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Effects of chemical cues on orientation of blue crab, *Callinectes sapidus*, megalopae in flow: implications for location of nursery areas

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Abstract Megalopae (postlarvae) of the blue crab *Callinectes sapidus* Rathbun use flood-tide transport (FTT) for movement into and up estuaries. Since they settle around the time of slack water at the end of flood tide during FTT, it was predicted that orientation toward primary nursery areas of aquatic vegetation occurs at this time. This study tested the hypotheses that megalopae locate nursery areas by swimming upstream in the presence of chemical odors from potential nursery areas and avoid adverse microhabitats by swimming downstream when predator or adverse environmental odors are present. Megalopae were tested in a flume where they were exposed to the sequence of cues mediating FTT (i.e. 2 psu increase in salinity followed by an increase and a decrease in current speed and turbulence). The flume contained odor water either from the developmental area (offshore water), nursery area vegetation (seagrass, *Zostera marina*; salt marsh cord grass, *Spartina alterniflora*), predators (fiddler crab, *Uca pugilator*; mud crab, *Panopeus herbstii*; grass shrimp, *Palaemonetes pugio*), or chemicals associated with adverse environments (ammonium). Vertical positions of premolt and intermolt megalopae were similar in water devoid of estuarine chemical cues (offshore water) and water containing seagrass odor. Upstream swimming behavior (orientation) of intermolt megalopae was also similar in these waters. However, there was an ontogenetic behavioral change, as the proportion of premolt megalopae oriented upstream generally increased as the concentration of seagrass and salt marsh cord grass odor increased and as current speed decreased. Upstream orientation of premolt megalopae in response to seagrass odor decreased significantly (i.e. downstream swimming increased) in the presence of odor from *U. pugilator*, *P. pugio*, and ammonium, but not from *P. herbstii*. Thus, the hypothesis was supported. These results suggest premolt megalopae orient toward nursery areas by swimming upstream in response to odors from aquatic vegetation as current speeds decrease at the end of nocturnal flood tides. Moreover, these results also indicate that megalopae may discriminate among microhabitats and avoid adverse settlement habitat, as orientation toward nursery areas is reversed by predator odors and ammonium.

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Introduction

After completing larval development in offshore areas, megalopae (postlarvae) of the blue crab *Callinectes sapidus* are transported shoreward, where they enter estuaries, settle, and metamorphose to the first crab stage (reviewed by Epifanio and Garvine 2001; Forward and Tankersley 2001). Megalopae use flood-tide transport (FTT) for movement into and up estuaries to settlement sites (reviewed by Forward et al. 2002). During FTT, megalopae are abundant in the water column during nocturnal flood tides and in low numbers at all other times, presumably because they are on or near the bottom (Dittel and Epifanio 1982; Brookings and Epifanio 1985; Mense and Wenner 1989; Little and Epifanio 1991; De Vries et al. 1994; Olmi 1994). Settlement sites consist of areas of submerged vegetation, such as seagrass beds (Orth and van Montfrans 1987; Olmi et al. 1990) and tidal marshes (Weinstein and Brooks 1983; Orth and van Montfrans 1987; Morgan et al. 1996), and metamorphosis is accelerated by chemical cues from estuarine vegetation (Wolcott and De Vries 1994; Forward et al. 1994, 1996, 1997b; Brumbaugh and McConaugha 1995; Forward et al. 2001).

Flood-tide transport is hypothesized to result from behavioral responses to a sequence of exogenous environmental cues. Megalopae have a negative phototaxis in estuarine water, which keeps them on or near the bottom during the day (Forward and Rittschof 1994; Tankersley et al. 1995). The current model for the behaviors underlying nocturnal FTT (Welch and Forward 2001; Forward et al. 2002) is that megalopae ascend during flood tide in response to a continuous rate of increase in salinity (Tankersley et al. 1995), associated with rising tide. Once in the water column, megalopae are transported with a parcel of water and salinity either remains constant or changes very slowly. This presents a problem because behavioral responses of crab larvae to salinity changes only last for several minutes (Latz and Forward 1977), yet megalopae remain in the water column for several hours during which they are transported by flood tides. The cue for sustained swimming in the water column is water turbulence (Welch et al. 1999; Welch and Forward 2001). This swimming does not need to be directional, as an increase in activity generally causes zooplankton to ascend in the water column (Rudjakov 1970). Even though megalopae can swim into currents (positive rheotaxis), the speeds of flood currents often exceed their maximum swimming velocity. Luckenback and Orth (1992) found that the maximum sustained swimming speed of blue crab megalopae was about 12.6 cm s^{-1}, whereas flood-tide currents can range from about 60 to >100 cm s^{-1} during neap and spring tides. Thus, even if megalopae swim into the current during flood tides, their net movement will be in the direction of current flow.

Megalopae settle out of the water column at the end of flood tide, when current speeds decline around the time of slack water (Tankersley et al. 2002). Since they can swim upstream at current speeds below 4.8 cm s^{-1}, they have the ability to actively search for settlement sites (Luckenback and Orth 1992) during times of low current flow. The implications of the foregoing model are that megalopae use FTT for large-scale horizontal movement throughout estuaries, but actual orientation toward and settlement in nursery areas occurs around the time of slack water, at the end of flood tide. The behaviors involved in locating a settlement site and settlement remain unknown. Clearly, megalopae can discriminate between settlement sites based on chemical cues. Welch et al. (1997) found that blue crab megalopae settle preferentially on passive collectors containing seagrass. However, settlement was reduced if collectors contained predatory crustaceans, including fiddler crabs and grass shrimp. In the laboratory, megalopae avoid odors from predatory crabs (Welch et al. 1997) and fish, but are not attracted to odor from seagrass (Diaz et al. 1999).

The present study was undertaken to determine how blue crab megalopae locate nursery sites, by testing their behavioral responses in flow and in the presence of odors associated with nursery areas and predators. The test hypotheses were that megalopae locate nursery areas by swimming upstream (positive rheotaxis) in the presence of odors from nursery areas and avoid adverse microhabitats by swimming downstream (negative rheotaxis) when predator or adverse environmental odors are present. Blue crab megalopae were tested in a flume in the presence of odors from aquatic vegetation, crustacean predators, and ammonium.

Materials and methods

Megalopae of the blue crab *Callinectes sapidus* Rathbun were collected in plankton tows (0.6 m diameter net; 505 μm mesh) from August to November 2001 about 1.5 km inside the entrance to the Newport River estuary (Beaufort, N.C., USA) during nocturnal flood tides. They were transferred daily into offshore water (see description below) with a salinity adjusted with deionized water to 33 psu, as measured with a refractometer (AO; accuracy 0.5 psu) and fed newly hatched brine shrimp nauplii (*Artemia franciscana* Kellogg), which had recently hatched in the same water. Megalopae were maintained at 23°C on a light:dark cycle approximating the natural photoperiod. Immediately prior to being used in experiments, megalopae were sorted according to molt stage into intermolt and premolt megalopae (Aiken 1973; Anger 1983; Stevenson 1985).

FTT and presumptive nursery site location occurs at night, but all experiments were performed during the middle 8 h of the light phase of the light:dark cycle. This inconsistency would be a problem if FTT were based on an endogenous rhythm in activity related to tidal times in the field. However, blue crab megalopae do not have a tidal rhythm in activity (Tankersley and Forward 1994; Forward et al. 1997a), and FTT is based upon behavioral responses to a sequence of environmental cues (Forward et al. 1995, 2003; Welch and Forward 2001). Moreover, previous experiments indicated that the responsiveness of megalopae to environmental cues (e.g. salinity change) does not vary significantly throughout the day (Tankersley et al. 1995).

Responses of megalopae to seven types of test water were determined. Offshore water (35 psu) was collected about 25 km seaward of Beaufort Inlet, the entrance to the Newport River estuary. This collection site was beyond the estuarine plume and in an area where blue crab megalopae were collected previously in the neuston (Forward and Rittschof 1994). This water was collected at about weekly intervals and stored in acid-washed glass and polycarbonate carboys.

Nursery habitat odor waters contained odors from the seagrass *Zostera marina* and salt marsh cord grass *Spartina alterniflora*, because megalopae settle in seagrass beds (Orth and van Montfrans 1987; Olmi et al. 1990) and tidal marshes (Weinstein and Brooks 1983; Orth and van Montfrans 1987; Morgan et al. 1996). Prior to the development of odor water, *Z. marina* was collected in the Newport River estuary and maintained in the laboratory in aerated ambient estuarine water (salinity: 33–35 psu; 23–25°C), which was changed every 2 days. Although the seagrass blades were relatively free of epiphytes, the bryzoan *Bugula neritina* was removed from the blades before they were used to produce odor water. *Z. marina* odor water was developed by incubating a fixed weight of seagrass in 6 l of aerated offshore water for 18 h. The seagrass was blotted dry to remove estuarine water before weighing. After incubation, the seagrass was removed and the water was filtered to remove particles > 5 μm. Groups of seagrass blades were used only once to establish odor water. New odor water was prepared daily and added to offshore water in the flume (see description below) to achieve target concentrations of 0.1, 0.75, 2.0, and 5.0 g l^{-1}. These concentrations were based on levels previously shown to affect metamorphosis of *C. sapidus* megalopae (Forward et al. 1996) and their orientation to visual and chemical cues (Diaz et al. 1999). A range of concentrations was tested to determine whether behavioral responses were dose dependent.

The salt marsh cord grass *S. alterniflora* was collected daily and the epiphytes and sediments removed from the stalks, which were cut into about 6 cm sections. *S. alterniflora* odor water was developed using the same procedure as described for seagrass odor water. Test concentrations in the flume (0.03, 0.3, and 3.0 g l^{-1}) were based on previous studies of effects on blue crab metamorphosis (Forward et al. 1996).

Potential estuarine predators from different habitats included the fiddler crab *Uca pugilator*, the mud crab *Panopeus herbstii*, and the grass shrimp *Palaemonetes pugio*, all of which readily feed on blue crab megalopae (Welch et al. 1997). *U. pugilator* is found on sand flats, *P. herbstii* is common in oyster areas, and *P. pugio* occurs in salt marshes (Williams 1984). Blue crab megalopae settle in salt marsh areas (Weinstein and Brooks 1983; Orth and van Montfrans 1987; Morgan et al. 1996) and perhaps oyster reefs (Eggleston et al. 1998). Although settlement on sand flats has not been reported, this habitat frequently occurs adjacent to seagrass beds and salt marshes.

Effect of odor from a predator alone was first tested by incubating *U. pugilator* for 1 h in 6 l of offshore water and then adding filtered (5 μm) odor water to the offshore water in the flume to produce a final concentration of 1 g l^{-1}. The short incubation time (1 h) was used to reduce the build-up of metabolic waste products (e.g. ammonia/ammonium) and associated bacteria in the incubation water. Since this procedure failed to induce a significant behavioral response (see "Results"; Fig. 6), the procedure was changed to determine whether predator odor could reverse the responses observed upon exposure to seagrass odor.

Seagrass odor water was developed for 18 h for a flume concentration of 2 g l^{-1}. For the last hour of seagrass incubation, predators were added to the water at specific concentrations, after which the water was filtered (5 μm) and added to the flume for testing. Final test concentrations of *U. pugilator* (0.1, 0.5, and 1.0 g l^{-1}), *P. herbstii* (0.5, 1, and 2.0 g l^{-1}), and *P. pugio* (0.1 and 1.0 g l^{-1}) were based on preliminary experiments on the orientation of blue crab megalopae to chemical cues in a choice tube (R. Tankersley, unpublished data). Since these crustaceans could potentially add ammonia/ammonium to the test solutions, the levels of ammonia/ammonium were measured in the most concentrated crustacean solution (technique 1.4 for determination of ammonia; Parsons et al. 1984). Measurements were made of offshore water, seagrass water and seagrass plus crustacean water. The latter two waters were measured at the end of the incubation period.

Ammonium is a potential habitat cue found in seagrass beds (Kenworthy et al. 1982) and oyster reefs (Fitt and Coon 1992). This chemical cue was tested by adding ammonium chloride (Fisher Scientific) to filtered seagrass odor water to achieve final concentrations in the flume of 2 g l^{-1} for seagrass odor and ammonium chloride concentrations of 10, 50, or 100 μmol l^{-1} (μM). Ammonium chloride concentrations were based on levels known to affect metamorphosis (Forward et al. 1997b). At the normal pH of seawater (8.0), 96% of the added ammonium exists as ammonium ion and 4% as ammonia (Bower and Bidwell 1978).

Experiments were conducted in a recirculating, paddle-wheel-driven, racetrack flume (see Welch et al. 1999 for a detailed description). The elliptical flume was 2.4 m long and 1.3 m wide, with a 15×15 cm cross-section flow channel. The 1-m diameter, 16-blade paddle wheel was driven by a 0.75 horsepower DC motor, controlled by an analog speed controller. This flume setup can produce water flow speeds from 0.25 to 50 cm s^{-1}. The flume was placed inside a light-tight room, and the speed control and video equipment for monitoring megalopal behavior were placed outside.

Measurements of flow characteristics were made with an acoustic Doppler velocimeter (Sontek ADV field: described in detail in Welch 1998). The sensor was positioned in the middle of the flume channel (7.5 cm from the bottom and walls), which permitted direct measurements of current speed and calculations of turbulent kinetic energy (TKE; Marrase et al. 1990; Peters and Gross 1994). Measurements at different locations in the flume indicated that flow speeds were within 1 cm s^{-1} of mean velocity at 1 cm from the flume walls (J.M. Welch, unpublished data). The TKE values at different current speeds in the flume have been reported previously (Welch et al. 1999). The present study used current speeds ranging from 4 to 12 cm s^{-1}, with TKE values ranging from 0.2 to 1.4 cm^2 s^{-2}. These values are comparable to those in the Newport River estuary (Welch 1998), where the blue crab megalopae were collected.

During experiments, the vertical position and swimming direction of the megalopae were recorded using a closed-circuit video system (NEC model T1-22A video camera; Panasonic model AG-7350 video recorder). Although experiments were conducted in darkness, megalopae were illuminated using far-red light (maximum transmission: 774 nm), which was imperceptible to the megalopae (Cronin and Forward 1988). The video camera was aligned horizontally to view a 15×12 cm section of the flume that included the entire water column (top to bottom).

For experiments, offshore water (35 psu; 23°C) was added to the flume to a depth of 15 cm. If an odor was tested, then 6 l of odor water was added to achieve a final volume of 130 l. These waters were mixed for 5 min by circulating the flume water at the maximum flow rate. Flow was then reduced to 4 cm s^{-1}, and intermolt or premolt megalopae were added to the flume. Megalopae had been acclimated at 33 psu for at least 12 h, but were introduced into 35 psu water to simulate the salinity increase that induces an ascent from the bottom at the beginning of FTT (reviewed by Forward et al. 1995, 2003). During FTT, megalopae ascend in response to a continuous rate of salinity increase (De Vries et al. 1994; Tankersley et al. 1995), not a step increase as used in the present study. Nevertheless, a step increase in salinity was used because Welch and Forward (2001) found that behavioral responses of blue crab megalopae to step increases and continuous rates of increase were the same in the flume.

At the beginning of each trial about 250 ml of acclimation water (33 psu) containing 100 megalopae was added to the flume water (35 psu), which had a current speed of 4 cm s^{-1}. This procedure was used because during FTT megalopae ascend in response to a salinity increase when currents are flowing during flood tide and are stimulated to continue swimming by turbulence (Welch et al. 1999; Welch and Forward 2001). Thus, megalopae were exposed to both a salinity increase and turbulence. The room lights were extinguished shortly after the megalopae were added to the flume, and the current speed was increased and decreased in 2 cm s^{-1} steps (4, 6, 8, 10, 12, 12, 10, 8, 6, 4 cm s^{-1}) at 7-min intervals. This sequence simulated the increase and decrease in current speed and turbulence a megalopa would encounter during nocturnal flood tides. The maximum test current speed (12 cm s^{-1}) was below the maximum speeds encountered by megalopae during FTT, but does induce a pronounced swimming response by blue crab megalopae (e.g. Welch et al. 1999). The lowest test speed (4 cm s^{-1}) occurs at the beginning and end of flood tide (Welch et al. 1999).

The total time (70 min) for the simulated tidal sequence was much shorter than the time for flood tide (~6.2 h) in an estuary with semi-diurnal tides. The justification for using this short sequence was first that megalopae respond to a sequence of cues during FTT. Second, Welch and Forward (2001) demonstrated that megalopal behavioral responses to a simulated flood tide sequence in the flume (a salinity increase followed by an increase and decrease in current speeds) over 60 min and 4 h were the same.

The behavior of megalopae was recorded continuously during the increase and decrease in current speeds. At the end of each replicate, megalopae were removed by filtration with plankton netting and a new group of 100 megalopae was introduced to the flume for the next replicate. Each experiment was replicated five times using a different group of megalopae. Thus, megalopae were only used once. At the end of each day's trials and upon a change in treatment conditions, the flume was drained and rinsed with distilled water to remove salt residue and test water odor.

Ideally the test water should have been changed with each trial. This procedure was impractical because of the water volume of the flume (130 l). Since there was no apparent change in responsiveness of megalopae between the first and fifth replicates, it was assumed that the chemical composition of the test water remained relatively constant over the 6 h test period. The salinity of the flume water was not measurably decreased by the fifth replicate, as measured with a refractometer.

For analysis of megalopal behavior on videotape, the vertical water column was separated into three equal 5 cm sections on the video screen. The number of megalopae in each section and their orientation (i.e. upstream or downstream) were recorded as they passed though the field of view. If a megalopa was swimming upstream (positive rheotaxis) but was being carried downstream because the current speed was faster than its swimming speed, it was counted as oriented upstream. In this situation, megalopae were swimming upstream but appeared to move downstream more slowly than the water. Counting began 3 min after the beginning of each test speed and continued until 75% of the flume water had passed the viewing section. Thus, the data recording time was different for each current speed, but the same volume of water was sampled. This procedure prevented megalopae from being counted more than once at each current velocity. The number of megalopae counted at each current velocity was about 60–90. The proportion of megalopae observed in the three vertical sections of the flume and their orientation (upstream vs. downstream) were calculated at each current speed. For the initial experiments, megalopal position and orientation were determined at all current speeds, whereas later experiments only analyzed the declining phase of currents (12–4 cm s^{-1}).

For initial experiments, the behaviors of premolt and intermolt megalopae in the presence of offshore water and *Z. marina* odor waters were compared using a repeated-measures analysis of variance (ANOVAR), with two between-group factors (molt stage and chemical odor) and one within-group factor (flow speed). The proportion of megalopae oriented upstream and the proportion of megalopae swimming in each of the three vertical sections (vertical distributions) were analyzed separately. Since the swimming behavior of intermolt megalopae and vertical position of both intermolt and premolt megalopae did not change significantly in response to chemical cues from *Z. marina* (see "Results"), only the swimming direction of premolt megalopae was examined in the subsequent experiments and the resulting statistical analyses included only one between-group factor (i.e. chemical odor). Comparisons among treatment groups at each flow speed were made using post hoc directed contrasts (Rosenthal and Rosnow 1985). All proportions were arcsine, square-root transformed prior to analysis, and back-transformed values are reported in the figures.

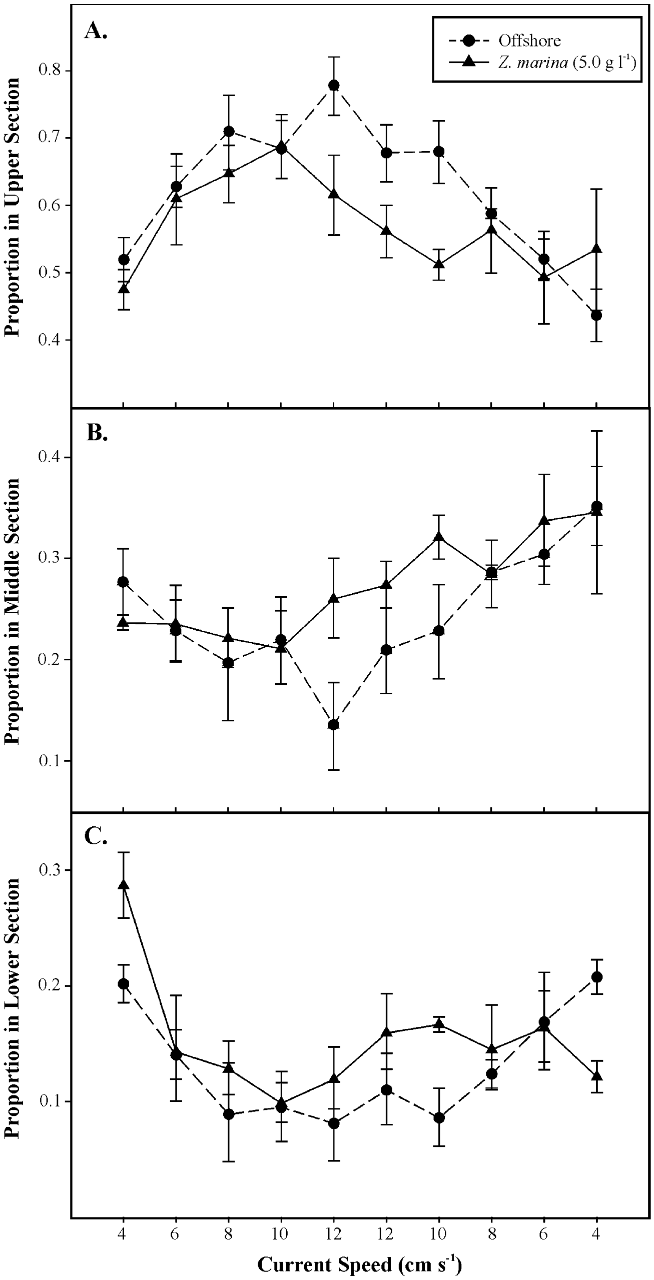

Fig. 1A–C *Callinectes sapidus*. Proportion ($\bar{X} \pm SE$) of intermolt megalopae in the upper (**A**), middle (**B**), and lower (**C**) sections of the flume during the current speed sequence, when tested in offshore water or *Zostera marina* odor water (5 g l^{-1}) ($n = 5$)

Results

Swimming behavior in response to seagrass odor

Responses of intermolt and premolt megalopae were compared in offshore water and *zostera marina* odor water (5 g l^{-1}) to guide the analysis of responses to other habitat and predator odors. The vertical distribution of intermolt (Fig. 1) and premolt (Fig. 2) megalopae in the flume was similar in offshore water and *Z. marina* odor water at different flow speeds. In general, the proportion of megalopae in the upper section increased as current speed increased and decreased as current speed declined (Figs. 1, 2).

The proportions of intermolt megalopae swimming upstream in offshore water and *Z. marina* odor water (Fig. 3A) were also similar ($F = 4.77$, $df = 1,8$, $P > 0.05$) and increased in both waters as the current speed declined ($F = 8.77$, $df = 9,72$, $P < 0.001$). However, the responses of premolt megalopae were very different (Fig. 3B). In offshore water, the proportion of premolt megalopae oriented upstream remained relatively constant at all current speeds. In *Z. marina* odor water, the proportion oriented upstream was also relatively constant upon the increase in current speed but increased dramatically, and was significantly different from levels in offshore water, as the current speed declined from 12 to 4 cm s^{-1} (Fig. 3B; $F = 49.11$, $df = 1,8$, $P < 0.001$). These results indicated that premolt megalopae discriminated between odors in the flume and the most obvious response was the change in the proportion oriented

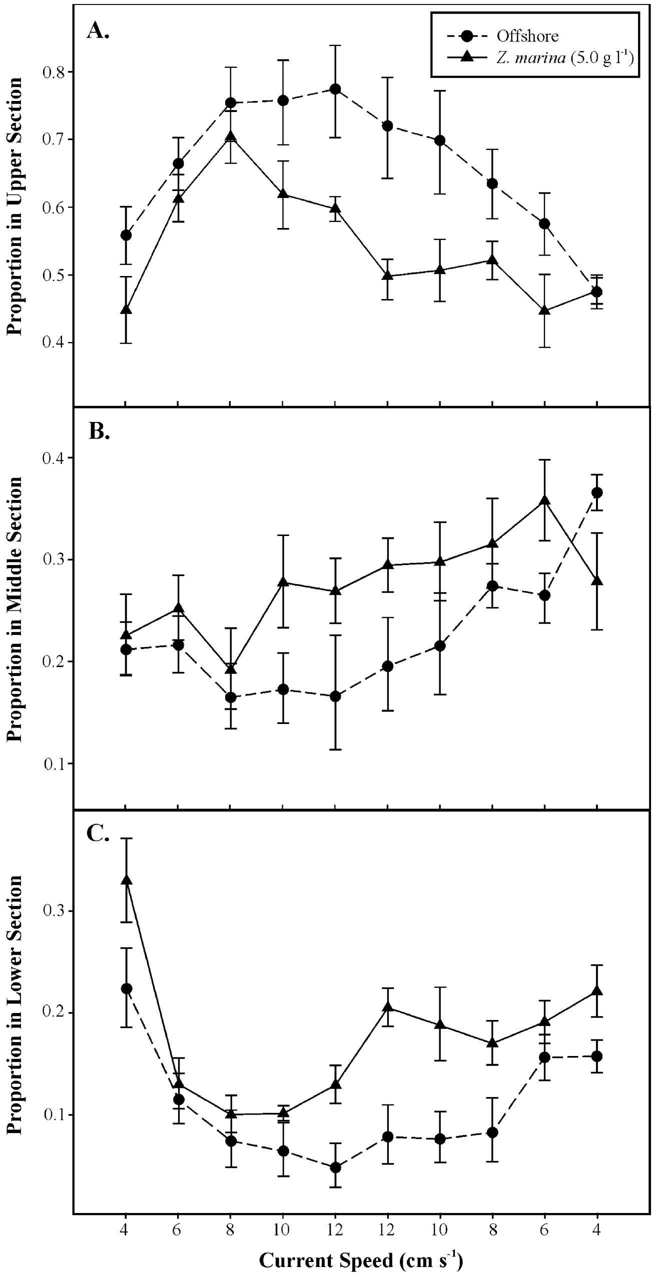

Fig. 2A–C *Callinectes sapidus*. Proportion ($\bar{X} \pm$ SE) of premolt megalopae in the upper (**A**), middle (**B**), and lower (**C**) sections of the flume during the current speed sequence, when tested in offshore water or *Zostera marina* odor water (5 g l^{-1}) ($n = 5$)

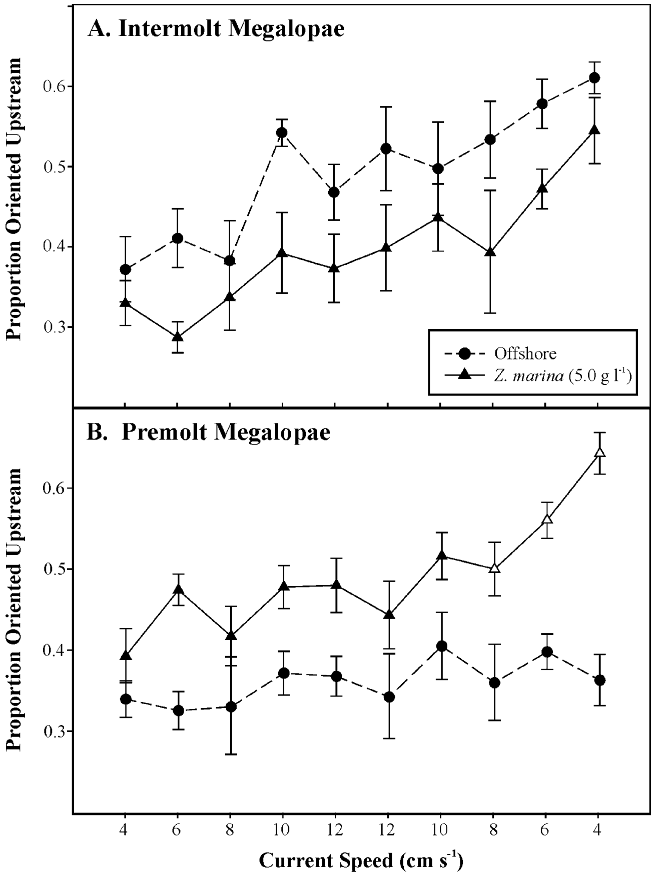

Fig. 3A,B *Callinectes sapidus*. Proportion ($\bar{X} \pm$ SE) of intermolt (**A**) and premolt (**B**) megalopae oriented upstream during the current speed sequence, when tested in offshore water or *Zostera marina* odor water (5 g l^{-1}) ($n = 5$). *Open symbols* indicate the response in *Z. marina* odor water was significantly different ($P < 0.05$) from the response in offshore water at the same current speed ($n = 5$)

upstream upon a decline in current speed. Thus, all future experiments only examined the response of premolt megalopae and compared the proportion of megalopae oriented upstream as current speeds decreased from 12 to 4 cm s^{-1} which simulated the decrease in current speeds at the end of flood tide.

Responses to odors from aquatic vegetation

When exposed to *Z. marina* odor water, the proportion of premolt megalopae oriented upstream generally increased as the current speed decreased (Fig. 4; $F = 11.84$, $df = 4{,}80$, $P < 0.001$). Since responses were significantly greater than those in offshore water at all concentrations of *Z. marina* (0.1–5 g l^{-1}) and almost all current speeds (except for three speeds at 0.75 g l^{-1}), the lowest concentration to evoke a significant response (threshold concentration) was at or below 0.1 g l^{-1}. In general, the responsiveness of megalopae increased as *Z. marina* concentration increased, but there was not a perfect gradient. Since a concentration of 2 g l^{-1} consistently evoked a high response (Fig. 4), this concentration was used to establish an upstream-orientation response for experiments involving predator odors.

Premolt megalopae exposed to salt marsh cord grass (*Spartina alterniflora*) odor exhibited behavioral responses (Fig. 5) similar to those observed in the presence of *Z. marina* odor. The proportion of premolt megalopae swimming upstream was significantly greater than that in offshore water at all test concentrations (0.03–3.0 g l^{-1}), for almost all flow speeds (two exceptions;

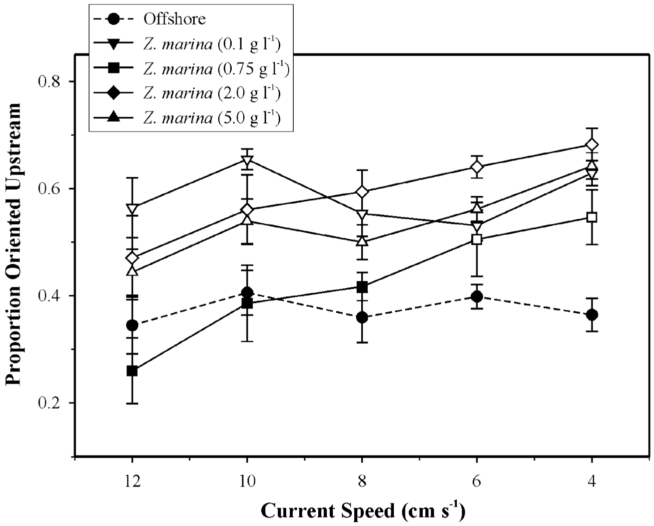

Fig. 4 *Callinectes sapidus*. Proportion ($\bar{X} \pm \mathrm{SE}$) of premolt megalopae oriented upstream in the flume upon a decline in current speed (12–4 cm s^{-1}), when exposed to either offshore water or *Zostera marina* odor water at concentrations of 0.1–5 g l^{-1}. *Open symbols* indicate the response of megalopae in *Z. marina* odor water was significantly different ($P < 0.05$) from the response in offshore water at the same current speed ($n = 5$)

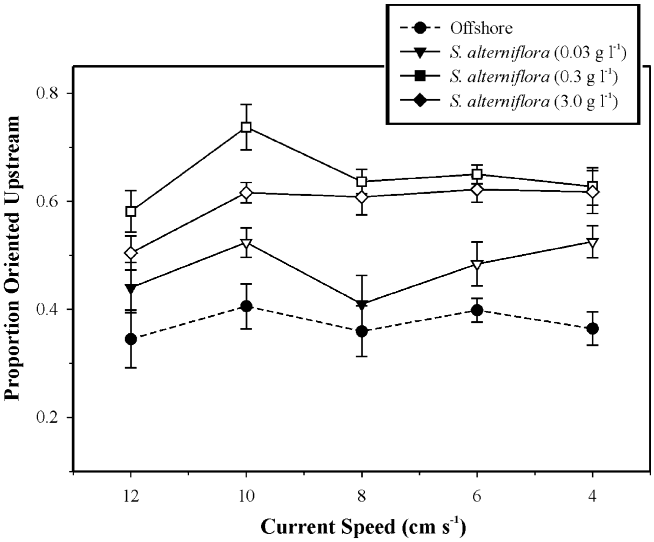

Fig. 5 *Callinectes sapidus*. Proportion ($\bar{X} \pm \mathrm{SE}$) of premolt megalopae oriented upstream in the flume upon a decline in current speed, when exposed to either offshore water or *Spartina alterniflora* odor water at concentrations of 0.03–3.0 g l^{-1}. *Open symbols* indicate the response of megalopae in *S. alterniflora* odor water was significantly different ($P < 0.05$) from the response in offshore water at the same current speed ($n = 5$)

Fig. 5). The response increased significantly as the current speed declined ($F = 5.53$, $df = 4{,}64$, $P < 0.001$) and decreased as the *S. alterniflora* concentration declined (Fig. 5; $F = 32.76$, $df = 3{,}16$, $P < 0.001$). Thus, as with *Z. marina* odor, the response of megalopae to *S. alterniflora* odor was dose dependent.

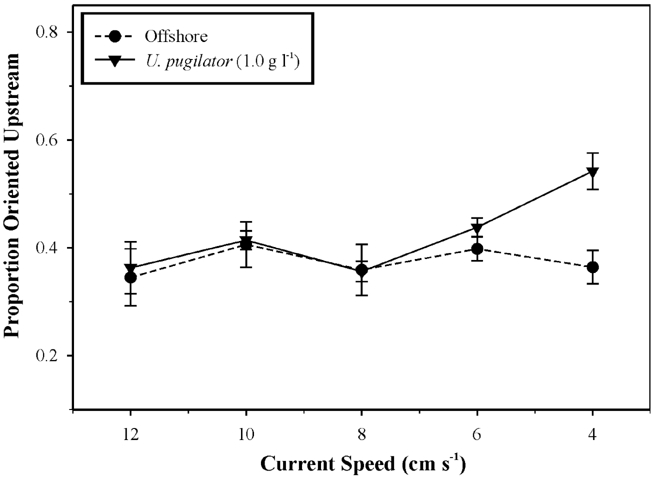

Fig. 6 *Callinectes sapidus*. Proportion ($\bar{X} \pm \mathrm{SE}$) of premolt megalopae oriented upstream in the flume upon a decline in current speed, when exposed to either offshore water or *Uca pugilator* odor water at a concentration of 1.0 g l^{-1}. The response of megalopae in *U. pugilator* odor water was not significantly different from the response in offshore water at all current speeds ($F = 4.47$, $df = 1{,}8$, $P > 0.05$) ($n = 5$)

Predator odor

When fiddler crab (*Uca pugilator*) odor water (1 g l^{-1}) was added to offshore water, there was no significant change in the proportion of premolt megalopae oriented upstream compared to levels in offshore water alone (Fig. 6; $F = 4.47$, $df = 1{,}8$, $P > 0.05$). Thus, an alternative experimental design was used in which predator odor was combined with an odor inducing upstream orientation (i.e. 2 g l^{-1} *Z. marina* odor water; Fig. 4). A significant reduction in the proportion oriented upstream would indicate that predator odor affects swimming and potentially settlement by inducing a reversal in orientation relative to current.

When odor from *U. pugilator* was combined with *Z. marina* odor water, the proportion of premolt megalopae oriented upstream decreased significantly as the concentration of fiddler crab odor increased (Fig. 7; $F = 9.25$, $df = 3{,}16$, $P < 0.001$). Significantly lower responses were evident at the two highest *U. pugilator* odor concentrations (0.5 and 1.0 g l^{-1}), but not at 0.1 g l^{-1}. The most significant response was observed at the highest *U. pugilator* concentration (1.0 g l^{-1}). Thus, predator odor from *U. pugilator* reduced upstream swimming, and the response was graded with concentration.

In the presence of odors from *Z. marina* and the predatory crab *Panopeus herbstii*, there was no significant decrease in the proportion of premolt megalopae oriented upstream at any concentration tested (0.5–2 g l^{-1}; Fig. 8; $F = 1.38$, $df = 1{,}18$, $P > 0.05$). Either odor from *P. herbstii* does not affect swimming behavior and settlement or the test concentrations of the odor were above or below the range inducing behavioral responses.

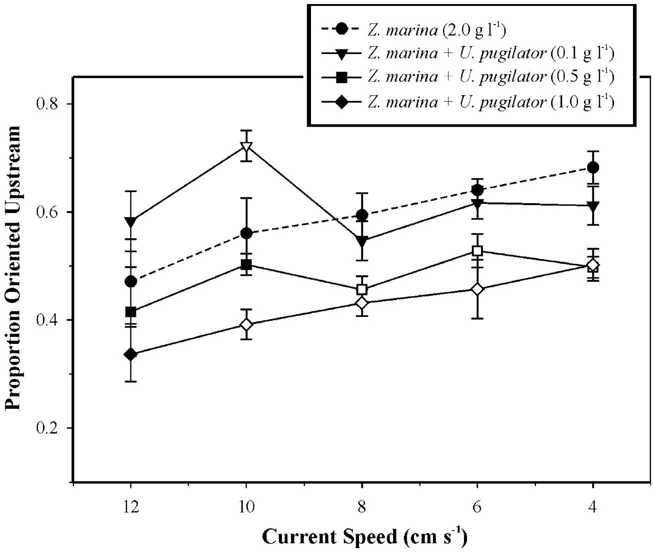

Fig. 7 *Callinectes sapidus*. Proportion ($\bar{X} \pm SE$) of premolt megalopae oriented upstream in the flume upon a decline in current speed, when exposed to either *Zostera marina* odor water at a concentration of 2.0 g l^{-1} or *Z. marina* odor water combined with *Uca pugilator* odor water at concentrations of 0.1–1.0 g l^{-1}. *Open symbols* indicate the response in *U. pugilator* odor water is significantly different ($P < 0.05$) from the response in *Z. marina* odor water alone at the same current speed ($n = 5$)

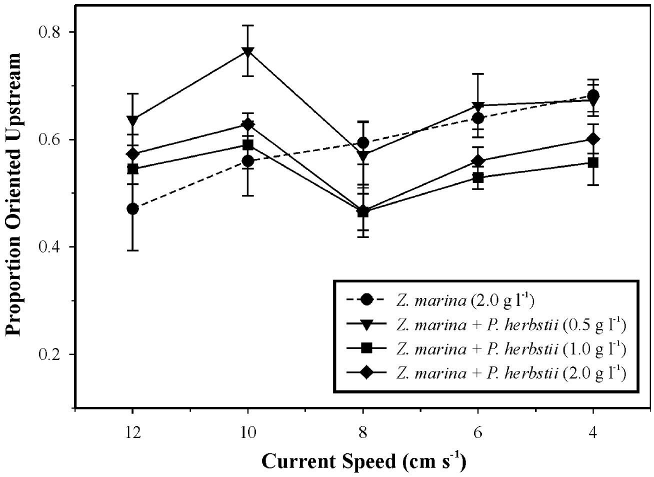

Fig. 8 *Callinectes sapidus*. Proportion ($\bar{X} \pm SE$) of premolt megalopae oriented upstream in the flume upon a decline in current speed, when exposed to either *Zostera marina* odor water at a concentration of 2.0 g l^{-1} or *Z. marina* odor water combined with *Panopeus herbstii* odor water at concentrations of 0.5–2.0 g l^{-1}. Responses of megalopae to all odor waters were not significantly different ($F = 1.38$, $df = 1{,}18$, $P > 0.05$) ($n = 5$)

In contrast, exposure to odor from the grass shrimp *Palaemonetes pugio* at 1.0 g l^{-1} significantly reduced the proportion of premolt megalopae oriented upstream (Fig. 9; $F = 9.55$, $df = 2{,}12$, $P < 0.01$) at the three lowest current speeds. The response depended upon concentration, as responsiveness of the megalopae did not change significantly at the lowest test concentration (0.1 g l^{-1}; Fig. 9).

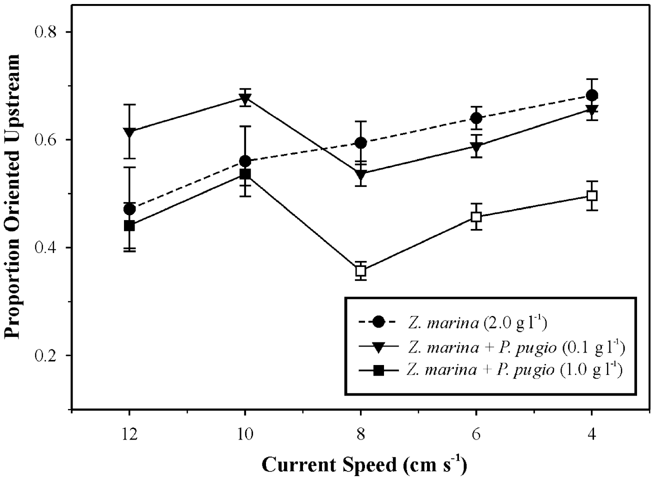

Fig. 9 *Callinectes sapidus*. Proportion ($\bar{X} \pm SE$) of premolt megalopae oriented upstream in the flume upon a decline in current speed, when exposed to either *Zostera marina* odor water at a concentration of 2.0 g l^{-1} or *Z. marina* odor water combined with *Palaemonetes pugio* odor water at concentrations of 0.1 or 1.0 g l^{-1}. *Open symbols* indicate the response in *P. pugio* odor water was significantly different ($P < 0.05$) from the response in *Z. marina* odor water alone ($n = 5$)

Ammonia/ammonium was measured in: (1) offshore seawater, (2) offshore seawater plus 2 g l^{-1} seagrass, and (3) seagrass odor water plus the highest incubation concentrations of the three crustaceans, in order to determine whether the above responses could be due to the presence of ammonia/ammonium. In all cases but one, the levels of ammonia/ammonium were below the limits of detection (0.3 μM). The exception was when 1 g l^{-1} of *P. pugio* was incubated with the seagrass; in this instance the mean ($n = 3$) ammonia/ammonium concentration was 0.85 μM.

Habitat chemical cues: ammonium

Ammonium is present in estuarine habitats where megalopae can potentially settle. When added to *Z. marina* odor water at concentrations of 50 and 100 μM, there was a significant decrease in the proportion of premolt megalopae swimming upstream at speeds of 8 cm s^{-1} and lower (Fig. 10; $F = 6.20$, $df = 3{,}16$, $P < 0.005$). This response was concentration dependent, because it was not evident at a concentration of 10 μM (Fig. 10).

Discussion

Blue crab, *Callinectes sapidus*, megalopae use FTT for entrance into estuaries and transport to nursery habitats (reviewed by Forward et al. 2002). Field studies indicate that during transport, megalopae ascend in the water column during nocturnal flood tide (Dittel and Epifanio 1982; Brookings and Epifanio 1985; Mense and Wenner 1989; Little and Epifanio 1991; De Vries et al. 1994; Olmi 1994) and settle out of the water column during slack water before ebb tide (Tankersley et al. 2002). The

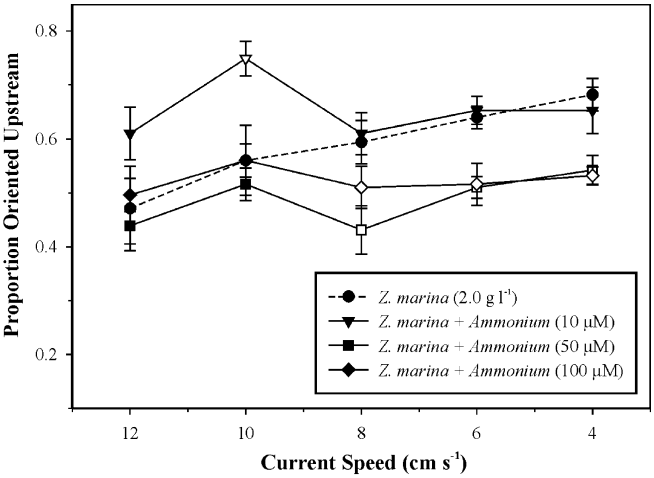

Fig. 10 *Callinectes sapidus*. Proportion ($\bar{X} \pm$ SE) of premolt megalopae oriented upstream in the flume upon a decline in current speed, when exposed to either *Zostera marina* odor water at a concentration of 2.0 g l^{-1} or *Z. marina* odor water combined with ammonium chloride to produce concentrations of 10–100 μM. *Open symbols* indicate the response in ammonium chloride odor water was significantly different ($P < 0.05$) from the response in *Z. marina* odor water alone at the same current speed ($n = 5$)

behavior underlying FTT consists of responses to a sequence of environmental cues.

The rate of increase in salinity triggers the ascent during flood tide (Tankersley et al. 1995). Although water turbulence induces sustained swimming in the water column during flood tide (Welch et al. 1999; Welch and Forward 2001), the relatively slow swimming speeds of megalopae (Luckenback and Orth 1992) compared to the speed of flood-tide current indicates that positive or negative rheotaxis would have little effect on horizontal movement. Megalopae will be transported with flood-tide currents until the end of flood tide, when current speed and turbulence decline and they settle out of the water column and attach to substrate (Tankersley et al. 2002). This sequence leads to the prediction that nursery site location occurs at the end of flood tide, when current speeds decline to levels that allow megalopae to orient and swim upstream to nursery areas of aquatic vegetation (Luckenback and Orth 1992; Tankersley et al. 2002). Thus, the test hypothesis for the study was that megalopae orient and swim upstream (i.e. positive rheotaxis) in the presence of odor from nursery areas. It was also predicted that megalopae can select between microhabitats and avoid those with abundant benthic predators and adverse environmental factors, such as ammonium. Thus, the related test hypothesis was that blue crab megalopae avoid these areas by swimming downstream (i.e. negative rheotaxis) when predator odor and ammonium are present.

The experimental design simulated the sequence of cues during FFT in an estuary. If the hypotheses are correct then rheotactic responses associated with nursery habitat location should be most evident as current speeds decline at the end of the simulated flood tide. In general, megalopae ascended in the flume as current speeds increased and descended as speeds declined, which agrees with results of previous studies (Welch et al. 1999; Welch and Forward 2001). However, the vertical positions of premolt and intermolt *C. sapidus* megalopae did not change upon exposure to water devoid of estuarine chemical cues (offshore water) and water containing odor from vegetation from the primary nursery habitat (seagrass beds; Orth and van Montfrans 1987; Olmi et al. 1990). These results agree with Welch et al. (1999), who found no difference between the vertical distribution of blue crab megalopae in offshore and estuarine water upon changes in turbulence in the flume. In addition, these results indicate that megalopae do not descend in the water column during FTT if they encounter odor from a seagrass bed. Thus, the cue for the descent is a decline in turbulence.

However, the swimming orientation of megalopae relative to current did change with odor and molt stage. Habitat odors were prepared by incubating plants and crustaceans for specific time periods, after which they were removed. The identities of the active chemical cues are unknown. Although whole organism wet weight to water volume ratios were used for concentrations, the release rates of the active chemicals from specific organisms probably varied. Thus, an exact dose–response relationship between incubation concentration and megalopal swimming behavior may be variable. Another confounding problem is that dose–response curves for chemical cues usually show an increase in response up to a particular concentration and then a decline at higher concentrations (e.g. Pettis et al. 1993; Rittschof 1993). Thus, a decline in responsiveness at higher concentrations could be due to differences in chemical release rates at different incubation ratios or to reduced responsiveness of megalopae to very high concentrations. Also, since the relationship between the test odor concentrations and those in estuarine habitats is unknown, the experiments can only demonstrate that the odors induce behavior associated with location of nursery habitats.

The proportion of intermolt megalopae oriented upstream was similar in the presence of offshore and seagrass odor water, but premolt megalopae showed a significant increase in orientation upstream in seagrass odor water as current speeds declined. This ontogenetic change in behavior with molt stage is logical, since intermolt megalopae occur in offshore and coastal areas where larvae undergo development (Wolcott and De Vries 1994) and are transported into estuaries (reviewed by Epifanio and Garvine 2001). Once in contact with chemical cues in estuarine water, events leading to metamorphosis are accelerated (Forward et al. 1994, 1996, 1997b), and there is a limited time for locating and settling in a suitable nursery area. This sequence predicts that behaviors underlying settlement site location should be more pronounced in premolt megalopae. The field study of Welch et al. (1997) agrees with this prediction, as premolt, but not intermolt, megalopae discriminated between passive collectors with and without vegetation from nursery areas.

Odor from seagrass and salt marsh cord grass induced an increase in upstream orientation by premolt megalopae, which generally increased as the concentration increased. This result conflicts with the study of Diaz et al. (1999), which found no evidence that blue crab megalopae oriented toward seagrass odor in a flow-though chemical choice apparatus. The implication of this difference is that megalopae require the sequence of cues encountered during FTT for orientation toward nursery habitats. Furthermore, the general increase in the proportion of megalopae showing positive rheotaxis as current speeds decline supports the prediction that nursery site location occurs at the end of flood tide. Since current speeds at the beginning of the subsequent ebb tide are slow enough to permit upstream swimming, chemically induced positive rheotaxis can be used to locate settlement habitats at the end of flood tide and continues into the beginning of ebb tide. This is supported by the observation that settlement of megalopae on passive collectors begins shortly before slack water prior to ebb tide and continues into the beginning of ebb tide (Tankersley et al. 2002).

As settlement approaches, megalopae could be positioned correctly to descend into aquatic vegetation at the end of flood tide. Alternatively, they could be located downstream of aquatic vegetation and use a chemically cued positive rheotaxis to swim upstream to the vegetation. Assuming a maximum sustained swimming speed of 12.6 cm s^{-1} (Luckenback and Orth 1992) and a net ground speed of half this value (6.3 cm s^{-1}) during positive rheotaxis, then they could move upstream 227 m in 1 h. Thus, they have a limited area over which to locate appropriate nursery habitat.

Orientation of blue crab megalopae was modified by exposure to predator odors. The proportions of megalopae orienting upstream in *Zostera marina* odor water were reduced by exposure to odor from the fiddler crab *Uca pugilator* and the grass shrimp *Palaemonetes pugio* (Fig. 9), but not from the mud crab *Panopeus herbstii*. Thus, for two of the predator odors, downstream swimming of megalopae increased as the concentration of predator odor increased. All of the crustaceans tested are known to prey upon blue crab megalopae, and their presence has been shown to reduce settlement on passive collectors in the field (Welch et al. 1997). These results agree with the study of Diaz et al. (1999), which found that blue crab premolt megalopae avoided predator odor in a chemical choice apparatus.

Concentrations of ammonium chloride at 50 and 100 μM caused a decrease in the proportion of blue crab megalopae oriented upstream, whereas 10 μM had no effect. Since the concentrations of ammonia/ammonium in the highest crustacean odor solutions were < 1 μM, the responses to crustacean odor were not due to excretion of ammonia/ammonium. Levels of ammonium in estuaries vary with time of day, tidal phase, and location. In the study estuary (Newport River estuary), levels in water samples taken at night during flood-tide range from 3 to 5 μM (Litaker et al. 1993), which are below the concentrations that induced a shift in the orientation of megalopae (> 10 μM). Ammonium concentrations in the upper 3 cm of the surface of seagrass beds in the Newport River estuary are about 50 μM (Kenworthy et al. 1982). Also, ammonium concentrations in excess of 100 μM have been reported for samples taken from the surface of oysters and the crevices between oysters (Fitt and Coon 1992), which may serve as alternative nursery habitat for *C. sapidus* juveniles (Eggleston et al. 1998). These latter values are in the range (50–100 μM) that reduced upstream orientation of blue crab megalopae, suggesting crabs avoid oysters and the sediment surface in seagrass beds. The latter response further suggests that settlement occurs on or near seagrass blades. The possible functional significance is that ammonium concentrations are inversely related to oxygen levels under natural conditions (Fitt and Coon 1992). Thus, by avoiding areas with high ammonium concentration, blue crab megalopae also avoid areas with low oxygen levels. Tankersley and Wieber (2000) found that hypoxia increased mortality and the time to metamorphosis of blue crab megalopae.

Thus, the test hypotheses were confirmed, as blue crab megalopae oriented upstream in the presence of odors from seagrass and salt marsh cord grass, but the response was only present in premolt megalopae. Although settlement site location is probably a very complex process, these results suggest that orientation toward nursery areas occurs as current speeds decline at the end of nocturnal flood tides, when premolt megalopae swim upstream in response to odors from aquatic vegetation. Megalopae, however, appear capable of discriminating between microhabitats based on chemical odor, since orientation upstream in the presence of seagrass odor was reduced when odor from crustacean predators and ammonium were present.

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References

Aiken DE (1973) Proecdysis, setal development and molt prediction in the American lobster (*Homarus americanus*). J Fish Res Board Can 30:1337–1344

Anger K (1983) Moult cycle and metamorphosis in *Hyas araneus* larvae (Decapoda, Majidae), reared in the laboratory. Helgol Meersunters 36:285–302

Bower CE, Bidwell JP (1978) Ionization of ammonia in seawater: effects of temperature, pH and salinity. J Fish Res Board Can 35:1012–1016

Brookings KG, Epifanio CE (1985) Abundance of brachyuran larvae in a small coastal inlet over 6 consecutive tidal cycles. Estuaries 8:60–67

Brumbaugh RD, McConaugha JR (1995) Time to metamorphosis of blue crab *Callinectes sapidus*: effects of benthic macroalgae. Mar Ecol Prog Ser 129:113–118

Cronin TW, Forward RB Jr (1988) The visual pigments of crabs. I. Spectral characteristics. J Comp Physiol A 162:463–478

De Vries MC, Tankersley RA, Forward RB Jr, Kirby-Smith WW, Luettich RA Jr (1994) Abundance of estuarine crab larvae is associated with tidal hydrologic variables. Mar Biol 118:403–413

Diaz H, Orihuela B, Forward RB Jr, Rittschof D (1999) Orientation of blue crab, *Callinectes sapidus* megalopae: responses to visual and chemical cues. J Exp Mar Biol Ecol 233:25–40

Dittel AY, Epifanio CE (1982) Seasonal abundance and vertical distribution of crab larvae in Delaware Bay, USA. Estuaries 5:197–202

Eggleston DB, Etherington LL, Elis WE (1998) Organism response to habitat patchiness: species and habitat-dependent recruitment of decapod crustaceans. J Exp Mar Biol Ecol 223:111–132

Epifanio CE, Garvine RW (2001) Larval transport on the Atlantic continental shelf of North America: a review. Estuar Coast Shelf Sci 52:51–77

Fitt WK, Coon SL (1992) Evidence for ammonia as a natural cue for recruitment of oyster larvae to oyster beds in a Georgia salt marsh. Biol Bull (Woods Hole) 182:401–408

Forward RB Jr, Rittschof D (1994) Photoresponses of crab megalopae in offshore and estuarine water: implications for transport. J Exp Mar Biol Ecol 182:183–192

Forward RB Jr, Tankersley RA (2001) Selective tidal stream transport of marine animals. Oceanogr Mar Biol Annu Rev 39:305–353

Forward RB Jr, Frankel DAZ, Rittschof D (1994) Molting of megalopae from the blue crab *Callinectes sapidus*: effects of offshore and estuarine cues. Mar Ecol Prog Ser 113:55–59

Forward RB Jr, Tankersley RA, De Vries MC, Rittschof D (1995) Sensory physiology and behavior of blue crab (*Callinectes sapidus*) postlarvae during horizontal transport. Mar Freshw Behav Physiol 26:233–248

Forward RB Jr, De Vries MC, Rittschof D, Frankel DAZ, Bischoff JP, Fisher CM, Welch JM (1996) Effects of environmental cues on metamorphosis of the blue crab *Callinectes sapidus*. Mar Ecol Prog Ser 131:165–177

Forward RB Jr, Swanson J, Tankersley RA, Welch JM (1997a) Endogenous swimming rhythms of blue crab, *Callinectes sapidus*, megalopae: effects of offshore and estuarine cues. Mar Biol 127:621–628

Forward RB Jr, Tankersley RA, Blondel D, Rittschof D (1997b) Metamorphosis of the blue crab *Callinectes sapidus*: effects of humic acids and ammonium. Mar Ecol Prog Ser 157:277–286

Forward RB Jr, Tankersley RA, Rittschof D (2001) Cues for metamorphosis of brachyuran crabs: an overview. Am Zool 41:56–70

Forward RB Jr, Tankersley RA, Welch JM (2003) Selective tidal-stream transport of the blue crab *Callinectes sapidus*: an overview. Bull Mar Sci (in press)

Kenworthy WJ, Zieman JC, Thayer GW (1982) Evidence for the influence on the benthic nitrogen cycle in a coastal plain estuary near Beaufort, North Carolina (USA). Oecologia 54:152–158

Latz MI, Forward RB Jr (1977) The effect of salinity upon phototaxis and geotaxis in a larval crustacean. Biol Bull (Woods Hole) 153:163–179

Litaker W, Duke CS, Kenny BE, Ramus J (1993) Short-term environmental variability and phytoplankton abundance in a shallow tidal estuary. II. Spring and fall. Mar Ecol Prog Ser 94:141–154

Little KT, Epifanio CE (1991) Mechanism for re-invasion of an estuary by two species of brachyuran megalopae. Mar Ecol Prog Ser 68:235–242

Luckenback MW, Orth RJ (1992) Swimming velocities and behavior of blue crab (*Callinectes sapidus* Rathbun) megalopae in still and flowing water. Estuaries 15:186–192

Marrase C, Costello JH, Granata T, Strickler JR (1990) Grazing in a turbulent environment: energy dissipation, encounter rates, and efficacy of feeding currents in *Centropages hamatus*. Proc Natl Acad Sci USA 87:1653–1657

Mense DJ, Wenner EL (1989) Distribution and abundance of early life history stages of the blue crab *Callinectes sapidus* in tidal marsh creeks near Charleston, South Carolina. Estuaries 12:157–168

Morgan SG, Zimmer-Faust RK, Heck KL Jr, Coen LD (1996) Population regulation of blue crabs *Callinectes sapidus* in the northern Gulf of Mexico: postlarval supply. Mar Ecol Prog Ser 133:73–88

Olmi EF III (1994) Vertical migration of blue crab *Callinectes sapidus* megalopae: implications for transport in estuaries. Mar Ecol Prog Ser 113:39–54

Olmi EF III, van Montfrans J, Lipcius RN, Orth RJ, Saddler PW (1990) Variation in planktonic availability and settlement of blue crab megalopae in the York River, Virginia. Bull Mar Sci 46:230–243

Orth RJ, van Montfrans J (1987) Utilization of seagrass meadow and tidal creek by blue crab, *Callinectes sapidus*. I. Seasonal and annual variation in abundance with emphasis on post-settlement juveniles. Mar Ecol Prog Ser 41:283–294

Parsons TR, Maita Y, Lalli CM (1984) A manual for chemical and biological methods for seawater analysis. Pergamon, New York

Peters F, Gross T (1994) Increased grazing rates of microplankton in response to small-scale turbulence. Mar Ecol Prog Ser 115:299–307

Pettis RJ, Erickson BW, Forward RB Jr, Rittschof D (1993) Superpotent synthetic tripeptide mimics of the mud-crab pumping pheromone. Int J Pept Protein Res 42:312–319

Rittschof D (1993) Body odors and neutral–basic peptide mimics: a review of responses by marine organisms. Am Zool 33:487–493

Rosenthal R, Rosnow RL (1985) Contrast analysis: focused comparisons in the analysis of variance. Cambridge University Press, Cambridge

Rudjakov JA (1970) The possible causes of diel vertical migration of planktonic animals. Mar Biol 6:98–105

Stevenson JR (1985) Dynamics of the integument. In: Bliss DE (ed) The biology of Crustacea, vol 9. Integument, pigments and hormonal processes. Academic, New York, pp 1–42

Tankersley RA, Forward RB Jr (1994) Endogenous swimming rhythms in estuarine crab megalopae: implications for flood-tide transport. Mar Biol 118:415–423

Tankersley RA, Wieber MG (2000) Physiological responses of postlarvae and juvenile blue crabs *Callinectes sapidus* to hypoxia and anoxia. Mar Ecol Prog Ser 194:179–191

Tankersley RA, McKelvey LM, Forward RB Jr (1995) Responses of estuarine crab megalopae to salinity, pressure and light: implications for flood tide transport. Mar Biol 122:391–400

Tankersley RA, Welch JM, Forward RB Jr (2002) Settlement time of blue crab (*Callinectes sapidus*) post-larvae during selective tidal-stream transport. Mar Biol 41:863–875

Weinstein MP, Brooks HA (1983) Comparative ecology of nekton residing in a tidal creek and adjacent seagrass meadow: community composition and structure. Mar Ecol Prog Ser 12:12–27

Welch JM (1998) Behavior of blue crab (*Callinectes sapidus*) megalopae during transport and settlement site selection: effects of turbulence and chemical cues. PhD dissertation, Duke University, Durham, N.C.

Welch JM, Forward RB Jr (2001) Flood tide transport of blue crab, *Callinectes sapidus*, postlarvae: behavioral responses to salinity and turbulence Mar Biol 139:911–918

Welch JM, Rittschof D, Bullock TM, Forward RB Jr (1997) Effects of chemical cues on settlement behavior of blue crab *Callinectes sapidus* postlarvae. Mar Ecol Prog Ser 154:143–153

Welch JM, Forward RB Jr, Howd PA (1999) Behavioral responses of blue crab, *Callinectes sapidus*, postlarvae to turbulence: implications for selective tidal stream transport. Mar Ecol Prog Ser 179:135–143

Williams AB (1984) Shrimps, lobsters and crabs of the Atlantic coast of the eastern United States, Maine to Florida. Smithsonian Institution Press, Washington, D.C.

Wolcott DL, De Vries MC (1994) Offshore megalopae of *Callinectes sapidus*: depth of collection, molt stage and response to estuarine cues. Mar Ecol Prog Ser 109:157–163