Metamorphosis of the estuarine crab *Rhithropanopeus harrisii*: effect of water type and adult odor

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ABSTRACT Larvae of many estuarine crabs are transported to coastal/offshore areas where they develop and subsequently return to the estuary as postlarvae (megalopae), which settle and metamorphose. For these species, it is important for megalopae to be able to differentiate between offshore and estuarine areas as sites for metamorphosis. In contrast, larvae of the crab *Rhithropanopeus harrisii* (Gould) are retained in estuaries near the adult habitat throughout development. Although previous studies have demonstrated that the rate of metamorphosis of the megalopal stages of several estuarine crabs is accelerated when exposed to estuarine cues, it was hypothesized that metamorphosis of *R. harrisii* postlarvae is not affected by exposure to offshore and estuarine waters but is accelerated by exposure to adult odor cues. Metamorphosis of *R. harrisii* megalopae was accelerated upon exposure to estuarine water and delayed in offshore water at salinities ranging from 5 to 25 PSU. Time to metamorphosis in both water types increased as the salinity decreased. Adult odor also accelerated metamorphosis. Thus, the test hypothesis was only partially supported, and the results indicate that differentiation between offshore and estuarine areas for metamorphosis is common among estuarine crabs regardless of their patterns of larval development.

KEY WORDS: Megalopae · Metamorphosis · Settlement · Habitat selection · Chemical cues · Adult odor · *Rhithropanopeus harrisii*

INTRODUCTION

Larvae of many estuarine crabs are transported seaward, undergo development in coastal areas and return to the estuary as postlarvae (megalopae), which settle and metamorphose in appropriate estuarine sites. In estuaries along the east coast of the United States, this sequence is probably best studied in the blue crab *Callinectes sapidus* (e.g. Sandifer 1975, McConaugha et al. 1983, Epifanio et al. 1984, Goodrich et al. 1989, De Vries et al. 1994, Olmi 1994) and fiddler crabs *Uca* sp. (Christy 1982, Christy & Stancyk 1982, Lambert & Epifanio 1982, Epifanio et al. 1988). The developmental sequence also underlies the hypothesis proposed by Wolcott & De Vries (1994) that metamorphosis is delayed by exposure to offshore water but is accelerated by chemical cues associated with estuaries. This hypothesis was developed for blue crabs but applies equally to other brachyuran crab species with similar life histories.

In the case of *Callinectes sapidus*, metamorphosis is delayed by exposure to offshore water (Forward et al. 1994, 1996, 1997, Wolcott & De Vries 1994, Brumbaugh & McConaugha 1995) but accelerated by exposure to estuarine water (Forward et al. 1994, 1996), lower salinity (Forward et al. 1994), humic acids (Forward et al. 1997), and cues from estuarine aquatic vegetation such as seagrasses (Forward et al. 1994, 1996), saltmarsh cord grass (Forward et al. 1996) and macroalgae...

Larvae of the estuarine mud crab *Panopeus herbstii* do not develop as far seaward as blue crab and fiddler crab larvae and are retained in high salinity regions of estuaries and coastal areas near estuaries (Dittel & Epifanio 1982, Jones & Epifanio 1995). Nevertheless, metamorphosis of *P. herbstii* agrees with the hypothesis of Wolcott & De Vries (1994) in that metamorphosis is delayed in offshore water and accelerated by exposure to sediment from the adult habitat, adult chemical odors, and the estuarine macroalgae *Fucus vesiculosus* (Weber & Epifanio 1996).

Alternatively, the entire life cycle of the xanthid mud crab *Rhithropanopeus harrisii* is completed within estuaries. Adults occur in brackish water areas of estuaries (Williams 1984), while larvae are abundant in the low salinity areas of estuaries (Bousfield 1955, Pinschmidt 1963, Tagatz 1968, Sandifer 1973, 1975, Goy 1976) and rarely found in coastal waters (Nichols & Keney 1963, Dudley & Judy 1971). Intensive sampling by Cronin (1982) indicated that all larval stages are retained within the estuary near the adult habitat due to a tidal rhythm in vertical migration (Cronin & Forward 1979). Since they are rarely exported to coastal areas *for larval development*, differentiation between ocean and estuarine areas as sites for metamorphosis is unnecessary. However, since juvenile and adult *R. harrisii* are gregarious (Forward pers. obs.) postlarvae should settle and metamorphose in areas with existing adults. Thus, the present study tested the hypothesis that the length of the megalopal stage of *R. harrisii* is unaffected by exposure to offshore and estuarine waters but is shortened by exposure to adult odors. This hypothesis was partially supported, as metamorphosis was accelerated by exposure to both estuarine water and adult odor cues.

**MATERIALS AND METHODS**

**Collection of adults and rearing of larvae.** Ovigerous females of the crab *Rhithropanopeus harrisii* (Gould) were collected from the Neuse River (North Carolina, USA) between June and August 1997 and maintained in estuarine water (20 PSU; 23°C) on a light:dark cycle that approximated ambient conditions. A salinity of 20 PSU was used because it is near the optimum for larval development (Costlow et al. 1966). ‘Estuarine water’ was collected off Pivers Island in Beaufort, North Carolina, USA, which is located about 1 km from the inlet to the Newport River Estuary. Dilutions were made by the addition of deionized water and salinity was measured with a refractometer (American Optical Co.; accuracy ±0.5 PSU). Once hatched, zoeae were transferred to glass culture bowls (19 cm diameter) containing new 20 PSU estuarine water. Zoeae were fed brine shrimp nauplii *Artemia franciscana* Kellogg, which had recently hatched in offshore water. Each brood was maintained in a separate bowl and large broods were divided into 2 or 3 bowls. Larvae were maintained on a 14 h light:10 h dark cycle at 25°C using a controlled Environment Chamber (Sherer-Gillett, Model CEL-4). Zoeae were kept in the chamber at all times, except for a brief period daily when they were placed in new 20 PSU estuarine water and fed recently hatched brine shrimp nauplii.

Larvae were reared in estuarine water for the first 8 d of development. The majority of larvae reached Stage III zoeae within this time. They were then transferred to 20 PSU ‘offshore water’ for the remainder of development to the megalopal stage. The offshore water was collected between 13 and 16 km seaward of the Newport River Estuary Inlet and diluted to 20 PSU with deionized water. This water was collected from a region beyond the estuarine *plume* and has previously been shown to lack cues found in estuarine water that accelerate the rate of postlarval development in other crab species (Forward et al. 1994, 1996, 1997). Offshore water was collected at the beginning of each experiment and stored at room temperature in carboys. Thus, during development of the last zoal stage (IV), larvae were assumed to be in water devoid of estuarine and adult odor cues that could influence the time to metamorphosis of the megalopal stage.

**General procedure.** The majority of zoeae molted to the megalopae stage between 11 and 13 d after hatching. Each day, newly molted megalopae from each brood were placed together in a single culture bowl. These megalopae were mixed and haphazardly distributed into experimental treatments. This procedure helped ensure that megalopae from all broods were distributed relatively evenly among the treatments.

The general procedure for all experiments was to test 54 megalopae in each treatment condition. A single megalopa was placed in its own compartment (3.4 × 3.4 × 3 cm) of a plastic box. Each box had 18 compartments, and each compartment was filled with approximately 15 ml of water containing the test chemical cues (test water) and approximately 30 to 50 brine shrimp nauplii. The megalopae were maintained in the environmental chamber at the same conditions used for rearing (see ‘Collection of adults and rearing of larvae’). The test water in the compartments was changed daily and new brine shrimp nauplii were added. The plastic test boxes were washed with distilled water, rinsed, and allowed to air-dry overnight.
During all experiments, megalopae were monitored for metamorphosis at 8 h intervals (08:00, 16:00, and 24:00 h). Experiments continued until all megalopae had molted to the first crab stage (instar). Mortality was rare in the first 2 experiments, as it was below 10% in each treatment. The last 2 experiments were carried out later in the summer, and mortality in each treatment was about 30%. The time to metamorphosis was measured as the duration of the megalopall stage, i.e., time between molting to the megalopall stage and metamorphosis to the first crab stage. Since no megalopae molted earlier than 3 d, monitoring postlarvae for metamorphosis to the first crab stage began 3 d after the beginning of each experiment.

Statistical analysis. The effects of various environmental factors on the time to metamorphosis of *Rhithropanopeus harrisii* megalopae were determined using 1- and 2-factor analysis of variance (ANOVA). For most experiments, time to metamorphosis values could not be transformed to meet the assumptions of normality and homogeneity of variances among groups. Therefore, the data were rank-transformed prior to analysis using the procedures outlined by Conover & Iman (1981). Since descriptive statistics of ranked values are often difficult to interpret, means (± SE) for the original (non-transformed) data are plotted in the figures. When ANOVA results indicated significant treatment effects, treatments were compared using either a Tukey test (for comparison of all treatment combinations) or a Dunnett multiple comparison test (for comparisons of controls with all other treatment groups; Zar 1996). For comparisons using the Dunnett test (i.e., Expts 3 and 4), 1-tailed tests were conducted since initial experiments (i.e., Expt 1) indicated a decrease in the length of the megalopall stage was expected when postlarvae were incubated in the presence of chemical odors from adults.

RESULTS

Effect of estuarine and adult odor cues

Results of a 1-way ANOVA revealed that water type (offshore, estuarine, and offshore plus adult odor) had a significant effect on the rate of metamorphosis of *Rhithropanopeus harrisii* megalopae (*F* = 12.11; *df* = 2, 151; *p* < 0.0001). Time to metamorphosis was significantly shorter in both 20 PSU estuarine water and 20 PSU offshore water + *R. harrisii* adult odor compared to 20 PSU offshore water (Tukey multiple comparison test; Fig. 1). There was no significant difference between the rate of metamorphosis of megalopae in estuarine water and offshore water containing *R. harrisii* adult odor cues (Fig. 1). These results suggest
there are chemical cues present in estuarine water that
decrease the duration of the megalopal stage in *R. harrisii*
and that adults also release a chemical odor that
has a similar effect on the time to metamorphosis.

**Effect of water type and salinity**

Since the first experiment demonstrated that *Rhithropanopeus harrisii*
megalopae metamorphose faster in estuarine water as compared to offshore water, the
second experiment determined whether the effect of
water type on the time to metamorphosis was influ-
enced by salinity (Fig. 2). Results of a 2-way ANOVA
indicated that both water type (offshore and estuarine)
and salinity (5, 15, and 25 PSU) had a significant
influence on the rate of metamorphosis (water type:
\( F = 19.45; \) df = 1.285; \( p < 0.0001 \); salinity: \( F = 72.59; \)
\( df = 2.285; \) \( p < 0.0001 \)). For both water types, the time
to metamorphosis decreased with increasing salinity
and there was no significant interaction between the
2 factors (Fig. 2; \( F = 0.136; \) df = 2.285; \( p > 0.5 \)). Thus,
megalopae responded in similar ways to changes in
salinity, regardless of water type. A rise in salinity
resulted in a decrease in the time to metamorphose to
the first crab stage (Fig. 2) in both estuarine and off-
shore water and times were shortest in estuarine water.

**Effect of adult odor cues**

Megalopae were found to shorten their time to meta-
morphosis when exposed to 20 PSU offshore water
containing odors from *Rhithropanopeus harrisii* adults
(Fig. 1). The final 2 experiments measured the re-
response of postlarvae to declining concentrations of
adult odors. Since it was assumed that odor concen-
tration was proportional to crab weight, different con-
centrations were generated by incubating different
weights of crabs in a fixed volume of offshore water.
The control for these experiments was exposure to off-
shore water, in which crabs were not incubated.

A dose response curve was obtained for adult odor
cue concentrations ranging from 1 g of adult \( 1^{-1} \) to 10 g
\( 1^{-1} \) (Fig. 3). As expected from Expt 1 (Fig. 1), the addi-
tion of adult odors to offshore water had a significant
effect on the rate of metamorphosis of Rhithropanopeus harrisii megalopae ($F = 15.56$; df = 4.193; $p < 0.0001$).

Results of a 1-tailed Dunnett comparison test revealed that the time to metamorphosis was significantly shorter in all treatments containing adult odors compared to offshore water alone (control).

Since all treatments in Fig. 3 significantly accelerated the time to metamorphosis, the experiment was repeated with lower concentrations (0.1 to 1.0 g of adult 1-l) of adult odor, in an attempt to determine the minimum concentration (threshold) necessary to induce a significant decrease in the time to metamorphosis (Fig. 4). Again, there was a significant overall treatment effect for the adult odor cue ($F = 6.50$; df = 3, 163; $p < 0.001$). A Dunnett multiple comparison test (1-tailed) indicated that the rates of metamorphosis for all treatments were significantly different ($p < 0.05$) from the control (offshore water). These results suggest that the threshold concentration for the adult odor cue is less than 0.1 g 1-l.

**DISCUSSION**

Adult blue crab Callinectes sapidus (e.g. Sandifer 1975, McConaughy et al. 1983, Epifanio et al. 1984, Goodrich et al. 1989, De Vries et al. 1994, Olmi 1994) and fiddler crabs Uca sp. (Christy 1982, Christy & Stancyk 1982, Lambert & Epifanio 1982, Epifanio et al. 1988) inhabit estuaries (Williams 1984) but their larvae develop in coastal/oceanic areas. Transport back into estuaries occurs during the megalopae stage, which subsequently metamorphose in appropriate estuarine sites. Thus, for these species it is important that megalopae be able to differentiate between coastal/oceanic and estuarine areas as sites for metamorphosis. Hence, Wolcott & De Vries (1994) hypothesized that metamorphosis of blue crab megalopae is delayed in offshore water and accelerated by cues associated with estuaries.

Since larvae and megalopae of the mud crab Rhithropanopeus harrisii are retained in low salinity areas of estuaries (e.g. Cronin 1982), there is no necessity for megalopae to differentiate between offshore and estuarine areas. Hence, Wolcott & De Vries (1994) hypothesized that metamorphosis of blue crab megalopae is delayed in offshore water and accelerated by cues associated with estuaries. Since larvae and megalopae of the mud crab Rhithropanopeus harrisii are retained in low salinity areas of estuaries (e.g. Cronin 1982), there is no necessity for megalopae to differentiate between offshore and estuarine areas. Thus, it was hypothesized that the rates of development of R. harrisii megalopae would be similar in offshore and estuarine waters. The first experiment (Fig. 1) clearly falsified this hypothesis, since metamorphosis was delayed in offshore water and accelerated in estuarine water. This result indicates that even though R. harrisii larvae are retained in estuaries, the postlarval stage can differentiate between offshore and estuarine waters and accelerate the time to metamorphosis in response to chemical cues in estuarine water. This species may have the ability to differentiate between offshore and estuarine waters because megalopae may be accidentally transported seaward and delay metamorphosis until they are transported back to an estuarine location. Possible estuarine chemical cues responsible for the accelerated development of R. harrisii postlarvae include odors from aquatic vegetation (e.g. Forward et al. 1996) and humic acids (Forward et al. 1997).

The accelerating effect of estuarine water was also evident when megalopae were exposed to different salinities of estuarine and offshore waters (Fig. 2). The time to metamorphosis was always faster in estuarine than offshore water but time decreased as the salinity increased for both water types. Since there was no statistical interaction between salinity and water type (see ‘Results’), the decrease in the time to metamorphosis as the salinity increased was due to salinity and not water type. This pattern appears to be consistent among Rhithropanopeus harrisii larval stages, since Costlow et al. (1966) observed a similar increase in the time of development from the first zoeal stage to the first crab stage with a decrease in salinity from 25 to 5 PSU. This relationship may reflect the adverse effects of salinity on development, since mortality also increased as the salinity decreased over the same salinity range (Costlow et al. 1966). This effect of salinity on metamorphosis is the opposite of that for Callinectes sapidus, for which the time to metamorphosis decreased as the salinity of estuarine water decreased (Forward et al. 1994).

Ovigerous Rhithropanopeus harrisii were collected in wire mesh traps containing mollusc shells (Forward
et al. 1982). *R. harrisii* appear to live gregariously because traps contained large numbers of males and females of all sizes (Forward pers. obs.). Both the initial experiment (Fig. 1) and the dose/response relationship depicted in Figs. 3 & 4 indicated that metamorphosis was accelerated by exposure to adult odor. Megalopae were very sensitive to adult odor, since metamorphosis was accelerated by exposure to concentrations of adult odor as low as 0.1 g of adult L-1 (Fig. 4). These observations suggest that megalopae settle and metamorphose in the presence of conspecifics.

Adult odor also accelerates metamorphosis in *Uca pugilator* (O'Connor 1991) and *Panopeus herbstii* (Weber & Epifanio 1996) but not in *Callinectes sapidus* (Forward et al. 1994). These species differences may result from adult distributions. *Rhithropanopeus harrisii*, *U. pugilator*, and *P. herbstii* live as adults in benthic aggregations, whereas *C. sapidus* is a swimming crab that ranges throughout estuaries (Williams 1984).

Thus, the test hypothesis was partially supported in that metamorphosis of *Rhithropanopeus harrisii* was accelerated by exposure to adult odor and estuarine water but was delayed in offshore water. Since larval development of *R. harrisii* takes place in estuaries and does not typically include a coastal/oceanic phase or an up-estuary migratory phase, the pattern of delaying metamorphosis in ocean water and acceleration in response to estuarine chemical cues appears to be a common pattern for estuarine crabs regardless of their developmental sequence.

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