Facilitative and Inhibitory Interactions Among Estuarine Meiobenthic Harpacticoid Copepods (Commensalism, Benthos, Louisiana).

George Thomas Chandler II
Louisiana State University and Agricultural & Mechanical College

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FACILITATIVE AND INHIBITORY INTERACTIONS AMONG ESTUARINE MEIOBENTHIC HARPACTICOID COPEPODS

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FACILITATIVE AND INHIBITORY INTERACTIONS AMONG
ESTUARINE MEIOBENTHIC HARPACTICOID COPEPODS

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

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August 1986
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Sincere thanks to the Louisiana Universities Marine Consortium and its director, D.F. Boesch, for the generous equipment and facilities support provided throughout my dissertation research. My dissertation advisor and good friend, John Fleeger, contributed a great deal of insight and personal effort towards my research, and to him I am especially grateful. Several persons deserve special thanks for technical assistance: Buddy Steffens for photomicrography, Harold Silverman for histological assistance, and Eden Phillips and Alan Decho for field support.
FOREWORD

The following manuscript, "Facilitative and Inhibitory Interactions Among Estuarine Meiobenthic Harpacticoid Copepods", and appended publications, "Tube-building by a Marine Meiobenthic Harpacticoid Copepod" and "High Density Culture of Meiobenthic Harpacticoid Copepods Within a Muddy Sediment Substrate", are three chapters comprising my doctoral dissertation research in zoology at Louisiana State University. The culture techniques were developed in 1983 and later published in the Canadian Journal of Fisheries and Aquatic Sciences. These techniques aided the discovery and subsequent description of tube-building by the harpacticoid copepod *Pseudostenhelia wellsii* (published 1984 in Marine Biology). Both studies in turn led to the experimental portion of my dissertation research concerning facilitative and inhibitory interactions of harpacticoid copepods.
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ABSTRACT

In Louisiana, USA, marshes, the harpacticoid copepods *Scottolana canadensis* and *Pseudosthenelia wellsi* are community co-dominants with similar lifestyles. *Scottolana canadensis* is a semi-sessile burrow-dweller capable of subsurface suspension and deposit feeding. *Pseudosthenelia wellsi* is a semi-sessile tube-building copepod that continuously corkscrews back and forth within its tube and appears to graze on the inner tube walls. Monospecific patches of both species (250 · 5 cm$^{-2}$) were generated in laboratory microcosms to determine their effects on colonization by two completely errant, burrowing harpacticoids, *Nitocra lacustris* and *Cletocamptus deitersi*, that have similar foraging and burrowing behaviors and similar effects on sediment structure. *Pseudosthenelia wellsi* tube patches facilitated colonization by *S. canadensis* and *N. lacustris*, but strongly inhibited colonization by *C. deitersi*. *Scottolana canadensis* patches were significantly unattractive to *N. lacustris* and inhibited their immigration. Mechanisms of facilitation were tested with colonization experiments offering patches of sterile sediments, natural *P. wellsi* tubes, *P. wellsi* agar tube mimics, and patches of pure mucin-enriched sediments. These experiments showed that both mucus and inert meiofaunal-sized tube structure act as strong facilitants to *N. lacustris* copepodites and adults overall. Patch colonization by *Nitocra lacustris* adult females and *S. canadensis* copepodites and adults was not facilitated by mucus or tube structure alone, but was strongly facilitated by natural *P. wellsi* tubes. These experiments demonstrate that species interactions may influence spatial patterns in harpacticoid communities, but assemblages are not consistently
predictable based on similarities/dissimilarities in species' functional effects on muddy sediment structure.
INTRODUCTION

The potential for interactions between species is great, spanning from doubly detrimental in the case of competition to doubly beneficial in the case of mutualism. Four interactive states may be defined along this continuum (Starr 1975), but their designations are not absolute nor constant and often transgress into adjacent states during the lifetimes of individuals or communities. These states are amensalism (interaction decreases survivorship of one pair member), agonism (interaction decreases survivorship of one pair member at the benefit of the other), neutralism (interaction has no effect on pair survivorship), and commensalism (interaction increases survivorship of one pair member). Mutualism and the interactions listed above may help to organize communities, potentially having greater effects than exploitative competition (Hutchinson 1975, Levine 1976; May 1982, Boucher 1982). In particular, agonistic interactions such as predation, parasitism and interference competition have recently been supported as strong determinants of structure in species assemblages (Underwood and Denley 1984, Faeth 1984, Dayton 1984).

In marine soft-bottom macrobenthic communities, sediment-mediated amensalism, (Rhoads and Young 1970, 1971, 1974), interference competition (Levin 1981, 1982, Brenchley 1982, Wilson 1983) and to a lesser extent exploitative competition (Peterson 1977, 1979, Fenchel 1975a,b, Connell 1983) are known to have deterministic effects on community structure. The importance of more benign interactions such as mutualism, commensalism and facilitation (i.e. presence of one species aids or improves recruitment/colonization of another; sensu
Egler 1954) is not well understood. Taxonomically diverse groups of sediment-dwelling macrofauna often affect sediment structure, chemical composition, oxygen profiles, etc., in functionally similar ways (Woodin 1976; Woodin and Jackson 1979), making the habitat more or less desirable to other organisms and facilitating or inhibiting their settlement or immigration. For example, sedentary fauna that stabilize sediments by building sediment-binding tubes (but see Eckman 1983) may be more likely to facilitate colonization by other sedentary tube-builders but inhibit settlement or survivorship of errant burrowing species (Brenchley 1982). In this way tube builders can have an ongoing deterministic effect on community structure that may persist as long as the tube-dominated assemblage is viable and undisturbed. The fidelity and predictiveness of macrofaunal functional groupings have been challenged, however, on the grounds that species-specific biotic interactions can generate community patterns that appear causally related to functional groupings but on closer examination are not (e.g. Weinberg 1984). Additionally hydrodynamic effects on larval recruitment and faunal resuspension may override functional group effects (Jumars and Nowell 1984).

The mechanisms and intensity of species interactions in the microscopic metazoan communities of marine sediments are virtually unknown. Yet the meiofauna (microzoans < 1.00 mm in body length) are the most abundant multicellular animals inhabiting marine sediments, and are very diverse taxonomically and morphologically in even the most homogeneous sedimentary environments (Swedmark 1964, Fenchel 1978, Hicks and Coull 1983). Unfortunately their small size, cryptic lifestyles and poor tolerance of laboratory conditions have precluded
easy observation and manipulation of meiofauna densities and community
composition in the field and laboratory.

The estuarine intertidal meiofauna community of Terrebonne Bay,
Louisiana (USA), is unusual in that it is dominated for much of the
year by high densities of a sedentary tube-building harpacticoid
copepod, *Pseudostenhelia wellsi* Coull and Fleeger, and a similar
semi-sedentary, burrow-dwelling copepod, *Scottolana canadensis*
Other harpacticoids in this community are completely errant,
sediment-disrupting species that probably contribute less to the
structural characteristics or complexity of the benthic habitat. The
sharply contrasting behaviors and sedimentary effects of *P. wellsi* and
*S. canadensis* with those of the more typical errant free-burrowing
species in this community, encouraged two questions previously
investigated only for macrofaunal communities: "Do those meiofauna
taxa that alter their sediment substrates in a given fashion (e.g.
tubes, burrows, sediment floc, etc.) facilitate, inhibit or have no
effect on immigration by other meiofauna with similar or dissimilar
substrate effects?"; and "What are the mechanisms of
facilitation/inhibition if they occur?". To answer these questions,
manipulative experiments were conducted with cultured meiobenthic
harpacticoid copepods in sediment microcosms in the laboratory. The
use of microcosm manipulations for the study of meiofaunal
interactions is unique, but proved to be a viable direct approach for
controlling hydrodynamics and sediment characteristics, and allowed
the fewest alternative explanations for these experimental outcomes.
Logistic difficulties and high susceptibility to hydrodynamic
disturbance have precluded similar experimental manipulations of the microscopic meiofauna in the field.
MATERIALS AND METHODS

Experimental Design

Five two-species interaction experiments were designed to test the attractiveness of high-density patches of one harpacticoid copepod species to a second potential colonizing species inhabiting the sediments surrounding each patch (Table 1). Three of the five experiments used the tube-building harpacticoid, *Pseudostenhelia wellsi*, as the patch-inhabiting species, one used the semi-sessile burrow-dweller, *Scottolana canadensis*, as the patch species, and one used the completely errant burrowing species, *Nitocra lacustris* (Schmankevitsch). The three *P. wellsi* patch experiments tested patch facilitation/inhibition to colonization by *N. lacustris*, *S. canadensis* and *Cletocamptus deitersi* (Richard), another errant burrowing species behaviorally very similar to *N. lacustris*. The *S. canadensis* patch experiment tested patch facilitation/inhibition to *N. lacustris*, and vice versa for the *N. lacustris* patch experiment.

All five experiments followed a randomized block design with three treatment levels tested in triplicate muddy-sediment microcosms. Treatment levels consisted of three manipulations: (1) a high-density patch manipulation where colonizer densities were measured in patches established with another pre-seeded resident species, (2) a control manipulation where colonizer densities were measured in initially azoic patches, and (3) a background manipulation where colonizer densities were measured in the sediments surrounding both types of patch areas. Densities of the original resident patch-species were also measured in the three manipulations as a check on patch (i.e.
Table 1. Designs of the two-species interaction experiments conducted in this study.

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treatment) integrity. All experiments tested for species interactions after a 2 day period.

One additional interaction experiment was designed to follow *Nitocra lacustris* colonization of *Scottolana canadensis* patches over time. A two-level 2x5 factorial design was used, where colonizer densities were measured in high-density *Scottolana canadensis* patches and azoic patches at five time intervals (2, 4, 8, 16, and 24 h).

Because our experiments found that colonization of *Nitocra lacustris* and *Scottolana canadensis* was enhanced by *Psedostenhelia wellsi* tube-patches, the mechanisms of facilitation were studied. A randomized block design was followed for both experiments and used four patch-treatment levels tested in triplicate microcosms. The treatments consisted of (1) high-density *P wellsi* patches, (2) azoic mucin-enriched patches, (3) azoic, *P. wellsi* tube-mimic patches, and (4) undisturbed azoic control patches. Colonizer densities were measured in each patch manipulation after 24 h of interaction.

All experiments were conducted in muddy-sediment microcosms under controlled laboratory conditions (21-23°C; 12:12 LD; 15 o/oo salinity). Large numbers (> 40,000) of the three colonizing harpacticoid species, *Nitocra lacustris*, *Scottolana canadensis* and *Cletocamptus deitersi*, (hereafter generally referred to as colonizers) were axenically cultured in separate sediment microcosms and used in these experiments. Primarily 1<sup>st</sup>-3<sup>rd</sup> generation offspring (copepodites and adults) were used to prevent spurious results caused by culture-induced changes in behavior, physiology, etc. that may result from long-term population inbreeding (Smol and Heip 1974). To promote rapid acclimation of colonizers to experimental microcosms,
all experiments were done under the identical microcosm conditions (i.e. sediment structure, organic content, flushing rates, etc.) as used for their original culture.

Copepod Culture and Microcosm Design

Copepod culture and experimentation were carried out in hexagonal glass microcosms ($65 \text{ cm}^2$ surface area) filled with a dense 3-cm base layer of azoic mud overlain by a 1-cm deep, flocculent muddy surface layer (after Chandler 1986). A flocculent surface layer was generated within each microcosm because most mud-inhabiting harpacticoids live almost exclusively in the flocculent, top cm of muddy sediments (Coull and Bell 1979, Chandler and Fleeger 1983). Culture sediments had a median grain diameter (MGD) of 0.02 mm and total organic matter (TOM) content of 7% (natural sediments = 0.04-mm MGD; 10% TOM). Each microcosm received a "once-through" supply of fully oxygenated ASW through small-bore (20 gauge), polyethylene catheters. Fresh microcosms were flushed at a rapid drip-rate ($3.0-5.0 \text{ ml.min}^{-1}$) for 24 h before copepods were added. Established microcosms received ASW more slowly at a continuous rate ($0.5 \text{ ml.min}^{-1}$).

Monocultures of Nitocra lacustris, Scottolana canadensis and Cletocamptus deitersi were started with 100 or more adult females, and were fed a mixed diet of chrysophytic (Isochrysis galbana) and chlorophytic (Dunaliella tertiolecta) algae, one diatom species (Thalassiosira weissflogii) and spinach fragments ($< 45 \text{ um}$). Mature cultures (4000 to 12000 individuals) were given 30 mL of an equal, four-part suspension of each of the three algal types and the spinach solution every 2 d.

General Methods for Two-Species Interaction Experiments
For each interaction experiment, triplicate microcosms (65 cm$^2$) were constructed as above and covered with aluminum foil up to the sediment-water interface. The foil excluded light from the sediment-glass interface and minimized confounding phototactic edge effects on copepod distributions. Six isolated patches were generated within each microcosm sediment base by pushing six, 5 cm$^2$, clear polypropylene core tubes (7.0 cm long) uniformly into the centers of each hexant of the hexagonal microcosms (Fig. 1A). The core tubes were pushed completely flush against the microcosms' Plexiglas bases. Each core tube extended 0.5 cm above the microcosm air-water interface, but was fitted 1 cm below the water line with a circular 0.063-mm mesh-covered channel to promote water circulation. The core tubes' above-water extensions prevented the copepods from swimming or crawling in or out of the patches, and their flush bases prevented underburrowing.

Within each replicate microcosm, three of the core-defined patches were seeded with 250 copepods each, and three were left empty as placebo controls. The three copepod and three control patches occupied 23 % of the total sediment volume/surface-area respectively. The surrounding background sediments accounted for the remaining 54 %. The control patches were each given a 3-ml placebo dosage of the 45-um filtered ASW in which the copepod inoculants were retained before seeding. The placebo was intended to inoculate similar levels of ciliates and bacteria into the empty control patches as were inoculated into the seeded patches.

The density of 250 copepods per 5 cm$^2$ patch was chosen because it is a natural "high end" value commonly seen in field samples of the
Fig. 1. Microcosm experimental design employed for the two-species interaction experiments. (A) Close-up surface view of core-barrier implants in the *Pseudostenhelia wellsi* - *Scottolana canadensis* patch colonization experiment. Dark pits in the central sediments are *Scottolana* burrows. (B) Microcosm appearance 3 hr after core-barrier removal in the *P. wellsi* - *Cletocamptus deitersi* experiment. (C) Patch appearance 24 hr (i.e. microcosm 1) and 3 hr (i.e. microcosm 2) after core-barrier removal in the *S. canadensis* - *Nitocra lacustris* experiment. (D) Patch-core template (No. 1) used in all two-species interaction experiments to record positions of contiguously placed core tubes (No. 2).
same area at various times when the four species peak in Louisiana marshes (Chandler and Fleeger 1983, Fleeger 1985b, Phillips and Fleeger 1985). Additionally, the patch densities were kept constant over experiments to ease cross-species comparisons of experimental results and to avoid disparate, density-dependent outcomes resulting from large differences in patch densities. Chandler and Fleeger (1983) showed that 24-48 h were necessary for complete colonization of azoic sediments by some of these species in the field; therefore the patch-enclosed copepods were allowed to work the sediments and establish themselves for 48 h before the core-tube barriers were removed.

Twenty-four hours after seeding the patch species, approximately 1030 individuals of a given colonizer species were pipetted into the background sediments surrounding the core-tube barriers (and enclosed patches) of each replicate. This value was chosen as a base density (156 • 10cm²) well within the ranges observed for all colonizers in field sediments (Chandler and Fleeger 1983, Fleeger 1985b, Phillips and Fleeger 1985). Colonizers were allowed to acclimate to the microcosm sediments for 24 h before removing the barriers separating the respective species pairs. The core tubes were slowly lifted vertically from the sediments leaving behind intact patches of undisturbed sediments and copepods (Fig. 1B). Both species were allowed to interact, emigrate or immigrate for 48 h before their densities and distributions were sampled.

In every interaction experiment, the distinct circular patch boundaries (Fig. 1B) became indiscernable 24-48 hr after core barrier removal (Fig. 1G-1). Sediment disruption from copepod burrowing and
grazing "erased" the circular depressions left by the core barriers, making it difficult to obtain precise cores directly from a given patch area. Therefore a grid coordinate system was employed. Copepod densities within treatment patches and background sediments were determined by coring 52% of the sediment area with 61 small (0.57 cm$^2$) contiguously placed cores (Fig. 1D-2). Every microcosm was identical in shape, surface area, sediment volume and patch positions. Therefore all 61 cores could be recorded according to their position on a master template of all core/patch positions (Fig. 1D-1). Thus in each replicate, four cores were taken within each patch to yield 12 cores from treatment patches and 12 from control patches. Twelve cores were selected from 13 possible background positions having no overlapping bias with patch boundaries. Core tubes were inserted manually in rapid succession; as such some error in exact core placement may have occurred. However, a runs test for randomness of residual errors showed that residuals were not significantly different from random over the various patch-core positions, and systematic error in core placement probably did not occur ($Pr=0.95$).

Treatments (i.e. seeded patches) and controls (i.e. azoic placebo patches) were interdispersed uniformly in each replicate microcosm; i.e. every other hexant contained the same treatment (Fig. 1A). With only six positions per replicate, a random treatment assignment recommended by many statistical tests would likely have generated multiple side-by-side sets of identical patches simply due to random sampling error. Such side-by-side patches probably would not have behaved as discrete experimental units (see Hurlburt 1984, for a discussion of this problem), but may have acted as a collective
"mega-patch" and biased the results. Uniform interdispersion of treatments and controls prevented this problem by enhancing patch isolation.

In each two-species interaction experiment, patch copepods were fed a daily algal ration of *Dunaliella tertiolecta* (1.0 ml·d⁻¹: 20,000 cells · ml⁻¹) until the tube barriers were removed (48 h). Empty control patches were given 0.5 ml·d⁻¹ as a placebo. Colonizers were fed maximally (see culture methods) 24 h before their inoculation into background sediments, but received no algal food during the total experimental timecourse (72 h).

Over 5300 copepods were used in each interaction experiment. Random collections of cultured colonizer copepodites and adults were separated from pure bulk collections as follows: (1) The bulk collections were immobilized via cooling to 0°C. (2) 15 lots of 206 individuals each were counted into Petri dishes and randomly combined into three 5-lot collections. (3) One 5-lot collection was then slowly pipetted into the center of each microcosm. Patch species were similarly cooled and counted into 18 lots of 125 individuals each. The lots were randomly combined two at a time and then pipetted into the core barriers of nine designated treatment patches.

The predominant patch species, *Pseudostenhelia wellsi*, could be easily maintained for several weeks in sediment microcosms, but it could not be cultured above maintenance densities. All but two of the interaction experiments used *P. wellsi* as the seeded patch species, therefore high numbers had to be sieved from field sediments, returned to the lab, and separated from the sediment debris. Intense fiber
optic illumination was used to chase the photonegative *P. wellsi* (and others) copepodites and adults from the debris and into clean water where they could be pipetted out. These rough multi-species collections were then cooled, and *P. wellsi* were randomly sorted and counted into dishes. Before *P. wellsi* were added to the core-tube barriers, they were transferred through three ASW rinses to remove adhering sediments and debris which may have been attractive to other species.

**Scottolana - Nitocra Time Series Experiments**

The colonization rate of *Nitocra lacustris* into patches of *Scottolana canadensis* was determined over a 24 h period. Triplicate microcosms were set up and seeded with the two species as described above. Changes in patch densities over time were followed with serial, non-destructive sampling at 2, 4, 8, 16, and 24 h. Two patches per treatment were randomly selected from among the three microcosms at hours 2, 4, 8, and 16, and cored with four cores per patch. The remaining two treatment and control patches were cored at 24 h. Non-destructive sampling was done by inserting the 0.57 cm$^2$ core tubes flush against the Plexiglas base-plate of a given patch, capping them off with rubber plugs, and then letting them (and their captured contents) remain upright in the patches until the experiment was completed (at 24 h). No background cores were taken from the surrounding sediments as this would have disturbed the muddy-sediment habitat and source of colonizing *N. lacustris*.

**Mechanisms of Facilitation Experiments**

Triplicate octagonal microcosms (100 cm$^2$) were designed similarly to the hexagonal ones used above, but a 1-cm thick, hard agar base was
cast in the bottom of each replicate. Uniformly spaced circular wells (5 cm² x 1 cm deep) were carved into the center of each octant (Fig. 2A). Four of the wells in each replicate microcosm were filled with wet autoclaved sediments (5 ml) of the same preparation and composition as the sediments used to culture the colonizers. Two wells were filled with pure-mucin enriched sediments (0.125 % dry wt mucin * wet wt sediments⁻¹), and two were filled with 3-ml azoic sediments overlain by a 2-ml network of 2 %-agar *P. wellsi* tube mimics. Mucin-enriched sediments were prepared by dissolving 50-mg pure bovine submaxillary mucin in 15-ml distilled H₂O and mixing thoroughly with 40-g wet autoclaved sediments. *Pseudostenhelia wellsi* tube mimics were formed by suspending 5-g < 0.045-mm sediment particles in 20-ml 2 % hot agar, and then extruding the mixture through a 23-ga Luer stub adapter immersed in 0°C ASW. The hot agar-sediment slurry solidified almost instantly upon contact with the cold ASW and formed solid, 3-4 cm tubes of similar morphology and diameter as *P. wellsi* tube segments (mimic diameter = 0.20-0.40 mm; *P. wellsi* tube diameter = 0.15-0.30 mm). The agar mimics were vortexed for 5 s to break them into shorter, more natural tubes (predominantly 0.3 to 1.0 cm). Twenty drops of suspended autoclaved sediments (30 % vol/vol sediments:ASW) were pipetted over the 2-ml aliquot of agar tube mimics to fill up the interstitial spaces between the tubes.

*Pseudostenhelia wellsi* patches were generated in each microcosm within two of the four wells filled with wet autoclaved sediments alone. The other two azoic wells served as unmanipulated patch controls. Clear polypropylene core tubes (5 cm² as above) were inserted flush against the agar walls of each *P. wellsi* designated
Fig. 2. (A) Microcosm experimental design used to test mechanisms of *Pseudostenhelia wellsi* facilitation. (B) Visual evidence of *Scottolana canadensis*’ strong attraction to *P. wellsi* tube patches, designated by arrows. Notice *P. wellsi* patches appear 1.5 X larger than the other three patches in the photo. High numbers of *S. canadensis* crowded sediments out onto the well edges. (T=tube patch, C=azoic control patch, M=mucin-enriched patch).
well. The core tubes extended 6 cm above the hard agar bases and 0.5 cm above the air-water interface. Each microcosm was then filled with ASW delivered (3 ml min\(^{-1}\)) via 20 ga microcatheter tubing. ASW was pipetted into each core tube until the enclosed water level reached the tube's distal mesh openings. Careful ASW delivery prevented hydrodynamic disruption of the soft sediment patches. 1500 *P. wellsi* copepodites and adults were sorted and counted as before, and six lots of 250 each were then added to the six core-tube enclosed sediment patches. The 250 *Pseudostenhelia wellsi* were allowed to burrow into the sediments and build tube networks for 24 h (Tube building commences within 5 min after contact with the sediments -- Chandler and Fleeger 1984) before the tube barriers were removed.

A modified latin-square style treatment dispersion was used to assign the four replicated patch types to the eight positions within each microcosm. The four patch types were randomized with respect to nearest neighbor type and then duplicated in each well position directly opposite on the octagon. The diagonal dispersion patterns were therefore equivalent, but had no identical side-by-side patches. The treatment sequence of four patches was randomized within an arbitrarily designated first "arc-row" of four positions, and then duplicated for the next four positions around the octagon. For example, if four patches were randomly designated as ABCD, then a typical treatment dispersion may have been ABCDABCD for the eight wells of replicate 1, CDBACDBA for replicate 2, and DACBDACB for replicate 3. Again with only eight patch positions, a complete randomization over all eight may have generated clusters of identical treatment patches (Hurlburt 1984).
After 24 h the *Pseudostenhelia wellsii* core barriers were removed. A core tube was then immediately placed upright on the center of each hard agar base, and either 1000 *Nitocra lacustris* (i.e. for *Nitocra* facilitation experiment) or 750 *Scottolana canadensis* (i.e. for *Scottolana* facilitation experiment) were pipetted inside. The colonizers were allowed to settle to the bottoms of the barriers and recover from transfer for 10-15 min. The barriers were then slowly lifted vertically to keep from affecting the copepods' initial selection of direction.

The colonizers were allowed to select and colonize patches for 24 h before sampling. At 24 h, the overlying ASW was completely suctioned from the center of each microcosm and retained in 500-ml side-arm flasks. In this way, the eight remaining patches were effectively isolated from cross-patch emigration/immigration. The patches were then quickly suction-sampled into separate 500-ml flasks. After each suction, the aspiration pipette and hose were flushed with 250-ml ASW to prevent cross-sample contamination. All patch samples and overlying waters from each microcosm were sieved on 0.063 mm mesh, preserved in 5 % buffered formalin, stained with Rose Bengal red, counted, staged and sexed. Copepods were not fed during either experiment due to the short experimental durations (48 h and 24 h respectively for *Pseudostenhelia wellsii* and colonizer species).

Adult *Scottolana canadensis* are 25 % - 33 % larger than adult *Nitocra lacustris*. Therefore, since suitable habitat space was limited (40 cm² sediments • microcosm⁻¹) by the absence of background sediments in these two experiments, fewer *S. canadensis* were used. This reduced the probability of density-dependent rejection of
individuals from highly desirable patches. (Consequently this was fortuitous as > 250 S. canadensis colonized some P. wellsi patches and literally crowded sediments out onto the peripheral well edges. This made the P. wellsi patches appear 30 % larger. -- Fig. 2B)

**Statistical analyses**

Copepod densities from the five two-species interaction experiments and the *Scottolana canadensis* - *Nitocra lacustris* time series experiment were not normally distributed. A non-parametric Kruskal-Wallis 2-way ANOVA was therefore used to test for significant treatment and microcosm (block) effects in the interaction experiments. The RANK and GLM procedures of SAS (SAS 1982) were used to rank the densities and obtain rank sums of squares (SS) for the treatment and block levels of the model, and to compute total mean SS. Hypotheses of no significant differences among treatment means or replicate chamber means were tested by computing the Kruskal-Wallis test statistic (H) which equals Rank SS Treatments (or Blocks) / Rank MS Total (Conover 1980). If a significant overall level effect was found, then individual orthogonal contrasts were performed on partitioned rank SS to determine which treatments or blocks were significantly different.

A Kruskal-Wallis ANOVA was also used to analyze the time series results. The copepod densities were ranked as above, and rank SS were computed for each treatment level and time period (i.e. a 2x5 factorial design). Orthogonal contrasts were computed when overall level effects were significant. Differences in microcosms (i.e. "block" effects) could not be tested with this design because every microcosm was not sampled at each time period.
Copepod densities from the two "mechanisms of facilitation" experiments were normally distributed and met the assumptions for parametric ANOVA. A two-way block design ANOVA model consisting of four treatment levels and three replicate blocks (microcosms) was used to test for treatment and block effects in both experiments. Orthogonal contrasts were used to compare means of individual treatments and replicate microcosms (i.e. blocks). The SAS GLM procedure was employed to test the models.
RESULTS

Two Species Interaction Experiments

Dense monospecific patches of the tube-building copepod, *Pseudostenhelia wellsi*, had contrasting species-specific effects on colonization by other harpacticoids. The extensive mucous tube networks in pre-seeded patches elicited a moderate to strong facilitation to colonization by *Nitocra lacustris* and *Scottolana canadensis*, but strongly inhibited colonization by *Cletocamptus deitersi*. Mean copepod densities per standardized 5 cm² of available sediment space are presented in Fig. 3 for the three *P. wellsi* interaction experiments. Densities in ambient sediments were corrected to a standard value of 41.5% of original per-core densities to allow treatment comparisons on an equal available-space basis. Ambient sediment space was over twice as abundant as azoic or *P. wellsi* patch space. Orthogonal contrast results are depicted (Fig. 3) by letters on top of each block, where blocks having two or more identical letters are not significantly different. All statistical analyses were performed on raw core densities. Kruskal-Wallis 2-way ANOVA results are presented in Table 2 for each two-species interaction experiment. The errant, burrowing harpacticoid, *Nitocra lacustris*, immigrated readily into *P. wellsi* tube patches over 48 hr, but avoided azoic (control) patches (control density was 1.5 X less). The semi-sessile, burrow-dweller, *Scottolana canadensis*, showed an even stronger attraction to *P. wellsi* tube patches (2.5 X control density) than *N. lacustris*. *Cletocamptus deitersi* is also a free burrowing species yet it showed a dramatic reverse interaction with
Fig. 3. Mean densities of *Pseudostenhelia wellsi* and each harpacticoid colonizer species in the treatment manipulations and ambient sediments of the three *P. wellsi* interaction experiments. Mean densities for ambient sediments are given on a standard available-habitat-area basis (see text). Means sharing the same letter(s) across treatments are not significantly different.
Table 2. Descriptive statistics and results of Kruskal-Wallis two-way ANOVA on raw core densities from the five two-species interaction experiments. Lilliefors' D is a test of the assumption of normality. Mean ranks are given for non-parametric treatment comparisons only. Coefficients of variation are provided as measures of variability for the actual treatment means depicted in Figs. 3 and 4.
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Pseudostenhella wellsi. Densities of C. deitersi were almost three
times less in P. wellsi patches than in control patches; and control
densities were not significantly different from ambient levels (Fig.
3).

In all three experiments, P. wellsi patch integrity was
consistently high through 48 hr. Overall 83% of the seeded copepods
remained within the P. wellsi designated patches, and only 12%
emigrated to control patches. Thus treatment levels remained
consistent throughout each experiment. Microscopic observation of P.
wellsi patches in preliminary experiments showed that beyond 48 hr
patch integrity gradually dissipated.

Results from 48 hr interaction experiments with Scottolana
canadensis and Nitocra lacustris, where one species and then the other
were pre-seeded into dense patches, were inconclusive. Neither
species retained its original patch integrity for 48 hr. After core
barriers were removed, both species dispersed out into the surrounding
ambient and control sediments, and by 48 hr showed no significantly
different densities in any of the pre-defined areas (Fig. 4). The
only significant colonizer response was by N. lacustris which avoided
azoic control patches and S. canadensis patches. Nitocra densities
were twice as high in ambient sediments (unstandardized) than in
either patch treatment, suggesting an avoidance to any manipulated
patch area, possibly caused by patch generation procedures.
Scottolana canadensis patch integrity in the Nitocra interaction
experiment (Fig. 4) remained distinct for at least 8 hr; based on
visual observation of S. canadensis burrow emigration from the
circular patch boundaries. By 24 hr, however, distinct high density
Fig. 4. Mean patch densities of *Nitocra lacustris* and *Scottolana canadensis* in their reciprocal patch interaction experiments. The six blocks on the left depict results of the *S. canadensis* patch experiment, and those on the right depict results of the *N. lacustris* patch experiment. *: Means are expressed on a standard available habitat basis (see text). Means sharing the same letter(s) across treatments are not significantly different for a given experiment. **: This mean is significantly greater than the others in its row. Its value is less only because of the area standardization.
patches were no longer visible, as the burrows had dispersed. The only significant "block" effect in any experiment was seen for *S. canadensis* densities in the *N. lacustris* patch experiment (Table 2). This among-replicate microcosm inconsistency in *S. canadensis* immigration into pre-seeded *N. lacustris* patches was probably caused by *Scottolana*'s variable responses to *Nitocra*'s errant, unpredictable distribution after 48 hr of patch emigration.

**Scottolana-Nitocra Time Series Experiments**

Because *Scottolana canadensis* patch integrity could not be maintained for 48 hr, short-term effects on colonization by *Nitocra lacustris* were tested over 24 hr. The *Scottolana-Nitocra* interaction experiment was repeated, but patch densities were sampled at 2, 4, 8, 16 and 24 hr. Serial sampling provided an estimate of *S. canadensis* patch integrity over time, and an overall test of *N. lacustris* attraction/repulsion to *S. canadensis* patches with patch integrity over time considered. Kruskal-Wallis 2X5 factorial ANOVA found a significant overall treatment effect for *N. lacustris*, but non-significant time and treatment*time interaction effects (Table 3). Densities of *N. lacustris* were significantly lower in azoic control patches than in *S. canadensis* patches irrespective of sampling time or time*treatment*combination. *Scottolana canadensis* showed significant treatment and time effects (note however P=0.94 for time effect; Table 3), but no significant treatment*time interaction. Orthogonal contrasts of sampling times (Table 3) showed that *S. canadensis* densities were significantly less in pre-defined *Scottolana* patches and control patches at only one sampling time, 16 hr. Strangely, samples taken at 24 hr were not significantly different from 8 hr
Table 3. Descriptive statistics and Kruskal-Wallis ANOVA results based on raw core densities from the *Nitocra lacustris* - *Scottolana canadensis* 24-hr time series experiment. Treatment means are depicted in Fig. 5.
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Note: Pr > D and Pr > H values are significance levels for the variables with Pr > D values of 0.01 or 0.001 and Pr > H values of 0.01 or 0.001, respectively.
samples, suggesting the 16 hr samples may have been biased by a microcosm (i.e. block) effect. Block effects could not be tested, however, with this experimental design (see Methods and Materials).

After only 2 hr, *Scottolana* patch integrity declined to 53 % of original density (250 copepods * patch^−1*) (Fig. 5). Patch densities of *S. canadensis* stabilized by 4 hr and were not significantly different through 24 hr (excepting 16 hr). Although *S. canadensis* densities declined to as low as 20 % of original pre-seeded densities, they were always significantly more abundant in pre-defined *Scottolana* patches than in azoic control patches (73 % more abundant averaged over all time periods). In contrast, *S. canadensis* densities in the analogous 48 hr experiment were not significantly different in ambient sediments, azoic control patches and pre-defined *Scottolana* patches after 48 hr. Based on the time-series results, a distinct *Scottolana* patch treatment was probably not applied beyond 24 hr; and a significant patch avoidance by *N. lacustris* in response to *S. canadensis*' presence was therefore not found.

**Mechanisms of Facilitation Experiments**

*Nitocra lacustris* and *Scottolana canadensis* have sharply contrasting behaviors yet both were attracted to dense patches of *Pseudostenhelia wellsi*. *Pseudostenhelia wellsi* increased the structural complexity and mucus content of the muddy sediment patches by building extensive networks of intertwining mucous tubes. Several mechanisms associated with *P. wellsi*'s tube-building behavior may have facilitated patch colonization (see Discussion), and two were tested -- mucus enrichment and increased habitat complexity. Therefore, in separate experiments, *N. lacustris* and *S. canadensis* were offered
Fig. 5. Mean patch densities of *Scottolana canadensis* and *Nitocra lacustris* in *Scottolana* patches and azoic control patches at 2, 4, 8, 16, and 24 hr of the *Scottolana* - *Nitocra* time series experiment. Means sharing the same letters across treatments are not significantly different.
choices of azoic sediments, mucous-enriched sediments, sediments seeded with networks of agar tube mimics, and sediments seeded with 250 P. wellsii. Results of 2-way ANOVA for each experiment, and orthogonal contrasts for each treatment comparison, are provided in Table 4.

Overall, Nitocra lacustris was attracted equally well to patches enriched with mucus alone, patches with increased structural complexity, and patches enhanced by both factors (i.e. natural P. wellsii mucous-tube patches). Densities were not significantly different in mucin-enriched patches, tube-mimic patches and P. wellsii patches, but were significantly less (25 % overall) in unmanipulated azoic patches. Nitocra exhibited a significant sexual difference in patch selection however. Nitocra adult females colonized mucin-enriched and tube-mimic patches only as well as azoic controls, and were significantly more abundant only in the P. wellsii patches (60 % of Nitocra in P. wellsii patches were adult females; 40 - 42 % were females in the other three patch-types; Fig. 6). Scottolana canadensis showed no significant sexual differences in patch colonization, but was attracted very strongly to the "real thing", P. wellsii patches; mean density of S. canadensis was over 50 % higher for P. wellsii patches than for mucin-enriched or tube-mimic patches. The magnitude of S. canadensis' facilitation by P. wellsii can be seen in Fig. 7B where the P. wellsii patches appear 1.5 X larger than the other three patch-types. High Scottolana densities displaced sediments out of the well-depressions and onto the peripheral edges of each P. wellsii patch. Scottolana densities in azoic control patches, mucin-enriched patches and tube-mimic patches were not significantly
Table 4. Descriptive statistics and parametric ANOVA results based on whole patch densities in the *Pseudostenhelia wellsi* mechanisms of facilitation experiments. Shapiro-Wilk's W is a test of normality recommended when total sample size is less than 50 (Conover, 1980). *Scottolana canadensis* densities were log10(X + 1) transformed to meet the assumption of normality. Treatment means are depicted in Fig. 6.
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<td>1</td>
<td>0.440</td>
<td>0.440</td>
<td>7.71</td>
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<td></td>
<td>Mucus vs Tubes</td>
<td>1</td>
<td>0.316</td>
<td>0.316</td>
<td>5.53</td>
<td>0.03</td>
</tr>
</tbody>
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*After Log10 transformation
Fig. 6. Mean patch densities of *Scottolana canadensis* (all stages) and *Nitocra lacustris* (all stages) in the mechanisms of *P. wellsi* facilitation experiments. Densities of *N. lacustris* adult females are depicted on the block faces as standard percentages to ease comparisons, but orthogonal contrasts were performed on raw patch densities. Means sharing the same letter(s) across treatments are not significantly different. Lower case letters refer only to means of adult female *N. lacustris*. 
S. CANADENSIS

AZOIC PATCH 92.8  
MIMIC PATCH 126.2  
MUCOUS PATCH 117.5  
P. WELLSI PATCH 129.8

SPECIES

FEMALE COPEPODS

TOTAL COPEPODS

TREATMENTS

N. LACUSTRIS

90.1  
61.3  
70.7  
151.7
different (Table 4).

*Pseudosten helia wellsi* patch integrity was higher for these shorter (24 hr) facilitation experiments than for the original two-species interaction experiments. Almost all (96 %) of the seeded *P. wellsi* individuals remained within their original pre-seeded patches throughout both experiments. Emigration was likely discouraged by the inpenetrable agar isolating each patch and the shorter timecourse of each experiment.
DISCUSSION

Pseudostenhelia wellsi and Scottolana canadensis are abundant, codominant species throughout the year in the meiofaunal communities of southern Louisiana marshes (Fleeger 1985a,b, Phillips and Fleeger 1985). Both species are often patchily distributed at variable densities over both heterogeneous and homogeneous intertidal and subtidal muddy bottoms. P. wellsi is a unique copepod in that it is one of only two species known to build mucous tubes (Chandler and Fleeger 1984). It leads a sedentary to semi-sessile lifestyle largely confined to its tube, but it must occasionally abandon or be swept from its tube to seek mates and to colonize open habitats such as trays of defaunated sediments (Chandler and Fleeger 1983). Tube networks can be extensive and interconnected throughout patches of surface sediments (Fig. 7A,B), but rarely extend below 1 cm depth. P. wellsi nauplii, copepodites and adults spend much of their time corkscrewing back and forth within their tubes, presumably grazing the inner walls. The back and forth corkscrew may act as a pump to bring water and oxygen into the tube network.

Similarly, Scottolana canadensis is a semi-sessile burrow dweller by day (Chandler, manuscript in preparation). It suspension feeds within its burrow 1-5 mm below the sediment surface so long as suspended foods (phytoplankton, detritus, etc.) are available. When suspended foods are absent or become depleted, S. canadensis copepodites and adults change feeding strategies and become errant deposit-feeding burrowers. At night, individuals (esp. adult females)
Fig. 7. Composite photo taken 3 hr after core-barrier removal showing the extensive effects that dense *Pseudostenhelia wellsi* tube networks have on sediment structure. (A) Overview of empty control patches (No. 1) and seeded *P. wellsi* patches (No. 2). (B) Close-up of both patch types. Each bump on the *P. wellsi* patch is a tube-cap which may extend as far as 1 mm above the sediment surface. Notice also that *P. wellsi*’s patch integrity and fidelity are high. (C) High magnification lateral view of a *P. wellsi* tube patch showing four tube-caps (arrows) extending above the sediment water interface.
emerge into the water column where they have been collected in shallow plankton tows (J. Howze personal communication). Scottolana canadensis nauplii are obligately planktonic (Heinle 1969). Vertical surface-burrows made by S. canadensis while suspension feeding are ephemeral, usually lasting less than 24 hr, while deeper horizontally connecting burrows persist for days but do not appear to have mucous reinforcement. However, when S. canadensis suspension feeds, burrow walls become compacted and well flushed by strong feeding currents generated by Scottolana’s setose pereiopods. Burrow flushing generates attractive halos of oxidized microhabitats around burrow walls of macrofauna in subsurface anoxic sediments (Aller and Yingst 1978) that are facilitative to many meiofauna (Reise 1981). Feeding and movement by S. canadensis (and also P. wellsi) must generate micro-oxidized zones of enhanced microbial production around their burrows as well. These zones may be facilitative to other meiofaunal bacteriovores and to meiofauna sensitive to hypoxic conditions.

Cletocamptus deitersi is also common year round and abundant in late summer/early fall (Fleeger 1985a, Phillips and Fleeger 1985). Nitocra lacustris is more ephemeral in distribution and abundance, occurring at high densities most often in disturbed habitats (Fleeger et al. 1982). Both species have completely errant lifestyles, incessantly burrowing and grazing surrounding sediment particles. They produce no lasting burrows or trails however. Both are generalist feeders with flexible food requirements (Lee et al. 1976, Chandler 1986).

Scottolana canadensis and Nitocra lacustris have sharply contrasting ecologies (i.e. sedimentary effects, feeding strategies,
reproductive strategies, burrowing abilities, etc.), yet patch colonization by both species was strongly facilitated by dense tube patches of *Pseudostenhelia wellsi*. Conversely, *Cletocamptus deitersi* which is an ecological equivalent to *N. lacustris*, exhibiting similar burrowing and feeding behaviors and sedimentary effects, was strongly inhibited by *P. wellsi* tube patches. Dense patches of *S. canadensis* were characterized by visible burrows somewhat similar to *P. wellsi* tubes, but they were a strong inhibition to colonization by *N. lacustris*.

Tube building by *Pseudostenhelia wellsi* has two major effects on the muddy sediment habitat that may be facilitative or inhibitory to other species. A substantial amount of mucus is added to the sediment providing organic enrichment and binding the silt/clay particles, and sediment structural complexity and surface area are increased by tubes protruding above and below the sediment-water interface (Fig. 7C). However, when sediments were enriched with pure mucin or seeded with inert, *P. wellsi* tube mimics, they did not facilitate colonization by *S. canadensis* (Fig. 6). The mechanisms of *S. canadensis' facilitation may be more subtle than these obvious main effects, as *S. canadensis* was very specific in its overwhelming attraction to *P. wellsi* patches alone. Possibly *S. canadensis* is attracted to some component of *P. wellsi* mucus per se; or perhaps *P. wellsi* tubes induce facilitative changes in sediment chemistry and/or microflora. Of course a synergism of effects could be operating, or an evolved mutualistic or commensalistic relationship is possible. Unfortunately, the tube-mimic patches could not duplicate the effects on sediment microflora and chemistry that a constantly moving/grazing copepod
would generate inside and outside of its tube.

*Scottolana canadensis* and *Nitocra lacustris* are not good bacteriovores, but selectively ingest and assimilate planktonic and sediment diatoms (Decho submitted manuscript). Therefore facilitative attraction to elevated microbial production around tubes of *Pseudostenhelia wellsi* is questionable, but attraction to enhanced microphytal production seems likely for both *S. canadensis* and *N. lacustris*. *Scottolana canadensis* even prefers planktonic diatoms over benthic naviculoid types when offered a choice; suggesting *P. wellsi* tube networks may in some indirect way(s) improve *S. canadensis*' suspension-feeding efficiency. Possibly *Scottolana* burrow construction and integrity are more efficient and persistent in sediments bound with *P. wellsi* tubes than in azoic sediments.

*Nitocra lacustris* overall was less discerning in its preference for specific *P. wellsi* tube effects, and it was inhibited by *Scottolana canadensis*. Colonization by *Nitocra* was facilitated equally well by mucous enrichment alone, inert tube structures alone, and both combined as natural *P. wellsi* tube patches. *Nitocra* is a generalist feeder/forager and therefore not surprisingly facilitated by both major sedimentary effects. *Nitocra* may ingest mucus directly or indirectly via bacterial production, and tube structures may provide attractive grazing substrates. Adult females, however, were more selective for natural *P. wellsi* tube patches. The combined mucous enrichment (potential food) and structural complexity (potential shelter from resuspension and/or grazing sites -- Fleeger et al. 1984, Bell and Coen 1982, Alongi 1985) of natural tubes may have been more attractive to gravid females and females soon to
produce egg sacs because naupliar survival/growth may be enhanced.

The specific attraction of *P. wellsi* tubes to *Scottolana canadensis* overall and *Nitocra lacustris* adult females in particular may have been caused by "microfloral gardening" by *P. wellsi*. Many nematodes and at least one harpacticoid, *Diarthrodes nobilis*, produce copious mucous trails or bags that entrap bacteria, detritus, diatoms, feces, etc., which may be later reingested with the mucus (Riemann and Schrage 1978, Hicks and Grahame 1979). The nematode *Praeacanthonchus punctatus* (Bastian) even produces mucous trails that become enriched with high density monocultures of the non-motile phase of *Tetraselmis*, a chlorophytic alga (Warwick 1981). *Praeacanthonchus* may selectively concentrate *Tetraselmis* in some way, such as by chemical attractants exuded with its mucus, or by targeting and encouraging active growth by one or a few *Tetraselmis* species. Some solitary homing limpets similarly produce mucous exudates that stimulate growth of preferred microalgae within their mucous trails (Connor and Quinn 1984).

The way *Pseudostenhelia wellsi* meets its nutritional needs is unknown. The copepod spends most of its time corkscrewling within its tube and appears to graze the tube walls; it has never been observed grazing outside of its tube. When forced to leave its tube, it immediately builds another and again begins to corkscrew. *P. wellsi*’s behavior suggests that it may have a microfloral "entrapment" or "gardening" trophic-strategy as well, either by particle entrapment as water moves in and out of its tube, or by providing a mucous substrate and exudates for intensive algal and/or bacterial production. If so, then the specific attraction by *S. canadensis* and *N. lacustris* to natural tube patches may be due to locally high concentrations of
entrapped or gardened food. It is unlikely however that *S. canadensis* or *N. lacustris* could utilize foods grown or cached within the tubes because of their larger body size and *P. wellsi*'s aggressive tendencies (Chandler and Fleeger 1984); but more likely would use microflora growing on or around tube exteriors if at all.

Despite its semi-sedentary lifestyle and sedimentary effects similar to those of *P. wellsi*, *Scottolana* did not facilitate colonization by *N. lacustris*. *Nitocra* densities were overall significantly lower in *P. wellsi* tube patches (Fig. 3). Several contrasting characteristics between *S. canadensis* and *P. wellsi* exist and probably generated the disparate outcomes. *Scottolana* provided no organic structure per se, but only open burrows without mucus enrichment above or below the sediment surface. They were sedentary while suspension feeding but flocculated and resuspended 0-3 mm of the surface sediment layer; they were more mobile while deposit feeding and generated extensive burrow networks in deeper sediments. Colonization by *Nitocra* was best facilitated by a more stable sediment fabric bound with mucus or interlaced with tubes; even inert tube-mimics were facilitative despite their obvious impedance to free burrowing.

*Cletocamptus deitersi* showed an inhibition response to *Pseudostenhelia wellsi* tube patches that was stronger than the facilitation responses of either *Nitocra lacustris* or *Scottolana canadensis* (Fig. 3). This was unexpected for a colonizer species with feeding and burrowing behaviors and sedimentary effects so similar to *N. lacustris*. Fleeger (1985a), however, found a significant inverse correlation ($R^2=0.4$; Pr=0.98) between densities of *P. wellsi* and *C. wellsi*. 
deitersi in predator exclusion cages where C. deitersi increased and
P. wellsi decreased relative to ambient control levels. Possibly
enhanced sediment cohesion and complexity from P. wellsi mucus and
tubes are amensalistic to some errant meiofauna and may have
interfered with grazing or burrowing by C. deitersi. A chemical
repulsion by some component of P. wellsi mucus or other exudates is
also possible, especially if both species are competitors for food or
space, or if C. deitersi can use mucopolysaccharides as food.
Cletocamptus deitersi in high density culture has been observed
grazing on the mucous tubes of spionid polychaetes, and gradually
decomposes them (Chandler personal observation). It is unknown
whether C. deitersi ingests mucus directly or indirectly after
bacterial breakdown (e.g. Callow 1984).

Meiofauna communities in general and harpacticoid copepods in
particular often exhibit moderate to extreme horizontal and vertical
patchiness in their spatial distributions (McLachlan 1978, Thistle
Fleeger, 1985). Patchiness occurs in physically demanding benthic
environments, such as high energy beaches (McLachlan 1978), as well as
in relatively stable environments such as the deep sea (Thistle 1978).
Causes of patchiness are not well known, but hydrodynamics,
animal-sediment interactions, patchy food resources, biogenic
structures, predation, intra/interspecific competition, aggression and
local bursts of reproduction have been implicated as probable causes
(see Hicks and Coull 1983 review). Facilitative influences of one
meiofaunal species on another have not been suggested as a probable
cause of small scale patchiness, but my results clearly show that such
interactions do occur and they can be surprisingly strong. An abundant species in a meiofaunal community, such as *P. wellsi*, could function as a key "host" for other species capable of deriving commensalistic (or even mutualistic) benefits from some aspect of its biology (e.g. tube building in this study).

The mechanisms behind spatial patterns of natural populations of such small, rapidly growing/changing organisms as harpacticoid copepods are probably synergisms of the causes listed above. Biotic interactions among harpacticoids are no doubt important, as shown by this study, but corroborative evidence is sparse. Bell and Coull (1980) showed a significant inverse correlation between densities of permanent and temporary meiofauna (i.e. polychaete larvae), as did Watzin (1983) for turbellarians and temporary meiofauna. Fleeger and Gee (1986) manipulated densities of two harpacticoid species predictably separated on a fine-scale vertical zonation to determine if interference competition for space occurs. They found no evidence that the parapatric vertical zonation was caused by competition, but suggested that the pattern was due to physical factors in the interstitial habitat. Much more evidence is available that shows meiofaunal distributions are strongly influenced positively and negatively by macrofaunal/floral effects on the sedimentary habitat (e.g. Hummon et al. 1976, Reise 1981, Bell et al. 1978, Osenga and Coull 1983, Creed 1983). Macrofaunal predation/disturbance are also important as they generate low density patches of meiofauna over broad expanses of sediment (e.g. Coull and Bell 1978, Thistle 1980, Reidenauer and Thistle 1981).
Outside of the present study, examples of meiofaunal facilitation and inhibition of colonization by other meiofauna are not known, but available evidence shows that macrofauna can facilitate colonization by other macrofauna. Gallagher et al. (1983) found that three species of tube-building surface deposit feeders (2 polychaetes and 1 tanaid crustacean) when seeded separately into 10 cm² azoic sediment patches and implanted into an intertidal sandflat facilitated colonization by at least six species inhabiting the surrounding natural sediments. Even simulated tubes (sticks) inserted vertically into azoic patches were also facilitative to oligochaetes and tanaids, suggesting the tube structure alone may have attracted the other species. However, passive hydrodynamic facilitation via enhanced rates of larval recruitment around tube structures (sensu Eckman 1979) could not be ruled out as a cause of the facilitative pattern. Paradoxically, Macoma balthica, a free-living, surface deposit feeding, tellinid bivalve, facilitated patch colonization by a tube-building polychaete but inhibited tube-building tanaid crustaceans.

Tube-building surface deposit feeders frequently show an opportunistic early dominance in successional patterns of defaunated areas after natural and artificial disturbances (Grassle and Grassle 1974, McCall 1977). Tubiculous macrofauna can generate dramatic changes in sediment chemistry (Aller and Yingst 1978, Aller 1982), sediment structure (Rhoads 1974, Rhoads and Boyer 1982) and sediment erodability (Mills 1969, Eckman et al. 1981) by their direct feeding/foraging activities and by their effects on hydrodynamics of near-bed flow. These sediment and hydrodynamic characteristics are no doubt important in determining how long and what kinds of early
colonists will persist (Mills 1967, 1969, Grassle and Grassle 1974) and what succession of species will follow in soft-bottom communities (McCall 1977, Gallagher et al. 1983).

Predictable successional patterns have not been seen in meiofaunal communities and probably do not occur after small-scale defaunations ($cm^{-2}m^{-2}$). Temporal patterns are obscured and irreproducible within small areas of open habitat because most meiofauna are rapid colonists and reach pre-disturbance densities and species composition in hours (Sherman and Coull 1980) to 1-2 days (Chandler and Fleeger 1983). Active emergence and migration are common for demersal meiofaunal species (Alldredge and King 1980), but passive hydrodynamic resuspension and transport are probably more important effectors of rapid en masse dispersal to open habitats (Bell and Sherman 1980, Palmer and Brandt 1981, Chandler and Fleeger 1983, Fleeger et al. 1984, Palmer 1984).

In benign benthic flow regimes, such as those found in Terrebonne Bay's low-amplitude tidal marshes, faunal resuspension is low; and biotic interactions must be important determinants of meiofaunal spatial distributions and community structure. On a meiofaunal size scale, Pseudostenhelia wellsi may have as strong an effect on sediment characteristics as larger tube-building macrofauna. If so, their patchy distribution and protection from resuspension provided by their tube-dwelling existence make them likely candidates as attractive nucleating sites for other meiofauna species; especially deeper-dwelling species able to avoid resuspension and individuals settling out of suspension after periodic hydrodynamic disturbances (e.g. spring tides, storms, etc.). Current velocities up to 15 cm
sec\(^{-1}\) occur during spring tides in Louisiana marshes (Fleeger et al. 1984) but cause no reduction in \textit{P. wellsi} sediment densities. However they can reduce densities of other meiofauna. Dense mucous tube patches may function as attractive protected microhabitats for other species less resistant to mild erosion and resuspension.

Mucus production by meiofauna is common (Riemann and Schrage 1978, Hicks and Grahame 1979, Coull and Grant 1981, Warwick 1981, Chandler and Fleeger 1984), but tube building is not and is generally unknown among permanent meiofauna taxa. Most meiofauna live freely in the interstices of sand or as burrowers in non-capillary sediments (Swedmark 1964, Fenchel 1978, McIntyre 1969, Hicks and Coull 1983). Tube building by meiofauna may be more common than generally thought, however, because meiofaunal-sized tubes are small, delicate and easily destroyed by standard sampling techniques; and \textit{in situ} observations of live meiofauna have been logistically difficult. Other members of \textit{P. wellsi}'s harpacticoid family, the Diosaccidae, are commonly represented in muddy sediments from the intertidal (Fleeger 1979) to the deep sea (Thistle 1978). At least two other genera in the family, \textit{Stenhelia} and \textit{Melima}, have similar morphologies and appendages to \textit{Pseudostenhelia wellsi}, and all have ellipsoid nauplii (a proposed adaptation for uninhibited, intra-tube mobility -- Chandler and Fleeger 1984). If these similarities are mutual adaptations for tube-dwelling lifestyles, then tubiculous harpacticoids may be more common in meiofaunal communities than previously thought, and could have local facilitative and inhibitory effects on other more motile species due to their functional effects on sediment structure.

Rhoads and Young (1970) found that filter-feeding macrofauna as a
group are functionally excluded from muddy sediments by the amensalistic feeding activities (e.g. burrowing and fecal pellet deposition) of deposit feeders. Deposit feeders often disrupt sediments and contribute to resuspension which in turn may foul filter organs of filter-feeders. In turn, filter-feeders often form dense tube-mats that impede free burrowing macrofauna; therefore tubiculous filter-feeders can also be amensalistic to mobile deposit feeders (Brenchley 1982). "Trophic group amensalism" has since been reported in numerous studies of macrofauna communities (Young and Rhoads 1971, Bloom et al. 1972, Whitlatch 1977, McCall 1977), and generalized as a "functional group hypothesis" (Woodin 1976, Woodin and Jackson 1979) where groups of species that alter or use the environment in similar ways should coexist if each can survive and reproduce in the presence of the others. Functional groupings of soft-sediment macrofauna depend heavily on the way species affect sediment structure, resuspension and erosion. Sediment compactness/cohesion influence the kinds of burrowing fauna that can immigrate, and suspended sediment loads affect the survivorship of many filter-feeders. Organismal interactions such as exploitative competition, mutualism, commensalism, etc. are species-specific and considered by the hypothesis to be lesser effectors of the composition of functionally similar assemblages. However adult-larval interactions such as predation and selective settlement/recruitment are important (Woodin 1976).

The functional group hypothesis assumes that sediment-mediated facilitative interactions have strong structuring effects among groups of functionally similar species, while competition has a stronger

The functional group hypothesis has proven generally predictive of the kinds of fauna inhabiting many soft and hard substrate communities (Woodin 1976, Jackson 1977, 1979, Woodin and Jackson 1979), but several recent studies reject its utility (Jumars and Nowell 1984, Weinberg 1984). For example, Weinberg (1984) tested the hypothesis that small, filter-feeding bivalves with large young should reach high densities with tube-builders of any trophic strategy because large young could escape predation (By functional group expectations small larvae should be ingested by deposit-feeders). Gemma gemma (Totten), a filter-feeding bivalve with large larvae, and
Clymenella torquata (Leidy), a tubiculous deposit-feeding polychaete) coexist at high densities (Sanders et al. 1962) because C. torquata feeds at depths unoccupied by G. gemma. The large size of G. gemma's larvae is unrelated to its paradoxical coexistence with C. torquata (Weinberg 1984).

The character and extent of meiofaunal functional effects on their sediment habitats are largely unknown. Sand dwelling meiofauna likely have little or no effect on sediment structure or erodability as the sand grains are typically as large or larger than the meiofauna dwelling in their interstices. However, in fine muddy sediments, the meiofauna can have dramatic effects on sediment appearance (Neumann et al. 1970, Cullen 1973, Severin et al. 1982, Chandler and Fleeger 1984). Known effects range from construction of mucous tubes to compacted burrows and mucous trails. Presently functional groupings of muddy sediment meiofauna seem of little utility, especially in light of our experimental findings and because so little is known of meiofauna behavioral ecology. For our experiments, predicted outcomes based on functional group expectations were that the tube-building harpacticoid, Pseudostenhelia wellsi, would facilitate colonization by the semi-sessile burrow-dweller Scottolana canadensis, but strongly inhibit both Cletocamptus deitersi and its behavioral equivalent Nitocra lacustris. Nitocra lacustris recall was attracted to P. wellsi while C. deitersi was strongly repelled. Yet intact patches of P. wellsi's functional mate in these experiments, S. canadensis, paradoxically repelled N. lacustris.

Our results suggest that hypotheses which explain meiofaunal associations based on functional group expectations may lose their
utility by being too general. Hypotheses and experiments which incorporate species behaviors and ecologies into their designs are usually more functional than "whole group" approaches in explaining and predicting species associations in time and space. However, as meiofaunal effects on sediment structure, chemistry and microflora become better understood, functional groupings may be refined and made more predictive; or they may prove biologically meaningless in meiofaunal communities due to multiple species-specific idiosyncrasies in response to "functional groups".
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G. Thomas Chandler
Ph.D. Candidate

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APPENDICES

(see pocket)
Tube-building by a marine meiobenthic harpacticoid copepod

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Abstract

Pseudosthenhelia wellsi Coull and Fleeger is a meiobenthic harpacticoid copepod inhabiting muddy, estuarine sediments. All individuals observed, and all ages including nauplii, build and inhabit elongate, mucous tubes, which may extend to a depth of 3.9 mm into the sediment. Many of the narrow tubes, 0.27 mm in diameter, have a tube-cap extending 0.32 mm above the sediment-water interface. The tubes are a matrix of fine silt, sand and detritus cemented with a mucopolysaccharide, as shown by the Periodic Acid Schiff stain, apparently secreted from glands in the ventrolateral margin of the cephalothorax. Upon addition to sterile, homogeneous sediment, P. wellsi quickly (within 1 to 2 h) transform the upper 0.4 cm to a cohesive conglomerate of tubes, silt-clay particles and mucous filaments.

Introduction

Tube-building and tube-dwelling behaviors are common for many marine crustaceans (e.g. ampeliscid amphipods, Mills, 1967; Barnes, 1980), but are generally unknown among the Copepoda (B. C. Coull and G. Hicks, personal communication). Benthic, infaunal copepods are reported to live freely in the interstices of sands or as burrowers in non-capillary sediments (Hicks and Coull, 1983). However, an observation of copepods associated with sand tubes in algal mats exists (Neumann et al., 1979). Earlier discovery of its tube-building nature was prevented because the delicate tubes are easily destroyed during sample sieving and processing.

Material and methods

Pseudosthenhelia wellsi Coull and Fleeger was collected from a shallow, tidal pond surrounded by Spartina alterniflora marsh near Cocodrie, Louisiana, USA. Muddy sediment (median grain size of 38 μm) was scooped into buckets, and rinsed through a graded sieve series. Sediments and meiofauna retained on a 252-μm sieve were aerated and transported to the laboratory. Individuals were separated from sediments under a dissection stereomicroscope, and placed, at a density of 50 per cm², into Plexiglas observation chambers (2.5 cm² in area) containing a 2-cm deep layer of sterile sediment. This sediment was obtained from the same graded sieve series, and normally consisted of smaller particles (median grain size of 19 μm; 7% organic matter). Additional sterile substrates, mostly detrital aggregates, retained on the 125- and 250-μm sieves, were also used. Observations and photographs were made from above and through the chamber side walls with a Nikon dissection stereomicroscope.

Histochmical staining of Pseudosthenhelia wellsi sections and of the sediment-tube matrix used the Periodic Acid Schiff (PAS) technique (Pearse, 1968). Positive staining with PAS indicates an acid mucopolysaccharide. Copepods were fixed in 2% glutaraldehyde and post-fixed in 1% OsO₄, embedded in L.R. White resin and sectioned (1 μm) with an ultramicrotome before staining. Tube caps were removed from the sediment with forceps, placed on a microscope slide and PAS stained. For SEM, copepods were fixed in Bouin's medium, dehydrated, and then critical point dried. They were sputter-coated with Au-Pd.
and examined on a JEM 100CX STEM equipped with an ASID scanning device.

Quantitative field sampling was conducted in an intertidal marsh near Port Fourchon, Louisiana. A total of 80 sediment cores (2.54-cm inner diameter) were collected at four tidal stages: low tide (no standing water), flooding tide, slack high and ebbing tide. Cores were fast frozen in liquid nitrogen, sectioned at 2-cm intervals in the laboratory, and extracted with LUDOX (e.g. Fleeger and Chandler, 1983). Pseudostenhelia welshi copepodites and adults were enumerated under a stereomicroscope.

Results and discussion

Upon addition to the sediment chambers, Pseudostenhelia welshi immediately seeks and enters the sediment irrespective of grain size or type (silt-clay or detrital). Tube building begins at once, and within 30 min the first tubes appear. In 1 to 2 h, a maze of horizontal and vertical tube channels (Fig. 1A) proliferates throughout the upper 0.4 cm of sediment. All tubes are constructed from a matrix of small sediment particles and detritus bound together with an acid mucopolysaccharide (mucin), as
shown by positive staining with the PAS stain. Many tubes extend laterally to form a continuous infaunal network that markedly transforms the free surface sediments to a cohesive mucus-sediment mat. This mucus-sediment matrix is slightly elastic so the tube stretches and/or bends as the copepod moves through it. Many tubes have a "tube-cap", a vertical extension of the tube 0.32±0.05 mm in height above the sediment-water interface (Fig. 1B). Tubes have an open mouth with no operculum or mechanism for closing off the tube. Tubes are elongate, extend as deep as 3.9 mm, and are slightly narrower than the copepod occupant. Tubes of adults average 0.27±0.04 mm in total width with an inner diameter of 0.17 mm. The maximum width of adults is 0.20 mm.

Because the tubes are slightly narrower than the occupant, they quake or shake when a copepod travels through them. *Pseudostenhelia wellsi* touches the tube wall at all times with its prosome dorsally, with its fifth leg (a flat, spatulate structure, Coull and Fleeger, 1977) laterally or with its cephalothorax and first four swimming legs ventrally. Appendages apparently "rub" the mucous wall as the occupant bustles through, "working" or smoothing the lining of the tube.

*Pseudostenhelia wellsi* nauplii, copepodites, adults and ovigerous females (Fig. 1A) all build tubes. Nauplii build tubes (0.08-mm diameter) almost immediately after hatching and occur alone in tubes much too small for adult construction. The nauplii are well adapted for tube-dwelling because they have an ellipsoid body shape with minimal protuberances that allows easy lateral movement through the tube [see Bresciani, 1960 for a description of the nauplii of the closely related *Stenhelia (Delavailla) palustris*]. As the copepod molts and grows, the tube is either abandoned and built anew, as with nauplii, or enlarged as seen with later-stage copepodites.

The behavior of *Pseudostenhelia wellsi* in the tubes is predictable. When an individual reaches the opening of a tube cap, it remains motionless for a brief time (8 to 28 s, mean = 18), with antennules, antenna and eye protruding into the water column. No appendage, e.g. mouth part, movement occurs. The copepod then suddenly does a backwards somersault at the mouth of the tube, protruding its ventral surface including swimming legs from the tube. The copepod then quickly proceeds head first down the tube. Individuals do not routinely stop until they reach the blind pocket at the sediment end of the tube. They again remain motionless for a time, then do a backwards somersault and return to the tube cap. The time elapsed from leaving the tube cap until return averages 19 s (standard deviation = 7.9). Some individuals probably visit more than one tube cap, but on only two occasions were individuals observed to meet within the tube network. In both cases, one was pushed backwards after a brief aggressive encounter, and eventually was expelled from the tube. The expelled individual immediately sought another tube cap and entered quickly.

Mucus secretion by *Pseudostenhelia wellsi* distinctly changes the character of the upper 0.4 cm of sediment. The sediment initially added to the experimental chambers was homogeneously mixed (Fig. 1C). Within 1 to 2 h after addition of *P. wellsi*, the surface was extensively worked into a cohesive matrix of mucous tubes and filaments (Fig. 1D). Other investigators (Holland *et al.*, 1974; Eckman *et al.*, 1981) have shown or suggested that mucus stabilizes sediment, and *P. wellsi*, as well as other mucus producing meiofauna such as nematodes (Cullen, 1973), probably have this effect. Mucus production by *P. wellsi*, which reaches densities of 150×10^3 cm^-2 in Louisiana, may have great stabilizing effects on the easily resuspended, predominantly silt- and clay-sized sediment in which it lives. The role of meiofauna, relative to microflora and macrofauna, in sediment stabilization is unknown.

Fluorescence microscopy of *Pseudostenhelia wellsi* revealed three paired areas (one on the ventral margin of the cephalothorax and two in the urosome) that autofluoresce and appear glandular. Although many glands are present in harpacticoids (Fahrenbach, 1962; Hicks and Grahame, 1979; Coull and Grant, 1981), PAS staining revealed at least three paired glands which contain acid mucopolysaccharides. One such paired gland is located anterodorsally to the labrum, and is similar to that described in *Diarthrodex nobilis* (Hicks and Grahame, 1979). SEM revealed no anterodorsal surface pores connected to these glands. Another pair of mucous glands is located on the ventrolateral margin of the cephalothorax. These glands are composed of large cells, approximately 18 μm in diameter, presumed to be secretory. SEM revealed a pore (2.0±0.2 μm) opening on the ventral margin of the cephalothorax in the region of the first maxilla in close proximity to these glands (Fig. 1E). A mucoid discharge was observed near these pores, which are continuous with a sigmoid, fissure-like articulation running perpendicular to the longitudinal axis of the cephalothorax (Fig. 1F). This sigmoid articulation may open or close depending on the bend of the cephalothorax. Extending posteriorly 4.7±0.5 μm from each pore there is a narrow ventral groove leading into an oval cuticular concavity that frequently contains mucoid residues. No other pores were observed, and sensilla from pit-like structures were the only other noteworthy integumentary features. A ventral pore near the fifth swimming leg or on the caudal rami is assumed; however, observation in this area is obscured by leg segmentation and setation.

Tube-dwelling undoubtedly has a great influence on the natural history and ecological relationships of *Pseudostenhelia wellsi*. We never observed this species foraging outside of its tube in any way, nor does it appear to generate water currents necessary for suspension feeding. The mode of nutrition in *P. wellsi* is unknown. Although individuals were not directly observed grazing on tubes, *P. wellsi* may use the tube or its microbial enrichment as a food source. The mucus-trap mode of an animal feeding on its own mucus after bacterial or algal enrichment has been inferred for other mucus-producing meiofauna (Riemanne and Schrage, 1978; Hicks and Grahame, 1979; Warwick,
Tube-dwelling may also restrict dispersal as many meiofaunal copepods in muds have been shown to disperse via the water column during tidal flushing (Hagerman and Rieger, 1981; Palmer and Brandt, 1981; Chandler and Fleeger, 1983). Unlike many mud-dwelling harpacticoids (see Palmer and Brandt, 1981), *P. wellsi* shows no reduction in sediment density with increasing current velocities (Table 1), indicating that tubes may offer protection from passive suspension. Tube-building in *P. wellsi* may dictate the nature of competitive interactions with other meiofauna. Macrofaunal tube-builders competitively interact with burrowers/bioturbators because of their contrasting effects on sediment structure, i.e. tube-building and subsequent sediment binding may be incompatible with burrowing and sediment destabilization (Woodin and Jackson, 1979; Brenchley, 1981, 1982; Wilson, 1981). If similar contrasting sedimentary effects occur among meiofauna species, then functional group interactions must have significant influences on meiofaunal community structure.


### Table 1. *Pseudostenhelia wellsi*. Changes in the density with changing tidal conditions. Data are given as mean ± standard error. Tidal velocities are in cm s⁻¹. Analysis of variance indicates no significant difference in density among tidal states, F=0.48; d.f.=4, 12; P=0.68.

<table>
<thead>
<tr>
<th>Tidal State</th>
<th>Low</th>
<th>Flooding</th>
<th>High</th>
<th>Ebbing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density × 10 cm⁻³</td>
<td>3.6±0.86</td>
<td>5.5±1.3</td>
<td>3.8±0.82</td>
<td>4.8±1.7</td>
</tr>
<tr>
<td>Tidal velocity</td>
<td>0</td>
<td>3.0</td>
<td>0</td>
<td>15.0</td>
</tr>
</tbody>
</table>

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**Acknowledgements.** We wish to thank M. Blackwell for use of a photostereomicroscope, H. Silverman for histochemical analyses, W. Steffens for electron microscope assistance, and J. Howze, H. Silverman, W. Sikora and J. Sikora for reviewing the manuscript.

**Literature cited**


G. T. Chandler and J. W. Fleeger: Tube-building by a copepod


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High-Density Culture of Meiobenthic Harpacticoid Copepods Within a Muddy Sediment Substrate

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Difficulties in producing a muddy substrate which does not easily foul have made previous efforts to culture mud-inhabiting, estuarine, and marine harpacticoid copepods unsuccessful. Natural, organic-rich muds are unsuitable as a long-term culture medium because they generate detrimental bacterial blooms in stagnant and periodically flushed culture systems. I present some simple procedures to (1) sort muddy sediments into a <125-μm size class, (2) flush away most dissolved organics, (3) sterilize the sediments providing a moderately foul-free culture medium, (4) generate a life-like, flocculent surface layer, and (5) allow easy observation above or below the sediment surface. Five harpacticoids were cultured within 45–90 d to densities 4–11 times their natural field maxima (per 10 cm²): Scottolana canadensis (372), Paronychocamptus huntsmani (380), Onychocamptus mohammed (448), Cletocamptus deitersi (1259), and Nitocra lacustris (1662). Since most mud-inhabiting harpacticoids are larger than 125 μm, simple sieving on a 125-μm screen eliminates culture sediments leaving hundreds of clean, easily collected harpacticoids.

Le fait qu’il soit difficile de produire un substrat vaseux qui ne devient pas fétide facilement a fait échouer les efforts antérieurs visant à éléver des copepodes estuariens et marins du groupe des harpacticoïdes qui vivent dans la vase. Les vases naturelles, riches en matières organiques, sont peu appropriées comme milieux de culture à long terme, car elles sont le siège de proliférations de bactéries nuisibles dans les milieux d’élevage stagnants et nettoyés périodiquement. J’ose présenter quelques techniques simples pour (1) classer les sédiments vaseux dans une catégorie de taille <125 μm, (2) éliminer la plupart des substances organiques dissoutes, (3) stériliser les sédiments en fournissant un milieu de culture passablement exempt de matières fétides, (4) produire une couche superficielle flocculeuse qui semble vraie et (5) permettre une observation aisé au-dessus et en-dessous de la surface des sédiments. On a élevé cinq harpacticoïdes pendant 45 à 90 j à des densités de 4 à 11 fois supérieures au maximum rencontré dans leur habitat naturel (par 10 cm²), soit les espèces Scottolana canadensis (372), Paronychocamptus huntsmani (380), Onychocamptus mohammed (448), Cletocamptus deitersi (1259) et Nitocra lacustris (1662). Comme la plupart des harpacticoïdes qui vivent dans la vase sont plus gros que 125 μm, un simple tamisage à l’aide d’une grille de 125 μm élimine les sédiments du milieu de culture en laissant des centaines d’harpacticoïdes propres qu’on peut recueillir facilement.

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(O1211)
and Fleeger 1984; Marcotte 1984). This is unfortunate, as strong substantiation of inferences from field data is best supported by direct observational evidence.

Very few truly sediment-dwelling harpacticoid species have been cultured successfully, and rarely at densities very much above inoculation (i.e. maintenance) levels. Their strong dependence on a sand or mud substrate of a particular size, texture, microfaunal/microfloral composition, etc., for food, reproduction, and protection has precluded easy cultivation in the laboratory. My purpose was to find simple, efficient, and economical ways to (1) cleanse and sterilize muddy sediments for use in normal or high-density culture of mud-inhabiting harpacticoids, (2) reconstruct a foul-free, reasonably life-like imitation of the natural sediment structure to promote natural copepod behaviors and life histories, and (3) photograph surface/subsurface behaviors and biogenic effects on a controlled, predetermined sediment structure. Harpacticoids cultured by such techniques would then be available for trophic or maricultural studies, as well as controlled laboratory studies of muddy-bottom meiofaunal activities.

**Materials and Methods**

**Preparation of Culture Medium**

Copepods were cultured in a muddy sediment medium prepared as follows. Two 19-L buckets of fresh, predominately silt—clay surface (0—3 cm) sediments were collected by hand from a mudflat in a *Spartina alterniflora* marsh near Cocodrie, Louisiana, and refrigerated or frozen until ready for use.

Sediments were washed with deionized water through a 0.5- and 0.125-mm sieve set (30-cm diameter) stacked flush over a cylindrical Pyrex® jar (30.5 × 305 cm; 17 L). Most of the silt and fine-sand fraction passing through the 0.125-mm sieve was captured in the jar, but the lighter clay and detrital particles and unwanted organics (humic acids, tannins, etc.) were flushed away by allowing the washwater to slowly overflow the jar.

After sieving and washing, the sediments in the jar were allowed to settle for 1 h until a distinct precipitation band formed between the uppermost layer of remaining clay-size particles and the heavier silt and fine-sand fraction. The lighter clay-size fraction was aspirated away to the precipitant band and discarded. The jar was refilled with deionized water, stirred vigorously, allowed to settle for 90 min, and again aspirated to the precipitant band.

The remaining heavy fraction was condensed to 50% original volume by heating in a drying oven for 24 h at 120—140°C. The medium was stirred every 6—8 h and removed when the 50% reduction was achieved. Perforated aluminum foil was placed over the bell jar to control the evaporation rate, thus reducing surface dehydration and clumping. The condensed medium usually contained shrinkage cavities filled with unwanted organic leachate. After cooling, the leachate was aspirated away.

The cooled medium was vigorously blended using an electric cake mixer until a consistency like pudding was achieved. This ensured that all sediment particles were dispersed uniformly. Portions (600 mL) of blended sediment were then ladled into 1-L beakers, covered with heavy duty foil, autoclaved (107°C, 4.5 kg·cm⁻², 40 min), and stored indefinitely under refrigeration. Yield of culture medium was 50—60% by weight of original natural sediments with a median grain diameter (MGD) of 0.02 mm and total organic matter (TOM) content of 7% (natural sediments = 0.04-mm MGD; 10% TOM).

Autoclaving semisolidified the medium, but it was rehydrated to natural texture by blending with artificial seawater (ASW) 1 to 1 or less to make "wet" medium. The proportion of medium to ASW that yielded the most natural reconstitution had to be determined experimentally, as the final texture of the autoclaved medium depended upon the original composition of the natural source sediment.

Artificial generation of a flocculent surface layer was necessary to achieve a realistic imitation of the natural sediment structure. Most mud-inhabiting harpacticoids live almost ex-
ethylene catheters (Fig. 1B). Flow through the catheters was gravity fed and controlled by standard aquarium-gang valves of 1.0-2.0 mL min⁻¹ before fauna were added. A fluorescent very slowly to each vessel through small-bore (20 gauge) poly­

The 130 mL of prepared floe was then stirred vigorously and reproducing in it, and even filtering the floe

The Na(PO₄)₆ neutralizes electrostatic bonding of clay particles and aids dispersion (see Buchanan 1971). After soaking, the sediments settled out of suspension and the remaining supernatant was aspirated away and replaced with aerated ASW. The mixture was stirred and sonicated for 10 min to complete the preparation.

Preparation of Culture Vessels for Surface/Subsurface Observation

Copepod culture and experimentation was carried out in hexagonal observation vessels (75 mm high × 100 mm wide; surface area = 66 cm²) made from glass and Plexiglas (Fig. 1A). A 3.0-cm-deep layer of wet medium was poured into the culture vessels and covered by a 1-cm-thick flocculent layer prepared as follows. Dry medium (20 g) was placed into a 150-mL beaker, brought up to 130 mL with 5% Na(PO₄)₆ in ASW, and stirred vigorously. It was then sonicated for 15-20 min and placed in a refrigerator to soak for 4 h. The Na(PO₄)₆ neutralizes electrostatic bonding of clay particles and aids dispersion (see Buchanan 1971). After soaking, the sediments settled out of suspension and the remaining supernatant was aspirated away and replaced with aerated ASW. The mixture was stirred and sonicated for 10 min to complete the preparation.

Feeding and Harvesting

Harpacticoids were fed a mixed diet of chrysophytic (Isochrysis galbana) and chlorophytic (Dunaliella tertiolecta) algae, one diatom species (Thalassiosira weissflogii), and spinach fragments. The spinach fragments were prepared by blending 10 g of frozen spinach at high speed in 500 mL of ASW for 3 min, washing it through a 45-μm screen, and collecting the sieved fraction in a 1-L beaker. Any foamy residues remaining on the surface of the spinach solution were aspirated away. Young cultures of 200 copepods or less were fed 10 mL of an equal, four-part suspension of each of the three algal types and the spinach solution every 3 d. Mature cultures were given 30 mL every 2 d. If food accumulated on the sediment surface, cultures were not fed until it disappeared; otherwise, fouling would occur. Algae were cultured on F/2 medium (Guillard 1972) in 500-mL Erlenmeyer flasks.

High-density cultures were nondestructively harvested as follows. A 3-mm-bore Pasteur pipette was connected to a 500-mL side-arm vacuum flask with 1 m of gum-rubber tubing...
<table>
<thead>
<tr>
<th>Species (habitat)</th>
<th>Culture medium</th>
<th>Culture apparatus</th>
<th>Food</th>
<th>Inoculation density</th>
<th>Maximum density</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paronychocamptus nanus (sand)</td>
<td>70 mL asbestos-filtered NSW with 50% replaced bidaily</td>
<td>Glass tubes (10 x 3.5 cm) kept at 10°C in water bath</td>
<td><em>Tetraselmis suecica</em> and <em>Phaeodactylum tricornutum</em> (0.2 mL each) and a few drops of lyophilized detritus; fed every 3-4 d</td>
<td>1 gravid·tube⁻¹</td>
<td>—</td>
<td>Smol and Heip 1974</td>
</tr>
<tr>
<td>Tachidius discipes (sand/muddy sand)</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Microlaophonte sp. (muddy sand)</td>
<td>Corn meal agar mixed 1/2 strength with NSW, scored, and covered with NSW film</td>
<td>Sealed Petri dishes (9.3 x 1.7 cm) kept on 12:12 LD for 3 mo</td>
<td>Incident microflora/fauna</td>
<td>“A few gravid”</td>
<td>100% survival of 20 adults and 20 larvae for 2 wk</td>
<td>George 1975</td>
</tr>
<tr>
<td>Thompsonula hyanae (sand)</td>
<td>Dried autoclaved sand from natural habitat and filtered NSW</td>
<td>Covered finger bowls (8-cm dia) kept on 12:12 LD in summer, 10:14 LD in winter</td>
<td>Mixture of 2 naviculoid diatom spp. and incidental bacteria</td>
<td>—</td>
<td>—</td>
<td>Sellner 1976</td>
</tr>
<tr>
<td>Nitrocra typica (salt-marsh aufwuchs)</td>
<td>Solidified ersdschreiber covered with Millipore-filtered (0.45 μm) NSW containing antibiotics and antimycotics (penicillin, 10 000 U·mL⁻¹; streptomycin, 10 g·mL⁻¹; fungizone, 25 μg·mL⁻¹); stocks transferred every 3 wk</td>
<td>Petri dishes (10 x 1.5 cm) kept at 25°C on 18:6 LD</td>
<td><em>Cylindrotheca closterium</em> (1 x 10⁴ cells·dish⁻¹)</td>
<td>2 gravid·dish⁻¹</td>
<td>159·dish⁻¹ (all stages)</td>
<td>Lee et al. 1976</td>
</tr>
<tr>
<td>Scottolana canadensis (mud/sandy mud)</td>
<td>100 mL Millipore-filtered (0.45 μm) NSW (10%)</td>
<td>125-mL Erlemeyer flasks kept in darkness</td>
<td><em>Isochrysis galbana</em> and <em>Thalassiosira pseudonana</em> (10 mL·wk⁻¹)</td>
<td>(batch inoculation of nauplii)</td>
<td>—</td>
<td>Harris 1977</td>
</tr>
<tr>
<td>Paramphiascea vararensis (sand)</td>
<td>Unsorted, acid-cleaned, autoclaved beach sand overlain by 80 mL coarse-filtered and autoclaved NSW</td>
<td>Covered evaporating dishes (100 mL) illuminated fluorescently on 12:12 LD at 18°C</td>
<td>Mixture of ground herring, crab, mussel meat, baker’s yeast, cottage cheese, urchin gonads, <em>Ulva</em>, <em>Asterias</em>, and Tetra Min fish food; all given in slight excess</td>
<td>—</td>
<td>—</td>
<td>Rieper 1978</td>
</tr>
<tr>
<td>Aselopsis intermedia (sand)</td>
<td>NSW and metazoan-free beach sand (3-grain thickness), agitated by aeration, and renewed every 5 d</td>
<td>Polyethylene specimen tubes with bases and lids replaced by 75-μm mesh; suspended in flow-through aquaria</td>
<td>“Probably bacterial epigrowth on sand-grains”</td>
<td>—</td>
<td>30-tube⁻¹ maintained up to 9 mo</td>
<td>Hardy 1978</td>
</tr>
<tr>
<td>Tachidius discipes (sand/muddy sand)</td>
<td>200 mL filtered NSW (26%)</td>
<td>(reared in darkness with NSW changed daily)</td>
<td><em>Tetraselmis suecida</em></td>
<td>50 detached egg sacs; unhatched sacs removed after 2 d</td>
<td>—</td>
<td>Teare and Price 1979</td>
</tr>
<tr>
<td>Species (habitat)</td>
<td>Culture medium</td>
<td>Culture apparatus</td>
<td>Food</td>
<td>Inoculation density</td>
<td>Maximum density</td>
<td>Reference</td>
</tr>
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</tr>
<tr>
<td>Amphiascoides sp. (sand)</td>
<td>100 mL coarse-filtered NSW, heated 10 min at 95°C, and changed weekly</td>
<td>100 mL covered crystallizing dishes</td>
<td>2 cm² boiled lettuce pieces</td>
<td>17 mL⁻¹ (all stages)</td>
<td>60–80 mL⁻¹ (all stages)</td>
<td>Walker 1979</td>
</tr>
<tr>
<td>Huntemannia jadensis (sand)</td>
<td>3-mm layer of metazoan-free sand from natural habitat covered by NSW (29%) and replenished every 2 wk</td>
<td>Petri dishes (3.5 × 1.0 cm) kept under continuous fluorescent illumination</td>
<td>Mixture of pinnate/centric diatoms detached from approx. 20 cm² of natural surface sand</td>
<td>1 gravid dish⁻¹ (attempts at mass culture failed)</td>
<td>—</td>
<td>Feller 1980</td>
</tr>
<tr>
<td>Microarthridion littorale (mud)</td>
<td>10 mL Millipore-filtered (0.45 μm) NSW (30%), 0.1 mL autoclaved resuspended sediments from collection site, and 0.1 mL fresh detrital filtrate (&lt;44 μm) as a bacterial source; 66% of mixture replaced bimonthly</td>
<td>Petri dishes (6.0 × 1.5 cm) stored in wet plastic boxes (31 × 24 cm) to minimize evaporation</td>
<td>Navicula sp. and incidental bacteria</td>
<td>— (several gravids and copulating pairs)</td>
<td>—</td>
<td>Palmer and Coull 1980</td>
</tr>
<tr>
<td>Heteropsyllus pseudonunnii (muddy sand)</td>
<td>50 mL glass-filtered NSW NSW (13%) with 50% replaced bimonthly</td>
<td>Erlenmeyer flasks kept in darkness</td>
<td>250 mg dried Juncus roemerianus stalks ground to 0.3 mm, suspended in 1 L filtered NSW for 2 wk</td>
<td>— (70% survival of 10 females kept in darkness for 65 d)</td>
<td>—</td>
<td>Ustach 1982</td>
</tr>
<tr>
<td>Nitrocris spinipes (sand/muddy sand)</td>
<td>50 mL ASW (35%); aerated without replacement</td>
<td>Round covered glass dishes (7 × 4 cm) kept in darkness</td>
<td>1 wheat grain per dish and carrot slivers</td>
<td>10 gravid disk⁻¹ (all stages)</td>
<td>105 ± 3.8 mL⁻¹ (all stages)</td>
<td>Kahan 1979</td>
</tr>
<tr>
<td>Schizopera elatensis (sand/gravel)</td>
<td>Same as for A. subdebilis</td>
<td>Same as for A. subdebilis</td>
<td>Same as for A. subdebilis</td>
<td>52 mL⁻¹ (all stages)</td>
<td>412 ± 73 mL⁻¹</td>
<td>Kahan et al. 1981</td>
</tr>
<tr>
<td>Amphiascoides subdebilis (sand)</td>
<td>2.5 L ASW (35%); aerated without replacement</td>
<td>Aquaria (30 × 16 cm) with constant fluorescent illumination</td>
<td>5 wheat grains and 5 g of 1–2 cm² fresh lettuce pieces per aquarium, replenished as needed</td>
<td>17 mL⁻¹ (all stages)</td>
<td>136 mL⁻¹ (all stages)</td>
<td>Kahan 1979</td>
</tr>
</tbody>
</table>

References:
- Kahan 1979
- Kahan et al. 1981
- Walker 1979
- Feller 1980
- Palmer and Coull 1980
- Ustach 1982
(1-cm diameter). Vacuum was applied to the flask and pipette via a sink aspirator so that the pipette tip could be used as a wand to draw 75–50% of the flocculent surface sediments and copepods into the flask. Only 25–50% of the surface was removed so that sufficient numbers remained to quickly repopulate a culture. After harvesting, it took approximately 2 wk for Nitocra and Cletocampus cultures to regain original densities.

Because the sediments were all <0.125 mm in diameter, they were completely washed through a 0.125-mm sieve leaving only the adults and copepods to be collected into Petri dishes. Nauplii, early copepodid stages, and very fine sands less than 0.125 mm in size were captured on a 0.63-mm sieve. This sample was not completely free of sediment aggregates, but it was still a very clean sample compared with natural sediments and was much more quickly and efficiently sorted.

Results and Discussion

Five local harpacticoid copepod species were kept in continuous (>6 mo) culture at densities 4–11 times their natural maxima (Table 1). All five are strictly estuarine and live in muddy, detrital-rich sediments. Nitocra lacustris, Cletocampus delfersi, Paronychoecampus huntsmani, and Onychocampus mohammed are holobenthic, infaunal burrowers and rarely emerge more than 1–2 cm above the sediment surface in natural density cultures. Scottolana canadensis is not strictly infaunal but does burrow 1 cm or more to feed and mate. Nitocra lacustris and C. delfersi are the most prolific and easily maintained harpacticoids cultured thus far, with cultures having a routine longevity of over 12 mo and needing only food, and fresh additions of culture floc every 4–5 mo. Scottolana canadensis, P. huntsmani, and O. mohammed reached considerably lower maximum densities (Table 1) than N. lacustris or C. delfersi, but they still could be maintained at densities high enough for most laboratory applications (2000–4000 copepods per culture).

Several mud-inhabiting harpacticoids could not be cultured using these techniques. For example, repeated attempts to culture the tube-building harpacticoid Pseudostenhelia wellsi (see Chandler and Fleeger 1984) have failed, as well as single attempts to culture Enhydrosoma woodini and Nanomastus palustris. Pseudostenhelia wellsi and N. palustris appear to have very specific nutritional requirements not met by this feeding regime, and E. woodini has not been collected at densities greater than 30 gravids per collection trip, making it difficult to get a critical inoculation density.

Culture substrates composed of azoic or freshly collected sands and muds have been used by others to culture sediment-dependent harpacticoids but without much success (Table 2). For example, Smol and Heip (1974) found it difficult to locate and count live copepods among sand grains that were at least as large as the copepods themselves, and abandoned its use. Hardy (1978) found that the sand grains and vessel walls of stagnant Asellus intermedia cultures became quickly overgrown with bacterial films, eventually entrapping and killing the copepods. Techniques for high-density culture of mud-inhabiting meiofauna have also been unsatisfactory, especially within a natural, muddy-sediment substrate; Muddy-sediment particles are much smaller than sands and many are electrically charged. This makes it much more difficult to wash away the organic contaminants that feed dense bacterial blooms without washing away the sediment particles themselves. Only Palmer and Coull (1980) have succeeded in culturing low densities of an epibenthic mud-dweller, Microarthridion littorale, in very small amounts of autoclaved mud (Table 2).

Cleaned and sorted muds are better than sands as a culture substrate because all of the sediment particles are less than 0.125 mm. Adult and later-stage copepods of harpacticoids are usually larger than 0.125 mm; therefore, simple sieving on a 0.125-mm sieve eliminates sediments and the searching and counting problems encountered with natural sands and muds. Most other investigators have grown harpacticoids with no natural substrate and only periodic (daily to monthly) water changes. Therefore, only a few mud-inhabiting harpacticoids have been cultured, and none at the densities achieved using these techniques.

Much more success has come from sediment-free flask, dish, and tank cultivation of epibenthic/fouling (e.g. Nitocra spinipes (Kahan 1979)), phytil/demersal (e.g. Schizopera elatensis and Amphiascella subdebelis (Kahan 1979; Kahan et al. 1981), Tisbe spp. and Tigrionus spp. (see Kinne 1977 review), and planktonic (Euterpina acutifrons (Neunes and Pongolini 1965; Haq 1972)) species. These species do not require a sediment substrate for a place to feed and reproduce, and they have broadly flexible nutritional requirements making them more amenable to batch cultivation. For these reasons, planktonic and semiplanktonic species have been the most commonly used harpacticoids in finfish mariculture (Fujita 1973, 1977).

The key advantages that my culture techniques have over others are that natural, muddy sediments can be sorted (all <0.125 mm), cleansed, and reconstituted to a reasonably like sediment structure allowing high harpacticoid densities without runaway bacterial growth. A broad-based diet with autotrophic and detrital energy components is provided for the copepods, and a constant influx of fully oxygenated seawater keeps metabolite (and bacterial) accumulation to a minimum. Cultures remain viable for many months with no apparent growth inhibition other than available space. The constant ASW influx may flush out growth inhibiting factors keeping their effects to a minimum (Walker 1979; Pava and Crotti 1979).

The thin-walled, hexagonal culture vessels provide good resolution and multiple angles for direct observation (photography) of behaviors occurring on or below the sediment surface (e.g. see Chandler and Fleeger 1984), and with minimal disturbance to the animals and sediment structure. Such observation is often difficult or impossible in muddy field settings.

The ability to rapidly grow high-density stocks may have potential value for fisheries as larval fish food, or for laboratory studies or population genetics/dynamics, physiology, ecology, toxicology, etc. Mariculture applications would no doubt require larger total numbers of harpacticoids than were cultured here, but fortunately the techniques are simple and inexpensive enough that they probably could be adapted easily to a larger scale.

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Education

1982 - Present: Ph.D. Candidate, Department of Zoology and Physiology, Louisiana State University
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Research Interests: Estuarine ecology; Commensalistic/mutualistic and competitive interactions of meio-benthic harpacticoid copepods; Culture of mud-inhabiting meiofauna; Meiofauna's role in benthic-pelagic coupling; Biogenic effects of meiofauna on muddy sediments.


1975 - 1979 University of North Carolina at Wilmington . . . B.Sc. HONORS, cum laude, Biology and Marine Biology

Professional Experience

Research Associate, University of Alaska at Juneau, April to July 1985: NOAA grant to UAJ to determine the relative importance of terrestrial and phytoplankton primary productivity to fish and kingcrab recruitment in coastal Alaskan bays.

Statistical Consultant, Department of Zoology, LSU, Summer 1983.

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Research Associate, Louisiana State University, Summer 1980: National Science Foundation Grant, W. B. Stickle, principal investigator: Study of the synergistic effects of temperature and salinity on energy budgets and indices of stress in the southern oyster drill, Thais haemostoma.

Teaching Assistant, Louisiana State University, September 1979 to present:
Graduate and Senior Level Courses -- Marine Ecology, Animal Ecology, and Marine Communities.
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Statistical Expertise:
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Research Societies
American Society of Limnology and Oceanography
American Society of Zoologists
International Association of Meiobenthologists
Ecological Society of America
Estuarine Research Federation
Gulf Estuarine Research Society

Publications


Conference Participation with Published Abstracts


Published Abstracts (continued)


Unpublished Conference Participation


Manuscripts in Preparation


Chandler, G. T. Feeding and burrowing behaviors of the common, estuarine, harpacticoid copepod, *Scottolana canadensis* (Willey), and their effects on muddy sediments. To be submitted to Mar. Behav. Physiol.
DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: George Thomas Chandler

Major Field: Zoology

Title of Dissertation: Facilitative and Inhibitory Interactions among Estuarine Melobenthic Harpacticoid Copepods

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June 23, 1986