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Role of Habitat Features and Chemical Cues in Substrate Selection by Blue Crab Megalopae: Evidence from Laboratory Experiments

Bruce Clare Layman

College of William and Mary - Virginia Institute of Marine Science

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ROLE OF HABITAT FEATURES AND CHEMICAL CUES IN SUBSTRATE SELECTION BY BLUE CRAB MEGALOPAE:
EVIDENCE FROM LABORATORY EXPERIMENTS

A Thesis
Presented to
The Faculty of the School of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment
Of the Requirements for the Degree of
Master of Arts

by
Bruce C. Layman
1992
This thesis is submitted in partial fulfillment of the requirements for the degree of Master of Arts.

Approved August 1, 1992
DEDICATION

To my grandparents and family for making this degree possible.
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</tbody>
</table>
ABSTRACT

Submerged aquatic vegetation (SAV) in lower Chesapeake Bay serves as a primary nursery habitat for juvenile blue crabs, *Callinectes sapidus* Rathbun, yet mechanisms regulating habitat selection by postlarvae remain unclear. Two experiments investigated the role of eelgrass (*Zostera marina*) characteristics in substrate selection by megalopae. Mesocosm experiments addressed the relative importance of physical and biological attributes of *Z. marina* in substrate selection. Macrophyte structure (both living and inert) appeared to influence substrate selection by megalopae; the addition of a periphytic community to *Z. marina* did not influence this response, refuting it as a proximal cue directing the behavior of megalopae. Microcosm experiments investigated the response of megalopae to species-specific chemical stimuli associated with *Z. marina* and several grass bed macrofauna. Positive orientation toward chemical cues associated with *Z. marina* in two of four trials suggests that exudates of *Z. marina* aid megalopae in remote habitat recognition and location. Neutral responses by megalopae to both soft-shelled clam (*Mya arenaria*) and toadfish (*Opsanus tau*) cues indicate little potential for influencing *in situ* behavior of megalopae. Conversely, conspecifics at high biomass per unit area, both extremely high juvenile densities and high adult densities, elicited a negative orientation in megalopae. However, these responses may have been due to artifactually high concentrations of excretory products or low dissolved oxygen concentrations, rather than avoidance of conspecific cues. Thus, chemical cues can initiate substrate selection in megalopae prior to physical contact and macrophyte structure appears to be of potential importance in substrate selection in close proximity to nursery habitats.
ROLE OF HABITAT FEATURES AND CHEMICAL CUES IN SUBSTRATE SELECTION BY BLUE CRAB MEGALOPAE: EVIDENCE FROM LABORATORY EXPERIMENTS
INTRODUCTION

Much research has been devoted to the importance of both post-settlement and pre-settlement processes in regulating marine benthic community structure. One view maintains that post-settlement processes, i.e. predation and competition, regulate community structure through inter- and intra-specific interactions (Connell & Orias 1964, Paine 1966 and references therein) while the other suggests pre-settlement processes, i.e. factors affecting recruitment dynamics, regulate community structure through spatial and temporal variation in supply of larvae (Dayton 1971 for review). Undoubtedly, both mechanisms interact to influence community structure; however, the relative impact of each is community specific (Menge & Sutherland 1987, for review).

Historically, competition and predation have been viewed as primary processes regulating benthic community structure, specifically in space-limited rocky-intertidal communities of the North Pacific (Connell 1961, Paine 1966, 1969, Dayton 1971, Paine 1974, Lubchenco 1985). More recently, factors such as meteorologic events, current patterns, food availability, and predator abundance, have been recognized as regulating community structure by
generating variation in recruitment success (Caffey 1985, Sale 1990 for review). Coral reef (Sale 1977, Williams 1983), rocky intertidal (Gains et al. 1985, Roughgarden et al. 1988), and kelp forest communities (Bernstein and Jung 1979) illustrate the diverse array of marine systems influenced by variation in recruitment.

Estuaries serve as crucial habitats in the life-histories of many marine organisms, including commercially important finfish (Adams 1976, Haedrich 1983) and shellfish (Heck and Orth 1980, Weinstein and Brooks 1983, Heck and Thoman 1984). The net seaward movement of surface waters, combined with tidal flushing, provides a mechanism for early life-history export from, or transport to, the estuary. Due to differential water circulation within estuaries, behavioral characteristics of larvae often dictate resultant retention or export. Tidal rhythms (upward migration in the water column during flood and downward migration during ebb tides) may facilitate retention in some decapod species (Sandifer 1975; Scheltema 1975; Cronin and Forward 1979, 1986), while behavioral responses to exogenous cues (i.e. barokinesis, phototaxis, or geotaxis) facilitate export and subsequent reinvasion in others (Sulkin et al. 1980, Sulkin and Epifanio 1986, Sulkin and Van Heukelem 1986, McConaugha 1988).
Zoeae (larvae) of the blue crab, *Callinectes sapidus*, display behavioral characteristics that facilitate export from Chesapeake Bay to the continental shelf (Sulkin et al. 1980, McConaugha et al. 1983, Sulkin 1984, McConaugha 1988). Female *C. sapidus* spawn at the Chesapeake Bay mouth; zoeae are subsequently transported in the seaward-flowing surface currents onto the continental shelf where they progress through seven zoeal stages. The combined wind-driven surface currents and larval behavioral characteristics retain larvae proximal to the Bay mouth. Here they metamorphose into the megalopal stage, which subsequently reinvades Chesapeake Bay (Epifanio et al. 1989, Goodrich et al. 1989).

Mechanisms believed to be used by megalopae to reinvade the bay include episodic wind-driven surface currents (Johnson et al. 1984, Goodrich et al. 1989), internal waves (Shanks 1983, 1985, 1988), residual upstream bottom currents (Sulkin & Epifanio 1986), or a combination of these (Sulkin & Van Heukelem 1986). Successful reinvansion relies, in part, upon behaviors of megalopae that regulate their vertical position in the water column, thus facilitating the utilization of the aforementioned mechanisms for reinvasion. Upon entering the bay, megalopae commence diurnal vertical migrations which facilitate transportation of megalopae upstream during nocturnal flood tides (Mense & Wenner 1989, Olmi unpubl. data). Combined, these behaviors and resultant
transport mechanisms are responsible for the conveyance of megalopae for distances of 10’s to 100’s of kilometers between continental shelf waters and shallow-water nursery habitats of Chesapeake Bay.

Submerged aquatic vegetation (SAV), relative to adjacent marsh creek and sand flat habitats within Chesapeake Bay, serves as the primary nursery habitat for C. sapidus postlarvae (Penry 1982, Orth & van Montfrans 1987, Williams et al. 1990). Settlement of megalopae is proposed in this study to establish this distribution within the nursery habitat; however, mechanisms facilitating realization of this distribution are poorly documented and remain unclear. Three hypotheses have been posed in the literature that summarize processes influencing the post-settlement association between megalopae and Zostera marina habitats (Orth & van Montfrans 1990). Megalopae may passively settle due to the reduced water velocities resulting from the baffling effects of Z. marina. Secondly, they could settle randomly, after-which differential post-settlement mortality could restrict them to the refugia in Z. marina beds. Thirdly, megalopae may actively settle in Z. marina beds due to some unique feature of the habitat.

Passive settlement frequently regulates larval and postlarval benthic distributions (Butman 1989, for review). The structure of Z. marina creates an environment conducive to passive settlement of larvae through reduction of below-
canopy water flow (Gambi et al. 1990); however, larvae of previously studied taxa (primarily polychaetes and gastropods) display limited mobility, in contrast to the highly mobile megalopae of *C. sapidus*. These megalopae are active swimmers, capable of three-dimensional spatial regulation and small-scale (meters or less) unidirectional locomotion (Sulkin et al. 1980, Luckenbach & Orth in press). Thus, passive settlement was not addressed as a primary factor directing the behavior of megalopae in the present study.

Differential post-settlement mortality often structures the distribution of early life-history stages (Keough & Downes 1982). Seagrass structure offers substantial refuge from predation for *C. sapidus* megalopae (van Montfrans, unpubl. data) and juveniles (Orth et al. 1984, Heck & Wilson 1987, Wilson et al. 1987), as well as a suite of other marine taxa (Nelson 1979, Stoner 1980b, Heck & Thoman 1984, Leber 1985). The transitional period between settlement and metamorphosis into the first instar crab is relatively short (12 to 24 hours; Metcalf & Lipcius 1992), suggesting that only intense predation could significantly influence the post-settlement distribution of megalopae. Laboratory studies have shown substantial predator-induced mortality via grass bed macrofauna (Olmi & Lipcius 1991); however, the importance of predation on megalopae in the field awaits documentation.
METHODS AND MATERIALS

Zostera marina habitats possess numerous characteristics of potential importance to habitat selection by megalopae. These include structural, biological, and chemical features of Z. marina and resident macrofauna. Consequently, two series of experiments were conducted during the megalopal recruitment season of C. sapidus (July through November). One series addressed the relative importance of physical vs. biological attributes of Z. marina in substrate preference behavior. The second series examined behavior of megalopae in response to chemical cues associated with Z. marina and several macrofaunal residents of the Z. marina community.

Mesocosm Experiments:

Experiments were initiated on 17 October and 7 November 1989 at the Virginia Institute of Marine Science (VIMS), College of William and Mary, located on the lower York River, Chesapeake Bay. Three 3.0 m³ cylindrical mesocosms (233 cm diameter x 91 cm depth) housed five substrate types to test the relative importance of various physical characteristics of Z. marina potentially influencing
substrate selection by blue crab megalopae. Large mesocosms were anticipated to reduce potential artifacts such as edge effects and confined hydrodynamic regimes normally associated with small aquaria.

Duplicate plots of five substrate types were randomly arranged in a circular configuration 30 cm from the tank wall on the sand bottom of each tank (Fig. 1). Substrate treatments included sand, fouled live Z. marina, unfouled live Z. marina, fouled artificial Z. marina, and unfouled artificial Z. marina. The fouled artificial treatment plots were placed in the York River for approximately two weeks prior to experimentation to acquire a degree of fouling comparable to live Z. marina. The periphytic community was removed from living blades of Z. marina by gently scraping with microscope slides.

Circular substrate plots (26 cm diameter: 0.071 m²) mimicking Z. marina turf were constructed to reflect in situ macrophyte density (approximately 1000 shoots m²; Orth and Moore 1986). Sixty fouled or unfouled shoots of either live or artificial Z. marina were secured with plastic-coated twist ties to each 5-mm mesh VEXAR® base. Vinyl tubing (Tygon®: 2.4 mm (3/32 inch) inside diameter) encased each shoot base, forming the artificial plant sheath. Each
FIGURE 1.

Top view of substrate arrangement on the bottom of each mesocosm. Positions are randomized in a circular pattern to eliminate orientation bias.
artificial shoot had 4 polypropylene leaves, a sheath length of approximately 42 mm, and an average leaf width and length of 2.5 mm and 193 mm, respectively. Artificial leaves were created by folding two 386 mm long ribbons to form four leaves per plant with a mean length of 193 mm. Leaf length varied from approximately 100 mm to 286 mm by off-setting the point at which the ribbons were folded, thus simulating the variation in length observed in natural leaves. Live plants were collected from cores taken near Sandy Point, lower York River, and processed within two days of experimentation.

York River water was sand filtered and circulated within each mesocosm as a closed system. An aerated 76 mm (3 inch) outside-diameter polyvinyl chloride (PVC) standpipe in the center of each tank created a standardized unidirectional water flow of approximately 4 cm s\(^{-1}\) over each substrate (Fig. 2). Heaters maintained mesocosm water temperatures near ambient river temperature (± 5°C, Table 1).

One-hundred-sixty megalopae (17 October) and 220 megalopae (7 November) were collected overnight and were introduced to each mesocosm the next morning, (quantity of experimental animals varied due to availability). A subset (N=15) of the megalopae was molt-staged prior to each trial using maxilliped morphology (Metcalf & Lipcius 1992), to determine mean developmental state. Twenty-four hours
FIGURE 2.

Cross-section of mesocosm with arrows indicating circulation pattern. Substrate plots and buried bucket lids are indicated on and within the sand bottom, respectively.
WATER CIRCULATION
WITHIN EACH MESOCOSM
TABLE 1.

Breakdown of megalopae used and the number (percentage) of animals responding to available substrates. Note: Only animals on the experimental substrates were enumerated.

<table>
<thead>
<tr>
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<th>TRIAL #1</th>
<th>TRIAL #2</th>
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<tr>
<td>Date:</td>
<td>17 October</td>
<td>7 November</td>
</tr>
<tr>
<td>Number of megalopae per trial (⅓ of total per mesocosm):</td>
<td>480</td>
<td>660</td>
</tr>
<tr>
<td>Total number of animals responding to substrates:</td>
<td>97 (23%)</td>
<td>61 (9%)</td>
</tr>
<tr>
<td>Total number of megalopae responding to substrates:</td>
<td>12 (12%)</td>
<td>21 (34%)</td>
</tr>
<tr>
<td>Total number of first instar crabs responding to substrates:</td>
<td>85 (88%)</td>
<td>40 (66%)</td>
</tr>
<tr>
<td>Ambient (river) temperature:</td>
<td>22°C</td>
<td>15°C</td>
</tr>
<tr>
<td>Mesocosm temperature:</td>
<td>25°C</td>
<td>20°C</td>
</tr>
</tbody>
</table>
later, a 21 liter (5 gallon) utility bucket with a 1 mm mesh-bottom was inverted over each plot and attached via two shock cords to a utility bucket lid that was buried under each substrate plot. Contents were sieved through 1 mm mesh screen and sorted in the laboratory. Only individuals on substrate plots were collected and statistically analyzed. Individuals not on substrate plots remained in the water column, on the mesocosm walls, or elsewhere on the mesocosm floor. Between trials, mesocosms were drained, filled with fresh water for seven days, completely drained, and allowed to dry to eliminate remaining individuals.

Response frequencies were standardized by treatment surface area and analyzed via one-way analysis of variance (ANOVA) (Sokal & Rohlf 1981). The assumption of homogeneity of variances was not met due to 0 variance associated with the sand treatment (i.e. no megalopae were associated with the sand treatment in any trial), however, statistical nonsignificance precluded problems associated with artificially inflated α (Sokal & Rohlf 1981). The power of the test was determined using power tables (Zar 1984).

Chemoreception Experiments:

Laboratory experiments were conducted between August and November, 1990 at VIMS. Experiments examined whether Callinectes sapidus megalopae differentially respond to the chemical milieu associated with several constituents of the
Z. marina community (i.e. eelgrass, Z. marina; soft-shelled clam, Mya arenaria; toadfish, Opsanus tau; and juvenile and adult C. sapidus).

Two sources of megalopae were used, including either naive individuals or those potentially experienced with experimental stimuli. Megalopae collected in the continental shelf neuston 15-20 km east of the Chesapeake Bay mouth (ca. 37° 03’ N, 75° 45’ W) were outside the influence of the bay plume and assumed naive to the chemical milieu of bay-associated habitats. These megalopae were used solely in experiments with Z. marina; it was assumed these had little or no prior experience with Z. marina before entering the Bay. The second group of megalopae was collected, as needed, 14 km upstream from the York River mouth (50 km from the bay mouth), off the VIMS Ferry Pier in overnight plankton samples. Lower York River shoals support fringing beds of Z. marina, hence pier-caught megalopae may have experienced the grass bed chemical milieu. These megalopae were used in the bivalve, toadfish, and conspecific treatments. Megalopae were held in 125 liter (30 gallon) aquaria in either filtered oceanic or river water, depending on their origin, exposed to ambient photoperiod and temperature, and fed cultured Artemia salina (Utah strain, Aqafauna Biomarine) nauplii prior to experimentation. Oceanic water was lowered to river salinity in 5 ppt. steps over a period of ca. 6 hours by
siphoning well water into the holding aquarium through vinyl tubing (Tygon®: 3.2 mm (1/8 inch) inside diameter).

Chemical preference experiments were conducted in opaque plexiglas apparati (Fig. 3). The central chamber was 13.7 cm in diameter, 7.0 cm deep, and 1.03 l in volume. The peripheral chambers were each 6.5 cm in diameter, 14 cm deep, and had a volume of 0.46 l. Two tubes (1.9 cm inside diameter) connected the central and peripheral chambers, allowing conditioned or unconditioned water entering the peripheral chambers to exit through a screened, central chamber stand-pipe.

Ten megalopae were placed in the center of each of five experimental apparati and allowed to respond to the test stimuli. Trials were run sequentially in complete darkness for three hours between ca. 21:00 - 24:00 and 02:00 - 05:00 hrs, the period during which in situ individuals are usually active in the water column and likely displaying behaviors conducive to testing the stated hypotheses. Total number of replicates varied from 10 to 20 depending upon the treatment. Water was pumped from the York River, filtered (10 μm), and delivered to two header tanks. One header tank contained conditioned water, the other contained unconditioned water (methods of conditioning are explained later). The water was then pumped at a constant flow rate of 2.9 liter hour⁻¹ via peristaltic pump (Harvard Apparatus, model 1210) from the conditioned header tank through silicon
FIGURE 3.

Plexiglas chemoreception apparatus with air-driven water circulation, arrows indicate water flow direction.
tubing to one of two randomly chosen peripheral chambers per experimental apparatus. The opposite peripheral chamber simultaneously received unconditioned water from the second header tank using the same methodology. A flow rate of 2.9 liter hour⁻¹ combined with a tangential airflow impinging upon the water surface within each central chamber created a standardized spiral stimulus gradient (Fig. 4), as determined from a previous dye study. The tubes connecting peripheral and central chambers were stoppered at the termination of an experiment and individuals were enumerated in each of the three chambers per experimental apparatus. Salinity and temperature were measured in each header tank at the beginning and end of each trial. Chambers were cleaned regularly with fresh water between sequential trials and were periodically cleaned (approximately every tenth trial) with dilute hydrochloric acid (and neutralized).

A control response by megalopae was quantified on September 14 and 15 by pumping unconditioned water from each header tank to both peripheral chambers per experimental apparatus. This quantified the distribution of megalopae resulting from the random dispersion of individuals over the experimental duration and served as the null model against which all subsequent treatments were compared. Proportional responses were angularly transformed to meet the assumptions of normality and homogeneity of variances. Assumptions were met as indicated by Hartley's F-max test and results were
FIGURE 4.

Waterborne stimulus circulation pattern and concentration gradient within experimental apparatus as determined from dye analysis.
SUBSTRATE FLOW WITHIN CHAMBER

Conditioned Water

Unconditioned Water
analyzed by one-way analysis of variance (ANOVA) at the $\alpha = 0.05$ level (Sokal & Rohlf 1981).

**Stimulus Preparation:**

*Zostera marina*

A 4-mm nylon mesh bag containing ca. 20 kg freshly harvested above-ground *Z. marina* was placed on the sand river bottom at 1.25 m mean low water (MLW). River water was drawn through a 26-cm-diameter funnel placed immediately above the bagged *Z. marina* and pumped at 8 liters min$^{-1}$ through 1.27 cm inner diameter polyethylene hose into the laboratory where it was filtered (10 $\mu$m) into a 160 l fiberglass flow-through header tank. This process, conceptually similar to that used to test habitat selection by coral reef fish (Sweatman 1988), attempted to mimic the ambient concentration of *Z. marina* stimulus, thus reducing artifacts associated with artificial mixtures of chemical stimuli (McLeese 1970, Linstedt 1971) and the negative behavioral feedback often associated with stimulus oversaturation (Shelton & Mackie 1971, Coon et al. 1990). The unconditioned treatment was similarly executed, except that source-water was pumped from 1 m below the surface from an offshore area (depth of 3.5 m MLW devoid of *Z. marina*).
Macrofauna

River channel water was pumped at 8 liters min.\(^{-1}\) through one hose into the laboratory (as previously described) where it was split into two 160 liter flow-through header tanks. The conditioned header tank contained the living stimulus source, while the other remained stimulus-free. Conditioning was initiated 30 minutes prior to experimentation and continued for the experimental duration with one of the following stimuli: O. tau (\(\bar{x} = 230 \text{ mm total length at } 12 \text{ m}^2\)); M. arenaria (\(\bar{x} = 20 \text{ mm total length at } 90 \text{ m}^2\)); juvenile C. sapidus (\(\bar{x} = 21.7 \text{ mm carapace width at } 90 \text{ m}^2\) and 300 m\(^2\)); and adult C. sapidus (\(\bar{x} = 140.0 \text{ mm carapace width at } 6 \text{ m}^2\)). Juvenile C. sapidus at 300 m\(^2\) represented equal volume displacement to that of the adult C. sapidus treatment. Specimens of O. tau and conspecifics were obtained by hook-and-line and dipnet, respectively, off the VIMS Ferry Pier. Cultured M. arenaria were obtained from the VIMS Wachapreague Laboratory.
RESULTS

Mesocosm Experiment:

The mean molt stages of the megalopae in both trials were 4.27 ± 0.14 and 4.33 ± 0.16 (± standard error: S.E.) out of 5.5, indicating an advanced state of premolt (Lipcius et al. 1990, Metcalf & Lipcius 1992; see discussion for importance of molt stage determination). The majority of individuals collected on experimental plots were first instar crabs; response frequency by megalopae was low (Table 1). Uncertainty in the relation between the present distribution of first instar crabs (Table 2) and their previous distribution as megalopae precluded using first instar individuals in the statistical analysis (i.e. substrate selection by megalopae may be different than that seen in first instar crabs). Twenty three percent of the megalopae initially introduced to each mesocosm responded positively to the substrates in the first trial (88% of those responding were first instar crabs). Nine percent responded to the substrates in the second trial (66% were first instar crabs). This apparent difference in metamorphosis rate between trials, as indicated by the greater proportion of megalopae responding in the second
TABLE 2.

First instar crab response frequencies per substrate type (unstandardized by substrate surface area).

<table>
<thead>
<tr>
<th>Substrate Type:</th>
<th>Trial #1</th>
<th>Trial #2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Zostera marina Treatments:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fouled Living</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Unfouled Living</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Fouled Artificial</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Unfouled Artificial</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>25</td>
</tr>
</tbody>
</table>
trial (see table 1), may be due to the notably lower ambient temperature during the later experiment (\(\bar{x}: 25^\circ C\) vs. \(20^\circ C\)). There was no interaction between trials (Two-way ANOVA; \(F = 1.88, df = 1, 50, P = 0.39\), Sokal & Rohlf 1981) or mesocosm units (Two-way ANOVA; \(F = 1.37, df = 5, 30, P > 0.50\), Sokal & Rohlf 1981), thus the data were pooled. Response by megalopae to the array of substrates, standardized by substrate surface area (Table 3), was not significant (One-way ANOVA; \(F = 1.67, df = 4, 55, P = 0.17\)). Power analysis determined the power of the test to be approximately 45% (\(\phi = 1.15, v_1 = 4, v_2 = 55, \alpha = 0.05\)) indicating that the data may suggest a trend in preference by megalopae for structured substrates, both inert and living, over sand alone (Fig. 5).

Chemoreception Experiment:

Under the experimentally established null model, 35.8% \(\pm 3.9\% (N = 10, \bar{x} \pm S.E.)\) of the megalopae responded to one peripheral chamber, while 64.2% were located in both the central and opposite peripheral chambers combined. Thus, during the treatments using conditioned water, 35.8% of the megalopae should respond to conditioned chambers due to random chance. Responses above or below this percentage would indicate attraction to or aversion from the conditioned water.
FIGURE 5.

Mean response frequency by megalopae, standardized by substrate surface area, to physical Zostera marina treatments (N=6). Mean response frequencies are less than unity due to standardization by treatment surface area. Vertical bars represent one S.E.
Response by Megalopae to *Z. marina* Treatments

- 
- 
- 
- 

Substrate Type

Frequency
TABLE 3.

Calculations used in standardizing the mean response by megalopae to substrate surface area (SA), given the \textit{in situ} and artificial grass shoot dimensions.

**Sand Plots**

Plot SA: $\pi (\text{radius})^2$

$= \pi (134 \text{ mm})^2$

$= 56,410 \text{ mm}^2$

**Living Zostera marina Plots**

Leaf SA: $2 (\text{leaf width})(\text{leaf length} - \text{sheath length})$

$= 2 (2.23 \text{ mm})(193 \text{ mm} - 45 \text{ mm})$

$= 660.08 \text{ mm}^2$

Sheath SA: $\pi (\text{radius})^2 (\text{sheath length})$

$= \pi (2 \text{ mm})^2 (45 \text{ mm})$

$= 565.5 \text{ mm}^2$

Plot SA: $(\text{leaf SA})(\text{no. leaves per plant}) + \text{sheath SA}) (\text{no. plants per plot}) + \text{sand SA}$

$= ((660.08 \text{ mm}^2)(4.1) + 565.5 \text{ mm}^2)(60) + 56410 \text{ mm}^2$

$= 252,720 \text{ mm}^2$

Thus, living \textit{Z. marina} plots have 4.48 times more SA than sand plots.

**Artificial Zostera marina Plots**

Leaf SA: $2 (\text{leaf width})(\text{leaf length} - \text{sheath length})$

$= 2 (2.5 \text{ mm})(193 \text{ mm} - 42 \text{ mm})$

$= 755 \text{ mm}^2$

Sheath SA: $\pi (\text{radius})^2 (\text{sheath length})$

$= \pi (2 \text{ mm})^2 (42 \text{ mm})$

$= 527.8 \text{ mm}^2$

Plot SA: $(\text{leaf SA})(\text{no. leaves per plant}) + \text{sheath SA}) (\text{no. plants per plot}) + \text{sand SA}$

$= ((755 \text{ mm}^2)(4.0) + 527.8 \text{ mm}^2)(60) + 56410 \text{ mm}^2$

$= 269,278 \text{ mm}^2$

Thus, artificial \textit{Z. marina} plots have 4.77 times more SA than sand plots.
Megalopae demonstrated varied responses to the *Z. marina* treatment (Fig. 6). Two (15 & 20 August) of four nonsequential trials demonstrated highly significant responses by megalopae toward the stimulus associated with *Z. marina* (one-way ANOVA; $F(s) = 23.2 & 27.7$, both $df = 1, 13$, $P < 0.001$). Response in the remaining two trials did not demonstrate significance. The time of day that trials were conducted, i.e. late night or early morning, appeared to have no effect on response by megalopae (Fig. 5). There was, however, a trend between the occurrence of flood tide and positive response by megalopae toward *Z. marina* stimulus. This trend was not evident in the control treatment where one trial occurred entirely during flood tide. The mean rate of metamorphosis during experimentation was $6.6\% \pm 1.1$ (S.E.) and tended to increase over successive trials.

Megalopae demonstrated no differential response to either *Opsanus tau* or *Mya arenaria* (Fig. 6). Similarly, juvenile *Callinectes sapidus* at high field densities ($90 \text{ m}^2$) elicited no response by megalopae; however, adult *C. sapidus* ($6 \text{ m}^2$) elicited a significantly negative response (one-way ANOVA; $F = 14.15$, $df = 1, 16$, $P = 0.002$). Equal juvenile volume to that of the adult treatment also elicited a comparably significant negative response (one-way ANOVA; $F = 5.41$, $df = 1, 16$, $P = 0.034$).
FIGURE 6.

Proportional response by *Callinectes sapidus* megalopae to various chemical stimuli (*Mya arenaria*, *Opsanus tau*, juvenile *C. sapidus*, adult *C. sapidus*, equal juvenile biomass, and *Zostera marina*). *Z. marina* treatments are displayed by trial date (N=20), 15 and 16 August represent one night of experiments and 19 and 20 August represent a second. The horizontal line at 0.33 proportional response indicates the null response (H₀). Vertical bars represent one S.E. * Denotes significantly different treatments at P < 0.05.
Megalopal Response to Chemical Stimuli

![Graph showing proportional response to different stimulus types.](image-url)
Megalopae of *C. sapidus* undergo a dramatic behavioral transition between those behaviors that promote retention in the neuston (i.e. positive phototaxis, negative geotaxis, and positive barokinesis) and those that promote settlement and the beginning of a benthic existence (i.e. negative phototaxis, positive geotaxis, and increased thigmotaxis) (Sulkin 1984, Sulkin & Van Heukelem 1986). A quantifiable morphological transformation accompanies these behavioral changes and is proposed to gauge the readiness of megalopae to settle (Lipcius et al. 1990, Metcalf & Lipcius 1992). The progression of internal morphological changes in the pleopods as megalopae approach ecdysis is described by a numerical scale (1 through 5.5). Megalopae nearing metamorphosis (i.e. the greater designations) are thought to be less selective in habitat choice. Present experiments considered substrate selection by megalopae, thus molt stage (or developmental state) was quantified and considered as a potential factor influencing observed behaviors. The interval between mesocosm experiments was 22 d; however, molt stages were comparable, suggesting that both groups of megalopae would be expected to demonstrate comparable
degrees of substrate selectivity. Conversely, individual chemoreception treatments were completed within 2 d, thus it was assumed that megalopae were of similar developmental states. Consequently, molt stage did not appear to stimulate behavioral variation among trials in either experiment.

Mesocosm Experiments

Combining megalopae with first instar crabs in the statistical analysis could severely affect experimental interpretations, if responses by each life-history stage differed between substrate types. As megalopae approach metamorphosis, they acquire behaviors similar to those of their benthic counterparts (i.e. increased thigmotaxis, negative phototaxis, and positive geotaxis). The megalopae used in both trials were in advanced developmental states, suggesting that behaviors may be similar to those of first instar crabs. However, the uncertainty in behavioral repertoires pre-empted combination of megalopae and first instar crabs in the statistical analysis.

Zostera marina comprises a major structural and biological component of seagrass communities in lower Chesapeake Bay. The prominence of Callinectes sapidus megalopal and early juvenile stages in Z. marina beds relative to other potential habitats (i.e. adjacent marsh mud and sand flat; Penry 1982, Orth & van Montfrans 1987,
1990, Lipcius and van Montfrans unpubl. data), suggests that active substrate selection by megalopae may, in part, account for observed field distributions.

Substrate structure plays an integral part in habitat selection by crustaceans (Barry 1974, Stoner 1980, Marx and Herrnkind 1985, Herrnkind and Butler 1986, Hacker and Steneck 1990). Since megalopae immigrate into subestuaries during nocturnal flood tides (Epifanio et al. 1984, Little & Epifanio 1991, E.J. Olmi III in prep.), Z. marina may be the first structure they encounter in the water column upon entering shallow Chesapeake Bay habitats. This is consistent with thigmotactic behavior displayed during recruitment of megalopae to tropical shallow-water habitats (Wehrtmann and Dittel 1990), suggesting a mechanism to facilitate an immediate association between Z. marina and megalopae.

The trend in responses by megalopae to Z. marina rather than bare sand, parallels the affinity for artificial habitat structure observed in two species of grass shrimp (Barry 1974, Hacker & Madin 1991) and Sargassum shrimp (Hacker & Madin 1991), suggesting the importance of macrophyte structure in substrate selection by C. sapidus megalopae. The positive trend under standardized data suggests that behaviors may reflect a response toward unique features of Z. marina (i.e vertical structure in the water column or characters amenable to thigmotaxis). In addition,
an energetic benefit may be gained from grass-shoot baffling, as suggested for lobster postlarvae (Johns & Mann 1982). Field studies failed to document comparable densities of megalopae in adjacent salt marshes which would presumably offer a similar baffling regime. It has been suggested that this is related to the degree of tidal inundation (Orth & van Montfrans 1987), however, this topic has not been experimentally addressed and provides a plausible mechanism for future research.

Though not addressed statistically, trends in the distribution of first instar crabs (Table 2) are indicative of a preference for a structured substrate rather than bare sand. This is in agreement with the responses by megalopae, strengthening the contention that megalopae of *C. sapidus* select *Z. marina* structure over bare sand. The presence of such a marked response to the fouled artificial treatment suggests that periphyton may influence the behavior of first instar crabs, however, mixed responses preclude a determination of the factors responsible for this distribution.

Chemoreception Experiment

Chemical mediation of behavior is well-known in practically all groups of living organisms (Lindstedt 1971, Brown 1975, Ali & Morgan 1990). Chemical cues are more thoroughly documented in the terrestrial realm; however,

Adult C. sapidus are acutely sensitive to minute concentrations of waterborne chemical stimuli (Pearson & Olla 1977), suggesting that chemoreception may be similarly important to megalopal behavior. Chemically-induced behavior by megalopae could result from source-specific ecological associations (i.e. predator/prey dynamics or reproductive interactions) or it could result from a
physiological response (i.e. to excretory products or fluxuations in dissolved oxygen). As a result, a neutral response by megalopae could indicate non-detection or indifference toward the stimulus, while positive or negative orientations are probably operant responses.

**Zostera marina Treatment:**

Habitat-specific chemical cues provide reliable mechanisms for habitat and substrate differentiation by finfish (Sweatman 1985, 1988), ascidians (Havenhand & Svane 1989), bivalves (Bonar et al. 1990), and crustaceans (decapods: Castro 1978, Derby & Atema 1980; hermit crabs: Rittschof et al. 1990, and references therein; and barnacles: Crisp & Meadows 1963). Aquatic macrophytes readily exchange nutrients with associated epiphytes and the surrounding environment through release of metabolic products such as carbon (50% of released carbon is > 10,000 Daltons), phosphorous, nitrogen (in the form NH$_4^+$), and water-soluble phenolic acids (McRoy & Goering 1974, Penhale & Smith 1977, Wetzel & Penhale 1979, Zapata & McMillan 1979, Frankignoulle & Distéche 1984). The amount of these chemicals released to the environment is dependent upon in situ compound concentration, light, temperature, macrophyte condition, and the presence of epiphytes (Wetzel & Penhale 1979). This chemical signature provides a medium for habitat differentiation in the absence of tactile and visual
cues. My results suggest that megalopae, like a grazing gastropod in a freshwater system (Bronmark 1985), may respond to macrophyte metabolites (two of four trials demonstrate significance; Fig. 6). The two remaining trials, however, did not demonstrate significance. Three possibilities exist to explain this inconsistency between trials: the positive response was real and an unknown mechanism influences behavioral expression, or the positive response was due to an unknown factor and there is no response to Z. marina chemical cues, or responses were a result of random movement. Due to the consistency of responses within each trial, the final option seems unlikely.

The realized tidal direction at the VIMS Ferry Pier deviates from the predicted tides (NOAA 1992) due to a gyre generated by an upstream riverine constriction (i.e. the current is typically upstream along the northern shore near the Ferry Pier). This constriction represents the present upstream limit of naturally occurring grass beds. Thus, due to the gyre, water origin (upstream without grass beds present or downstream within grass beds present (although sparse in 1990)) and chemistry is not certain. Both trials exhibiting significant responses commenced at slack low tide and continued for three hours into flood tide. Conversely, both nonsignificant trials commenced prior to, and spanned, a slack water period. In contrast, a lack of variant
responses between control trials, one of which commenced at slack low tide and the other of which occurred during ebb tide suggests that it is unlikely that ambient chemistry associated with tidal stage invoked both significant responses observed in the Z. marina treatment. Therefore, it appears that chemical cues associated with Z. marina may have influenced these responses. The two nonsignificant responses suggest the presence of additional factors that influence behavioral expression by C. sapidus megalopae. As such, this topic is inconclusive and requires further attention.

Grass Bed Resident Treatment:

Macrofaunal chemical output contributes to grass bed water chemistry; thus three abundant species associated with grass beds were used as experimental treatments (O. tau, M. arenaria, and juvenile or adult C. sapidus). None represent reliable indicators of grass bed location, since they are not restricted to grassbed habitats. Therefore, the response of megalopae was interpreted with respect to inferred effects on within-habitat distributions of megalopae.

M. arenaria and O. tau play important roles in C. sapidus population dynamics. M. arenaria represent a major food resource for juvenile and adult C. sapidus (Virnstein 1977, 1979; Peterson 1979; Holland et al. 1980; Blundon &
Kennedy 1982a, b; Lipcius & Hines 1986). *M. arenaria* densities to 600 m$^2$ have been reported in York River shoals. However, this abundance declines rapidly and is highly variable spatially and temporally (Lucy 1976). This indicates that a response to highly variable populations of *M. arenaria* could have important consequences for *in situ* populations of megalopae. *C. sapidus*, in turn, provide a food resource for *O. tau* (Gudger 1910, Wilson et al. 1982). These ecological associations are, however, of little immediate consequence for megalopae, since they are assumed insignificant for several months post-settlement. Neutral responses to both *M. arenaria* and *O. tau* support the contention that these species have no immediate ecological or physiological impact on *in situ* megalopal behavior.

Fish and invertebrate larvae frequently settle in the presence of conspecifics since juveniles or adults often indicate the presence of suitable settlement habitat (Scheltema 1974, Talbot et al. 1978, Highsmith 1982, Jensen 1989, Sweatman 1989). Unlike sessile invertebrates whose settlement distribution often reflects the requirements of the adult stage, mobile organisms may settle to satisfy the needs of the juvenile stages, particularly if juvenile needs are unrelated to adult habitat requirements. Juvenile *C. sapidus* could, therefore, denote favorable settlement habitat since SAV represents the primary nursery habitat within Chesapeake Bay. Conversely, juvenile *C. sapidus* are
known to be cannibalistic (Laughlin 1982, Peery 1989, Hines et al. 1990), thus resultant behavioral responses could integrate the relative importance of suitable settlement habitat vs. an increased threat of predation associated with high densities of juvenile conspecifics.

Jensen (1989) documented gregarious settlement of decapod megalopae in response to adult conspecifics in the rocky intertidal, however, similar behavior was not observed in the present study. Equality in body volume displacement, due to lack of information on an allometric excretion function, was used as a treatment to standardize juvenile and adult chemical output. Contrasting both the juvenile treatment neutral response and the unrealistically high juvenile density that elicits a negative response, it seems unlikely that in situ _C. sapidus_ juveniles could influence the distribution of megalopae by chemical means.

A moderately high density of adult _C. sapidus_, on the other hand, elicited a negative orientation by megalopae, suggesting a potential mechanism to impact within-habitat distribution. Adult _C. sapidus_ are voracious predators (Virnstein 1977; Blundon & Kennedy 1982a, b; Gibbons and Castagna 1985) and are known to be cannibalistic (Peery 1989), though it seem unlikely that megalopae serve as a prey item due to their agility and small size.
Low dissolved oxygen (DO) concentrations may have resulted from the experimental design and may have contributed to the response by megalopae to both the equal juvenile volume and adult *C. sapidus* treatments. It is difficult to separate respiratory (DO) impacts from excretory production without the direct measurement of either process. Consequently, due to lack of this data, the negative response by megalopae to *C. sapidus* adult chemical cues may be due to the properties associated with unquantified chemical factors (possibly urine or DO concentration). Response threshold and chemical composition were not specifically addressed in the present study and offer a topic for further research.

Due to experimental design constraints, it is unknown if observed behavioral responses to grassbed macrofauna were initiated immediately upon detection or if individuals responded slowly over the 3-h experimental period. This is critical for the proper interpretation of inferred *in situ* behavior by megalopae since *in situ* stimulus concentrations are likely ephemeral due to several factors including chemical source mobility, hydrodynamic variability, and temporal and volumetric variability in excretion rates. Thus, if detection threshold in megalopae is low and megalopae respond promptly to chemical cues, then behavioral modification could result in small-scale differential distribution.
In conclusion, this study addressed the behavior of megalopae in response to several features of Z. marina to determine their importance in substrate selection. Proximal to Z. marina, macrophyte structure (over no structure), appears important to substrate selection by megalopae; however, low statistical power is suggested to preclude significance. Chemicals released into the environment by Z. marina appear to influence the behavior of megalopae resulting in a positive chemotactic response; however, heterogeneity between trials suggests an additional factor may regulate this response. Specific macrofauna (i.e. adult conspecifics) have the potential to influence the behavior of megalopae within a grass bed, while others appear noninfluential (i.e. O. tau, M. arenaria, and juvenile conspecifics). This appears reasonable in light of the diversity, abundance, and seasonality of the taxa inhabiting seagrass beds in lower Chesapeake Bay. Additionally, these taxa impact the later developmental stages of C. sapidus and pose no immediate threat or benefit. Results herein suggest that megalopae of C. sapidus use a variety of sensory mechanisms in behavioral modification and that individual behaviors actively play a part in determining the fate of megalopae in an environment where physical processes often regulate larval/postlarval distributions.
LITERATURE CITED


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