane University, Louisiana, USA) provided fucoxanthin and lutein standards. Standards of Chl *a* and *b* were converted to pheophytin *a* and *b* by acidification. Chlorophyllide *a* was obtained from a culture of *Thalassiosira* sp. (diatom) by incubating algae in 50% acetone in the dark overnight. Chlorophyllide *a* was then purified by HPLC and converted to pheophorbide *a* by acidification. Standard solutions were quantified by spectrophotometry using the extinction coefficients listed by Jeffrey et al. (1997). A response factor was calculated for each standard from the linear relationship between the concentration and the corresponding peak area obtained by HPLC. Pheophytin *a* and pheophorbide *a* pigments that were spectrally similar to, but did not have the same retention time as standards were designated 'like'-pigments, and were quantified using the response factor obtained from standards.

**Statistical analysis**—Data were analyzed using Sigmatat 2.0 software. Two-way analysis of variance (ANOVA) was used to test variations of pigment concentrations versus both time and days, as well as gut-pigment contents versus both species and time. When data did not meet assumptions of ANOVA, rank transformation was applied. A posteriori

comparisons were performed using Tukey's multiple-comparison test. Pearson's test was used to determine correlations of pigment concentrations within microalgal communities, and between microalgal communities and  $^{14}\text{C}$  grazing rates. Multi-dimensional scaling (MDS) analysis of pigment composition was used to determine if sediment and water microalgal communities differed ('Primer Package' Clarke and Warwick 1994).

## Results

**$^{14}\text{C}$  grazing rates**—Consumption of  $^{14}\text{C}$ -labeled microalgae by *Coullana* sp. females and juveniles, *M. littorale*, and ostracods was significantly influenced by time of day (Table 1, Fig. 1). In all cases where significant temporal variation occurred, grazing activity was highest at noon, and noon was the only time during which grazing activity differed significantly from other times. Relative to the average of all other times, grazing at noon in dark incubations was elevated by a factor of 1.5 for *Coullana* females, 1.8 for *Coullana* juveniles, 2.9 for *M. littorale*, and 3.3 for ostracods. Grazing by *P. wellsi* and *Coullana* sp. males was not significantly influenced by time of day (Table 1).

Exposure to light significantly decreased consumption of  $^{14}\text{C}$ -labeled algae by *Coullana* sp. females, males, and juveniles and significantly enhanced the feeding activity of *M. littorale* (Table 1, Fig. 1). Light did not significantly influence the feeding activity of *P. wellsi* or ostracods (Table 1). There were no significant interactions between the effects of light and time on the grazing rates of any of the taxa considered (Table 1).

**Pigment composition of microalgal communities**—Mean Chl *a* concentrations ranged from  $1.8 \pm 0.1$  to  $5.5 \pm 0.2$   $\mu\text{g L}^{-1}$  in the water column, and from  $8.2 \pm 3.4$  to  $15.4 \pm 2$   $\mu\text{g g}^{-1}$  dry weight between 0 and 2 cm depth in sediment (Fig. 2). Significant increases in Chl *a* concentration were observed during the day (noon and dusk) in both the water column and the sediment surface (Table 1). In sediment, Chl *a* concentration in the upper 4 mm ranged from  $11.3 \pm 5.3$  to  $40.5 \pm 17.8$   $\mu\text{g g}^{-1}$  dry weight and decreased with depth (Table 2, Fig. 2). Chl *a* in the 4–8 mm depth horizon was less variable (from  $6.5 \pm 2.5$  to  $14.6 \pm 2.7$   $\mu\text{g g}^{-1}$  dry weight). No significant temporal variation in Chl *a* was observed below 8 mm (Table 1).

Water and sediment pheopigments were mainly comprised of pheophytin *a*, pheophytin *a*-like, pheophytin *b*, pheophorbide *a*, two pheophorbide *a*-like pigments, and chlorophyllide *a* (Table 2). No significant temporal variation in pheopigment concentrations was observed in water or sediment samples (Table 1). In the top 4 mm of sediment, total pheophytin *a* concentration (pheophytin *a* + pheophytin *a*-like) averaged 75% of the total pheopigment concentration. The Chl *a*/pheopigment ratio averaged 4 to 10 in the water column and 0.8 to 2 in the top 4 mm of the sediment over the sampling period.

Other than Chl *a*, the primary pigments in water and sediment samples were Chl *b*, lutein, zeaxanthin, peridinin, alloxanthin, and fucoxanthin (Table 2). In water samples, Chl *b* and lutein indicated that chlorophytes contributed to the

Table 1. Two-way ANOVAS of  $^{14}\text{C}$ -grazing rates by copepods and ostracods (light exposure and time of day), and of pigment concentrations in water and sediment (day and time of day) (0–4 mm depth for pigments other than Chl *a*). A posteriori tests (Tukey's) compare light (L) and dark (D) times of day (Da = dawn, No = noon, Du = dusk, Mi = midnight), and are listed in order of decreasing values. Different superscript symbols indicate significant differences. Where Light Exposure  $\times$  Time or Day  $\times$  Time interactions were significant, a posteriori comparisons for both days are shown. nsd = no significant differences.

Grazing			
Taxon	Factor		
	Light	Time	Light $\times$ Time
<i>Coullana</i> Females	0.003 D > L	0.03 No* Du†	0.149
<i>Coullana</i> Males	<0.001 D > L	0.670	0.815
<i>Coullana</i> Juveniles	<0.001 D > L	<0.001 No* Da† Mi† Du†	0.091
<i>M. littorale</i>	<0.001 L > D	<0.001 No* Da† Mi† Du†	0.076
<i>P. wellsi</i>	0.579	0.269	0.139
Ostracods	0.249	<0.001 No* Du† Mi† Da†	0.868
Pigment concentrations			
	Factor		
	Day	Time	Day $\times$ Time
Water			
Chl <i>a</i>	0.13	<0.001 Day 1: No* Du† Mi‡ Da‡ Day 2: No* Du*† Mi†‡ Da‡	<0.001
Chl <i>b</i>	0.029 1 > 2	<0.001 Day 1: Du* No† Mi‡ Da‡ Day 2: Du* Mi† No† Da†	0.045
Fucoanthin	<0.001 1 > 2	<0.001 Day 1: No* Du† Da† Mi† Day 2: No* Da† Mi† Du†	<0.001
Diadinoxanthin	0.74	<0.001 Day 1: No* Du† Mi† Da† Day 2: No* Da† Du† Mi†	<0.001
Zeaxanthin	<0.001 1 > 2	<0.001 Day 1: No* Du† Mi† Da† Day 2: nsd	0.001
Lutein	<0.001 1 > 2	<0.001 Day 1: No* Du† Mi‡ Da‡ Day 2: No* Da† Mi† Du†	<0.001
Peridinin	0.012 1 > 2	0.011 Day 1: Du* Da*† No*† Mi† Day 2: nsd	0.003
Pheophytin <i>a</i>	0.24	0.31	0.27
Pheophorbide <i>a</i>	0.95	0.53	0.09
Sediment			
Chl <i>a</i> (0–4 mm)	0.76	0.25 Day 1: No* Mi*† Da†‡ Du‡ Day 2: nsd	<0.001
(4–8 mm)	0.88	0.007 Du* Mi*† No† Da†	0.25
(8–12 mm)	0.50	0.036 Du* Mi*† No*† Da†	0.079
(12–20 mm)	0.90	0.84	0.44

Table 1. Continued.

Pigment concentrations	Factor		
	Day	Time	Day × Time
Fucoxanthin (0–4 mm)	0.63	0.12 Day 1: No* Mi**† Da† Du† Day 2: nsd	0.003
Diadinoxanthin (0–4 mm)	0.97	0.22 Day 1: No* Mi**† Da*† Du† Day 2: nsd	0.004
Zeaxanthin (0–4 mm)	0.19	0.73	0.19
Lutein (0–4 mm)	0.04	0.83	0.21
	1 > 2		
Pheophytin <i>a</i> (0–4 mm)	0.14	0.051	0.13
Pheophorbide <i>a</i> (0–4 mm)	0.56	0.17	0.12

planktonic microalgal community (Fig. 3). High zeaxanthin concentrations were also detected in water samples. Although zeaxanthin may be found as a minor pigment in chlorophytes, it is primarily a product of cyanobacteria (Brotas and Plante-Cuny 1998). Fucoxanthin may be produced by diatoms (Stauber and Jeffrey 1988) as well as other classes of brown microalgae (prymnesiophytes and chrysophytes). Biomarkers for prymnesiophytes and chrysophytes (19'-butanoyloxyfucoxanthin and 19'-hexanoyloxyfucoxanthin; Jeffrey et al. 1997) were, however, not detected in water or sediments, indicating that fucoxanthin mainly originated from diatoms. The presence of peridinin indicated that dinoflagellates contributed to the planktonic microalgal community. Some photosynthetic dinoflagellates may contain fucoxanthin (Jeffrey et al. 1997). However, peridinin was a minor pigment in water samples (Table 2) and, peridinin and fucoxanthin concentrations were not correlated over the sampling period ( $P = 0.67$ ), suggesting that dinoflagellates were not the primary origin of fucoxanthin. Finally, the presence of alloxanthin indicated that the planktonic microalgal community also included cryptophytes. Water concentrations of lutein (green microalgae), zeaxanthin (cyanobacteria), and fucoxanthin (diatoms) varied significantly over time (Table 1) and were correlated with Chl *a* concentration (analyses not shown). Relative percentages of the different classes of phytoplankton were determined using pigment ratios from pure cultures: Chl *a*/Chl *b* = 0.75 for green microalgae, Chl *a*/peridinin = 2.58 for dinoflagellates, Chl *a*/zeaxanthin = 1.7 for cyanobacteria, and Chl *a*/fucoxanthin = 1.4 for diatoms (Everitt et al. 1990). At noon, it was estimated that total planktonic algal biomass consisted of 38.5% diatoms, 35.5% cyanobacteria, 9.5% chlorophytes, and 5% dinoflagellates (Fig. 3); approximately 11.5% of the total Chl *a* pool originated from other microalgal groups including cryptophytes.

The primary non-Chl *a* pigments detected in sediments were fucoxanthin, diadinoxanthin lutein, and zeaxanthin (Table 2). Peridinin was not detected, suggesting that dinoflagellates did not contribute to the benthic community. Among

biomarker pigments for green microalgae, lutein was detected while Chl *b* was not. No significant diel variation of lutein or zeaxanthin in the top 4 mm of the sediment was detected (Table 1), suggesting that these carotenoids originated mainly from a sediment detritus pool. Because Chl *b* decays much faster than lutein (Bianchi et al. 1991), the absence of Chl *b* in sediment also suggests that live green microalgae were absent or poorly represented in sediment. Among biomarker pigments, significant diel variation was observed only in fucoxanthin and diadinoxanthin concentrations (Table 1). The Chl *a*/fucoxanthin ratio (Everitt et al. 1990) suggested that Chl *a* from diatoms represented between 70% (Da, Day-1) and 84% (No, Day-2) of the total Chl *a* pool in the top 4 mm of the sediment.

MDS analysis of pigments identified one sediment and two water microalgal groups (Fig. 4). The stress value was very low (0.02), indicating an excellent two-dimensional representation of similarities among samples. Chl *a* was the dominant pigment in each group. Accessory pigments that were responsible for most of the remaining variance in water groups were zeaxanthin, Chl *b*, fucoxanthin, and lutein, in that order. A small water group consisted of samples taken at Mi on Day-2, and differed from a larger water group (all other water samples;  $P = 0.001$ ) because of a lack of peridinin. A group consisting of all sediment samples differed from both water groups ( $P < 0.001$ ) because of the larger contribution of diatom pigments (fucoxanthin and diadinoxanthin). MDS analysis thus quantitatively confirms the qualitative differences in the composition of water and sediment communities illustrated in Fig. 3.

Correlations between diel variation in  $^{14}\text{C}$ -grazing rates and major water and sediment pigments (Table 4) illustrated that (1) grazing by adult female *Coullana* sp. was more strongly correlated with benthic than with planktonic microalgal biomass, while grazing by juvenile *Coullana* sp. was more closely associated with planktonic food, (2) feeding by *Coullana* sp. males showed no evidence of correlation with microalgal food availability, (3) feeding by *M. littorale* was not strongly correlated with microalgal pigments, (4)

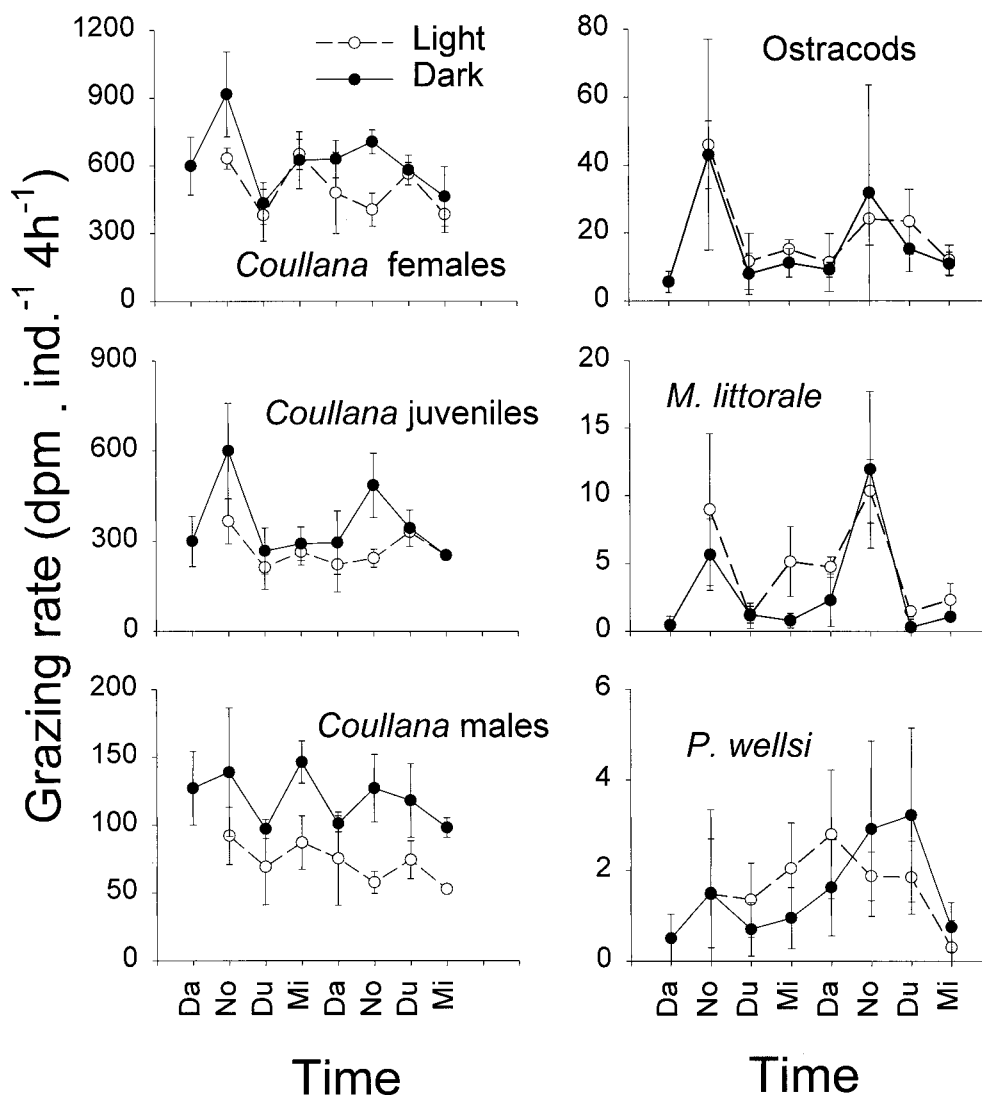


Fig. 1. Grazing by copepods and ostracods on  $^{14}\text{C}$ -labeled *I. galbana* at dawn (Da), noon (No), dusk (Du), and midnight (Mi), over a 48-h sampling period. Grazing is expressed in mean DPM per individual  $\pm$  SD ( $n = 4$ ), and was measured after incubation under artificial light and in the dark. Values for light incubations at dawn on Day-1 are missing because of malfunctioning lights at that time.

ostracod feeding was correlated with variation in benthic and planktonic microalgal biomass, and (5) exposure to light resulted in a closer association of grazing with benthic microalgae for *Coullana* sp. juveniles and adult males, but did not influence planktonic vs. benthic associations for other grazers.

**HPLC gut-pigment analyses**—Depending on the copepod species, 60 to 250 individuals per extract were required for HPLC detection of carotenoids. We were able to collect sufficient numbers of *Coullana* sp. for carotenoid analysis of copepods at both noon and midnight sampling times. *M. littorale* was sufficiently abundant to allow carotenoid analysis only at noon and midnight of Day-1. Analyses of chloropigments (Chl *a* and pheopigments) were, however, performed

for *Coullana* sp., *M. littorale*, and for *P. wellsi* at noon and midnight on both Days 1 and 2.

Fucoanthin was the major biomarker in *Coullana* sp. and *M. littorale* gut-pigment contents quantified from noon samples (Table 3, Fig. 5), indicating that these copepods fed on diatoms. For *Coullana* sp., Chl *b* was also present in Day-1 extracts (Fig. 6A). Because Chl *b* was detected in water but not in sediment (Figs. 6, 7), Chl *b* in *Coullana* sp. indicated that it fed at least in part on planktonic microalgae. However, zeaxanthin concentration in *Coullana* sp. guts never exceeded the values detected in starved copepods, suggesting that *Coullana* sp. did not consume the abundant planktonic cyanobacteria (Figs. 5, 6). Chl *b* was present only as a trace pigment in *Coullana* sp. from Day-2 samples, suggesting that consumption of planktonic algae

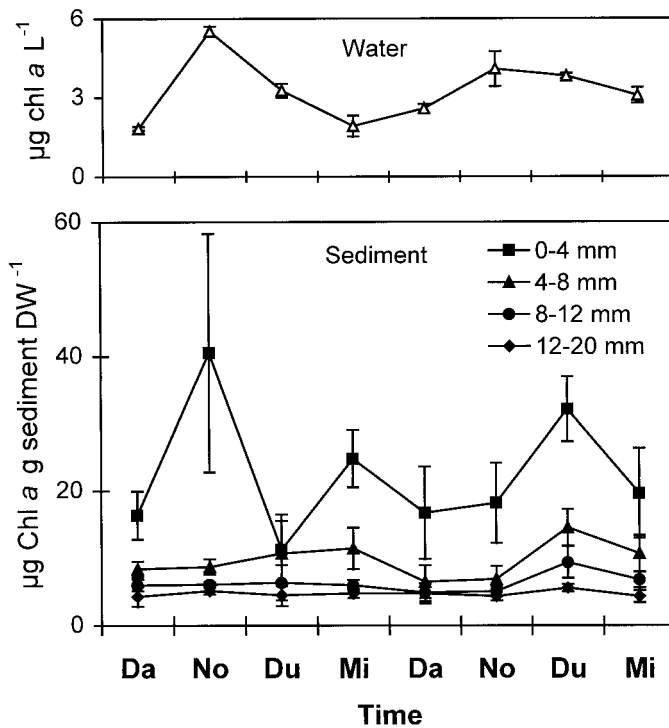


Fig. 2. Chl *a* concentrations in the water column and in sediment depth horizons, at dawn (Da), noon (No), dusk (Du), and midnight (Mi) over the 48-h sampling period. Sediment concentrations are normalized for sediment dry weight (DW). Error bars are  $\pm$  SD ( $n = 3$  for water samples,  $n = 4$  for sediment samples).

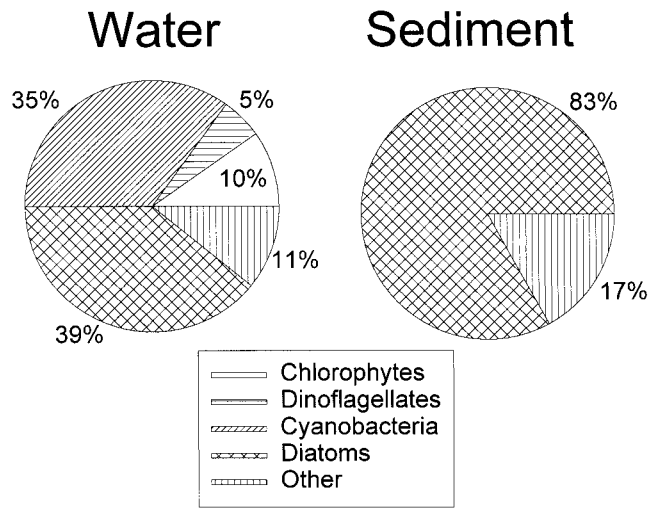


Fig. 3. Average community composition of phytoplankton (water) and benthic microalgae in the top 4 mm of the sediment surface (sediment), estimated for Day-1 noon sampling time ( $n = 3$  for water samples,  $n = 4$  for sediment samples).

was lower than on Day 1 (Fig. 6B). Fucoxanthin concentration in *Coullana* sp. was significantly higher at noon than at midnight ( $P < 0.001$ , Fig. 8), suggesting that *Coullana* sp. ingested diatoms at a higher rate during the day than at night.

Chloropigments in copepods were mainly represented by Chl *a*, pheophytin *a*, and pheophytin *a*-like, while pheo-

Table 2. HPLC analysis of major pigments in water and sediment extracts (0–4 mm). Pigments are listed following their elution order. Values are means  $\pm$  SD; highest and lowest mean concentrations over all sampling times are given. Likely algal sources were determined for the biomarker pigments according to the literature references listed in the table (Ref): 1, Jeffrey (1974); 2, Wright and Jeffrey (1987); 3, Stauber and Jeffrey (1988); 4, Wright et al. (1991); 5, Jeffrey et al. (1997). Probable algal sources (see text for rationale) are defined as follows: A, dinoflagellates; B, diatoms; C, green microalgae; D, detritus; E, cryptophytes; F, cyanobacteria; All, all microalgal classes. Symbols are as follows: \*, detected but not quantified; ND, pigment not detected. BDL = the detection limit.

No.	Pigment	Prob. source	Water concentration range ( $\mu\text{g L}^{-1}$ )	Prob. source	Sediment (0–4 mm) concentration range ( $\mu\text{g g}^{-1}$ )	Ref.
1	Chlorophyllide <i>a</i>				ND	
2	Peridinin	A	BDL–0.1 $\pm$ 0.01		ND	4
3	Fucoxanthin	B	0.3 $\pm$ 0.01–1.7 $\pm$ 0.2	B	6 $\pm$ 3–24 $\pm$ 11	2, 3
4	Neoxanthin	B	*		ND	5
5	Pheophorbide <i>a</i>		0.1 $\pm$ 0.01 $\ddagger$ –0.2 $\pm$ 0.01 $\ddagger$		2.3 $\pm$ 0.4 $\ddagger$ –4.9 $\pm$ 1.3 $\ddagger$	
6	Violaxanthin	B, C	*		ND	5
7	Diadinoxanthin	A, B	0.1 $\pm$ 0.01–0.5 $\pm$ 0.04	B	2 $\pm$ 0.8–5.5 $\pm$ 2.3	3, 4
8	Alloxanthin	E	*		ND	5
9	Diatoxanthin		ND	B	*	5
10	Lutein	C	0.1 $\pm$ 0.01–0.3 $\pm$ 0.02		1 $\pm$ 0.2–1.4 $\pm$ 0.1	1
11	Zeaxanthin	F	0.6 $\pm$ 0.02–1.1 $\pm$ 0.05	D	1 $\pm$ 0.3–1.8 $\pm$ 0.1	4
12	Chlorophyll <i>b</i>	C	0.4 $\pm$ 0.01–1 $\pm$ 0.05	D	ND	4
13	Chl <i>a</i> allomere		*		*	
14	Chlorophyll <i>a</i>	All	1.8 $\pm$ 0.07–5.5 $\pm$ 0.2		11 $\pm$ 5–41 $\pm$ 18	1
15	Pheophytin <i>b</i>		0.03 $\pm$ 0.03–0.06 $\pm$ 0.01	B	ND	
16	Pheophytin <i>a</i>		0.3 $\pm$ 0.03 $\ddagger$ –0.5 $\pm$ 0.1 $\ddagger$		8 $\pm$ 1.4 $\ddagger$ –13 $\pm$ 2.7 $\ddagger$	
17	$\beta$ -Carotene	All	0.1 $\pm$ 0.01–0.2 $\pm$ 0.01		0.5 $\pm$ 0.15–0.8 $\pm$ 0.17	1, 3
18	Pheophytin <i>a</i> -like $\ddagger$		*	B	*	

$\ddagger$  Two pheophorbide *a*-like compounds were additionally detected using the fluorescence detector, pheophorbide *a* quantification = pheophorbide *a* + pheophorbide *a*-like<sub>1</sub> + pheophorbide *a*-like<sub>2</sub>.

$\ddagger$  Pheophytin *a* quantification = Pheophytin *a* + Pheophytin *a*-like.

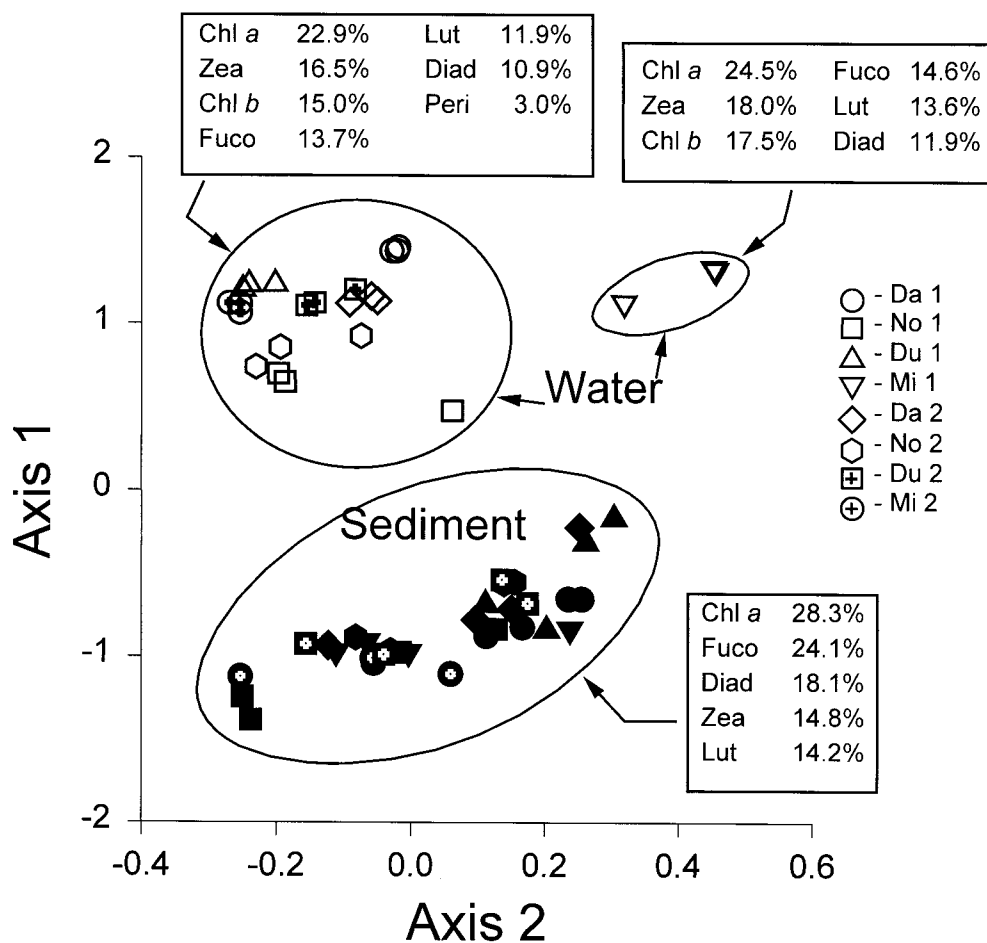


Fig. 4. Multi-dimensional scaling (MDS) analysis of photosynthetic pigments in sediment and water samples over two 24-h diel periods. Ellipses delineate three groups that were identified by the analysis. Open symbols are water, closed symbols are sediment. Values in boxes are variance explained by pigments in each group. Chl *a* = Chl *a*, Zea = zeaxanthin, Chl *b* = Chl *b*, Fuco = fucoxanthin, Lut = lutein, Diad = diadinoxanthin.

phorbide *a* was detected in *Coullana* sp. extracts only (Table 3, Figs. 5, 9). No significant temporal variation in pheophorbide *a* concentration was observed in *Coullana* sp. ( $P = 0.59$ ). In noon extracts, total pheophytin *a* concentration (pheophytin *a* + pheophytin *a*-like) averaged 61, 76, and

Table 3. Average gut pigment concentrations ( $\text{pg } \mu\text{g}^{-1}$  copepod DW) of adult-female, field-collected copepods collected at noon sampling times on days 1 and 2. ND: Not detected. Values are means  $\pm$  SD ( $n = 6$ ).

Pigment	<i>Coullana</i> sp.	<i>M. littorale</i>	<i>P. wellsi</i>
Fucoxanthin	26.2 $\pm$ 9.7	25.2 $\pm$ 1.4	No data
Pheophorbide <i>a</i>	1 $\pm$ 0.7	ND	ND
Lutein	7 $\pm$ 1	ND	No data
Chlorophyll <i>b</i>	1.7 $\pm$ 1.3	ND	ND
Chl <i>a</i> allomere	0.2 $\pm$ 0.1	4.2 $\pm$ 3.2	0.4 $\pm$ 0.8
Chlorophyll <i>a</i>	1.1 $\pm$ 1.1	15.2 $\pm$ 14.3	2 $\pm$ 1.9
Pheophytin <i>a</i>	2.5 $\pm$ 1.3	20.8 $\pm$ 8	33 $\pm$ 11
$\beta$ -Carotene	6.8 $\pm$ 3.7	1.8 $\pm$ 2.6	No data
Pheophytin <i>a</i> -like	2.9 $\pm$ 2.2	24.6 $\pm$ 11	68.7 $\pm$ 14.3

97% of total chloropigment concentration (Chl *a* + pheophytin *a* + pheophytin *a*-like + pheophorbide *a*) for *Coullana* sp., *M. littorale*, and *P. wellsi*, respectively. Total pheophytin *a* concentration varied significantly over time for all copepod species (two-way ANOVA,  $P < 0.001$ ), and concentrations were significantly higher at No than at Mi. No and Mi concentrations of Chl *a* in copepod guts did not differ significantly ( $P = 0.927$ ). Among species, Chl *a* content was significantly higher in *M. littorale* than in *Coullana* sp. and *P. wellsi* ( $P < 0.001$ , Fig. 9). Total pheophytin *a* was significantly more concentrated in *P. wellsi* ( $59.7 \pm 32.8 \text{ pg } \mu\text{g}^{-1}$ ) than in *M. littorale* ( $26.5 \pm 18.8 \text{ pg } \mu\text{g}^{-1}$ ), and both species contained more pheophytin *a* than did *Coullana* sp. ( $3.4 \pm 2.6 \text{ pg } \mu\text{g}^{-1}$ ) ( $P < 0.001$ , Fig. 9).

## Discussion

Our observations generally indicate midday peaks in meiofaunal consumption of microalgae, but that diel responses vary among taxa and demographic groups. Co-variation of environmental factors may complicate interpretation of diel-

Table 4. Pearson's correlations of  $^{14}\text{C}$ -grazing rates with sediment (0–4 mm) and plankton pigment concentrations over two 24-h diel periods. 'Dark incubations' indicate cores that were incubated in darkness; 'Light incubations' indicate cores that were incubated under fluorescent illumination. F = females, M = males, J = juveniles.  $n = 8$  for dark incubations,  $n = 7$  for light incubations.

Pigments	Taxon					
	<i>Coullana</i> F	<i>Coullana</i> M	<i>Coullana</i> J	<i>P. wellsi</i>	<i>M. littorale</i>	Ostracods
Dark incubations						
Sediment						
Chl <i>a</i>	0.718†	0.572	0.684*	0.386	0.095	0.682*
Fucoxanthin	0.714†	0.544	0.704*	0.439	0.141	0.708†
Zeaxanthin	0.229	0.533	-0.016	-0.025	-0.387	-0.078
Water						
Chl <i>a</i>	0.595	0.116	0.862†	0.507	0.572	0.882‡
Chl <i>b</i>	-0.127	-0.180	0.095	0.245	-0.139	0.159
Fucoxanthin	0.860†	0.363	0.970‡	0.311	0.687*	0.963‡
Zeaxanthin	0.461	-0.06	0.721†	0.68*	0.471	0.73†
Light incubations						
Sediment						
Chl <i>a</i>	0.785†	0.628	0.987‡	-0.123	0.313	0.849†
Fucoxanthin	0.761†	0.594	0.989‡	-0.126	0.332	0.857†
Zeaxanthin	0.796†	0.710*	0.723*	0.260	-0.084	0.469
Water						
Chl <i>a</i>	0.110	0.149	0.682*	-0.281	0.480	0.885‡
Chl <i>b</i>	0.0165	0.177	0.324	-0.204	-0.428	0.242
Fucoxanthin	0.269	0.320	0.603	-0.104	0.781†	0.911‡
Zeaxanthin	0.0344	-0.068	0.672*	-0.257	0.328	0.712*

\*  $0.10 \geq P > 0.05$ .

†  $0.05 \geq P \geq 0.005$ .

‡  $P < 0.005$ .

feeding patterns. For example, water cover may influence harpacticoid feeding rates (e.g., Decho 1986, 1988; Souza-Santos et al. 1995; Pace and Carman 1996). In our study, however, water cover was essentially constant throughout the study period. Temperature variation can also influence grazing rates (e.g., zooplankton, Dagg and Wyman 1983). In the present study, however,  $^{14}\text{C}$ -grazing experiments were conducted under constant temperature, and in situ temperature varied only from 29.4 to 32.2°C. Thus, temperature and water cover should not have influenced feeding rates. Our observations do indicate, however, that grazing rates were influenced by functional responses to variable food supply, exposure to light, and/or entrained diel-feeding rhythms.

Collective consideration of  $^{14}\text{C}$ -grazing rates and gut pigments, and their relationship to diel variation of benthic and planktonic microalgal biomass, suggest qualitatively different feeding strategies among taxa. *Coullana* sp.  $^{14}\text{C}$ -grazing rates peaked at noon, but were diminished by exposure to light (Fig. 1). Although we did not measure in situ penetration of light to the benthos, light penetration through the turbid water overlying sediments was probably substantially less than light conditions used in  $^{14}\text{C}$ -grazing experiments. Elevated gut pigments at noon relative to midnight in field-collected *Coullana* sp. supports the conclusion that light did not significantly diminish the daytime grazing peak in situ (Figs. 8, 9). Grazing by adult female *Coullana* sp. was more

strongly correlated with benthic than with planktonic microalgal biomass, while grazing by juvenile *Coullana* sp. was more closely associated with planktonic food (Table 4). Juvenile *Coullana* sp. spend more time in the water column than do adult females (Huys et al. 1986; Kern 1990), and thus their feeding affinities parallel their preferred habitats. The correlation of feeding activity with microalgal resources (benthic and/or planktonic) is consistent with the hypothesis that the diel-feeding pattern of *Coullana* sp. reflects a functional response to variation in available food. *Coullana* is negatively phototactic (Harris 1977), and the negative influence of light on feeding suggests that functional responses may be diminished, or preferences shifted from planktonic to benthic resources when light levels are high (e.g., midday low tides). *Coullana* sp. is most abundant in subtidal habitats (Fleeger pers. obs.), and its aversion to light may be a mechanism for maintaining a subtidal existence. In contrast to females and juveniles, feeding by *Coullana* sp. males showed no evidence of diel variation (Fig. 1) or correlation with microalgal food availability (Table 4). It is possible that *Coullana* sp. males focus primarily on mating, while juveniles and adult females devote more time to feeding to provide energetic requirements for growth and reproduction.

*M. littorale*  $^{14}\text{C}$ -grazing rates indicated a distinct midday feeding peak that was enhanced by exposure to light (Fig. 1). Although microalgal biomass was highest during daylight

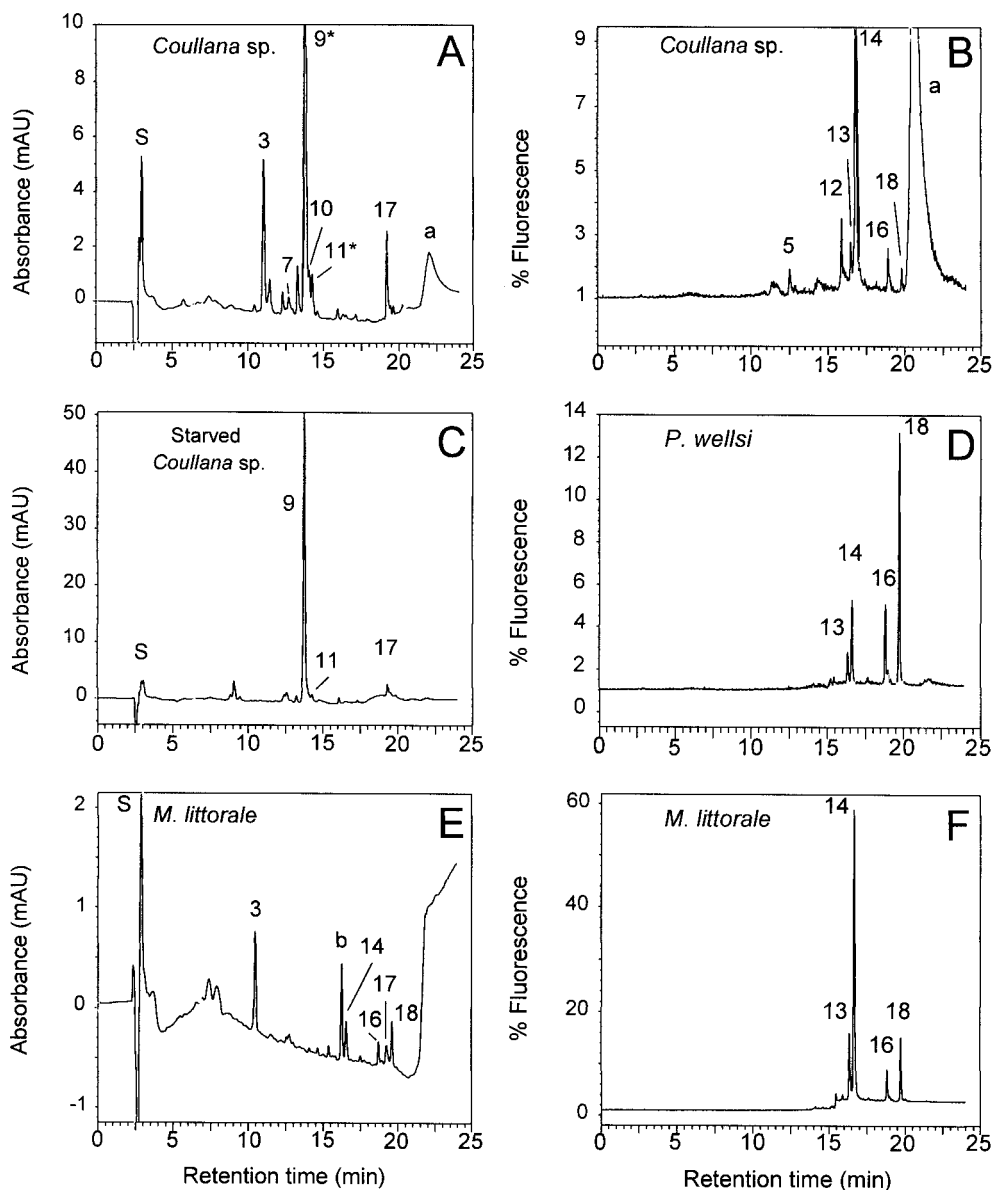


Fig. 5. HPLC chromatograms for extracts of adult-female *Coullana* sp., *M. littorale*, and *P. wellsi*. Absorbance chromatograms at 436 nm (A, E) and fluorescence chromatograms (B, D, F), from extracts of harpacticoid copepods collected at noon, during the first day (B, D, E, F) and the second day (A). Samples consisted of 200 females (A), 218 adults (E, F), 100 females (B), and 33 adults (D). Absorbance chromatogram "C" (436 nm) was obtained from the extract of starved *Coullana* sp. (258 females). For peak identification see Table 2. Symbol "\*" designates pigments that did not originate from gut content (see text). Letter "a" designates a wide artefact peak occurring on both absorbance and fluorescence chromatograms from *Coullana* sp. extracts only. Letter "b" designates a peak representing coelution between a Chl *a* derivative and an unidentified carotenoid. "S" = solvent peak. "mAU" = milli-Absorbance Units; "% fluorescence" = relative fluorescent units.

hours, feeding activity by *M. littorale* was not strongly correlated with general (Chl *a*) or specific (carotenoid) indicators of microalgal biomass (Table 4). This pattern contrasts with those observed for *Coullana* sp. females and juveniles, and suggests that a functional response to food availability was not the primary factor influencing *M. littorale* feeding; rather, its feeding pattern is more consistent with an en-

trained diel-feeding rhythm that is enhanced by exposure to light. At low tide, *M. littorale* feeds extensively on microphytobenthos and its distribution is positively correlated with sedimentary Chl *a* (Decho and Fleeger 1988); during periods of water cover, however, it makes frequent excursions into the water, where it presumably feeds (Decho 1988). This variable feeding behavior may have contributed to the lack

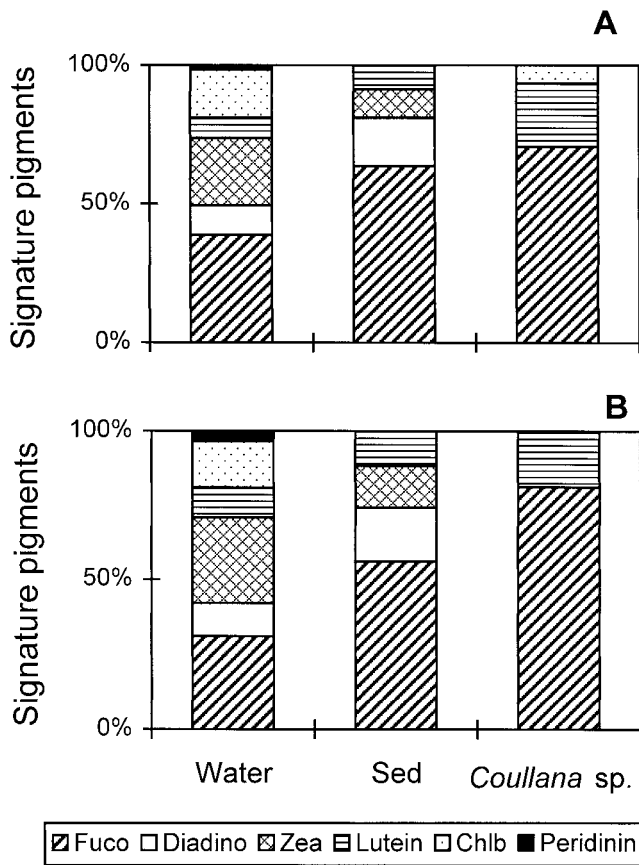


Fig. 6. Mean proportional abundances of pigments in water samples (water), 0–4 mm depth sediment samples (sed), and in field-collected, adult-female *Coullana sp.* Samples were collected at noon during the first (A) and second (B) sampling days. Fuco = fucoxanthin, Diadino = diadinoxanthin, Zea = zeaxanthin, and Chl b = Chl b. “mAU” = milli-Absorbance Units.

of strong correlation between feeding activity and benthic or planktonic microalgal resources. Nevertheless, as was observed in *Coullana sp.* and *P. wellsi*, pheophytin *a* in *M. littorale* guts was higher at noon than at midnight (Fig. 9), providing further evidence of a midday feeding peak. In contrast to *Coullana sp.* and *P. wellsi*, however, Chl *a* in *M. littorale* guts appeared to peak at midnight. We can only speculate that this inverse relationship between pheophytin *a* and Chl *a* may be indicative of relatively low digestive activity during nonpeak feeding periods, as has been observed in some calanoid copepods (Nott et al. 1985). It is clear, however, that *M. littorale*'s feeding strategy differs from *Coullana sp.*: Variable feeding by *M. littorale* appears to be primarily a response to entrained diel rhythms, while *Coullana sp.* feeding varies with food availability.

As was observed in this study, Pace and Carman (1996) detected no relationship between grazing by *P. wellsi* and available microalgal biomass (Chl *a*). The midday peak of pheophytin *a* in *P. wellsi* guts, however, indicated diel variation in feeding that was not reflected in <sup>14</sup>C-grazing experiments. *P. wellsi* lives in mucus tubes, and may be restricted to feeding on microorganisms growing on the inner walls of tubes (Chandler and Fleeger 1984). If so, it is likely

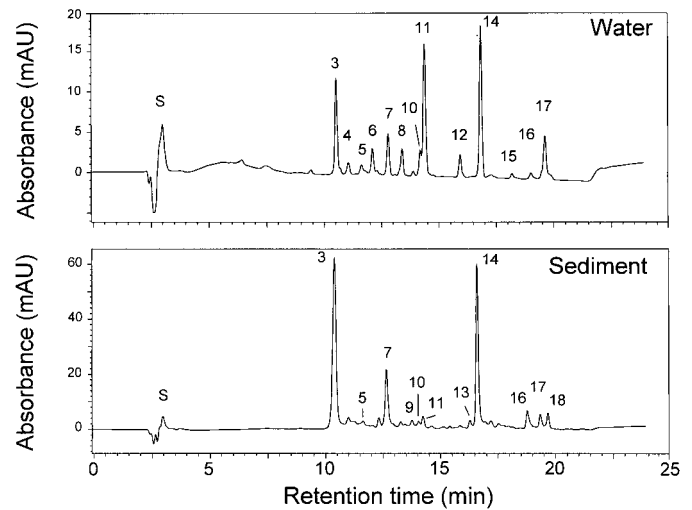


Fig. 7. Absorbance chromatograms of extracts from water samples (water) and 0–4 mm depth sediment samples (sediment) collected at noon during the first sampling day. For peak identification see Table 2. S = solvent peak. “mAU” = milli-Absorbance Units; “% fluorescence” = relative fluorescent units.

that labeled food added exogenously would not be extensively consumed (Pace and Carman 1996). As observed by Pace and Carman (1996), pheopigment concentrations in *P. wellsi* guts were considerably higher than in other species, which may reflect interspecific differences in digestive processing of ingested microalgae, or ingestion of a qualitative-

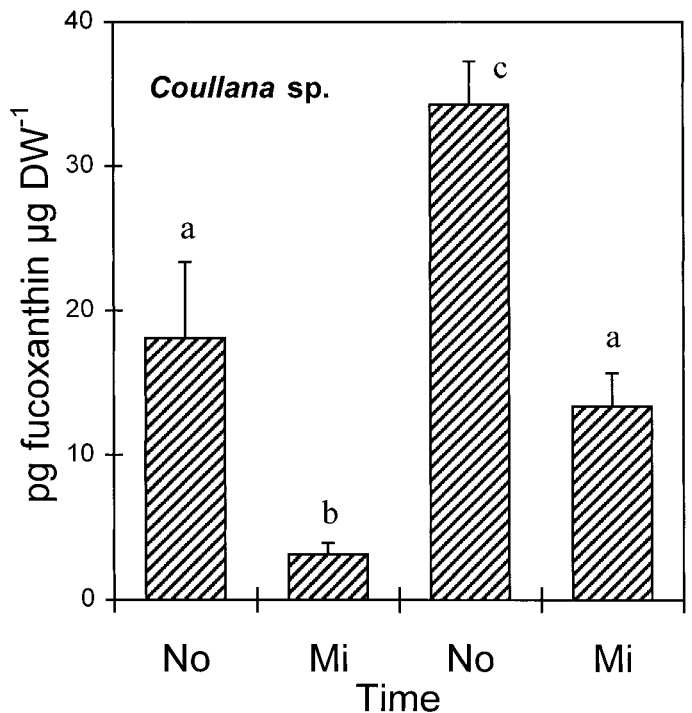


Fig. 8. Fucoxanthin gut content of adult-female *Coullana sp.* at noon (No) and midnight (Mi) over the 48-h sampling period. Different letters indicate significant differences among times ( $P < 0.05$ ). Error bars are 1 SD ( $n = 3$ ).

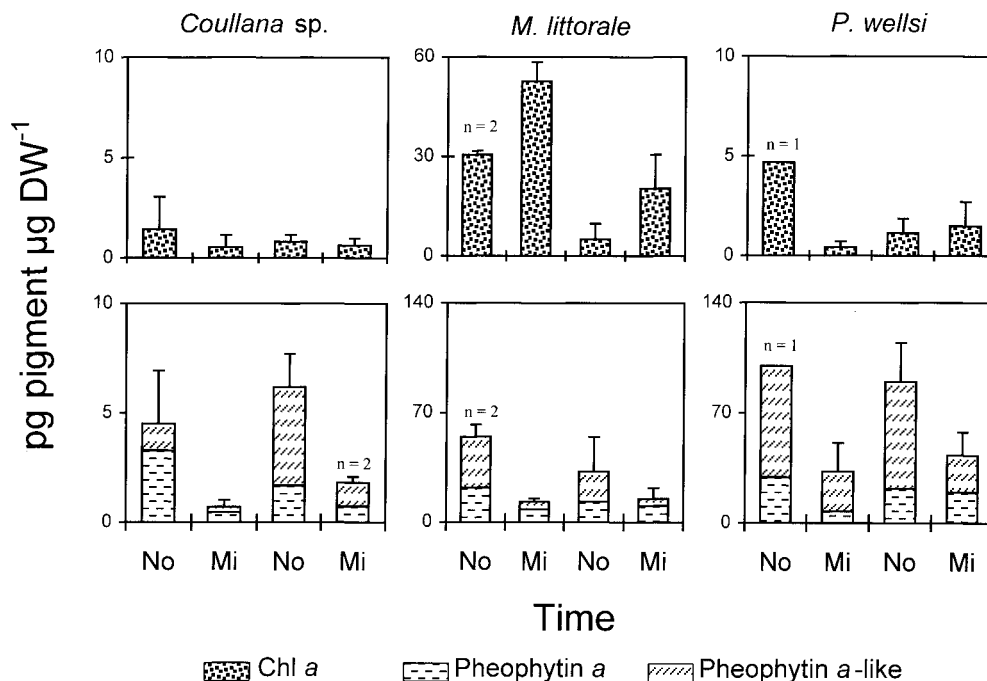


Fig. 9. Chl *a* and total pheophytin *a* (pheophytin *a* + pheophytin *a*-like) in gut contents of adult-female *Coullana* sp., *M. littorale*, and *P. wellsi* at noon (No) and midnight (Mi) over the 48-h sampling period. Error bars are 1 SD ( $n = 3$ ).

ly different food source (e.g., detrital material). Clearly, however, feeding by *P. wellsi* differs significantly from other copepod species and merits further study.

The midday feeding peak by ostracods was qualitatively similar to those observed for *Coullana* sp. (females and juveniles) and *M. littorale*. Ostracod feeding was also similar to *Coullana* sp. and differed from *M. littorale*, and *P. wellsi*, in that it was correlated with variation in benthic and planktonic microalgal biomass (diatoms in particular, Table 4), and thus suggestive of a functional response to food resources. Unlike *Coullana* sp. and *M. littorale*, ostracod feeding was not influenced by exposure to light. Despite the ubiquitous occurrence of ostracods, studies of their feeding habits are rare. Grant et al. (1983), however, reported that grazing on blue-green algae by the ostracod *Cyprinotus carolinensis* was not influenced by photoperiods. Ólafsson et al. (1999) observed that consumption of phytodetritus by ostracods represented 46% of total meiofaunal grazing, and recently completed quantitative feeding studies in our lab also indicate that feeding by ostracods can represent a substantial fraction of total benthic grazing (Goldfinch and Carman in prep.). Thus, ostracods may represent an important, but understudied component of benthic food webs.

Mathematical models predict (e.g., Dade et al. 1990), and experimental evidence (e.g., Karrh and Miller 1994) indicates that feeding rates of macrofaunal deposit feeders increase up to a saturation point with increasing food concentration. Although little is known about meiofaunal functional responses to food availability, harpacticoids and ostracods can exponentially increase grazing rates in response to increased microphytobenthic biomass (Montagna et al. 1995), but functional responses vary among species (Pace and Car-

man 1996). While our results are qualitatively consistent with these previous studies, the species-specific responses that we observed differed from those of Pace and Carman (1996), possibly as a consequence of differences in sampling time scales (6 h in the present study and 7–14 d in Pace and Carman 1996). Indeed, Bock and Miller (1999) showed that macrofaunal polychaetes respond to changes in resource availability within 1–2 h. Our data indicate that variation in feeding by ostracods and *Coullana* sp. females and juveniles is consistent with a functional response to food availability within a diel cycle. However, variation of *M. littorale*'s feeding activity was indicative of an entrained feeding rhythm, and *Coullana* sp. males displayed no apparent diel variation in feeding. Thus, responses to variation in food resources vary among and within taxa, and it would be inappropriate to draw the general conclusion that a functional response to resource availability is the predominant factor that influences meiofaunal feeding rates.

HPLC analysis of gut pigments has been successfully used in previous studies of dietary selectivity by planktonic (e.g., Kleppel et al. 1988; Head and Harris 1994) and benthic copepods (Buffan-Dubau et al. 1996), and is attractive because it allows an evaluation of grazer exploitation of the in situ microalgal community. Nevertheless, gut-pigment analysis of in situ feeding by copepods has been criticized because ingested pigments may be converted into colorless products (review by Head and Harris 1996). Peaks in pheophytin *a* and fucoxanthin concentrations in *Coullana* sp. and *M. littorale* guts (Figs. 8, 9) were coincident with peaks of <sup>14</sup>C-grazing rates (Fig. 1), and thus these pigments appear to be qualitative indicators of recent feeding activity. Chl *a*, however, was not a good indicator of diel variation in

grazing rates: Either no significant variation in Chl *a* was observed (*Coullana* sp. and *P. wellsi*) or Chl *a* variation was not consistent with grazing-rate variability (*M. littorale*).

HPLC analyses of water and sediment pigments indicated that microphytobenthos were strongly dominated by diatoms, while plankton was a mixture of diatoms, cyanobacteria, green microalgae, and dinoflagellates (Fig. 3). Diatom dominance in microphytobenthic communities of shallow-water muddy environments is common (MacIntyre et al. 1996). While resuspension of microphytobenthos (via tidal currents, wind-waves, and bioturbation) and settling of phytoplankton can blur the distinction between benthic and planktonic microalgal communities (MacIntyre et al. 1996; Blanchard et al. 1997), our results show that the composition of phytoplankton and microphytobenthos may be quite different even in shallow environments; this difference facilitated the use of pigment analyses to determine if benthic animals were exploiting planktonic resources. Gut-pigment analyses of copepods indicated that diatoms contributed significantly to the natural diets of *Coullana* sp. and *M. littorale* (Fig. 5), which is consistent with laboratory and field studies that have found diatoms to be an important food source for these copepods (Decho 1988), and implicates microphytobenthos as an important resource. The presence of Chl *b* in *Coullana* sp. guts provides field verification of previous studies (Decho 1986; Pace and Carman 1996) which indicate that *Coullana* sp. feeds on suspended microalgae in addition to microphytobenthos. The lack of zeaxanthin in *Coullana* sp. guts, however, suggests that it avoided ingestion of abundant planktonic cyanobacteria (Fig. 6A). While it is known that grazers may avoid consumption of toxic cyanobacteria (O'Neil and Roman 1994), we cannot be certain as to the mechanism(s) responsible for *Coullana*'s avoidance of cyanobacteria in their natural environment. Further study is needed to evaluate the quantitative importance of benthic vs. planktonic microalgae for meiofauna.

The diel cycles that we detected suggest that significant quantities of microphytobenthic biomass were being removed from the system on a daily basis (Fig. 2). There was very little water movement throughout the study period, and thus it is unlikely that advection contributed significantly to variability in microalgal biomass. In sediments, benthic diatoms can move vertically within the sediment and thus redistribute microphytobenthic biomass (MacIntyre et al. 1996; Paterson et al. 1998). In our samples, Chl *a* concentration in the top 4 mm of sediment was always much higher than in deeper layers (Fig. 2), and Chl *a* in the 0–4 mm layer was positively correlated with Chl *a* in deeper layers over time (statistics not shown); we therefore conclude that diel variation of microphytobenthic biomass was not primarily a consequence of vertical migration. Removal by grazing appears to be the most logical explanation for diel variation in microalgal biomass, and the midday peak in meiofaunal feeding rates (Figs. 1, 8, 9) is consistent with this explanation. Diel variability in planktonic microalgal biomass has been linked to variable grazing pressure and microalgal growth (e.g., Le Bouteillier and Herbland 1982), but no analogous observations have been made in benthic systems.

It is possible, however, that diel migration of diatoms within the top 4 mm of sediment influenced feeding by meiofauna. Specifically, a concentration of diatoms at the sediment-water interface during daylight hours (MacIntyre et al. 1996) would yield a more concentrated food source for grazers, which could increase their feeding efficiency and ultimately yield higher grazing rates. Similarly, dispersion of diatoms below the sediment surface at night could lead to reduced feeding efficiency and lower grazing rates. Thus, while we have suggested that the diel-feeding patterns exhibited by *M. littorale*, *Coullana* sp., and ostracods are consistent with entrained rhythms or functional responses to food availability, another plausible explanation is that feeding rates are constant, but that ingested microalgal biomass varies in response to its concentration in surficial sediments.

Trophic interactions between grazers and microalgae in coastal zoobenthos apparently differ qualitatively from those that occur in the plankton. Zooplankton feeding typically peaks at night, and is a consequence of diel patterns of vertical migration by grazers, which consume phytoplankton that remain in a relatively constant position in the photic zone (Durbin et al. 1990). It is well established that meiofauna migrate vertically within sediment (Foy and Thistle 1991) and between sediment and water (Bell et al. 1988; Shanks and Edmonson 1990), but there is no evidence that meiofaunal migration is related to variation in feeding activity. Our data show that, in contrast to zooplankton, meiofaunal feeding peaks during midday and is influenced by diel variation in food availability, which in turn may be influenced by mm-scale migrations of benthic diatoms.

The general covariation of microalgal biomass with elevated feeding activity that we observed is consistent with the hypothesis of grazer control of microalgal biomass (Carman et al. 1997). Thus, meiofauna in this system may contribute significantly to the regulation of microphytobenthic biomass, and by extension, as an important trophic link between primary production and higher-level consumers. If correct, this conclusion has important implications for understanding the food-web structure of coastal ecosystems. While stable-isotope studies implicate microphytobenthos as an important source of primary production for higher-level consumers in coastal food webs (Peterson and Howarth 1987; Sullivan and Moncreiff 1990; Currin et al. 1995), very little is known about how this production is transferred to higher trophic levels. If meiofaunal grazing regulates microphytobenthic biomass, and microphytobenthic production supports a large fraction of secondary production, meiofauna should represent a critical link between primary producers and higher trophic levels. Further, because meiofaunal consumption of microalgae varies over a diel cycle and is influenced by a variety of species and taxon-specific factors (i.e., functional responses, light exposure, and entrained rhythms), the transfer of primary production to secondary consumers may depend critically on meiofaunal community composition and prevailing environmental conditions such as midday low tides. Future research should examine the influences of environmental factors such as tidal fluctuation, light exposure, and temperature variation on diel- and longer-term feeding patterns. The costs (e.g., predation exposure) and benefits of midday feeding peaks by benthic consumers

should also be examined. More generally, a systematic, quantitative field study is needed to fully evaluate the relationships between microphytobenthos, benthic grazers, and higher-level consumers (Miller et al. 1996).

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