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Feeding and Microspatial Relationships of Meiobenthic Harpacticoid Copepods With Microbial Flora.

Alan William Decho
Louisiana State University and Agricultural & Mechanical College

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Feeding and microspatial relationships of meiobenthic harpacticoid copepods with microbial flora

Decho, Alan William, Ph.D.
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Feeding and Microspatial Relationships of Meiobenthic Harpacticoid Copepods With Microbial Flora

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

The Department of Zoology and Physiology

by

Alan William Decho

M.S., Ohio University, 1981
August 1987
ACKNOWLEDGMENTS

I dedicate this work to my parents, Susan and Mr. William Decho, who patiently and faithfully supported me in the pursuit and fulfillment of this endeavor, which was once only a child's dream. To this I plead eternal gratitude.

I thank my advisor and good friend, Dr. John W. Fleeger, who so often assisted, corrected and inspired me in my development and focus as a scientist. I also thank my dissertation committee members: Drs. William Stickle and Earl Weidner, who have been both friends and colleagues to me, Drs. Donald Boesch, James Geaghan, Marion Socolofsky and John Larkin who provided help and constructive support to this dissertation, and finally the many individuals who assisted me in countless ways during my five years at LSU pursuing this degree.
FORWARD

Harpacticoid copepods are small crustaceans (actual lengths are from 0.25-1.50 mm in size) which are commonly associated with the sediments (meiobenthic) of marine and estuarine environments. While they are typically the second most numerous taxon within the meiobenthos (nematodes are usually more abundant), they represent a potentially important link in the food webs and energy flow dynamics of marine systems. Their importance is two fold. First, (in the lower food web) they consume both bacteria and diatoms, and therefore are tightly coupled to grazing and detrital food chains. Second, they themselves are consumed (as a food resource) preferentially by the juvenile stages of many fish and macroinvertebrates, and therefore are important to higher trophic levels.

In understanding the role of these animals in food webs and overall marine energy flow, the basic mechanisms of the microbe→harpacticoid copepod→macroconsumer step must also be understood.

It is the purpose of this dissertation to focus on the lower portion of this step, involving microbe→harpacticoid copepod interactions. More specifically its purpose is to investigate the feeding processes of mudflat harpacticoids on microbial flora (i.e. the processes of ingestion and assimilation, and what factors influence these processes). Also, to investigate whether these feeding processes influence the microspatial patterns of harpacticoids in the field (and ultimately their potential availability to higher trophic levels).

This study is composed of four chapters, in the form of
submitted or published manuscripts, which are designed to address the following questions:

1) **What are the predominant microbial food resources ingested and assimilated by harpacticoids?** This question is addressed in the manuscript: Ontogenetic feeding shifts in the meiobenthic harpacticoid copepods *Nitocra lacustris*; submitted to Marine Biology.

2) **Are ingestion rates of food resources constant over different portions of a tidal cycle (Field Study)?** This question is addressed in the manuscript: How do meiofauna grazing rates differ over a tidal cycle?: Field verification using chlorophyll-pigment analyses; submitted to Limnology and Oceanography.

3) **How are feeding rates modified by the presence or absence of water-cover, i.e. simulated high-tide vs. low-tide (Laboratory Study)?** This question is addressed in the published manuscript: Water-cover influences on diatom ingestion rates by meiobenthic copepods. Marine Ecology Progress Series, 33:139-146.

4) **How do feeding processes affect the microspatial patterns of harpacticoids in the field (and ultimately the availability of harpacticoids to macroconsumers)?** The question is addressed in the manuscript: Microspatial distributional responses of meiobenthic harpacticoids to food resource patchiness; to be submitted to Journal of Experimental Marine Biology and Ecology.

In the first chapter, it is shown that diatoms are a major food resource ingested and assimilated by the adult stages of the harpacticoid *Nitocra lacustris*. Adult stages also secondarily ingest sediment particles, bacteria, and probably the mucus-exopolymer secretions of both bacteria and diatoms (coincidently ingested while
consuming diatoms). However, significant feeding shifts in food resource utilization occur between adult and naupliar stages. Naupliar stages do not ingest diatoms, but instead scrape the outside of frustules (ingesting attached bacteria, some sediment particles, and probably mucus exopolymer secretions associated with these microbial flora). Data derived from $^{14}$C- and $^3$H-radiolabelling experiments, and gut pellet analyses (showing the presence of diatom frustules) were used in this study.

In the second chapter, similar techniques were used to determine the utilization of diatoms by three harpacticoid species commonly occurring on an intertidal mudflat. Feeding rates on diatoms (as measured by ingestion of chlorophyll-pigments) were measured over different portions of a tidal cycle in the field.

In the third chapter, controlled laboratory feeding experiments using $^{14}$C-radiolabels were conducted to understand how the feeding strategies (benthic or planktonic) used by these species, are influenced by changes over a tidal cycle, and how these changes ultimately affect their feeding rates on diatoms.

In chapter four, microspatial (cm to mm) small-scale species dispersions were observed in field sediments during high-tide and low-tide by spatial autocorrelation. The degree of patchiness was then examined for associations with microscale patchiness of microbial food resources (i.e. diatoms), as measured by chlorophyll-pigment analysis through correlative associations. Laboratory preference experiments were conducted to determine if microbial food patches can cause formation of harpacticoid species patches, and if so, what is the mechanism of attraction (i.e. chemical exudates, etc.). These attractive responses to microbial food resources were
related to the feeding strategies used by the various harpacticoid species. These relationships would help determine under what conditions (i.e. tidal stage) food patchiness is a causal mechanism of harpacticoid species aggregations in natural sediments.
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Feeding processes and their relationship to microspatial patterns were examined for several harpacticoid species. Field evidences and laboratory radiolabel experiments suggest that diatoms comprise a major portion of the food resources ingested by harpacticoids. Bacteria and mucus-exopolymer secretions (associated with the bacteria and diatoms) are also ingested, coincidently while ingesting diatoms. Also, significant ontogenetic shifts in food resource utilization occur from naupliar to adult stages.

Field grazing rate studies were conducted on an intertidal mudflat over different portions of a tidal cycle using several harpacticoid species (*Scottolana canadensis*, *Microarthridion littorale*, *Paronychocamptus huntsmani*). Highest grazing rates for *S. canadensis* occurred just after the mudflat becomes exposed (i.e. early low-water ELW) with very low grazing rates during late low-water (i.e. after mudflat is exposed for several hours LLW). *M. littorale* also showed highest grazing rates during ELW. Similar grazing rates occurred during high-water (HW) and LLW.

Laboratory experiments indicated that *M. littorale* maintained similar grazing rates at HW and LLW by changing its food resource utilization. During HW, *M. littorale* feeds planktonically while swimming in the water column. During LLW it feeds benthically while actively crawling over the sediment surface. *S. canadensis* feeds during HW by drawing suspended plankton into its burrow. During LLW feeding is greatly reduced by the absence of water cover.

Microspatial (mm) patterns of harpacticoids in the field as determined by spatial autocorrelation indicate that at low-water high
density patches of the harpacticoid *M. littorale* were positively correlated with microbial food resource patchiness (as measured by chlorophyll-a concentrations). Laboratory experiments show that this harpacticoid actively seeks out sediment patches containing high concentrations of diatom food resources and their exudates. Microspatial patchiness of *M. littorale* may be regulated by patchiness of its microbial food resources. During HW patchiness patterns disappear as individuals leave the sediment to feed in the water column. The patchiness of *S. canadensis* is not correlated with benthic food abundances and this harpacticoid does not actively migrate to food patches in the laboratory. Its microspatial patchiness is probably regulated by other factors. Mechanisms of microspatial patchiness for intertidal mudflat harpacticoids vary depending on the species and portion of the tidal cycle examined.
CHAPTER ONE

TITLE: ONTOGENETIC FEEDING SHIFTS IN THE MEIOBENTHIC HARPACTICOID

COPEPOD Nitocra lacustris

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Running Head: Copepod Adult and Nauplii Food

submitted to Marine Biology
Differential ingestion and utilization of microbial food resources by adult and naupliar stages of the salt marsh harpacticoid copepod *Nitocra lacustris* were examined with laboratory and field techniques. 

$^{14}$C-radiolabelling experiments indicate that adult stages probably receive a large part of their nutrition by the ingestion and assimilation of certain diatoms. Observations of field-collected individuals support these findings, showing an abundance of empty diatom frustules in their gut-pellet contents. However, naupliar stages do not ingest diatoms in the laboratory, and field-collected individuals do not contain frustules in their gut-pellets. In the laboratory ingestion of diatoms appears to first occur during the second or third copepodite stage.

Both adults and nauplii ingest bacteria adhering to the outer diatom mucus coating (and probably ingest the diatom mucus itself) as evidenced by $^3$H-radiolabel experiments, and grazing experiments using bacterium-sized beads adhering to the diatoms. Adults ingest bacteria (and probably mucus exopolymer) coincidently while ingesting diatoms. The nauplii however, ingest these components by scraping the outer surface of the diatoms (SEM observations indicate that diatoms are not punctured by the nauplii during feeding). Preliminary available evidence indicates that while diatom-mucus and associated bacteria play a role (as yet unquantified) in the nutrition of the adult, these components may comprise the bulk of food-resources for naupliar stages.
INTRODUCTION

Studies of food resource utilization by the meiobenthos have progressed substantially in the past decade. The use of radioisotopes and other methodologies have allowed examination of feeding rates, ingestion selectivity and digestion selectivity (Chua & Brinkhurst, 1973; Tietjen & Lee, 1973; Brown & Sibert, 1977; Lee et al., 1977; Deutsch, 1978; Reiper, 1978; Reiper & Flotow, 1981; Vanden Berghe & Bergmans, 1981; Reiper, 1982; Montagna, 1984; Trotter & Webster, 1984; Carman & Thistle, 1985; Decho, 1986; Decho & Castenholz, 1986). Such studies are beginning to provide detailed information, and reveal the mechanisms by which energy flow through the meiobenthos may be more precisely understood.

Feeding studies have generally been confined to the adult stages, however. Problematic or unknown taxonomy for immature stages of many meiobenthic taxa, and the logistical problems associated with experimenting with such small organisms has slowed progress. Studies of other groups of marine organisms have noted that food sources may change throughout the course of development. This has been shown in planktonic copepods (Elster, 1936; Marshall & Orr, 1956; Lewis, 1967; Smyly, 1970; Paffenhoffer, 1971; Maly & Maly, 1974; Allan et al., 1977; Fernandez, 1979; Chow-Fraser & Wong, 1986), euphausids (Mauchline & Fisher, 1969), sand lances (Monteleone & Peterson, 1986), and many different fish (Sibert et al., 1977; Livingston, 1980; 1982) and other groups. Such dietary shifts are often a result of changes in 1) size and or 2) developmental needs.
Because adult distribution and abundance may ultimately depend on survival of early life history stages (Sellner, 1976; Weinberg et al., 1986), it is of interest to know whether feeding patterns in the meiobenthos change throughout the life history of a given species. We conducted laboratory and field studies to investigate the feeding and resource utilization of an estuarine meiobenthic harpacticoid copepod Nitocra lacustris (Schmankevitsch). Their purpose was to determine 1) what constitutes the food resources of this species; and 2) if adults ingest and assimilate the same food resources as nauplii.
METHODS

Individuals of the harpacticoid copepod *Nitocra lacustris* were collected from salt marsh sediments near the Louisiana Universities Marine Center (LUMCON) at Cocodrie, Louisiana, and maintained for several generations in laboratory culture (Chandler, 1986) until use. Additional specimens (both adult and naupliar stages) were also collected from field sediments and immediately frozen *in situ* in liquid nitrogen for later gut and fecal pellet analyses.

Feeding Experiments:

Feeding experiments were conducted to determine if the suspected microbial foods (diatoms and bacteria) were ingested and assimilated by *N. lacustris*. For diatom feeding experiments, sediment diatoms were isolated from the same local habitat as *N. lacustris*, and maintained in laboratory culture on Erdschreibers medium in 15% Instant Ocean Seawater (ASW) using 16h light 8h dark cycle at 20±2°C. The diatom chosen for feeding experiments was *Amphora tenerrima* (Aleem & Hustedt) because 1) it is commonly found in the local habitat of *N. lacustris*, and is often found in gut contents of individuals collected from field sediments; 2) it is a very small diatom (16 μm x 7.5 μm) relative to the size of the harpacticoid nauplii and therefore would reduce any size-selectivity effects; and 3) unialgal cultures of this diatom on agar plates can sustain vigorous growth rates of *N. lacustris* for many generations (Decho, personal observation). Diatoms were labelled in their log phase of growth with NaH$^{14}$CO$_3$ (New England Nuclear, Boston,
MA. 10μg/μCi; 50 μCi·l⁻¹ final conc.) for 24 h under constant light, washed and prepared according to the methods of Decho (1986), and placed in sediment microcosms.

The bacterial flora associated with the diatoms were labelled with ³H-methyl thymidine (New England Nuclear 50-80 Ci/mmol; 0.8 μCi·ml⁻¹ final conc.) for 4h. Uptake of label by bacteria and adsorption by non-bacterial components was monitored over the 4h period and compared with formalin-killed controls (Lessard & Swift, 1985). Estimations of bacterial numbers were made using the direct count method (Hobbie et al., 1977) as modified for sediments by Montagna (1984).

For feeding experiments 75 adult and 375 naupliar individuals of the harpacticoid copepod Nitocra lacustris were added to sediment microcosms containing labelled microbial flora and allowed to feed for 2h. At the conclusion of the 2h feeding period, the animals were quickly removed by sieving and washed. A portion of these animals were immediately fixed and placed in scintillation vials containing 1 ml Protosol (New England Nuclear) and digested in darkness (65°C) for 4h. Samples were later counted on a Beckman LS8000 liquid scintillation counter using 10 ml Econofluor (New England Nuclear) as the scintillation cocktail. Each vial contained either seven adults or 20 nauplii. Six replicates were used for each group (adult and nauplii). Quenching was corrected for using the external standards ratio method.

Another portion of animals (used in assimilation studies) were immediately transferred to "cold-feed" sediment microcosms containing unlabelled microbial flora of the same type as mentioned. These animals were allowed to feed for 2h, then removed and prepared for LSC.
Respiration of $^{14}$CO$_2$ during the labelled feeding incubations and cold-feed" incubations were monitored according to the methods of Hobbie and Crawford (1969) as modified by Decho (1986).

To control for uptake of label not due to ingestion of either diatoms or bacteria, adult and nauplii were placed in sediment containing filtered (0.22 µm) exudates from $^{14}$C-labelled diatoms or sterile SW containing $^3$H-methyl thymidine (at the same concentration used in feeding experiments). Results were analysed by ANOVA (SAS Institute Inc., 1982).

Bacterial-Sized Particle Ingestion:

Preliminary observation indicated that nauplii often scrape the outsides of diatom frustules while feeding. In order to determine if bacteria-sized particles, (adhering to the mucus coat on the outside of the diatom frustule) were ingested by the nauplii during feeding, bacteria-sized (0.75 µm) fluorescent beads (Fluoresbite, Polysciences Inc.) were used as an inert tracer. The beads were mixed with diatom (A. tenerima) cultures. The cultures were then washed by centrifugation (3,000 rpm for 5 min) to remove unattached beads, and mixed with sediment. Naupliar and adult harpacticoids were added and allowed to feed for 1h. Individuals were then removed, washed and observed using epi-fluorescence microscopy (excitation $\lambda$=458 nm; emission $\lambda$=540 nm) for the presence of fluorescent particles in gut-pellets.

Gut-Content Analyses:
To visually verify the results of radiolabelling experiments, adults and nauplii were collected from field sediments and the gut-pellet contents observed by light microscopy. Gut pellets were dissected out of various portions of the adult harpacticoid digestive tracts. Naupliar gut pellets, too small to be dissected out, were observed intact using squash preparations. Specifically noted were the presence or absence of diatom frustules in the pellets. Because the silica frustules are not totally destroyed (and sometimes completely intact) during digestive processes, the presence of these frustules in the gut- and fecal-pellets provides good observational evidence of diatom ingestion. Identification of *N. lacustris* nauplii from field samples was verified by comparisons with laboratory-hatched individuals.

Growth Stage Which Feeding Shift Occurs:

Preliminary experiments indicated that the diatoms were not ingested by nauplii. Therefore experiments were conducted to determine at which developmental stage ingestion of diatoms began. Single individuals of newly hatched *N. lacustris* (Naupliar stage I) were placed in agar petri dishes (35 x 10 mm) containing unialgal cultures of the diatom *A. tenerrima*. The dishes were examined for exuvia every 4-8 h in order to keep track of the number of molts. The gut pellets from several individuals of all naupliar and early copepodite stages were examined for the presence of diatom frustules.

Effect of Naupliar Grazing of Diatom Frustules:
Finally, to determine if the nauplii break open the diatom (then remove their contents) during feeding, scanning electron microscopy (SEM) was used to examine diatom frustules which had previously been grazed by nauplii. Thirty nauplii were added to an approx 5 x 5 mm patch of *A. tenerrima* diatoms and allowed to feed for 10 h. The diatoms were then prepared for SEM by fixation in 2% glutaraldehyde buffered with sodium cacodylate buffer (adjusting for osmolality), for 2 h, rinsed in buffer, then placed in 1% osmium tetroxide for 1 h. The specimens were rinsed twice in buffer (15 min. each), then in dH$_2$O, and dehydrated in 3 ml 2,2-dimethoxypropane (Sigma Chem. Co.) with 400 µl conc. HCl. The mixture was allowed to equilibrate to room temperature, then replaced with 3 changes of 100% acetone (HPLC-grade) for 15 min. Finally, the specimens were critical point dried, mounted and sputter-coated with Au/Pd and examined on a Hitachi S-500 Scanning electron microscope. A control, consisting of diatoms from the same culture which were not exposed to naupliar grazing, was also examined.
RESULTS

14C-bicarbonate (Diatom) Grazing Experiments:

Food concentrations of diatoms used in feeding experiments were 1,058,000 ± 34,500 cells·cm\(^{-2}\) sediment with specific activities = 4.3 ± 0.25 dpm·cell\(^{-1}\).

Results of 14C-bicarbonate (used to label diatoms) grazing experiments show that significant amounts of diatoms are consumed by adult N. lacustris. After 2h "cold-feeding" on unlabelled diatoms the copepods still retain a significant amount (70%) of label with 20% being removed by respiration of 14CO\(_2\) (Fig. 1).

Naupliar grazing indicates that a significant (P<0.05) amount of label is taken up above control levels (Fig. 1), however, this amount is quite small. After 2h cold-feeding, most (58%) of this label remains, indicating assimilation. Respiration accounted for 27% of the lost label.

Examination of harpacticoid gut-pellets used in feeding experiments show the presence of many diatom frustules in adult individuals however, no frustules were observed in nauplii gut pellets. These results coincide with radiolabel analyses.

3H-Thymidine (bacterial-uptake) Experiments:

Uptake of 3H-methyl thymidine was linear over the 4h incubation period when compared with formalin poisoned controls. Most of this uptake, correcting for adsorption, could therefore be
assumed to be due to bacterial incorporation (Lessard & Swift, 1985). Direct counts of bacteria indicated $5.43 \pm 0.13 \times 10^{-7}$ cells·cm$^{-2}$ sediment. Diatom densities were $873,670 \pm 8100$ cells·cm$^{-2}$ sediment. Specific activities of bacteria were $0.6 \pm 0.018$ dpm·cell$^{-1}$.

Results of grazing experiments show significant uptake of $^3$H-bacteria by *N. lacustris* adults (p<0.05). After 2h cold-feeding, most (78%) of this label is lost. These reduced levels however still differ from control levels (P<0.05).

Nauplii also show significant uptake (P<0.05) of labelled bacteria however retain a small, but significant (P<0.05) portion (18%) of this label after 2h cold-feeding (Fig. 1).

**Observational Results:**

Experiments using bacterial-sized fluorescent beads (adhering to diatom matrices) show the presence of these ingested bacteria-sized beads in gut and fecal pellets of adults and naupliar stages (Fig. 2).

Harpacticoid gut-pellets collected from field sediments show an abundance of diatom frustules (and sediment particles) in adult stages of *N. lacustris* (Fig. 3). Naupliar stages however show no frustules in the gut contents of the individuals examined (Fig. 3). Experiments observing the gut contents of naupliar and early copepodite stages for the presence of diatom frustules indicate that ingestion of diatoms (*A. tenerrima*) first begins at the second to third copepodite stage. Diatoms are regularly found in gut contents during subsequent later life stages.
Scanning electron microscopy of clusters of diatoms show no breakage of the diatoms frustules (Fig. 4) after they had been grazed upon by nauplii.
DISCUSSION

The process of ontogenetic feeding shifts has been demonstrated in a wide variety of vertebrates and invertebrates. Among the crustaceans, many euphausid nauplii do not feed due to the lack of a mouth (Mauchline & Fisher, 1969), while adults feed on a variety of both planktonic and benthic phygal foods. Some calanoid copepods switch from herbivorous to omnivorous feeding as they develop from nauplii to adult (Lewis, 1967; Smyly, 1970; Chow-Fraser & Wong, 1986). Feeding shifts in the meiohenthos have received little or no attention despite the potentially important role of these shifts in the maturation and subsequent reproduction of marine animals (Phillips, 1984) and in food-web interactions.

The results of the present study show significant shifts in the ingestion and utilization of microbial food resources between the adult and naupliar stages of the meiohenthic harpacticoid copepod Nitocra lacustris. Adults ingest and assimilate $^{14}$C-labelled diatoms in the laboratory. Observations of field-collected adults support this showing an abundance of empty diatom frustules in the gut-pellets and indicate that these diatoms may comprise a large part of the ingested food resources of the adult.

Naupliar stages, however, do not ingest diatoms. This is evidenced by both $^{14}$C-feeding experiments and observational data. Diatom frustules are not observed in gut-pellets (Fig. 3). A small amount of $^{14}$C-label is taken up by nauplii, however, several lines
of evidence further indicate that this uptake is not due to ingestion of diatoms or even breakage of the diatoms (and subsequent ingestion of intracellular contents) by the nauplii.

First, the $^{14}$C-bicarbonate label is not totally restricted to the diatom cell contents. Some of the $^{14}$C is quickly metabolized by the diatom and excreted as dissolved labelled-exudate products (i.e. amino acids, carbohydrates, etc.). Such $^{14}$C-products are quickly incorporated by bacteria (Bell & Sakshaug, 1980) growing in close association with these diatoms. Also, some of this metabolised $^{14}$C-label is incorporated into extracellular mucus (exopolymer) of the diatom. Ingestion of these labelled extracellular mucus-exopolymer and secondarily-labelled bacteria, or even the direct uptake of dissolved exudates can result in uptake of $^{14}$C label (originally derived from diatoms) by nauplii (and adults). Second, observations of *N. lacustris* nauplii show that although the mouthparts are well-developed, they can only manipulate and scrape the outsides of diatoms (like "eating corn on the cob") and cannot actually ingest the diatom cell (Decho, personal observation). The absence of diatom frustules in the gut-pellets and presence (in gut-pellets) of bacteria-sized fluorescent particles, which were experimentally affixed to the outer diatom mucus coat (from separate experiments) strongly support this scraping-ingestion behavior. Finally, SEM examination of diatoms previously grazed by nauplii for 10 h do not show any breakage of diatom frustules (Fig. 4). This indicates the nauplii do not break open the diatoms during feeding to remove their
intracellular contents, as have been observed in some nematodes (Jensen, 1982).

The naupliar (through adult) stages can be successfully cultured for many generations on monoxenic strains of the diatom, *Amphora tenerrima* (growing on agar plates). Lee et al., (1976) was able to grow a congener (*N. typica*) on agar plates containing different microalgal strains. Since the nauplii here, do not ingest the diatoms themselves, some associated component of the diatoms (i.e. mucus-exopolymer coats, associated bacteria, dissolved exudates, etc.), which is neccessary for substrained growth, must be utilized. In adults, diatoms appear to be a commonly ingested (and assimilated) food item in the diet, however, the potential relevance of other components (i.e. associated mucus, bacterial flora, etc.) may be equally as important. During adult feeding, for example, the ingestion of whole diatoms will coincidently include the mucus-exopolymer slime and numerous bacteria associated with these diatoms (Fig. 4).

Bacteria may act as important supplemental food components (Phillips, 1984) in the diets of many organisms, especially as an important source of specific nutrients, e.g. B-complex vitamins (Kutsky, 1981). Some harpacticoids (*Tigriopus californicus*) require the presence of bacteria for continued reproduction (Provasoli et al., 1959). For other harpacticoids (*Tisbe, Paramphiascella*), bacteria can represent the sole food source (Reiper, 1978; 1982), at least over short-terms. Our work with thymidine-labelled bacteria in grazing experiments indicate
ingestion of bacteria by both adult and naupliar stages. These results are supported by experiments examining the ingestion of bacteria-sized fluorescent beads experimentally attached to the diatoms (Fig. 2). Assimilation of these bacteria by the copepods is not indicated by the thymidine-grazing experiments. However, the pathways which the $^3$H-methyl group on the thymidine vary unpredictably (Azam & Holm-Hansen, 1973; Hollibaugh et al., 1980) and are difficult to quantify. Therefore the quick loss of $^3$H-thymidine (by both adults and nauplii) after 2h cold feeding does not exclude the possibility that bacteria (or at least a portion of the bacteria) are assimilated by these harpacticoids. It is noteworthy that live bacteria can be observed by light microscopy in fecal pellets of laboratory-reared nauplii (Decho, personal observation). These bacteria however, can either represent 1) ingested bacteria which have survived passage through the gut or 2) resident gut-flora which are periodically lost through defecation. While microautoradiographic studies (Moriarty & Pollard, 1982) show that nm concentrations of thymidine are not incorporated into eukaryote microalgae itself (i.e. diatoms), adsorption of some $^3$H-thymidine to mucus-exopolymer and subsequent ingestion of this mucus by harpacticoids is possible, and this could account for a portion of the thymidine uptake. It is not known, however, how much of the diet of adult *N. lacustris* is from bacteria.

The exact nature of the naupliar food resources (i.e. bacteria, mucus-exopolymer, dissolved exudate, etc.) is difficult
to distinguish for two reasons: First, the nauplii consume a small amount of food compared to adult stages. These amounts are close to the limits of resolution of radiolabelling techniques using these isotopes. Other radiolabels (i.e. $^{14}$C-glucose, $^{14}$C-acetate, etc.) would be incorporated into the exopolymer coat in addition to the bacterial cell itself, and therefore could not differentiate utilization of the bacterial cell versus its associated exopolymer. Secondly, the gut-retention times of nauplii are very fast (5-20 min) and are difficult to quantify, relative to adult stages (approx. 40 min at $20^\circ$C). Therefore metabolic losses (i.e. excretion, respiration of assimilated label) over a given time frame will be much greater than for adults.

A recent body of direct and indirect evidences is now accumulating which support the utilization of microbial mucus-exopolymer as a substantial food resource of marine invertebrates (Jennings & Deutsch, 1975; Deutsch, 1978; Cammen, 1980; Hobbie & Lee, 1980; Moriarty, 1982; Baird & Thistle, 1986; Rice et al., 1986; and present study). Mucus is secreted in copious amounts by many sediment diatoms such as *Amphora* (Daniel et al., 1980), including those (*A. tenerrima*) used in this study. Note however, that during SEM preparation (especially during the dehydration steps) the mucus coat associated with the diatoms is very reduced or effectively removed (Chang & Rittmann, 1986; Grant et al., 1986) because a large portion of the mucus consists of water (Sutherland, 1972). Many bacteria also secrete
mucus-exopolymer capsules and slime (Corpe, 1975). *N. lacustris*, especially the nauplii, may utilize these mucus exopolymer secretions. The idea is supported by both radiolabel experiments and observational information gained in this study. However, such observations cannot be used as proof. Further investigations and new methodologies will be required to quantitatively determine the significance of mucus as a potential food resource for both adult and naupliar stages.

Food resource utilization appears to be complex and variable, depending on the life stage. As stated by Hicks and Coull (1983): "It appears that the use of the terms diatom-feeder or bacteria-feeder will be far too general in scope to ultimately describe the utilization of food resources in the meiofauna." While some harpacticoids may be labelled as diatom-feeders or diatom-specialists (i.e. preferentially ingest only certain species of diatoms), these should not be considered their sole food resource. It is more likely that a wide variety of other organic components (diatoms, mucus-exopolymer, bacteria, etc.) are of supplemental importance in their nutritional requirements. Some of these components, such as specific fatty acids found in diatoms, may play key roles in the reproductive biology of the animals (Phillips, 1984). These requirements may change seasonally (Lee et al., 1976), or throughout the life of an individual, and should be considered when assessing trophic ecological relationships.
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Fig. 1. Results of $^{14}$C-bicarbonate (diatom) and $^3$H-thymidine (bacteria) grazing experiments using adult and nauplii of the harpacticoid *N. lacustris* (dpm=disintegrations per minute; $R=^{14}$C-respired; $U=^{14}$C-unaccounted for).
The image shows two graphs for different experiments:

**3H-bacteria**
- Y-axis: dpm / Copepod
- X-axis: Time (h) Cold-Feeding
- Graphs for **ADULT** and **NAUPLII**

**14C-diatoms**
- Y-axis: 0 to 700
- X-axis: Time (h) Cold Feeding
- Graphs for **ADULT** and **NAUPLII**
Fig. 2. Gut-pellets of (A) nauplii and (B) adult stages of the harpacticoid *N. lacustris* showing the presence of ingested bacteria-sized fluorescent beads using epifluorescence microscopy (fb= fluorescent beads; scale bar=30 μm).
Fig. 3. Photographs showing gut-contents of (A) nauplii and (B) adults of _N. lacustris_ collected from field sediments (gp=gut pellet; d=diatom frustule; scale bar=20 μm).
Fig. 4. Scanning electron micrograph showing clusters of diatoms (A. tenerrima) A) ungrazed (control) patches vs. B) previously grazed by the nauplii of N. lacustris. Note absence of broken diatom frustules in "grazed" patches. See text for explanation (b=bacterium, m=mucus-exopolymer; scale bar=5 μm).
CHAPTER TWO

TITLE: HOW DO HARPACTICOID GRAZING RATES DIFFER OVER A TIDAL CYCLE?: FIELD VERIFICATION USING CHLOROPHYLL-PIGMENT ANALYSES

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ABSTRACT

Four species of meio-benthic copepods were examined for diatom-feeding. Microscopic analysis of gut-pellet contents from field-collected individuals indicates very frequent ingestion of diatoms by three harpacticoids (Scottolana canadensis, Microarthridion littorale, Cletocamptus deitersi) while occasional diatom ingestion occurred in Paronychocamptus huntsmani. Frustules were usually empty and broken, indicating that contents were digested. Laboratory experiments using $^{14}$C-labelling showed assimilation of diatoms by the three species examined. Field grazing rate studies were conducted over different portions of a tidal cycle using fluorescent chlorophyll-pigment analysis of gut-contents. Highest diatom consumption ($P<0.05$) occurred just after the mudflat became exposed (i.e. Early Low-Water level ELW) for S. canadensis, while consumption at Late Low-Water (LLW; i.e. after mudflat is exposed for several hours) was very reduced. M. littorale showed a somewhat similar pattern in that highest consumption rates ($P<0.05$) also occurred during ELW. However, during High-Water (HW) and LLW a similar (but reduced) feeding rate was measured. P. huntsmani appeared to only ingest diatoms (i.e. Chl-pigments) during HW.

Relationships of feeding processes over a tidal cycle are discussed with regard to distributional patterns in intertidal and subtidal habitats.

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INTRODUCTION

Feeding activities of the meiobenthos have been quantified using both laboratory and field investigations. Laboratory feeding studies, however, can potentially introduce artifacts (see Hicks & Coull, 1983; for review) inherent to artificial systems. While conducting feeding studies in the field represents more realistic conditions, it is often difficult to control many of the necessary parameters, and some resolution in interpretation can be lost. Therefore, if possible, it is important to verify processes observed in the laboratory under actual field conditions.

Diatoms are known to be an important food resource of many meiobenthic harpacticoids from both laboratory and field studies (Lee et al., 1976; Sellner, 1976; Brown & Sibert, 1977; Harris, 1977; Lee et al., 1977; Vanden Berghe & Bergmans, 1981; Ustach, 1982; Admiraal et al., 1983; Montagna, 1984; Carman & Thistle, 1985; Lee et al., 1985; Chandler, 1986; Decho & Castenholz, 1986). Previously, laboratory studies (Decho, 1986) have indicated that certain harpacticoids consume diatom food resources at different rates over portions of a simulated tidal cycle. However, since these studies were conducted in laboratory microcosms, field verification of the observed feeding patterns, represents an important step in understanding harpacticoid feeding processes.

Radiolabels are frequently used to measure feeding rates, and have been applied effectively in a wide variety of studies under
field conditions. However, in order to examine feeding rates over a tidal cycle, radiolabels could not be effectively used in the field studies here due to associated hydrodynamic constraints. The dispersion of radiolabels must be contained, therefore, an enclosure (i.e. a capped core tube inserted into the sediment) is generally used. Any enclosure, however, reduces natural hydrodynamic perturbations and excludes routine macro-predatory disturbances (i.e. fish, crabs, shrimp, etc.) which may occur during high-water conditions (Eckman, 1979). These natural perturbations and disturbances would be expected to affect feeding rates of a benthic assemblage.

Instead, the chlorophyll gut-content method of Dagg and Wyman (1983) was employed. This technique measures the amount of chlorophyll and pheopigment (i.e. degradation products of chlorophyll) present in the alimentary canal of field-collected copepods by fluorescence. This method has been frequently used in water-column studies investigating calanoid copepod grazing on diatoms (Paffenhofe & Van Sant, 1985; Tande & Barnstedt, 1985; Stearns, 1986; Wang & Conover, 1986; Dagg & Walser, 1987; and others). By concurrently measuring gut-retention times under these same conditions (i.e. temperature, food concentration, tidal level), grazing rates on diatoms can be estimated over different parts of a tidal cycle.

This study was designed to: 1) determine if diatoms are ingested and assimilated as a food resource by three species of harpacticoids; 2) determine if grazing rates on these food resources vary in the
field over a tidal cycle; and 3) compare grazing rates with previous laboratory studies. In this paper I show that tidal influences can have a very pronounced effect on feeding rates. I thank Dr. M.J. Dagg, Louisiana Universities Marine Consortium (LUMCON), for technical assistance, consultation, and the use of his laboratory for chlorophyll-pigment analyses; the staff of LUMCON for use of their Marine Center and equipment during various portions of field experimentation; Dr. J.W. Fleeger for suggestions and helpful review on earlier versions of this manuscript; and Dr. M. Sullivan, Mississippi State University, for identification of benthic diatoms used in feeding experiments.

METHODS

Copepods used in laboratory and field experiments were collected from the lower regions of an intertidal mudflat near the Louisiana Universities Marine Center (LUMCON) at Cocodrie, Louisiana near the sampling site of Phillips and Fleeger (1985). To determine if diatoms were ingested and utilized as a food resource, several observational and experimental procedures were used:

**Harpacticoid Gut-Pellet Analysis**—— Harpacticoid gut-pellet analyses were conducted to determine the presence or absence of diatom frustules in copepod guts. The presence of diatom frustules in gut-pellets would indicate recent ingestion of diatoms by harpacticoids. Gut-pellets, from field-collected individuals fast-frozen in liquid nitrogen, were dissected out of the gut and observed by light microscopy under oil immersion.
Laboratory Feeding Experiments

Laboratory feeding experiments using radiolabelled diatoms ($^{14}$C) were conducted to determine if diatoms were ingested and assimilated by three harpacticoid species: Scottolana canadensis (Willey), Microarthridion littorale (Poppe), and Cletocamptus deitersi (Richard). Cultures of the sediment diatom Amphora tenerrima (Aleem & Hustedt) isolated from the local habitat of the harpacticoids, was grown in Erdschreiber media and labelled in their log phase of growth with 0.33 $\mu$Ci/ml NaH$^{14}$CO$_3$ (New England Nuclear, Specific Act.=10$\mu$g/$\mu$Ci) for 48 hours in constant light. At this time, unincorporated label was removed by multiple centrifugations, resuspending each time in sterile (15% salinity) artificial seawater (ASW). Food concentrations (measured by triplicate subsamples) and specific activities of diatoms used in feeding experiments are listed in Table 1. Each experiment was run in triplicate. For each experiment, 150 adults of each species were prestarved for no longer than 30 min, placed in sediment wells containing a known concentration of labelled diatoms with 1 cm water cover (ASW), and allowed to feed for 1 h. At this time the copepods were removed and washed in filtered (0.22$\mu$m) ASW. Twenty-five copepods were immediately fixed in 10% formalin in ASW and placed in scintillation vials (six individuals per vial) with 1.0 ml KOH (10%), heated for 2 h (45°C) and counted on a Beckmann LS8000 Liquid Scintillation Counter (LSC) using Universolv as the scintillation cocktail. Quenching was corrected for using the external standards ratio method. From the remaining copepods, 25 individuals were placed in "Cold-Feed" sediment wells containing sediment, ASW and unlabelled diatoms. Copepods were allowed to feed for 3 h in order to clear
their guts of labelled-diatom material. Twenty-four individuals were placed in 25 ml respiration flasks (6 ind. per flask) under similar conditions (i.e. food conc., etc.) as the cold-feed wells and allowed to feed for 2 h. Respiration of $^{14}$CO$_2$ was measured according to the methods of Hobbie and Crawford (1969).

To correct for uptake of $^{14}$C-label not due to the ingestion of labelled diatoms, controls, consisting of sediment wells containing the filtrate (0.22 μm) of $^{14}$C-diatoms incubated for 1 h (approx. feeding incubation period) were used. This filtrate contained $^{14}$C-exudates of the diatoms and leaked intracellular contents, which the copepods were exposed to during the 1h feeding experiments. Eighteen copepods were placed in these control wells and incubated for 1h, at which time they were removed, washed and prepared for LSC.

Field Grazing Rate Experiments: Field experiments were conducted during late March to measure consumption rates of diatoms and microalgae by harpacticoid species over three different portions of a tidal cycle: 1) High-Water (HW), when the mudflat was covered by water; 2) Early Low-Water (ELW), just after the mudflat was exposed; and 3) Late Low-Water (LLW), after the mudflat was been exposed for several hours. Consumption rates of diatoms were estimated by the gut chlorophyll-pigment method of Dagg and Wyman (1983) with slight modifications for sediment. All experiments were conducted at the lower portion of an intertidal mudflat. Harpacticoids were extracted by collecting sediment, quickly sieving it through 125 μm mesh and immediately freezing the retained fraction in liquid nitrogen (in darkness). Samples were kept frozen in dry ice
(in darkness) while transported back to the laboratory, and remained frozen for a maximum of four hours.

In the laboratory, the animals were extracted, washed, and separated according to species using minimal light exposure (to prevent degradation of chl-pigments within copepod guts). Only adults were used in subsequent pigment analyses. Twenty individuals of each species (*Scottolana canadensis*, *Microarthridion littorale*, and *Paronychocamptus huntsmani* (Willey)) were placed on replicate separate glass-fiber filters (Whatmann GF/F). For *S. canadensis*, only adult females were used because of the great sexual dimorphism in size (Coull, 1972). The samples (and filters) were homogenized in 15 ml of 90% acetone, and the fluorescence of the filtrate determined before and after acidification in 10% HCl using a Turner Designs Model 10 Fluorometer calibrated for chlorophyll-a. Chlorophyll-a and phaeopigment content of each copepod was calculated using equations modified from Strickland and Parsons (1968) by Dagg and Wymann (1983). Gut-contents were determined by summation of gut-chlorophyll and gut-phaeopigment, and expressed as "ng Chl-equiv. pigment per individual." All data on copepod fluorescence were corrected for the small amount of analytical interference from copepod tissues (control fluorescence). Controls represented copepods previously starved for 12 h in filtered SW. These copepods were observed before analysis to confirm the absence of gut-pellets. Six replicate samples were used for each treatment estimate (HW, ELW, and LLW), and controls for each species (20 individuals/ replicate). Grazing rates were calculated from these gut-pigment estimates.

Ambient concentrations of sediment chlorophyll-a were determined
from replicate 1 cc sediment core samples, corrected for the sediment dry weight. These concentrations are primarily due to diatoms, which are abundant on the mudflat at the time of the experiment. Slight overestimations in chlorophyll-a, due to small amounts of chlorophyll-b (Daemen, 1986), were assumed to be negligible since cyanobacteria (a major source of Chl-b) were not present in detectable abundances at the time of sampling. Concentrations of chlorophyll-a in the overlying water during High-Water (HW) conditions were also measured.

Separate gut-retention times were measured "in situ" for each harpacticoid species at each portion of the tidal cycle. To estimate gut passage times 3.5 cm diameter core tubes were placed in sediments and, 500 µl azo-carmine (Sigma Chem. Co.) in ASW solution (1% w/v) was added to each as an inert tracer. Core tubes were removed from the sediment and immediately frozen at 10 min. intervals, starting at 40, ending at 100 min. Later, the posterior gut of each species was examined for the location of azo-carmine with time to approximate gut-passage times for each tidal condition. Gut-passage times were used in conjunction with gut pigment contents to estimate grazing rates (Frost, 1972).

In order to standardize ingestion rates for the relative size differences between the three species of harpacticoids, adults (75) of each species were dried and weighed on a Mettler AE163 analytical balance. Five replicate samples were always used. These weights were necessary to calculate consumption rates/g copepod tissue (i.e. weight-specific grazing rates).

Results of gut-passage times and ingestion rates were analyzed
by ANOVA. Log (X+1) transformed data were used for analyses of gut passage times and arcsin transformed data were used for analyses of ingestion rates. If a significant difference was indicated, Bonferroni's test was used for a posteriori comparison of means (SAS Institute, 1986).

RESULTS

Gut-Content Analyses—— Analyses of gut-pellets, dissected from field-collected individuals, indicate that diatoms, sediment particles, and some bacteria-sized particles were ingested by three species of harpacticoids (S. canadensis, M. littorale, and C. deitersi). The diatom frustules comprise approximately 30-70% of the gut pellet (by volume). A fourth species (Paronychocamptus huntsmani) showed only occasional frustules, with the majority of the gut-pellet contents composed of sediment particles (with some bacteria-sized particles) and unidentified amorphous material.

Laboratory Feeding Experiments—— Food concentrations and specific activities of diatoms used in feeding experiments are given in Table 1. Feeding experiments indicate significant uptake of $^{14}C$-labelled diatoms after 1 h feeding by all three species of harpacticoids (Table 2). After 3 h of cold-feeding (on unlabelled diatoms), to clear the guts of labelled material, a significant portion of the ingested $^{14}C$-label still remained in all three species of harpacticoids indicating assimilation of $^{14}C$-diatoms. A large portion (25-38%) of the ingested label, lost during cold-feeding was accounted for through respiratory losses of $^{14}CO_2$, a fact which
further indicates assimilation of diatoms by harpacticoids.

Field Grazing Experiments------ Field-sampling occurred during the movement of a weather front into the local area. Temperatures during sampling remained relatively constant, ranging from 12-14°C, with strong north winds (20-25 knots) prevailing. Sediment chlorophyll-a and pheopigment values were highest at ELW (Early Low-Water conditions), (Table 3).

Control harpacticoids, prestarved for 12 h, contained 0.0117 ±0.004 ng Chl·ind⁻¹ and 0.1367 ±0.0137 ng Phaeopigment·ind⁻¹ for S. canadensis, 0.0083 ±0.0041 ng chl·ind⁻¹ and 0.11 ±0.004 ng Pheopigment·ind⁻¹ for M. littorale, and 0.0077 ±0.0041 ng Chl·ind⁻¹ and 0.1203 ±0.014 ng Pheo·ind⁻¹ for P. huntsmani.

S. canadensis showed the highest gut-pigment content during ELW conditions (0.69 ±0.13 ng Chl-equiv pigment·ind⁻¹), with significantly (P<0.05) lower values during HW conditions (0.51 ±0.06 ng Chl-equiv pigment·ind⁻¹) and LLW conditions (0.21 ±0.05 ng Chl-equiv pigment·ind⁻¹), respectively. Also, gut-retention times were similar during HW and ELW conditions, 62-65 min (P>0.05), but significantly (P<0.05) longer, 80 min., during LLW (Fig. 1). The resulting grazing rates, calculated from gut-pigment contents and gut-retention data, show highest consumption during ELW, 0.67 ng chl-equiv. pigment·ind⁻¹·h⁻¹ with significantly lower rates (P<0.05) during HW (0.47) and LLW (0.14), respectively (Fig. 2).

M. littorale showed highest gut-pigment contents during ELW (0.42 ±0.13 ng Chl-equiv pigment·ind⁻¹), with slightly lower values during HW and LLW conditions, ranging from 0.18 to 0.20 ng Chl-equiv pigment·ind⁻¹. Gut-retention times were similar at HW and ELW (50 and
52 min, respectively), however, but significantly longer (P<0.05) during LLW (76 min), (Fig. 1). Consumption rates of diatoms were greatest at ELW and significantly less (P<0.05) at HW and LLW (Fig. 2). *P. huntsmani* showed gut-pigment contents which did not differ from controls (P>0.05) except during HW when 0.13 ±0.02 ng Chl-equiv pigment·ind⁻¹ was present.

Mean tissue dry-weights (and lengths), used to standardize ingestion rates for species size differences are listed in Table 4. Resulting weight-specific ingestion rates (to remove the effect of relative size differences between the three harpacticoid species), indicate that all three harpacticoids consumed diatoms at similar rates (per g tissue) during HW (Table 5). Highest consumption for *S. canadensis* and *M. littorale* occurred during ELW. The only significant consumption for *P. huntsmani* occurred HW conditions.

**DISCUSSION**

Decho (1986), used laboratory studies, to determine that the presence (or absence) of water-cover dramatically affected the feeding rates and mode of feeding (benthic or planktonic) of certain harpacticoid species. Laboratory conditions were equivalent to High-Water (HW) and Late Low-Water (LLW), with no hydrodynamic or disturbance effects. In the present study, field ingestion rates of diatoms also varied dramatically over different parts of a tidal cycle (as measured by gut-pigment contents). These observed differences are species-specific and relate to the differing feeding
modes used by each harpacticoid. *S. canadensis* showed highest rates of feeding during Early Low-Water (ELW) conditions (i.e. just after the tide has receded but when the mudflat is still very wet). Its feeding rate was significantly lower during HW conditions (i.e. high-tide) then decreased drastically at LLW (i.e. after the mudflat has been exposed for several hours) (Fig. 2). *S. canadensis* utilizes planktonic diatoms (Chandler, 1986; Lonsdale & Levinton, 1986), but does build burrows (Chandler and Fleeger, in press) and feeds on cells in the immediate overlying water column (Decho, 1986). For *M. littorale*, approximately similar (though slightly lower) feeding rates were found at HW and LLW conditions. Laboratory studies show that *M. littorale* compensates for these changing water-level conditions by changing its mode of feeding (Decho, 1986). Feeding during HW occurs primarily while swimming and consists of mainly planktonically-suspended food resources. Feeding during LW (i.e. LLW) is benthic while crawling over and through the sediment.

Interestingly, highest gut contents and feeding rates occur during ELW for both *S. canadensis* and *M. littorale*. The immediate causes are unknown but may relate to several factors. First, there is an abundant water film covering the mudflat at ELW. The surface sediment is very flocculent which could facilitate the movement of meiobenthic copepods, allowing them to readily find and ingest food rich patches. Second, there is an absence at ELW of the disturbing wave actions or hydrodynamic influences typically present during HW (Eckman, 1983; Palmer & Gust, 1985; Palmer, 1986). Also small fish predators (Feller & Kacynski, 1975; Bell & Coull, 1978; Bell, 1980; Fitzhugh & Fleeger, 1985; Smith & Coull, 1987) which can potentially
affect feeding behaviors are absent. *S. canadensis*, for example, in laboratory microcosms moves very quickly to the lower portion of its burrow (approx. 1-1.5 cm vertical depth) in response to a predator-like physical disturbance at the surface (A.W. Decho, personal observation). Thirdly, the availability and accessibility of potential food (i.e. diatoms and flocculent aggregate material) may well be greatest at the sediment surface at this time.

Food availability on the surface of a mudflat is likely to fluctuate considerably over a tidal cycle. More than a 50% flux in particulate organic carbon and chlorophyll-a can occur in near-bottom water-layers during a tidal cycle (Roman & Tenore, 1978). The cause of this pattern is the tidal cycle itself. During high-water much of the sediment-floc, benthic diatoms, detritus, and phytoplankton (which had previously settled to the bottom mud) is resuspended into the water column. It settles out before the tide flat is exposed, causing higher concentrations at Low-Tide. Similar patterns occurred in the present study. Sediment chlorophyll and phaeopigments concentrations were highest during Low-Water (Table 3). At low-water, the settled material can benefit the benthic community as a whole because the nutritive value of the detrital material (and associated diatoms) may be enhanced via the previous resuspension and mixing (sensu Tenore, 1976). This provides a "new" composition of food for mudflat meiofauna on the mudflat with each tidal ebb. This "new" food is first available in high sediment concentrations during ELW (Jenness & Duineveld, 1985). Therefore, the high ambient food supply, flocculent nature of the sediment, and lack of various disturbances (normally imposed by water-cover) may combine to make ELW an ideal
feeding time for some mudflat meio-benthos.

Harpacticoid biology is apparently dependent on the stage of the tide and tidal currents. Sediment densities are highest at low-tide (Palmer & Brandt, 1981; Fleeger et al., 1984), feeding strategies change (Decho, 1986) and even patchiness differs (i.e. patchiness is most pronounced at low-tide (Decho & Fleeger, submitted)). Several of these activities could relate to the distribution of sediment or water column diatoms. During Low-Water, patchiness is most pronounced in *M. littorale* which actively forages and feeds at this time. This patchiness appears to result from aggregating around microbial food (diatom) clumps. In *S. canadensis*, which does not actively move or feed at Low-Water, patchiness is not in proximity to food clumps. During High-Water, some species utilize planktonic food resources by leaving the sediment (i.e. *M. littorale*, *C. deitersi*, also *S. canadensis* to some extent) and swimming in the water column. Other species (i.e. *S. canadensis*) construct burrows and filter suspended material above the burrows. Resuspension during times of water cover (whether active or passive) appears to be a common event for many harpacticoids in muddy habitats (Palmer and Brandt, 1981; Fleeger et al., 1984). It is not yet known if entry into the water column is good or bad for the harpacticoids (i.e. predation in the water column may be great). More empirical data on individual species is needed to fully evaluate how this relates to feeding processes. If many harpacticoids feed while in the water column on suspended benthic or water column resources, it might suggest that harpacticoids have evolved behaviors necessary to track phytal food resources over tidal cycles. Marcotte (1986) has
suggested a benthic (harpacticoid-like ancestor) to the Copepoda, which eventually radiated into the planktonic environment.

Many meiofauna show distributions related to tidal variation and amplitude (see Hicks & Coull, 1983; for review). Intertidal transition zones (i.e. mudflats), owing to their tidal influences can have a most pronounced effect on distributions via 1) extremes in physical conditions, 2) water-cover effects on feeding rates, and 3) hydrodynamic and biotic disturbances. In Louisiana marshes, these effects can be exaggerated. Tides are often wind-dominated, which can result in prolonged (i.e. several days to weeks) low-tides or high-tides. It is therefore not surprising than abundances of certain mudflat harpacticoids in Louisiana marshes follow these tidal anomalies on a seasonal scale. Highest densities of *S. canadensis* occur during summer and fall months (Phillips & Fleeger, 1985) when prevailing south winds cause HW conditions on the mudflat much of the time. Observations of *S. canadensis* indicate it requires water-cover (or at least a wet film over the sediment) to efficiently feed (Decho, 1986; Chandler & Fleeger, 1987). Abundances on the mudflat are greatly reduced during winter and early spring, when prevailing north winds keep the mudflat at low-tide conditions for weeks at a time. *P. huntsmani* appears to feed on diatoms (i.e. ingest chl-pigment) only during HW (although its ingestion of azocarmine during ELW and LLW (used to measure gut-passage time) indicates that it does ingest some particulate matter during these times). *P. huntsmani* is found primarily in subtidal areas. It is therefore exposed to Low-Water conditions only during occasional wind-driven extremes. Its range does not typically extend into mid- or high-intertidal regions.
of the mudflat

Gut-retention times, in the present study, also varied with tidal conditions supporting the arguments of Dagg and Walser (1987) stressing the importance of measuring gut-retention times under in situ conditions. It is also important to note that the use of a relatively inert tracer, such as azo-carmine and its first appearance in the feces, will provide a minimum gut-retention time. Observations suggest that significant mixing occurs in the anterior and middle portions of copepod guts during passage (personal observation). Gut retention times show a similar trend for all three harpacticoid species examined. Longest retention-times occurred consistently at LLW (Fig. 1). Relative changes in gut-retention times are a function of: 1) temperature (Kiorboe et al., 1982; Dagg & Wyman, 1983) (which remained relatively constant throughout the present field study), and feeding rate (Richman, 1966; Geller, 1975; Arashkevich, 1977; Baars & Oosterhuis, 1984; Simard et al., 1985). *S. canadensis* predictably showed longest gut-retention times during LLW when feeding rates were lowest, and much shorter retention times during HW and ELW (when feeding rates are much higher). *M. littorale* also follows this relationship, having longest retention rates during LLW (however feeding rates are only slightly lower than feeding rates at HW). Differences in the type of food (benthic vs. planktonic) ingested may affect the relative retention at these two times.

Size differences of copepod species also affect grazing rates. Adults of *S. canadensis* are typically two times larger than adults of either *M. littorale* or *P. huntsmani* (Table 4). These size differences certainly affect the observed consumption rates by *S. canadensis*.
relative to the other two harpacticoid species. However, weight-specific consumption rates (removing the effect of relative size differences among species) indicates approximately similar consumption rates for all three species during HW (Table 5). This implies that these three species consume similar amounts of chl-pigment (i.e. diatoms) per tissue weight, during HW. Also, both S. canadensis and M. littorale feed at similar rates during ELW (on a per weight basis).

The use of fluorescent gut-pigments as an index of herbivorous grazing has many potential applications in benthic studies. It is a conservative feeding estimate for two reasons. First, much of the benthic and near bottom food material (containing pigment), represent sinking scenedent phytoplankton, benthic diatoms, and detritus. A portion of their chlorophyll-derived pigments within this flora will be in various stages of decay, some of which are in non-fluorescent forms. Ingestion of these pigments will not be detected during subsequent gut-pigment analyses, and therefore will underestimate ingestion rates. Second, a portion of ingested chlorophyll (which is detectable) is converted by the digestive process to non-fluorescent forms, the exact mechanism of which is not known (Downs & Lorenzen, 1986). Conversion ranges from about 11% (Dagg & Walser, 1987) to over 34% (Shuman & Lorenzen, 1975) of the ingested chlorophyll in planktonic copepods. A correction factor, to compensate for these losses, was not applied to my data because the magnitude of these losses are not known for benthic harpacticoids. The lack of a correction factor, assuming it is a constant, however, should not influence the relative differences in grazing rates over the various
stages of the tidal cycle, and hence the trends in feeding which were
observed.

Many meiobenthic harpacticoids are known to utilize diatoms as a
major food resource. In the present study, analyses of gut-contents
from field-collected individuals, and laboratory radiolabel
experiments indicate the regular ingestion and utilization of diatoms
by four species of harpacticoids (S. canadensis, M. littorale, C.
deitersi, and P. huntsmani). Diatoms probably comprise a major
portion of the food resources for these species. However, as with
most harpacticoids which are labelled "diatom-feeders", the diatoms
probably do not constitute the sole (entire) food resource. Other
components are most likely utilized (perhaps in a supplemental
manner). For example, the bacteria, attached to the diatoms and
sediment particles are ingested (Decho & Fleeger, submitted), can be
an important source of certain vitamins (Phillips, 1984) and other
micronutrients (Morita, 1979). Some harpacticoids which typically
grow on diatoms, reproduce better with the additional presence of
bacteria (Provasoli et al., 1959). In some harpacticoids, bacteria
can even represent the sole food source (Rieper, 1978). The
extracellular secretions (sensu Hobbie & Lee, 1980), ingested
coincidentally with the bacteria and diatoms which secrete them, may
also be utilized in a similar manner.

The observed differences in feeding rates over different
portions of a tidal cycle provide further evidence that intertidal
meiobenthos do not feed at constant rates (and may even be
distributionally limited to some extent by their ability to feed)
over a typical tidal cycle. Also, food resource utilization (i.e.
benthic vs. planktonic) may change with changing tidal level. The frequent resuspension, mixing and settling of both meiobenthic animals and their food resources are imposed by the changing tidal levels. This implies that small-scale distributional patterns of some meiobenthic species and their food resources must develop relatively quickly, and are probably short-lived (i.e. less than the length of a tidal cycle).
LITERATURE CITED


Smith, and B.C. Coull. 1987. Juvenile spot (Pisces) and grass shrimp predation on meiobenthos in muddy and sandy substrates.


Table 1. Mean (±SE) food concentrations (no. cells·cm⁻²) and specific activities (dpm·cell⁻¹) of ^14C-diatoms (Amphora tenerrima) offered to harpacticoids in feeding experiments.

<table>
<thead>
<tr>
<th>HARPACTICOID SPECIES</th>
<th>FOOD CONCENTRATION</th>
<th>SPECIFIC ACTIVITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scottolana canadensis</td>
<td>775,000 ±16,300</td>
<td>3.1 - 4.3</td>
</tr>
<tr>
<td>Microarthridion litorale</td>
<td>510,760 ±14,875</td>
<td>1.8 - 2.6</td>
</tr>
<tr>
<td>Cletocamptus deitersi</td>
<td>611,400 ±21,634</td>
<td>2.6 - 3.5</td>
</tr>
</tbody>
</table>
Table 2. Results of laboratory feeding experiments on harpacticoid copepods using $^{14}$C-diatoms (Amphora tenerrima) as a food resource. Values expressed as dpms (disintegrations per minute and represent means ($\pm SE$) corrected for controls.

<table>
<thead>
<tr>
<th>HARPACTICOID SPECIES</th>
<th>$^{14}$C-INGESTED</th>
<th>$^{14}$C-REMAINING IN COPEPODS (3h Post-Feeding)</th>
<th>% LABEL RESPIRED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scottolana canadensis</td>
<td>186 $\pm$16</td>
<td>99 $\pm$12</td>
<td>25 $\pm$14</td>
</tr>
<tr>
<td>Microarthridion littorale</td>
<td>74 $\pm$12</td>
<td>37 $\pm$9</td>
<td>38 $\pm$18</td>
</tr>
<tr>
<td>Cletocamptus deitersi</td>
<td>106 $\pm$18</td>
<td>63 $\pm$15</td>
<td>32 $\pm$13</td>
</tr>
</tbody>
</table>
Table 3. Ambient sediment and water-column chlorophyll-pigment concentrations during field grazing experiments using harpacticoid copepods. HW= high-water conditions (i.e. high-tide); ELW= early low-water conditions (i.e. just after mudflat becomes exposed); LLW= late low-water (i.e. several hours after mudflat becomes exposed). Concentrations represent ng pigment:cm$^{-3}$ sediment (water).

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>TIDE</th>
<th>CHLOROPHYLL-a</th>
<th>PHEOPIGMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sediment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HW</td>
<td>1,705 ±633</td>
<td>11,630 ±1,977</td>
</tr>
<tr>
<td></td>
<td>ELW</td>
<td>2,466 ±591</td>
<td>18,980 ±5,320</td>
</tr>
<tr>
<td></td>
<td>LLW</td>
<td>2,245 ±786</td>
<td>15,864 ±3,015</td>
</tr>
<tr>
<td>Water-Column</td>
<td>HW</td>
<td>954 ±327</td>
<td>1,740 ±1,460</td>
</tr>
</tbody>
</table>
Table 4. Length and mean (±SE) dry weights for harpacticoids used in field grazing experiments.

<table>
<thead>
<tr>
<th>HARPACTICOID SPECIES</th>
<th>LENGTH (mm)</th>
<th>MEAN DRY WEIGHT (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scottolana canadensis</td>
<td>1.10 - 1.20</td>
<td>5.50 ±0.60</td>
</tr>
<tr>
<td>Microarthridion titoriare</td>
<td>0.50 - 0.72</td>
<td>2.64 ±0.18</td>
</tr>
<tr>
<td>Paronychocamptus huntsmani</td>
<td>0.40 - 0.60</td>
<td>2.38 ±0.12</td>
</tr>
</tbody>
</table>
Table 5. Standardized weight-specific ingestion rates of chlorophyll-equivalent pigments by harpacticoids. Values represent "ng chl-equiv. pigment·µg dry tissue·h". HW = high-water level; ELW = early low-water level (i.e. just after tide has receded); LLW = late low-water (i.e. several hours after tide has receded). Significance levels: ns = not significantly different from zero (P>0.05); * = significant (P<0.05); ** = significant (P<0.01).

<table>
<thead>
<tr>
<th>HARPACTICOID SPECIES</th>
<th>HW</th>
<th>ELW</th>
<th>LLW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scottolana canadensis</td>
<td>0.09 ±0.01*</td>
<td>0.12 ±0.02*</td>
<td>0.03 ±0.02ns</td>
</tr>
<tr>
<td>Microarthridion littorale</td>
<td>0.09 ±0.02*</td>
<td>0.18 ±0.05**</td>
<td>0.06 ±0.02*</td>
</tr>
<tr>
<td>Paronychocamptus huntsmani</td>
<td>0.09 ±0.01*</td>
<td>0.03 ±0.03ns</td>
<td>0.03 ±0.02ns</td>
</tr>
</tbody>
</table>
Fig. 1. Mean (±SE) gut-retention times for three species of harpacticoid copepods over different portions of a tidal cycle. Values given in minutes; (HW= high-water level; ELW= early low-water level; LLW= late low-water level). Letters on bars are results of Bonferroni tests; different letters signify differences for a given species at different tidal stages.
Fig. 2. Mean (±SE) ingestion rates of chlorophyll-equivalent pigment by three species of harpacticoid copepods over different portions of a tidal cycle. Values represent "ng chl-equiv. pigment· ind⁻¹·h⁻¹". HW= high-water level; ELW= early low-water level; LLW= late low-water level (see text for explanation). Letters on bars are results of Bonferroni tests; different letters signify differences for a given species at different tidal stages.
CHAPTER THREE

WATER-COVER INFLUENCES ON DIATOM INGESTION RATES BY MEIOBENTHIC COPEPODS

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Marine Ecology Progress Series
Water-cover influences on diatom ingestion rates by meiobenthic copepods

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ABSTRACT: Laboratory experiments on meiobenthic copepods using 14C-diatoms were conducted to investigate whether: (1) feeding rates or (2) food sources (planktonically-suspended foods or benthic sediment-associated foods) vary in response to the presence or absence of water-cover (i.e. simulated High-water vs Low-water conditions). Three diatom-feeding harpacticoids were examined. Scottolana canadensis feeds at significantly higher rates (2x) during High-water (HW) conditions (P<0.001), at which time it consumes planktonic foods; during Low-water (LW), feeding is greatly reduced. These feeding patterns are related to its burrow-dwelling and to its subtidal habitat. Cletocamptus deitersi remains virtually unaffected by changes in ambient water-cover, feeding at nearly equal rates during HW and LW conditions but always tending to consume more benthic diatoms. Microarthridion littorale consumes food at nearly equal rates during HW and LW conditions, but does so by shifting its feeding mode. During HW-times it makes excursions into the water column, feeding on planktonically-suspended foods. During LW-times it feeds benthically, moving over the sediment surface. Such feeding differences must affect meso-scale distributions of meiobenthos in the field, total benthic consumption and energy-flow estimates over a tidal-cycle, and the coupling of benthic and pelagic systems.

INTRODUCTION

Meiobenthic animals living at or near the intertidal limits of a salt marsh are exposed to a variety of physical conditions and potential food resources imposed by the fluctuating tidal cycle. During high-water conditions, the presence of water-cover over the sediment brings an influx of planktonic food resources (planktonic diatoms, detritus, heterotrophic bacteria) to the bottom and may permit meiofauna to enter the water column to better exploit these resources. Hydrodynamic effects on distribution (Eckman 1983, Jumars & Nowell 1984) and predation pressure by small fish (Bodicu & Villiers 1978) and invertebrates might be most intense on the meiobenthos during this stage (Fleeger 1980). At high-water, micro-algal grazers can potentially feed on (1) suspended planktonic food, (2) benthic (sediment-associated) food, or (3) both.

During low-water conditions, however, mudflat exposure may potentially limit feeding of some species. Much of the planktonically-suspended material has been removed in the outflow, some may now rest on the sediment surface. While predation can still be significant (Bell 1980), hydrodynamic effects are virtually non-existent due to lack of water cover. Grazing by meiobenthos, therefore, is limited to benthic (sediment) associated sources.

One might conclude that saltmarsh meiofauna, owing to their sediment-associated nature, feed at relatively constant rates over a tidal cycle. Recent studies, however, have shown that behavioral patterns affect both activity and movement (passive or active) of meiofauna over a tidal cycle, in a highly species-specific fashion (Fleeger et al. 1984, Palmer 1984). It therefore follows that these changes in activities or movements (with regard to tidal-level), may also influence both feeding rates and the type of food ingested (planktonic or benthic) at different parts of a tidal cycle.

The purpose of this study was to examine 2 aspects of meiobenthic harpacticoid copepods feeding on diatoms. First, to determine how the ingestion rate of diatoms by harpacticoids varies over simulated parts of a tidal cycle (presence or absence of water-cover; High-water vs Low-water conditions); second, to determine if these harpacticoids feed on (1) benthically-derived foods, or (2) planktonically-derived foods, or (3) both.
METHODS

Diatom culture. Two diatom species were used as food in feeding experiments. A planktonic species, *Thalassiosira weissflogii*, commonly found in salt marsh phytoplankton, was obtained from the Texas Algal Collection and grown in f-2 media (Guillard & Ryther 1962) in 15 % Instant Ocean Artificial Seawater (ASW) using 16 h light 8 h dark cycle at 20 °C. The benthic (sediment) diatom *Amphora tennerrima* (Alexis & Husteds) was isolated from salt marsh sediments and grown on Erdschreibers media under similar conditions. Cultures were kept under near-axenic conditions using Penicillin (10 000 U ml⁻¹ final conc.) and Vancomycin (0.2 mg ml⁻¹ final conc.). These 2 diatom species were chosen because they are of similar size, and are both ingested and assimilated by harpacticoids (*Scotolana canadensis*, *Cletocamptus deitersi*, *Microarthridion littorale*).

Labelling diatoms. Diatoms, in their log phase of growth, were labelled with NaH¹⁴CO₃ (New England Nuclear, Boston, Massachusetts) and centrifugation (6000 rpm for 10 min), resuspending in 40 ml sterile ASW. The final pellet was resuspended in 10 ml sterile ASW to yield a concentrated diatom suspension.

Quenching was corrected for using the external-standards ratio method. A second set of triplicate samples were cleared of unincorporated label (by centrifugation) with the pellet placed in scintillation vials and counted. Dark incubated controls were used to account for non-photosynthetic incorporation of label.

To measure the specific activity of ¹⁴C-diatoms, triplicate subsamples (100 µl) of diatoms were solubilized and counted by liquid scintillation counting (LSC). Diatom densities (cells ml⁻¹) were measured in triplicate by haemocytometer. Therefore, the specific activity (dpm cell⁻¹) could be measured. Specific activities of centrifuge-washed diatoms were measured at both the start and conclusion of the feeding experiment to examine if a significant loss of ¹⁴C-exudate loss during 1 h feeding experiments. Differences were analysed by ANOVA (SAS Institute Inc. 1982). The mean specific activity (from start and conclusion samples) was used later to calculate copepod ingestion rates.

Sediment preparation. Four separate treatments (Fig. 1) were constructed: 2 High-water treatments (HW-Tmt) and 2 Low-water treatments (LW-Tmt) using circular sediment microcosms (3.4 cm dia). The experimental animals and processed according to the methods of Chandler (1986). Each HW-Tmt contained 5 ml of ASW (final volume) overlying the sediment layer. LW-Tmts contained no overlying seawater cover in order to simulate an exposed mudflat at low tide.

Feeding experiment. For feeding experiments, harpacticoids were prestarved no longer than 15 min (maximum handling time) in order to reduce ingestion rate artifacts due to starvation effects (Hassett & Landry 1983, Murtagh 1984). Addition of food diatoms occurred in the following manner: for HW-Tmts, first, benthic diatoms (*Amphora tennerrima*) were added gently and close to the sediment, then allowed to settle and adhere to the sediment surface; second, 30 adult copepods of a given species were added; and third, immediately after, the planktonic diatoms (*Thalassiosira weissflogii*) were added. This set-up allowed minimal resuspension of sediment diatoms and also allowed planktonic diatoms to remain in suspension.

To one HW-Tmt (Fig. 1a) ¹⁴C-labelled planktonic diatoms and unlabelled sediment diatoms were added. ¹⁴C-label levels in the overlying seawater were measured in this Tmt at the start and conclusion of the feeding experiment to examine if a significant loss of label (due to settling of ¹⁴C-planktonic diatoms) occurred during this time. To a second HW-Tmt (Fig. 1b), unlabelled planktonic diatoms and ¹⁴C-labelled benthic diatoms were added. ¹⁴C-label levels of overlying water were also measured at the beginning and
Decho: Diatom ingestion rates by copepods

Fig. 1. Arrangement of 14C-labelled diatoms in High-Water (HW) and Low-Water (LW) treatments used in feeding experiments (ASW = Instant Ocean Artificial Seawater)

**RESULTS**

**Food concentrations**

Analysis of 14C-bicarbonate uptake by cells of both diatom species (Thalassiosira weissflogii, Amphora tenerrima) over a time series indicate linear uptake of label over the first 6 h of incubation. After 2 d incubation a near-homogeneous distribution of label among diatom cells was assumed. Dark incubated controls showed no significant uptake over a 48 h period. Specific activities of 14C-diatoms used in each of the feeding experiments are summarized in Table 1 and ranged from 0.4 to 3.4 dpm (diatom cell)-1. Food concentrations of labelled and unlabelled diatom were similar at each HW and LW-Tmt food concentration.

Analysis of HW-14C planktonic diatom Tmts (to determine if planktonic diatoms remained suspended in the water column during 1 h feeding experiments) indicated no significant (P>0.01) reduction in water column 14C-diatoms after 1 h. Analysis of water aliquots from 14C-benthic diatom Tmts showed no significant label in the water column indicating no appreciable suspension of 14C-sediment diatoms during 1 h feeding experiments. Therefore it could be assumed that during 1 h feeding experiments, planktonic-diatoms remained suspended in the water column and benthic-diatoms remained associated with the sediment.

Conclusion of the feeding experiment to ensure that benthic-diatoms were confined to the sediment and to examine if significant resuspension had occurred during the feeding experiments. To LW-Tmts (Fig. 1c & d) a similar format was followed in the additions of diatoms. For each feeding experiment, 3 pre-measured diatom food concentrations were used and are designated as Low, Medium, and High. Approximately equal concentrations of 14C-labelled and unlabelled diatoms were added to LW-Tmts. Proportionally higher concentrations of planktonic diatoms were added to HW-Tmts to account for the dilution of the overlying water.

Harpacticoid feeding time was always 1 h. This is less than the minimum gut-retention time as determined by azo-carmine appearance (Icely & Nott 1985) in the fecal pellets of the 3 species fed under similar conditions (i.e. temperature and food concentrations) (Decho unpubl.). Therefore, loss of ingested label via fecal pellets could be assumed to be zero. Also, as a second control, fecal pellets, collected from copepods used in feeding experiments were examined by LSC for the presence of 14C and compared to control fecal pellets (collected from copepods fed unlabelled diatoms). Ten fecal pellets were used for each sample. Samples were run in triplicate.

At the conclusion of the 1 h feeding period, individuals were removed from the sediment, washed in sterile ASW and transferred to scintillation vials using 200 μl ASW, prepared, and counted by LSC. Seven individuals were used in each replicate, with 4 replicates per treatment.

**Controls.** In order to control for harpacticoid uptake of 14C-label not as a result of ingesting diatoms, adults of each species were added to sediment containing the 14C-labelled diatom filtrate (0.8 μm Gelman filter). This filtrate was collected from 14C-labelled diatoms incubated in sterile ASW for 1 h (same time-length as feeding experiments). The diatom concentrations were of similar densities as those used in feeding experiments. The control copepods were incubated for 1 h, then removed and washed, and counted by LSC. This will more precisely control for 14C-diatom exudates, and leaky diatom intracellular products which the copepods are exposed to during feeding experiments than the standard NaH14CO3 in sterile SW controls (own obs.). Controls (dpm) were subtracted from Tmt level readings.

Respiration of 14CO2 during 1 h feeding experiments was measured in triplicate using separate 25 ml flasks, according to a modification of Hobbie & Crawford (1969). Each flask contained 7 individuals and was set up similarly to 1 of the 4 Tmts used in feeding experiments.

**Data analysis.** Ingestion rate data were analysed by 3-way ANOVA using General Linear Models (SAS, 1982). If a significant difference was indicated, Bonferroni's test was applied for comparison of means.
Table 1. Mean (± SE) food concentrations of diatoms (cells ml⁻¹) for each treatment (Tmt) used in feeding experiments for each food concentration. Specific activities (dpm cell⁻¹) of diatoms in parentheses.

<table>
<thead>
<tr>
<th>Harpacticoid</th>
<th>Food conc.</th>
<th>High-water Tmt</th>
<th>Low-water Tmt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Planktonic (T. weissflogii)</td>
<td>Benthic (A. tenerrima)</td>
</tr>
<tr>
<td>S. canadensis</td>
<td>Low</td>
<td>44,350 ± 1198 (1.8)</td>
<td>33,750 ± 1220 (0.44)</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>83,950 ± 1919 (1.6)</td>
<td>79,200 ± 1345 (1.5)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>161,450 ± 4032 (1.4)</td>
<td>148,600 ± 4460 (1.0)</td>
</tr>
<tr>
<td>C. deitersi</td>
<td>Low</td>
<td>17,680 ± 645 (1.7)</td>
<td>10,330 ± 620 (1.1)</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>48,240 ± 1887 (2.55)</td>
<td>49,440 ± 2070 (1.6)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>127,350 ± 6758 (2.2)</td>
<td>132,300 ± 4230 (3.1)</td>
</tr>
<tr>
<td>M. littorale</td>
<td>Low</td>
<td>52,580 ± 1800 (2.2)</td>
<td>29,020 ± 1350 (2.1)</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>108,064 ± 2090 (1.4)</td>
<td>61,600 ± 2246 (3.1)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>155,610 ± 3483 (1.03)</td>
<td>88,440 ± 4685 (2.6)</td>
</tr>
</tbody>
</table>

Water contents of sediments from LW-Tmts ranged between 1.51 and 1.63 ml equivalents of dh2O g⁻¹ dry sediment. These values are within the range of those observed at low-tide in the field habitat of the harpacticoids used in the experiments (own obs.).

Feeding experiments

Harpacticoid feeding experiments indicate that ingestion rates over the simulated parts of a tidal cycle vary significantly, depending on the harpacticoid species and the type of food (planktonic or benthic) ingested.

Scottolana canadensis showed greatest variation in diatom ingestion rates (Fig. 2) with regard to both ambient water level (HW vs LW) and diatom type (benthic vs planktonic). Highest total ingestion rates (P<0.001 by Bonferroni test) occurred during HW conditions with very low ingestion rates during LW conditions, over all 3 food concentrations. Ingestion rate increased with increasing food concentration (Fig. 2). Also, during HW conditions, 60 to 88 % of the diatoms consumed were planktonic (Thalasiosira weissflogii), indicating a 2x greater rate than the consumption rate of benthic-diatoms (Amphora tenerrima). During LW conditions however, ingestion of planktonic diatoms and benthic diatoms did not differ significantly (P>0.05).

Cletocamptus deitersi exhibited relatively similar ingestion rates at both HW and LW conditions, except low-food concentrations (Fig. 2). Here ingestion rates were significantly (P<0.05) greater during HW conditions.

Microarthridion littorale showed similar trends for total consumption of diatoms with slightly higher mean rates during LW conditions. However, during HW conditions, planktonic diatoms were consumed at much higher rates (P>0.001) than benthic diatoms (Fig. 2). This pattern was present over all 3 food concentrations. Ingestion rates of both planktonic and benthic diatoms increased with increasing food concentration.

Respiration of 14CO2 varied in magnitude depending on the species and quantity of food consumed. Approximately 15 to 28 % of the ingested 14C-diatom label was respired by the 3 harpacticoid species. Values at each Tmt food-concentration are given in Table 2.

Analysis of fecal pellets collected from copepods used in feeding experiments indicated no appreciable amounts of 14C above control levels could be detected for any of the 3 harpacticoid species. This indicates there was no appreciable loss or recycling of 14C-label via fecal pellets during the 1 h feeding experiments.

DISCUSSION

Significant variability in ingestion rates for meiobenthic copepods results from (1) the presence or absence of water-cover (HW vs LW), and/or (2) the type of diatom (planktonic-suspended or benthic) ingested.
Decho: Diatom ingestion rates by copepods

Two of the 3 harpacticoid species examined showed significant differences in ingestion rates of diatoms with regard to these parameters.

During High-water (HW), *Scottolana canadensis* ingested more diatoms than during low-water (LW) over all food concentrations. This increased feeding rate was primarily due to the increased consumption of planktonic diatoms (Fig. 2) at HW. As food concentration increased, a greater percentage of ingested food was planktonic. Ingestion of benthic (sediment) diatoms remained relatively constant (P > 0.05) between HW and LW.

Laboratory and field observations of *Scottolana canadensis* at HW and LW indicate it to be a semi-sessile burrow-dweller. During HW conditions, *S. canadensis* constructs a semi-permanent burrow in which it resides (although individuals occasionally enter the water column). By use of its appendages, water and particulate matter from the overlying water column are drawn into the burrow (G. T. Chandler pers. comm.). The majority of this material consists of suspended plankton and detritus, with also some sediment particles (and benthic diatoms) in proximity to the burrow opening. The material is concentrated into a bolus (Chandler pers. comm.), with some of the bolus being ingested, and the remainder being discarded out of the opposite end of the burrow. The mechanics of particle selection are unknown. Both short-range (i.e. tactile) and long-range chemoreception may contribute as in some planktonic copepods (Paffenhofer & Van Sant 1985). During LW conditions, *S. canadensis* is occasionally seen moving atop the sediment surface, but most often resides within its burrow. Feeding at this time is reduced to that material already within its burrow or in immediate proximity to its burrow, and feeding rates are significantly reduced.

Predelection of *Scottolana canadensis* toward planktonic food sources is characteristic of both adults and nauplii. Adults grow best in culture on a variety of

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**Table 2.** $^{14}$C-label respired (dpm copepod$^{-1}$ h$^{-1}$) during 1 h feeding experiments. Values expressed as mean ± SE (percentage of ingested label respired in parentheses)

<table>
<thead>
<tr>
<th>Harpacticoid</th>
<th>Food conc.</th>
<th>High-water Tmt</th>
<th>Low-water Tmt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Planktonic diatom</td>
<td>Benthic diatom</td>
<td>Planktonic diatom</td>
</tr>
<tr>
<td><em>S. canadensis</em></td>
<td>Low</td>
<td>21.0 ± 3.4 (22)</td>
<td>4.2 ± 2.4 (28)</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>16.0 ± 2.1 (19)</td>
<td>10.0 ± 2.4 (20)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>18.0 ± 4.0 (20)</td>
<td>2.4 ± 1.1 (27)</td>
</tr>
<tr>
<td><em>C. deitersi</em></td>
<td>Low</td>
<td>6.0 ± 1.1 (22)</td>
<td>9.0 ± 2.3 (24)</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>7.5 ± 1.6 (21)</td>
<td>19.0 ± 1.6 (20)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>7.9 ± 0.6 (19)</td>
<td>10.0 ± 0.4 (25)</td>
</tr>
<tr>
<td><em>M. littorale</em></td>
<td>Low</td>
<td>11.3 ± 2.0 (20)</td>
<td>10.8 ± 1.6 (23)</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>17.3 ± 2.6 (25)</td>
<td>7.4 ± 0.5 (27)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>13.0 ± 2.1 (21)</td>
<td>22.1 ± 1.9 (24)</td>
</tr>
</tbody>
</table>
planktonic diatoms (Harris 1977, Lonsdale & Levinton 1985, Chandler 1986) and can often be collected at night from the water column in plankton tows (J. Howze pers. comm.). Nauplii are pelagic (Lonsdale & Levinton 1985) and obligate planktivores (Harris 1977).

Diatom ingestion rates of *Cletocamptus deitersi* were slightly higher during HW conditions (at low and medium food concentrations), but more benthic diatoms were ingested regardless of water level. *C. deitersi* continuously crawls over the sediment surface and/or burrows throughout the sediment with intermittent periods of swimming through the water column. Movement is quite vigorous on the sediment during LW conditions. Swimming at HW can increase its foraging ability, especially at low food concentrations. This harpacticoid grows best in culture on a mixture of both planktonic diatoms and sediment diatoms (Chandler 1986).

*Microarthridion littorale* ingests similar amounts of total food between HW and LW conditions. Apparently it does so by shifting its feeding mode. During HW, it tends to feed on planktonic diatoms by spending a large portion of its time in the water column, first swimming upward, then ‘dive-bombing’ to the sediment surface (Chandler pers. comm.). *M. littorale* is easily suspended during HW and does not readily return to the sediment once suspended (Palmer 1984). At LW it feeds on either benthic diatoms or planktonic diatoms. During this time it actively plows across the sediment surface, apparently foraging. Its total ingestion rate increases slightly during LW. The increased consumption of benthic diatoms (and decrease in planktonic diatoms) during LW conditions is most pronounced at medium food concentrations. The reasons for this pattern are unclear. This copepod can be cultured on either planktonic diatoms (Chandler 1986) or benthic diatoms (Palmer & Coull 1980).

Respiration values of *14CO2* (Table 2) do not appear to reflect the relative activities (i.e. movements) of the harpacticoids during feeding but instead reflect only the amounts of *14C*-labelled food ingested.

The ability of a meiobenthic animal to feed at HW and LW may potentially affect its distribution in the field. Mesoscale (meters) distributional variations of salt marsh meiofauna have been documented by Phillips & Fleeger (1985). Distributional changes in species composition have also been shown to occur over a vertical tidal gradient and are well established in some areas (Coull et al. 1979, Fleeger 1985).

*Scotolana canadensis* feeds at considerably higher rates during HW (under laboratory conditions). Field distributions of *S. canadensis* in Louisiana marshes reveal that it is associated with areas and times of the year when water cover predominates (HW conditions) for extended periods (Phillips & Fleeger 1985). Here, it is commonly found in intertidal marsh ponds (Chandler & Fleeger 1983, Phillips & Fleeger 1985) and subtidal areas (Fleeger et al. 1983). High abundances in intertidal ponds tend to occur during late summer and fall, when predominant south winds override lunar tidal fluctuations, and the marsh remains at HW conditions for many weeks at a time (Whipple et al. 1981). During spring, when north winds predominate and the marsh is kept at LW conditions for extended periods, *S. canadensis* is typically found at low abundances intertidally (Phillips & Fleeger 1985) with higher abundances subtidally (own obs.). *S. canadensis* has a wide geographical range (Coull 1972) and is most commonly found in shallow subtidal and low-intertidal brackish-water areas (Coull et al. 1979, Phillips & Fleeger 1985) where water cover and plankton prevail.

Field distribution of *Cletocamptus deitersi* in Louisiana extends from subtidal to high intertidal. Its ability to ingest food diatoms under laboratory conditions indicates that it can feed equally well under both subtidal (HW) and high-intertidal (mainly LW) conditions.

The distribution of *Microarthridion littorale* extends from subtidal to high intertidal in South Carolina marshes (Coull et al. 1979) comprising 2 definable subpopulations differing in size and life history patterns (Palmer 1980). Its range of distribution from subtidal to high marsh documents its ability to exist under prolonged HW and/or LW conditions. In Louisiana, however, the range of *M. littorale* is confined to the low intertidal and primarily subtidal regions of marshes (Fleeger 1985). Reasons for this somewhat restricted distribution have been postulated by Fleeger (1985) but are still unknown. *M. littorale* can consume a relatively constant amount of diatoms at HW vs LW conditions by apparently shifting its feeding mode from pelagic to benthic. However, field verification of such shifts (observed in laboratory experiments) would be especially important because other factors may play a role. Predation, time of day (day vs dusk vs night) (Bell et al. 1984), and hydrodynamics during HW (Eckman 1983, Jumars & Nowell 1984) and associated tidal resuspension (Palmer & Brandt 1981, Fleeger et al. 1984) can influence the pelagic or epibenthic behavior of some meiofauna (Palmer 1984) ultimately restricting them to the sediment. Variable food supply (Bulthuis et al. 1984, Kirchman et al. 1984, Webb & Marcotte 1984, Laanbroek & Verplanke 1986) may also influence the behavior of meiobenthic species (Chandler pers. comm.). In my studies, keeping these other factors constant, ambient water-cover alone could significantly influence the ability of some meiobenthos to ingest food.

Meiobenthos are not confined to the sediment (Bell...
& Sherman 1980, Hagerman & Rieger 1981, Bell et al. 1984, Fleeger et al. 1984, Jumars & Nowell 1984, Palmer 1984, and others). Consumption of, and persistence on, planktonic food in the lab indicates for some meiofauna a potentially close coupling with the overlying plankton. More precise estimation of meiofaunal consumption effects on benthic and pelagic microbial communities in the field, and of the overall role of meiofauna in energy flow requires species-specific grazing-rate measurements (in conjunction with species abundance and production estimates) where logistically possible. Energy flow through the meiofauna may be underestimated (Admiraal et al. 1983, Montagna 1984, Strayer & Likens 1986) and have been largely confined to examining major taxa. The importance of these rate measurements in the field cannot be overstated with regard to quantifying the role of the meiofauna as consumers and producers.

Finally, the ability of a meiofaunal species to feed during HW and LW can potentially affect energy-flow estimates of meiofauna, depending on the portion of a tidal cycle measured. Also, the field distribution of a meiofaunal species, with respect to the total amount of time it is covered with water, should be in accordance with its ability to feed under those conditions if it is to maximize its energy intake. Field verification of these rate differences is required, as other factors – such as food availability, predation, and hydrodynamic effects – can either compound or reduce differences observed in the laboratory.

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LITERATURE CITED


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CHAPTER FOUR

TITLE: MICROSPATIAL DISTRIBUTIONAL RESPONSES OF MEIOBENTHIC HARPACTICOIDs TO FOOD RESOURCE PATCHINESS.

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ABSTRACT: Micro-scale (mm²) spatial patterns of harpacticoid copepods were measured on intertidal field mudflat sediments using contiguous core samples and spatial autocorrelation. Their associations with potential major food resources (diatoms) were further examined in laboratory studies. High density patches of the harpacticoid Microarthridion littorale were positively correlated with high concentrations of diatoms (as measured by chlorophyll-a conc.) during low-tide. Also, laboratory preference experiments show this species seeks out and locates sediments containing high concentrations of diatoms and/or their chemical exudates. During high-water, patchiness patterns in M. littorale disappear as individuals typically frequent the water column for feeding. A second harpacticoid, Scottolana canadensis, feeds at very reduced rates during low-tide, and is not associated with chl-pigments. S. canadensis is a burrow-dweller which feeds primarily during times of water-cover on suspended planktonic material, and laboratory preference experiments indicate it does not actively seek sediments containing high concentrations of sediment food resources. Micro-spatial distributions (patchiness) of intertidal meiobenthos are not regulated by any single factor, but regulation varies depending on the species in question, mode of feeding used (benthic or planktonic), and stage of the tidal cycle. Spatial pattern in most epibenthic mud-dwellers may be disrupted with each tidal cycle due to active and passive resuspension processes. Spatial patterns therefore, must develop relatively quickly, and may be influenced by processes occurring during the preceding tidal stage.
INTRODUCTION

Meiofauna are noted for their patchy spatial distributions at several spatial scales (see Fleeger & Decho, 1987; for review). Many causes for spatial patchiness have been proposed. Hydrodynamics (Bell & Coen, 1982a; Eckman, 1983; Chandler & Fleeger, 1983; Fleeger et al., 1984; Palmer, 1984; Thistle et al., 1984;), biotic structure (Bell et al., 1978; Thistle, 1978; Thistle, 1979; Thistle, 1980; Findlay, 1981; Bell & Coen, 1982b; Thistle et al., 1984; Warwick et al., 1986), reproduction (Heip, 1975; Findlay, 1981), predation (Bell, 1979; Thistle, 1980; Fleeger 1985a), microphysical topography (Hogue & Miller, 1981; Decho et al., 1985; interspecific (competetive and facilitative) interactions (Ivester & Coull, 1977; Hogue, 1978; Bell, 1980; Joint et al., 1982; Bell, 1983; Chandler & Fleeger, 1984; Marcotte, 1984; Fleeger. 1985a; Chandler & Fleeger, 1987), disturbance (Thistle, 1980; Reidenauer & Thistle, 1981), and food (microbial flora) patchiness (Gray, 1968; Gray & Johnson, 1970; Gerlach, 1977; Hicks, 1977; Lee et al., 1977; Ravenel & Thistle, 1981; Montagna & Spies, 1985; Decho & Castenholz, 1986; Carman & Thistle, 1985) are most often cited. These proposed causal effects, however, frequently remain untested because of the logistical difficulties in manipulating both the organisms and local conditions in the field or laboratory.

Response to small-scale (mm²-cm²) patchiness of microbial food resources has been considered one of the most plausible explanations of meiofaunal patchiness. However, this hypothesis is difficult to test for several reasons. First, one must accurately measure and
define what constitutes a microbial clump in natural sediments (i.e. are all microbial clumps the same?). Second, one must identify the appropriate size scale to sample in the field: Is it m², cm² or mm²? The sampling size should be a function of the meiofaunal clump size, which has been shown to be variable (Findlay, 1981; Hogue, 1978). Thirdly, it is not clear how a meiofaunal patch forms? (i.e. the mechanism of attraction) and how long does one last? (i.e. hours, days, until the microbial food is consumed, etc.). Lastly, as the microbial food in a clump diminishes (due to consumption by meiofauna) the microbial portion would no longer be detectable as a clump during sampling. Therefore, some meiofauna clumps would not be associated with corresponding microbial clumps.

How then, are correlative field data interpretable? The lack of a significant correlation cannot always be used as evidence that meiofauna do not respond to microbial patchiness. There are several reasons why a true relationship may not be detected: A) Attraction may be to specific microbial clumps rather than a generalized distribution of diatoms or total microflora. Microbial patches differ with age (species composition, and associated ciliates, bacteria, etc. are different on diatom patches of different ages) and meiofauna have been shown to be attracted to only certain types of microbial clumps depending on the species composition (Lee et al., 1977; Trotter & Webster, 1984). B) A temporal sampling artifact may occur. Meiofauna may be attracted to most clumps but simply have not located or colonized some of the clumps at the time of sampling). This seems unlikely because meiofauna (especially harpacticoids) can colonize sediments quickly. C) There is a sampling-size artifact. The size range of the clumps (both meiofaunal and microbial) is variable,
therefore clumps smaller than the size of the sampled area will very likely not be detected.

Microbial clumps may vary considerably in size as well as composition. For example, a lysed diatom cell may create a bacterial clump within the immediate "phycosphere." This may be only several micrometers in diameter (Bell & Mitchell, 1972; Bell et al., 1974) and is not detectable by most sampling methods. Positive correlations may also be spurious (i.e. no biologically meaningful relationship may be present). Thus it may prove necessary to examine the causes of field distributions using controlled laboratory experiments.

Here we report on a series of laboratory and field studies conducted to examine whether the patchiness of microbial (diatom) food resources can influence the small-scale spatial patchiness of meio-benthic copepods in the field. First, field distributions of harpacticoid copepods and their diatom food resources were examined at a microspatial scale (mm$^2$) using spatial auto-correlation among and between harpacticoids and chlorophyll-pigments (as a measure of diatom abundance). Then, two sets of laboratory preference experiments were conducted to determine if patches of diatoms (or their exudates) initiate aggregation of copepods, and if so, what the attractive mechanism is (i.e. how do diatom-feeding copepods find clumps of diatoms)? Laboratory experiments were designed to control the type of microbial flora in the clump, the clump size, and potential temporal sampling artifacts which might otherwise occur.
METHODS

MICROSPATIAL DISTRIBUTIONS:

Field sampling was conducted to determine if microspatial (mm²) patchiness of harpacticoid species could be associated with distributional patterns of their microalgal food (diatoms) as determined by chlorophyll content. Diatoms represent a major food resource for many of the harpacticoid species at the study site (Decho, submitted). Since chlorophyll-a is found in diatoms, this was chosen as a means of detecting microbial (food) patches. Both meiofauna and microbial determinations were made from the same sediment core samples. Sediment samples were collected at high-tide and low-tide using 16 contiguous cores (8 mm dia) arranged in a 4 x 4 matrix (core set). Three core-sets were taken at each sampling time. Plungers were not used in coring syringes since their presence can introduce a significant bow-wave artifact (Rutledge & Fleeger, submitted). Only the top 1 cm of sediment was used in analyses. Samples were collected on March 30, 1987 from the lower portion of an intertidal mudflat near the Louisiana Universities Marine Center (LUMCON) at Cocodrie, LA.

Samples were quickly (within 10 min) returned to the laboratory, where sediment cores were individually sieved (105 μm Nitex mesh) to remove harpacticoid copepodite and adult stages. The remaining detritus was returned to the sediment portion. The copepods were
fixed in 10% buffered formalin in SW in separate vials, stained in rose bengal, and later identified to species and enumerated. The sediment portion was centrifuged, then the supernatant filtered (Whatman GF/F) to retain microalgal cells still in suspension. The filter and sediment pellet was then added to 15 ml 95% acetone and homogenized. Chlorophyll and pheopigments were subsequently measured before and after acidification (10% HCl) on a Turner Model 110 Fluorometer. These methods ensured that the abundance of copepods and the concentration of chlorophyll could be measured from the same core sample, providing a small-scale (mm²-cm²) resolution of meiofauna and microbial distributions.

Harpacticoid species distributions were analyzed at both high-tide and low-tide conditions. Since preliminary studies indicated patchiness was most pronounced during low-tide conditions, chlorophyll-pheopigments were only analyzed during low-tide conditions. All chlorophyll-pheopigment results were standardized by sediment dry-weight.

Distributional analyses (to detect patchiness) of harpacticoid species and pigment concentrations were conducted by spatial-autocorrelation analysis (Jumars et al., 1977) using Interactive Matrix Language (SAS, 1985). Spatial autocorrelation measures the presence and relative magnitude of the aggregations. It locates aggregations in a two-dimensional area, and may be used to determine patch size. Several statistic are necessary to utilize spatial autocorrelation: Fisher's index of dispersion (s²/λ), Moran's I, and Geary's c (Cliff & Ord, 1973). Weights used for calculations of I and c represent distance⁻². Raw data counts were used in these analyses. Log (x+1) transformation of data counts (for assumptions of
normality) did not affect the resulting interpretations.

PREFERENCE EXPERIMENTS (LEVEL OF ATTRACTION):

Preference experiments were conducted to examine if diatom-feeding meiobenthic harpacticoid copepods were significantly attracted to 1) Diatom-sediment patches: containing sediment, diatoms, bacteria, diatom exudate products; 2) Exudate-sediment patches: containing sediment, diatoms exudates (but no diatoms), bacteria; 3) Background-sediment patches: containing bacteria, and some organics; and 4) Control-sediment patches (containing sediment pre-treated with 30% peroxide to remove most organics, and bacteria.

Three species of meiobenthic copepods were collected from a salt marsh mudflat near the LUMCON facility at Cocodrie, LA, by gentle wet sieving, and returned to the laboratory in Baton Rouge. They were kept in temporary culture (Chandler, 1986) until use.

Preference experiment chambers were circular (10.5 cm dia.) 5 cm. ht. The bottoms of the chambers were covered with 5% agar to approximately 2 cm in depth. From each of these chambers eight circular wells were cored near the periphery of the chamber (Fig. 1). Into the wells was placed an equal amount of sediment from one of four different treatments (Table I):

1) Control-Sediment: consisted of sieved (<125 μm) salt marsh sediment, digested in 30% peroxide to remove organics, then repeatedly washed in dH2O (5 times). This would serve as control sediment, with negligible organics and bacteria.

2) Background-Sediment: consisted of sieved sediment prepared according to the methods of Chandler (1986) to remove coarse detritus (>125 μm) and much of the humic acid. This sediment contained
bacteria, some detritus and organics, and served as a background organic sediment.

3) **Exudate-Sediment**: was composed of background-sediment with added diatom-exudate products filtered (0.22 μm) sterilized isolated from near-axenic cultures of the saltmarsh diatom *Amphora tenerrima* in the log-phase of growth. This treatment therefore contained diatom exudate products, bacteria plus background organic levels. Exudates here were isolated from similar densities of diatoms used in the Diatom-trts. Exudates were mixed homogeneously with sediments before addition to chamber wells. Amino acid analyses were conducted on subsamples of sediment filtrate to verify and quantify concentrations of these dissolved organic exudates before addition to chamber wells. Dissolved free amino acids were measured from centrifuged pore water samples using a Pico-Tag Workstation HPLC (Waters, Assoc.), with derivitization by PITC (phenylisothiocyanate) (Bidlingmeyer et al., 1984). Subsamples were desalted prior to derivitization using Sep-Pak C18 chromatography cartridges (Waters, Assoc.) (Pfeifer et al., 1983). The detection limit is 1 pmol per amino acid. The exudate-sediment treatment tested the effect of dissolved diatom exudate products in attracting meio-benthic harpacticoids.

4) **Diatom-Sediment**: was composed of background-sediment with known densities of the diatom *Amphora tenerrima* added. This treatment also contained bacteria associated with the diatoms and sediment, exudates released by diatoms, and background organics, and tested the effect of a diatom clump and its associated microflora in attracting harpacticoids. The four replicate patch treatments were randomly assigned to the eight positions (wells) within each chamber. The four patch types were randomized with respect to nearest neighbor, then
duplicated in each well position directly opposite on the octagon. Therefore there were no identical side by side patches. Three replicate chambers were used for each experiment. Care was taken to avoid psuedoreplication (Hurlbert, 1984).

In order to monitor the local microbial conditions within each treatment well, several parameters were measured. Each treatment was prepared as a single preparation, then aliquots were added to the corresponding wells. Bacterial biomass in the sediments was monitored using extractable lipid phosphate analysis (White et al., 1979). Lipid phosphate measured both prokaryote and eukaryote lipid (i.e. total biomass). Therefore, significant contamination by eukaryotes could only occur in the diatom-containing treatments. To obviate mismeasurement in the diatom treatment patches, lipid phosphate measurements were made from samples collected immediately before addition of diatoms. Added diatom densities were measured by direct counts using a haemocytometer. Total organic carbon of sediments was measured by ash-dry weight method of Conover (1966).

For a typical preference experiment, 600-650 adult harpacticoids of a single species (Scottolana canadensis Willey, Cletocamptus deitersi Richard, or Microarthridion littorale Poppe) were added to the center of a chamber. They were temporarily confined to the center by a clear plastic cylinder barrier. This allowed the animals to acclimatize, and allowed the uniform release of all animals at once. To begin an experiment, the barrier was slowly removed. All experiments were run for 2 h (22-23°C) in darkness, at which time the copepods were removed from the wells and enumerated. A red light was used for illumination during the loading and final extraction of animals. Other experiments indicated 2 h was sufficient time for
colonization of sediment by harpacticoids, but short enough to reduce artifacts due to overgrazing (Chandler & Fleeger, 1987).

Data was analyzed using a randomized block design (ANOVA) by SAS (1985). If the Block (between chamber) effect probability was >0.3 this effect was then pooled with trt*chamber interaction (Bancroft & Han, 1983). Also if the chamber* trt effect was >0.3 then this effect was pooled with the sampling error effect to increase the degrees of freedom. A posteriori comparison of treatment means was analyzed using Bonferroni tests (SAS, 1985).
RESULTS

FIELD MICROSpatial DISTRIBUTIONS:

Harpacticoid species densities were consistently lower in numbers in the sediment at high-water (Table II). The mean chlorophyll concentrations in sediments at low-water was 2.037 ±1.128 µg pigment·cm⁻³. Mean pheopigment and total pigment concentrations were 14.496 ±4.877 and 16.535 ±5.118 µg pigment·cm⁻³, respectively.

Analyses of species distributions using spatial autocorrelation indicate significant patchiness patterns for several harpacticoid species (see s²/¯x ratios, Moran's I, and Geary's c indices of aggregation -Table III). Microarthridion littorale and Pseudostenhelia wellsi showed most pronounced patchiness during low-tide, as indicated by s²/¯x, c and I indices. During high-tide, patchiness was not distinguishable. Chlorophyll-a data indicate significant patchiness patterns present for all replicate core sets at low-tide.

Results of log-log regression, using pooled core-sets of harpacticoid species abundance data and chlorophyll-pigment concentrations at low-tide, shows a significant (P<0.001) positive relationship between M. littorale and chlorophyll concentration (R²=0.41). Fig. 3 visually portrays the remarkable similarity in patch locations of M. littorale and pigment concentration in each replicate core-set. M. littorale was not significantly associated with total-pigment concentrations or abundances of any other harpacticoid species. S. canadensis low-tide abundances were not
significantly associated with chlorophyll concentration or total-pigment concentration (P>0.05).

During both low-tide and high-tide, *S. canadensis* was significantly (P<0.05) correlated with distributions of *P. wellsi* (R²=0.19 & 0.21, respectively). Other inter-species correlations were not significant. No other harpacticoid species analyzed showed significant correlations with either potential food concentration or other harpacticoid species.

LABORATORY PREFERENCE EXPERIMENTS:

Chemical and microbiological analyses of sediments used in preference experiment are summarized in Table IV. Control-sediments contained only trace amounts of organic carbon (<1.5% by wt) with negligible amounts of microbial biomass (as measured by lipid phosphate). Background-sediments contained organic carbon content (9.9-10.8%) considerably higher than control-sediments but similar to exudate- and diatom-sediments. Amino acid analyses of pore water exudate-treatment sediments showed: glutamic acid (1047-1286 pmol·ml⁻¹); valine (270-451 pmol·ml⁻¹); aspartic acid (43-79 pmol·ml⁻¹); glycine (208-386 pmol·ml⁻¹).

Analysis of variance using a randomized block design on log-transformed density counts indicated no significant (P>0.85) block effect (i.e. replicate chambers did not differ) for preference experiments. Therefore this error term associated with the block effect was pooled to increase the degrees of freedom. The resulting ANOVA indicates a highly significant (P<0.001) treatment effect for all three harpacticoid species examined colonizing sediment-altered treatments. A posteriori comparison of treatment-means using
Bonferroni tests indicate that *Scottolana canadensis* showed equal attraction to Diatom-trt, Exudate-trt, and Background-trt, with significantly less attraction to Control-trt patches. Approximately 3% of the individuals were not located in any patch trt (i.e. were free swimming at the time of sampling). *Microarthridion littorale* showed statistically similar attraction to both the Diatom-trt and Exudate-trt patches with significantly less attraction to Background-trt and Control-trt patches. Approximately 9% of individuals were not confined to any patch at the time of sampling. *Cletocamptus deitersi* showed highest attraction to Diatom-trt patches, significantly less attraction to Exudate-trt, and even less attraction to Background-trts. 2% of the individuals were free swimming at the time of sampling.

**DISCUSSION**

Densities in contiguous cores from an intertidal mudflat show a strong association of certain harpactioid species (i.e. *Microarthridion littorale*) with patches of their microalgal food resources (as measured by chlorophyll-a concentration) at a microspatial (mm$^2$-cm$^2$) scale. Patchiness in *M. littorale* was dependent on the stage of the tidal cycle and preference experiments suggest that it reflects feeding strategies. Clearly, our sampling method whereby copepod abundances and the concentration of chlorophyll were measured in the same core was important in allowing the use of sophisticated statistical techniques (i.e. spatial autocorrelation) to identify this relationship where others have
failed (Montagna et al., 1983). Abundance data and concentration data from the same cores makes interpretation more straight-forward by reducing alternative explanations.

HIGH-TIDE LOW-TIDE ABUNDANCE PATTERNS:

Copepod abundances in mudflat sediments show marked differences between high-tide and low-tide (Table II). Significantly (P<0.05) higher mean densities occur for most species during low-tide. This pattern is comparable with other studies (Fleeger et al., 1984) and has been shown to result from both the passive and active suspension of meiobenthic organisms into the water column during times of water-cover (i.e. high-tide). Both *Scortolana canadensis* and *M. littorale* follow this trend (Table II) and other studies have shown these species to frequently enter the water-column during high-water conditions (Fleeger et al., 1984; Palmer, 1984; Lonsdale & Levinton, 1985). A species that did not typically leave the sediment during high-water conditions was the harpacticoid *Pseudostenhelia wellsi*, which showed similar sediment densities at both high- and low-tides (P>0.05). *P. wellsi* is a mucus tube-dweller, and laboratory observations indicate it is generally confined to its tube much of the time (Chandler & Fleeger, 1984).

Tidal stage also affects patchiness. Aggregation was most pronounced at low-tide, (see spatial autocorrelation results, Table III) and was especially notable in *M. littorale*, *P. wellsi*, and chlorophyll (food) concentration (as measured by either Moran's I and Geary's c coefficients). Aggregation tendencies for most species disappeared at high-tide (chlorophyll concentration was not measured at high-tide). The size of the patches (during low-tide) for both
potential food (as determined by chlorophyll-pigments) and copepods was variable during low-tide, ranging from less than a cm to several cm in width.

Some tendency for aggregation during high-tide was present for *S. canadensis* with patch sizes probably less than 9 mm (approx. diameter of a single core sample) as indicated by significant $s^2/\bar{x}$ ratios (Table III). However, the I and c statistics, corresponding to these core-sets, indicate a very low level of patchiness. This implies that the sizes of the aggregations are probably smaller than one which would include two or more adjacent core samples, and therefore are not aggregated at the scale of sampling (Jumars et al., 1977). Low-tide core-set 3 for *M. littorale* (Table III) also shows this same pattern. Spatial patterns using I are best detected when abundances in several adjacent core samples deviate strongly from (above or below) the overall mean (Sokal & Oden, 1978). Chlorophyll (food) concentrations were also patchy at low-tide. Significant I values indicate the presence of a few "extreme" peaks present (Jumars et al., 1977) (Fig. 3).

A regular dispersion of harpacticoids in some core sets also occurred depending on the tidal cycle. A $s^2/\bar{x} < 1$ generally indicates a regular distribution (Sokal & Rohlf, 1981). This dispersion pattern, however, is further supported when values for I are less than E(I) = 0.066 and values for c are less than E(c) = 1.000 (for n=16 in present study). *S. canadensis* at low-water (core-sets 1 & 2), for *M. littorale* at high-water (core-sets 1 & 2) and low-water (core-set 1), and for *Paronychocamptus huntsmani* at both low-water (core-set 3) and high-water (core-set 3) display this pattern (see Table III).
Regular patterns are found in animals with territories or that have intraspecific interactions (e.g. spionid polychaetes (Levin, 1981). However, the ephemeral nature (only part of a tide) of harpacticoid distributions make it difficult to interpret.

The results of spatial autocorrelation indicate that dispersion patterns of meiofenthic individuals in the sediment can vary at several size levels within a relatively small area (i.e. less than 1 m$^{-2}$) on a mudflat. Some core-sets indicate highly contagious distributions with patch sizes ranging from less than 1 cm (i.e. S. canadensis during HW) to several cm (i.e. M. littorale at low-water), to distributions where a regular dispersion of individuals is apparent over an entire core-set (i.e. M. littorale - core sets 1 & 2 at HW). In other studies, Heip (1976) found patch sizes of ostracods approximately 26 cm in diameter with evidence of smaller aggregations within each patch. Nixon (1976) found sandy beach gastrotrichs aggregations to range from 2 to 6 cm, and the aggregations were most stable at low-tide, while Hogue (1978) found similar patch sizes (4-10 cm) in subtidal gastrotrich assemblages. These studies show that patchiness patterns can vary considerably in size, and detecting their presence at these various levels is highly dependent on the sampling size scale used (Findlay, 1982).

**SPECIES-FOOD ASSOCIATIONS:**

Regressions of harpacticoid abundances with microbial food abundances on a core by core basis, show the significant association of M. littorale (P<0.001) with chlorophyll-pigment (food) concentration at a microspatial scale ($R^2=0.41$). Laboratory and field
feeding experiments indicate that diatoms (which contain chlorophyll) comprise a substantial part of the diet of M. littorale (Decho, submitted). Contour plots (Fig. 2) of raw data show the striking correspondence in the distribution of this copepod and chlorophyll (food) concentrations over all three core-sets.

Associations of other harpacticoid species (such as S. canadensis) with potential food resources are not significant at low-tide. Reasons for this may be related to the manner in which it feeds. S. canadensis feeds primarily during high-water and just after the water has receded from the mudflat (Decho, submitted). Its food resources are mainly planktonic (Decho, 1986). Preference experiments, using S. canadensis showed no differences (P<0.05) in its attraction to patches containing either diatoms, diatom-exudates, or background-sediments, indicating it does not actively seek areas of high food concentrations when choosing a substrate for settling. The lack of attraction of S. canadensis to control patches, however, indicates that some organic matter is necessary for settling and subsequent burrowing of S. canadensis. While in its burrow, the food resources of S. canadensis include a large amount of planktonically suspended material (which is sucked into the burrow) (Decho, 1986). Since its food is suspended material, specific organics in the sediment (i.e. diatom patches, etc.) may not be a prerequisite for its colonization of sediment. Other laboratory studies (Chandler & Fleeger, 1987) have shown that organic enrichment of sediment is not attractive to S. canadensis.

LABORATORY PREFERENCE EXPERIMENTS:

Feeding preference experiments, used to investigate attractive
processes, have been conducted for a wide variety of meiobenthic harpacticoids (Gray, 1968; Hicks, 1977; Lee et al., 1977; Vanden Berghe & Bergmans, 1981; Rieper, 1982) and nematodes (Lee et al., 1970; Wilt et al., 1973; Lee et al., 1977; Tietjen & Lee, 1977; Trotter & Webster, 1984). These studies have shown species-specific responses in selectivity of microbial flora under these conditions.

In the present study, laboratory preference experiments indicate *M. littorale* is significantly attracted to diatom-patches and patches containing their chemical exudates. Its significant attraction to exudate patches indirectly indicates that this harpacticoid may utilize long-range chemosensory abilities to find food, especially during high-water. Its preferential attraction to diatoms and exudates of diatoms strongly supports field microdistributional results.

*C. deitersi* was not present in large enough abundances in field samples to analyze statistically. The response of *C. deitersi* in preference experiments however, also showed a significantly large attraction to diatom patches (*P<0.05*) with significantly less attractions to exudate- and background-patches, respectively. *C. deitersi* consumes and assimilates diatoms in the laboratory (Decho, submitted). Its predelective attraction to diatom-patches show it is able to effectively forage and choose food areas. Laboratory observations show it often enters the water column to feed and swim, especially when food concentrations are low (Decho, 1986). Field data from other studies indicate it is a rapid colonizer of recently disturbed sediment areas, such as sediment near experimentally-clipped *Spartina* shoots (Fleeger et al., 1982).
SPECIES-INTERACTION ASSOCIATIONS:

In field-collected cores, several harpacticoids were patchy, however aggregation in only *M. littorale* appears to respond to microalgal patchiness. Patchiness in other species may relate to other factors. Species interactions among meiofauna have been suggested to regulate patchiness for some species (see Chandler & Fleeger, 1987; for work on the same species studied here). Correlative evidence from the present study supports the laboratory investigations of Chandler and Fleeger (1987). The microspatial distribution of *S. canadensis* correlates significantly (*P*<0.05) with the microdistribution of *P. wellsi* (*R^2*=0.32) at both low-tide (*R^2*=0.49) and high-tide (*R^2*=0.31). Chandler and Fleeger (1987) suggest that the tube-building activities of *P. wellsi* adds cohesiveness to the sediment microstructure and this significantly attracted the burrow-dwelling *S. canadensis*, over mucin-enriched patches and patches enriched with agar-tube mimics, and azoic sediment alone. The exact mechanism of the biogenic facilitative attraction of these two species observed by Chandler and Fleeger (1987) is still unknown. Therefore, it appears that *S. canadensis* does not actively seek sediment areas containing abundant food resources (since it largely feeds planktonically) but rather may choose areas based on sediment factors facilitatively enhanced by other species (such as *P. wellsi*).

PREVIOUS STUDIES:

The spatial patchiness of meiobenthic animal distributions has been observed in a wide variety of habitats (see Heip et al., 1982;
and Hicks & Coull, 1983; for reviews). As sampling techniques and associated statistical inference have become more refined it has become apparent that meiobenthic distributions can vary at several levels. Variability at the meso- and macro-scale (Barnett, 1968; Bell, 1979; Fleeger, 1980; Phillips & Fleeger, 1985) has been generally attributed to changes in physical habitat parameters, though seasonal changes in food abundances may play a role in some systems (Lee et al., 1976; Montagna et al., 1983). At much smaller scales, however, biotic influences are thought to predominate (Findlay, 1981) although microphysical gradients, often influenced by biogenic processes (Findlay, 1981; Thistle et al., 1984; Fleeger & Gee, 1986) can be primary effectors, depending on the local habitat conditions.

Most studies of distributional patchiness have been conducted at low-water in intertidal sandy sediments. Where water-cover influences have been studied, Thistle et al., (1984) found single grass blades can influence copepod and microbial distributions via hydrodynamic conditions. These variations can occur on the scale of mm and can have profound effects in seagrass beds. At a slightly larger scale (several cm²) differences in microbial flora have been shown to correlate with abundances of various harpacticoid species in sandy sediments (Ravenel & Thistle, 1981; Decho & Castenholz, 1986).

**CAUSES OF PATCHINESS:**

Since a large porportion of meiobenthic animals (especially harpacticoids) is resuspended with each tidal cycle (Palmer & Brandt,
1981; Fleeger et al., 1984) in muds, patches at low-water must develop relatively quickly and are probably short-lived. Most studies examining patchiness in intertidal areas have been conducted during low-tide conditions. Low-water sampling in the present study was conducted approximately 3-4 hours after exposure of the mudflat. However, patchiness observed at a low-tide may be influenced by processes which have already taken place. A large reshuffling of harpacticoids, as well as their potential food resources occurs with each tidal cycle, via resuspension and mixing (see Roman & Tenore, 1978). Therefore, sediment distribution patterns must be reorganized, either actively or passively, with each subsequent low-tide. If settling of both animals and food (suspended diatoms, detrital particles, etc.) after an ebb tide is largely passive, then aggregations of both animals and food resources will tend to occur in small localized areas (i.e. small patches) of reduced current flow. This would mean that meiofauna-microbial associations on a micro-scale can be initially mediated through microhydrodynamic processes. This is more emphatically seen in sandy bottom marshgrass (Eckman, 1983) and seagrass (Thistle et al., 1984) environments. Artificial seagrass shoots were used to create micro eddies and areas of passive settlement. Aggregations of both high microbial numbers (Thistle et al., 1984) and certain harpacticoid species (Eckman, 1983; Thistle et al., 1984) occurred in small regions of reduced current flow behind the seagrass shoots. While on a typical mudflat, there are no seagrass shoots present, there are innumerable irregularities in the sediment surface topography. These irregularities may initially contribute (via differential hydrodynamic settling) to the initial
aggregation of settling microalgal flora and meio-benthos as the tide recedes from the mudflat. However, the field studies of Lee et al., (1977), Kern and Taghon (1986), and the present laboratory preference experiments, indicate that some harpacticoids actively seek areas of high-food concentrations (i.e. *M. littorale* and *C. deitersi*). These processes contribute significantly to small-scale patchiness patterns. If meio-benthic patchiness results from feeding-related processes, then patchiness should be most pronounced during- or shortly after times of highest feeding rates. Some meio-benthic harpacticoids (i.e. *M. littorale* and *S. canadensis*) have been shown to feed at highest rates, just after a mudflat has become exposed (i.e. when the mudflat is still very wet), when compared to other parts of a tidal cycle (Decho, submitted). *M. littorale* was shown by laboratory experiments to feed at similar rates during a simulated High-water and Low-water conditions (Decho, 1986). However, at high-water it feeds more on planktonic sources while swimming in the water column. At low-water it feeds benthically (crawling over the sediment). Field patchiness patterns for *M. littorale* are most pronounced during these subsequent times of low-water conditions.

No single factor appears to universally control spatial patchiness in the meio-benthos. Rather, these patchiness patterns are species-specific in nature, and may vary depending on the portion of the tidal cycle. Furthermore, differences due to sediment type may appear as interstitial species (in sandy sediments) will not likely respond to tidal events as do those in muds. Resuspension of both meio-benthic animals and their food are strongly influenced by the tidal cycle. The foraging ecology and feeding habits of the animal
may reflect this. The patchiness of *M. littorale*, for example, appears to be food-related during low-tide. During HW, this pattern dissipates as individuals spend considerable time in the water column. Both laboratory and field studies support this. Conversely, the patchiness of *S. canadensis* does not correlate with its food abundance at LW. Laboratory experiments indicate it does not actively seek benthic food patches. Also, *S. canadensis* feeds at a very reduced level during low-water (Decho, 1986; Decho & Fleeger, submitted). Its macro-scale distribution is usually confined to shallow subtidal or low-intertidal areas where high-water predominates. Instead, the micro-spatial distribution of *S. canadensis* appears to correlate more strongly with the distribution of other species (such as *P. wellsi*) which may facilitate its burrow-dwelling habits. Finally, spatial patchiness is variable in size. The sizes of these patches may ultimately relate to the various causes which initiate them (i.e. food-patchiness, species interactions, microphysical conditions, etc.).
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Fig. 1. Diagram showing arrangement of (A) 4 x 4 contiguous core samples used in field sampling; and (B) sediment wells for laboratory preference experiments. Refer to Table I for treatments (TB= temporary barrier).
Fig. 2. Contour plots of contiguous core-sample sets from field mudflat sediments showing abundances (and concentrations) of the harpacticoid species *Microarthridion littorale* and its associated food resource (as indicated by chlorophyll-a concentration). Harpacticoid abundances represent raw counts per core sample (see text). Chlorophyll concentrations represent ng Chl-pigment per core.
Table showing the composition of different treatment wells for laboratory preference experiments using three harpacticoid copepod species (*precleaned with 30% peroxide to remove organics- see text for explanation).

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>COMPOSITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Control-Sediment</td>
<td>sediment*</td>
</tr>
<tr>
<td>2) Background-Sediment</td>
<td>bacteria, organics, sediment</td>
</tr>
<tr>
<td>3) Exudate-Sediment</td>
<td>diatom-exudates, bacteria, organics, sediment</td>
</tr>
<tr>
<td>4) Diatom-Sediment</td>
<td>diatoms, diatom-exudates, bacteria, organics, sediment</td>
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</tbody>
</table>
TABLE II

Mean (±SE) abundances for harpacticoid species at high-tide (HW) and low-tide (LW) during microspatial field sampling. Abundances expressed as no. per 10 cm$^{-3}$.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>LW</th>
<th>HW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scottolana canadensis</td>
<td>2248.2 ±470</td>
<td>1406.3 ±538</td>
</tr>
<tr>
<td>Microarthridion littorale</td>
<td>343.8 ±261</td>
<td>70.8 ±71</td>
</tr>
<tr>
<td>Pseudostenhelia wellsi</td>
<td>139.6 ±163</td>
<td>116.6 ±131</td>
</tr>
<tr>
<td>Paronychocamptus huntsmani</td>
<td>168.7 ±133</td>
<td>43.7 ±54</td>
</tr>
<tr>
<td>Enhydrosoma woodini</td>
<td>45.8 ±65</td>
<td>37.5 ±53</td>
</tr>
<tr>
<td>Ectinosomid sp.</td>
<td>52.1 ±65</td>
<td>29.1 ±45</td>
</tr>
<tr>
<td>Halicycllops coulli</td>
<td>68.7 ±80</td>
<td>14.6 ±35</td>
</tr>
<tr>
<td>Cletocamptus deitersi</td>
<td>16.6 ±37</td>
<td>14.5 ±41</td>
</tr>
</tbody>
</table>
TABLE III

Summary statistics of spatial autocorrelation analyses using harpacticoid copepod species and potential food (as measured by chlorophyll-a). Each core-set represents 16 contiguous core samples. Expected values: E(I)= -0.067 and E(c)= 1.00. See text for explanation of indices: I, c, s^2/\bar{x}. Levels of significance: ns= not significant P 0.05; *= P 0.05; **= P 0.01; ***= P 0.001. (n= no. ind. per core set).

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>INDEX</th>
<th>HIGH-WATER Core-Set</th>
<th>LOW-WATER Core-Set</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Scottolana canadensis</td>
<td>n</td>
<td>216</td>
<td>233</td>
</tr>
<tr>
<td></td>
<td>(s^2/\bar{x})</td>
<td>2.31**</td>
<td>2.01*</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>-0.015 ns</td>
<td>-0.143 ns</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>0.966 ns</td>
<td>1.230*</td>
</tr>
<tr>
<td>Microarthridion littoreale</td>
<td>n</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>(s^2/\bar{x})</td>
<td>0.56 ns</td>
<td>0.62 ns</td>
</tr>
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<td></td>
<td>I</td>
<td>-0.099 ns</td>
<td>-0.246 ns</td>
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<tr>
<td></td>
<td>c</td>
<td>1.071 ns</td>
<td>1.143*</td>
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<tr>
<td>Pseudostenhella wellsii</td>
<td>n</td>
<td>22</td>
<td>17</td>
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<td>(s^2/\bar{x})</td>
<td>1.57 ns</td>
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<td>Paronychocamptus huntsmani</td>
<td>n</td>
<td>6</td>
<td>5</td>
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<tr>
<td></td>
<td>(s^2/\bar{x})</td>
<td>0.65 ns</td>
<td>1.16 ns</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>-0.079 ns</td>
<td>-0.183 ns</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>1.000 ns</td>
<td>1.242*</td>
</tr>
<tr>
<td>Chlorophyll-a concentration (Food)</td>
<td>(s^2/\bar{x})</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>
TABLE IV

Selected parameters of sediments used in preference experiments for harpacticoid copepods. Diatom concentrations expressed as "no. cells:cm$^{-2}$; Microbial biomass expressed as: "umole (lipid phosphate):g dry sediment$^{-1}$."

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>%C/g DRY SEDIMENT</th>
<th>MICROBIAL BIOMASS</th>
<th>DIATOM CONC.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control-Sediment</td>
<td>0.87-1.23</td>
<td>0.01</td>
<td>---</td>
</tr>
<tr>
<td>Background-Sediment</td>
<td>9.93-10.82</td>
<td>0.19-0.22</td>
<td>---</td>
</tr>
<tr>
<td>Exudate-Sediment</td>
<td>9.95-10.67</td>
<td>0.21-0.24</td>
<td>---</td>
</tr>
<tr>
<td>Diatom-Sediment</td>
<td>10.43-10.98</td>
<td>0.23-0.26</td>
<td>768,350-883,900</td>
</tr>
</tbody>
</table>
SUMMARY

Meiobenthic harpacticoid copepods utilize a variety of food resources, the main component of which are diatoms for most species. While utilization of food resources differs between species, changes also occur ontogenetically, from naupliar to adult stages. Ingestion rates of diatom food resources (i.e. consumption of microbial food: microbe--harpacticoid) are strongly dependent on changing tidal stage for some species. Most species show highest ingestion rates at early low-water (ELW- i.e. just after the tide has receded from the mudflat). This may be due to 1) the relatively high abundance of food resources (both benthic and settled planktonic); 2) the high water content of the sediment (which facilitates the movement and feeding of certain harpacticoids); and 3) the lack of physical disturbance normally imposed by water-cover. Scottolana canadensis feeds primarily during times of water-cover (i.e. HW high-tide) or when the mudflat is still water-saturated (ELW), while feeding at late low-water (LLW- i.e. several hours after exposure of the mudflat) is greatly reduced, as evidenced by both laboratory and field experiments. It therefore shows no significant responses to food patchiness at low-water. Since it is a semi-sessile burrow-dweller, which feeds primarily on planktonically suspended food resources, its patchiness is probably regulated by other factors (such as facilitative interactions with Pseudostenhelia wellsii). This is supported by both laboratory and field data.

Other species, such as Microarthridion littorale, are able to switch food resource utilization depending on the tidal stage, and therefore can maintain a relatively constant feeding rate over a
tidal cycle. At LLW, this species actively feeds and tends to show close correlations with patches of microbial food resources. Therefore, food resource patchiness at this time appears to regulate the patchiness of *M. littorale* in the sediment. These patchiness patterns however, may be initiated by processes occurring during the previous tidal stage (i.e. high-water). At high-water, responses to food patchiness in sediments will be less clear since most meiofaunal species (and their food resources) are suspended (either actively or passively) into the water column, and feed on suspended material while swimming. Meiobenthic harpacticoids may have evolved these feeding strategies to utilize the abundant planktonic resources at this time. Animals which show patchiness (in sediments) during HW are probably responding to other parameters such as species interactions and micro-hydrodynamic gradients. Microspatial patchiness patterns for most species are disrupted with each tidal cycle via resuspension. Therefore, patchiness patterns at low-tide conditions must develop relatively quickly, and may be influenced by events (i.e. settling, microhydrodynamic conditions) during the preceding tidal stage.
25 May 1987

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I thank you for your attention to this matter.

Sincerely,

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CURRICULUM VITAE

ALAN WILLIAM DECHO

Born: 3 May 1956; New Haven, Connecticut
Marital Status: Single
Address: Dept. of Zoology & Physiology, Louisiana State University
Baton Rouge, La. 70803
Phone: (504)388-1132

EDUCATIONAL BACKGROUND:

1982-1987 Louisiana State University - Ph.D.
Major: Zoology; Minor: Statistics-Microbiology
Dissertation Topics: "Feeding and microspatial relationships of meiofaunal harpacticoid copepods with microbial flora."
Advisor: J.W. Fleeger

1979-1982 M.S. Department of Zoology & Microbiology, Ohio University

1979 B.A. Department of Geology, Eastern Connecticut State College

TEACHING EXPERIENCE:

Louisiana State University: (graduate teaching assistant)
Introductory Zoology
Introductory Biology
Marine Ecology

Ohio University: (graduate teaching assistant)
Zoology 151
Introductory Zoology 101

Eastern Connecticut State College: (teaching assistant)
Limnology
Taxonomy of Animals
Introductory Geology
Geology Field Methods

ACADEMIC-RELATED EXPERIENCE:

Graduate student representative for Graduate Committee
Campus Judicial Hearing Board, graduate student representative, 1986.
RESEARCH GRANTS & FELLOWSHIPS:

1987 Fulbright-Hays Post-Doctorate Fellowship (Australia) with Dr. D.J.W. Moriarty, CSIRO Laboratory to investigate microbial-exopolymer interactions with marine animals.

1986 Research Associate for Dr. Thomas Shirley (Univ. Alaska-Juneau) for "Association of Primary Production and Recruitment in Subarctic Ecosystems" (APPRISE) project investigating benthic-pelagic coupling processes.

1983 National Science Foundation Dissertation Improvement Grant, Biological Oceanography (OCE-8313109) on "The interactions of microbial flora and dissolved organic compounds with estuarine meiofauna".

1983 Sigma Xi Grants-In-Aid of Research Grant for: "The role of bacteria and DOM in the microspatial patterns of meiofauna; A field manipulative study".

1983 Marine Biological Laboratory, Woods Hole, Mass., summer fellowship for graduate course-research at MBL (summer 1983).


1980 H. Roy Wilson Fellowship (Ohio University) to attend Duke University Marine Laboratory (summer).

1980 Research Assistant for Dr. W.D. Hummon, analysing meiofauna and sediments from Scotland Gastrotrich Survey.

1978 Scholarship Award (Eastern Conn. State College) to attend Bermuda Biological Station for Research (summer course in Oceanic Island Ecology).

BIOCHEMICAL TECHNIQUES USED:

Fatty Acid Analysis by capillary gas chromatography for quantitative microbial analyses.
Quantitative Amino Acid Analysis of sediment pore water by HPLC (OPA derivitization).
Radioisotope labelling techniques used in feeding ingestion and assimilation studies.
Electron Microscopy, scanning and transmission.
RESEARCH INTERESTS:
Microbial-exopolymer secretions as a food resource.
Animal-microbial interactions;
Meiofauna feeding relationships;
Mechanisms controlling patchiness in benthic animals.

PUBLICATIONS:


Decho, A.W. 1986. Water-cover influences on diatom ingestion rates by meio-benthic copepods. Marine Ecology Progress Series, 33:139-146.


STATISTICAL EXPERTISE:
Extensive experience in regression techniques (simple to multiple), multivariate techniques (discriminate, principle components, factor, cluster analyses, spatial auto-correlation), most non-parametric procedures. Proficiency in SAS, SAS-Graph, BMDP, and the Cornell Program Series in Ecology (Ordiflex, Decorana, etc.).
PROFESSIONAL SOCIETY MEETING PRESENTATION:

1986. Sixth International Meiofauna Conference. Tampa, Fla.
1980. Ohio Academy of Science, Toledo, OH.

PROFESSIONAL SOCIETIES:

- International Society of Meiobenthologists
- American Society of Limnology & Oceanography
- American Association for the Advancement of Science
- Sigma Xi

REFERENCES:

Dr. Earl Weidner, Dept. Zoology & Physiology, Louisiana State University, Baton Rouge, LA. 70803. 504 388-1132.

Dr. William Stickle, Dept. Zoology & Physiology, Louisiana State University, Baton Rouge, LA. 70803. 504 388-1132.

Dr. John W. Fleeger, Dept. Zoology & Physiology, Louisiana State University, Baton Rouge, La. 70803. 504 388-1132.

Dr. Thomas Shirley, School of Fisheries and Sciences, University of Alaska-Juneau, Juneau, AK. 99801. 907 789-4441.
DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Alan William Decho

Major Field: Zoology

Title of Dissertation: Feeding and microspatial relationships of meiobenthic harpacticoid copepods with microbial flora

Approved:

[Signatures]

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

[Signatures]

Date of Examination: July 7, 1987

[Signatures]