

THE CIRCATIDAL RHYTHM IN VERTICAL SWIMMING OF FEMALE BLUE CRABS, *CALLINECTES SAPIDUS*, DURING THEIR SPAWNING MIGRATION: A RECONSIDERATION

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ABSTRACT Ovigerous blue crabs, *Callinectes sapidus* Rathbun, undergo ebb-tide transport (ETT) during the spawning migration from estuaries to coastal areas where they release larvae. An initial field study found that only females with mature embryos underwent the spawning migration and that postspawned females switched to flood-tide transport for movement back into and up estuaries. Alternatively, a more recent field study found ETT occurred in females throughout embryo development and continued after larval release. An endogenous rhythm in vertical migration underlies ETT during the spawning migration. A past study found the circatidal rhythm was only present in females with mature embryos but not in females with immature embryos or after larval release. The present study reinvestigates this rhythm in vertical migration. Both females with early embryos and postlarval release females entrained in the field have circatidal rhythms, in which they swim vertically at the time of consecutive ebb tides at the collection site or on alternate ebb tides. These results support the field study that ovigerous females undergo ETT throughout embryo development and after larval release. Thus, females will be transported seaward and release subsequent larval clutches in coastal areas that increase the likelihood that larvae will be transported to offshore developmental areas.

KEY WORDS: blue crabs, *Callinectes sapidus*, circatidal rhythms, spawning migration, ebb-tide transport

INTRODUCTION

The blue crab, *Callinectes sapidus*, is a swimming crab that migrates between coastal and estuarine areas at different phases of its life cycle. Ovigerous females occur in high salinity areas of estuaries and migrate seaward to release larvae during the second phase of their spawning migration (Forward et al. 2003b). In the seminal field study of this migration, Tankersley et al. (1998) observed ovigerous females swimming at the surface of an estuary only during nocturnal ebb tides and concluded they were undergoing ebb-tide transport (ETT) for migration to coastal larval release areas. Subsequently, postspawned females were observed swimming at the surface during nocturnal flood tides, which indicated they were undergoing flood-tide transport (FTT) for movement into and up the estuary (Tankersley et al. 1998). ETT by ovigerous females was further verified by following females with attached sonic tags during their spawning migrations (Carr et al. 2004).

In a related study Hench et al. (2004) tethered female blue crabs in an estuary and interpreted their selective tidal stream transport pattern from miniature internally recording pressure sensors. Ovigerous females swam vertically on ebb tides during both day and night throughout embryo development and remained on the bottom during flood tide. In contrast to the observations of Tankersley et al. (1998), Hench et al. (2004) found that postlarval release females continued ETT and did not switch to FTT.

Forward et al. (2003a) and Forward and Cohen (2004) studied the behavior underlying ETT and observed that ovigerous females had an endogenous rhythm in vertical migration. Under constant conditions in the laboratory, females with mature eggs had a circatidal rhythm in which they have frequent bouts of swimming to the surface of a vertical column during the time of ebb tide in the field, and they remain on the bottom during the time of flood tide in the field (Forward et al. 2003a, Forward & Cohen 2004). The rhythm was not present in females with immature eggs, disap-

peared after larval release under constant conditions (Forward et al. 2003a) and was not affected by the light:dark cycle (Forward & Cohen 2004).

Thus, there are two inconsistencies in results from the laboratory study of endogenous rhythms in vertical migration (Forward et al. 2003a) and the field study by Hench et al. (2004). First, the field study found that ovigerous females vertically migrated throughout embryo development not just with mature embryos as was observed in the laboratory. Second postlarval release females continued ETT in the field (Hench et al. 2004), whereas the rhythm in vertical swimming disappeared after larval release in constant conditions (Forward et al. 2003a).

The present study was undertaken to re-examine the endogenous rhythm in vertical migration of: (1) females with immature embryos and (2) postlarval release females. A circatidal rhythm in vertical swimming that would contribute to ETT was observed for females at both life cycle stages.

MATERIAL AND METHODS

Female blue crabs, *Callinectes sapidus* Rathbun, were collected at night during July through October 2002 and 2004 from a tidal flat near the Duke University Marine Laboratory in the Newport River Estuary, North Carolina (34°43'N; 76°40'W). Ovigerous females were classified according to the developmental stage of their egg masses (De Vries et al. 1983) by examining small groups of eggs (10–20) under a dissecting microscope. Crab egg masses were grouped based on yolk content and embryo eye development. Only females with early-stage eggs that were yellow/orange in color and contained embryos with >85% yoke (Stages 1–2 of De Vries et al. 1983) were used in experiments with ovigerous crabs. Nonovigerous females that had released larvae (postlarval release) were collected in the field and identified by morphologic characteristics including the inability to fully close the abdomen, remnants of the egg mass attached to the pleopods and mature ovaries visible through the area near the dorsal spines.

Endogenous rhythms in vertical swimming behavior were monitored under constant conditions in the laboratory using pre-

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vious techniques (Forward et al. 2003a, Forward & Cohen 2004). The experimental chambers were cylindrical tubes (1.23 m × 44 cm dia.), which were filled with ambient estuarine water (32–35 psu; 25°C). The estuarine water was filtered to remove particles >5 μm and was aerated throughout the experiments. Tubes were illuminated continuously with red fluorescent lamps (40 W) covered by red cellophane. Blue crab visual pigments (e.g., Cronin & Forward 1988) predict they should have low visual sensitivity to red light. After collection, crabs were maintained on the ambient light: dark cycle and experiments begun at the time of the next sunset in the field. Crabs were placed individually in tubes under constant conditions and not fed during experiments. Their behavior was monitored for at least 6 tidal cycles with a video camera (RCA Model TC1005) and recorded with a time-lapse video recorder (Panasonic Model AGRT600A).

There were three sets of experiments. First, 19 females with early-stage eggs were placed under constant conditions in the tubes to determine whether the rhythm in vertical migration was present. Second, eight females that had released larvae under constant conditions in the laboratory were placed individually in plastic baskets suspended at a depth of about 3 m (at high tide) at the Duke University Marine Laboratory (Beaufort, North Carolina). Semidiurnal tides occur at this site. They remained in the baskets from 2 to 8 days and then were placed under constant conditions in the tubes. Because females become arrhythmic after larval release under constant conditions (Forward et al. 2003a), this experiment determined if the vertical migration rhythm could be reactivated by exposure to tidal cycles in the field. For the third experiment, six crabs that had released larvae were collected from the field and tested for a rhythm in vertical migration.

In the tubes, crabs either remained on the bottom or swam upward toward the surface of the water. They continued to swim at the surface for up to 2–3 min and then returned to the bottom. Behavior was quantified by counting the number of vertical swimming bouts in each 30-min interval. A crab was considered to undergo a swimming bout if it ascended above the lower 1/3 of the tube (41 cm high). Because there was agreement between the rhythms of crabs in the same conditions, representative figures will be presented with information about the other crabs.

Time series of behavioral rhythms were analyzed using a combination of autocorrelation analysis and maximum entropy spectral analysis (MESA) algorithms (Levine et al. 2002). Autocorrelation analysis was used to determine if each time series had significant periodicity; autocorrelation plots with peaks exceeding the 95% confidence interval of $\pm 2/\sqrt{N}$ (N = sample size) were considered to have statistically significant periodicity ($P < 0.05$; Chatfield 1989). The period estimates for time series with statistically significant periodicity were determined using MESA, which fits an autoregressive model to the data and uses Fourier analysis of the autoregressive coefficients to construct a spectrum. Only those peaks in the MESA plots that corresponded to large peaks (i.e., exceeding the 95% confidence intervals) in the autocorrelation plots were considered to be significant.

Synchrony between swimming bouts and the tidal cycle in the field was determined using cross-correlation analysis (MATLAB software; Levine et al. 2002). Tide height relative to mean lower low water (MLLW) was obtained from a tides program (Nautical Software) for the nearest location to the collection sites. Because crabs migrate horizontally and tidal times change throughout the estuary, tidal times and heights only approximate the specific tidal regimen the field caught crabs encountered prior to collection.

Plots of cross-correlation as a function of lag interval (1 lag = 0.5 h) were used to compare behavior to tide stage. Thus, peaks at positive or negative lag intervals indicated that maximum swimming activity occurred that many hours after (+lags) or before (–lags) high tide. Cross-correlations exceeding the 95% confidence intervals ($\pm 2/\sqrt{N}$) were considered to be statistically significant (Chatfield 1989).

RESULTS

Under constant conditions, crabs had either: (1) a circatidal rhythm in vertical migration with displays at the time of consecutive ebb tides in the field; (2) one display each lunar day at the time of ebb tide in the field or (3) no rhythm. Among the 19 ovigerous crabs tested with early-stage eggs, 16 had circatidal rhythms with an average period length of 12.55 h (SD = 0.58 h) (Fig. 1A). Cross correlation analysis found that the mean time of peaks was 1.34 h (SD = 1.22 h) after high tide in the field. The other 3 ovigerous crabs had rhythms with an average period length of 24.67 h (SD = 1.11 h) (Fig. 1B). Peaks in activity all occurred

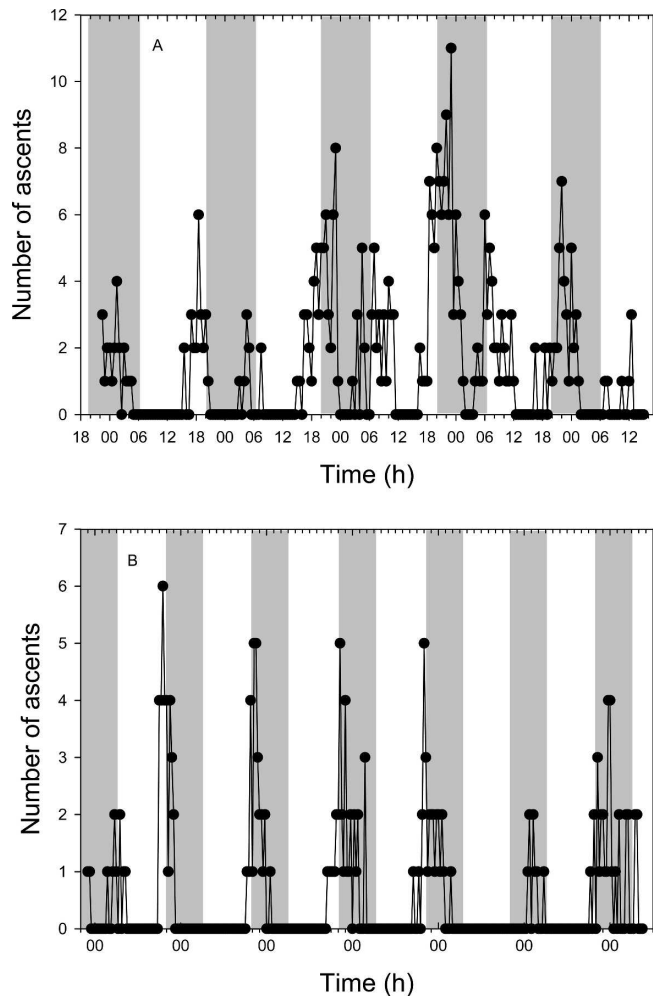


Figure 1. Actographs of the number of swimming bouts at 0.5 h intervals for two representative ovigerous *Callinectes sapidus* with early-stage embryos that are active at the time of consecutive ebb tides in the field (A) or on alternate ebb tides (B). The free running period length in A is 12.7 h and in B is 25.1 h. The time of peaks occurred 2.5 h (A) and 0.5 h (B) after the time of high tide in the field at the collections site.

during the time of twilight or night in the field and had an average time of 0.5 h (SD = 2.17 h) before the time of high tide. In most cases the first two peaks occurred about 12 h apart after which only one peak occurred each lunar day (e.g., Fig. 1B). Thus, vertical migration peaks occurred at high tide or during ebb tide in the field, which is consistent with ebb tide transport. The relationship of activity peaks to high tide did not result because experiments were conducted when high tide occurred at the same time of night or because most crabs were tested in only one experiment. About equal numbers of crabs (3 or 4) were tested on 5 occasions. The times of nocturnal high tide at the collection site at the times of experiments were 00:35, 00:37, 03:07, 05:05 and 05:12 h.

The eight test females that had released larvae and were then placed back in the estuary for 2 to 8 days displayed all 3 types of rhythms. One crab was arrhythmic, whereas four crabs (Fig. 2A) had circatidal rhythms with an average period length of 11.75 h (SD = 0.6 h). The average time of peak activity was 1.25 h (SD = 1.89 h) after the time of high tide in the field. The three other crabs had one peak in activity each lunar day (Fig. 2B) with

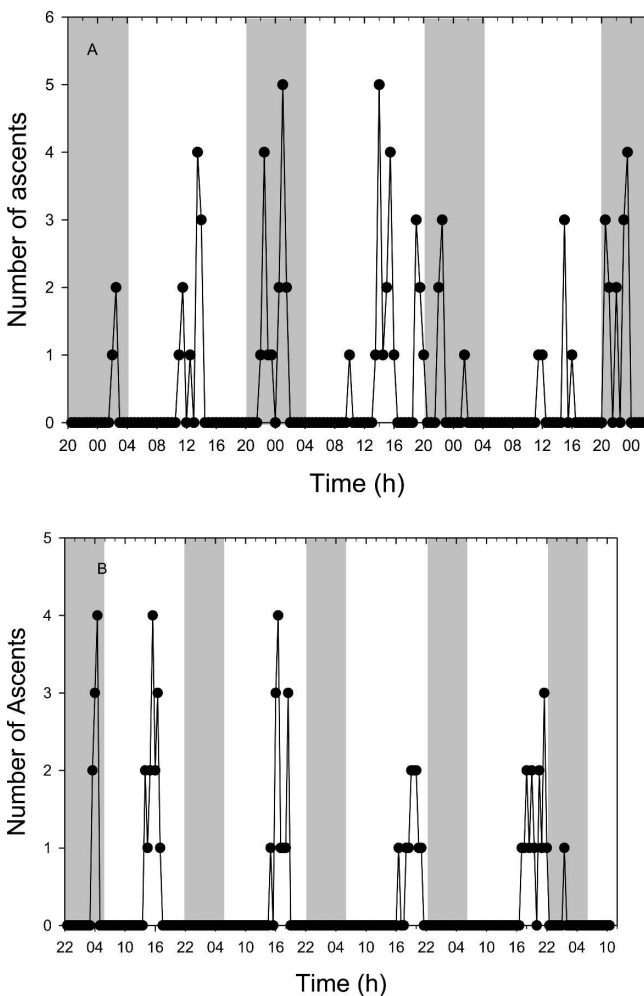


Figure 2. Actographs of the number of swimming bouts at 0.5 h intervals for two representative *Callinectes sapidus* that were exposed to semidiurnal tidal conditions in an estuary for about 3 (A) or 6 (B) days after larval release. Crab A had a free running period of 11.7 h with the time of peaks corresponding to the time of high tide in field. Crab B had a free running period of 24.7 h with peaks occurring at 2 h after the time of high tide in the field.

an average period length of 24.23 h (SD = 0.57 h). The peaks had an average lag of 1.33 h (SD = 1.15 h) after the time of high tide in the field.

The six crabs collected in the field that had released larvae displayed only two of the rhythm types. Three crabs were arrhythmic. The other 3 crabs had circatidal rhythms (Fig. 3) with a mean period of 12.23 h (SD = 0.21 h). Peaks for these 3 crabs occurred at about the time of high tide in the field (mean = -0.17 h; SD = 1.76 h). Experiments with nonovigerous crabs tested three to four crabs on four dates, each of which had different times of high tide at night at 21:39, 23:39, 03:45 and 04:15 h. Thus, the lags were unrelated to a specific time of night.

DISCUSSION

Ovigerous female blue crabs, *Callinectes sapidus*, migrate seaward to release larvae near the entrance to estuaries or in coastal areas. The majority of seaward movement occurs though ebb-tide transport (ETT) in which females swim in the water column during ebb tides and remain on the bottom during flood tides (Tankersley et al. 1998, Carr et al. 2004). The inconsistencies of past field studies of the spawning migration are that Tankersley et al. (1998) observed ovigerous females with mature embryos undergoing ETT followed by flood-tide transport (FTT) by postrelease females. In contrast, Hench et al. (2004) found ovigerous females migrated vertically during ebb tides throughout embryo development and after larvae release. A reversal in swimming from ebb to flood tide was not observed after larval release.

ETT could be based on an endogenous rhythm in vertical migration or responses to environmental cues associated with the different tidal phases (Forward & Tankersley 2001). Laboratory studies found that female blue crabs with mature embryos have a circatidal rhythm in vertical migration, in which they make vertical ascents into the water column during the time of consecutive ebb tides in the field at their collections sites (Forward et al. 2003a, Forward & Cohen 2004). Rhythmic vertical migration disappears immediately after larval release under constant conditions in the laboratory (Forward et al. 2003a). The inconsistencies with the field study of Hench et al. (2004) were that the circatidal rhythm

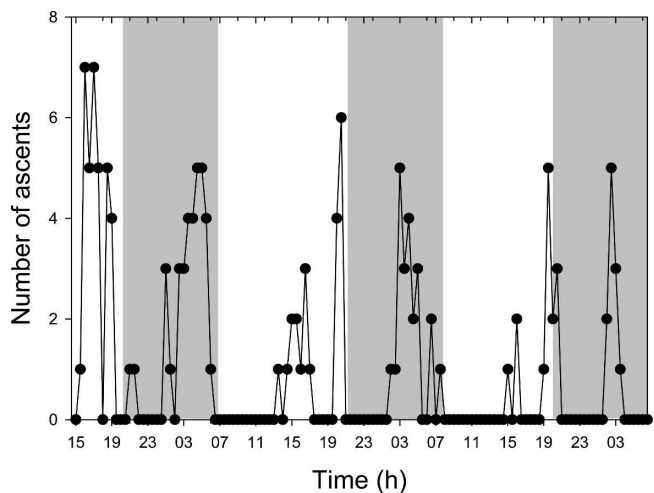


Figure 3. Actographs of the number of swimming bouts at 0.5 h intervals for a representative postlarval release *Callinectes sapidus* female collected in the field. The free running period was 12.0 h and the time of peaks corresponded to the time of high tide in the field.

in vertical migration was not present in females with immature embryos or in females after larval release (Forward et al. 2003a).

The present study was designed to reinvestigate the endogenous rhythms in vertical migration of female blue crabs with immature embryos and of postlarval release females. The results support the field study of Hench et al. (2004). Most females with immature embryos had a circatidal rhythm in vertical migration, in which they swam at the time of consecutive ebb tides in the field. The average time of peak swimming was 1.34 h after the time of high tide in the field. A minority of crabs had a circatidal rhythm, in which they only vertically migrated at the time of alternate ebb tides. The average free running period length was close to that for a lunar day and the average time of peak activity was 0.5 h after the time of high tide in the field. These rhythms were not observed in our previous study of females with immature embryos (Forward et al. 2003a). We have no explanation for the absence of the rhythms in the previous study. Since ebb currents begin about 0.5 h after the time of high tide at the collection site, a peak in activity shortly after high tide would insure seaward transport during ebb tide.

Females that were placed in semidiurnal tidal conditions for 2 to 8 days after larval release and then tested in constant conditions also had the two types of endogenous rhythms observed for ovigerous crabs. Average peaks in vertical migration occurred about 1.3 h after the time of high tide in the field. In contrast, postlarval release females collected from the field were more variable, as three crabs had the circatidal rhythm and three were arrhythmic. Because the time between larval release and testing was not known, it was possible that the arrhythmic crabs had just released larvae. Forward et al. (2003a) found that the crabs were arrhythmic just after larval release under constant conditions, and Hench et al. (2004) observed that vertical migration at the time of ebb tide disappeared immediately after larval release.

Thus, the observed sequence in endogenous rhythms agrees with the field study of Hench et al. (2004). Ovigerous crabs with immature embryos have a circatidal rhythm in vertical migration that underlies ETT. Females either swim on consecutive ebb tides or only during alternate ebb tides in the field. The rhythm is very pronounced in females with mature embryos but does not continue immediately after larval release (Forward et al. 2003a, Forward &

Cohen 2004). If postlarval release females are exposed to semidiurnal tidal conditions for 2 to 8 days, the rhythm becomes re-entrained and is present in constant conditions.

Hench et al. (2004) observed that females with immature embryos either made vertical ascents during consecutive ebb tides or only during nocturnal ebb tides and the frequency of the ascents increased as the time of larval release approached. Vertical migration abruptly decreased immediately after larval release and increased in frequency during the week after release. Because Hench et al. (2004) found that peak vertical migration bouts corresponded to rates of decrease in hydrostatic pressure during ebb tide, the cue for re-entrainment of the endogenous rhythm could be the tidal cycle in hydrostatic pressure associated with changes in water depth as has been found for other marine animals (Palmer 1995).

Neither the present laboratory rhythm studies nor the Hench et al. (2004) field study found a switch in the time of vertical migration from ebb to flood tide after larval release as predicted from the field study of Tankersley et al. (1998). A minority of crabs may undergo this reversal but none were used in the present study or by Hench et al. (2004). The implications are that on becoming ovigerous, a female undertakes ETT and migrates seaward. Transport wanes immediately after larvae release but if females are still in estuarine areas, they continue ETT thereafter and move out of estuaries to coast/offshore areas. Females mate only once and store sperm for fertilization of multiple egg broods (Hines et al. 2003). Thus after migrating seaward, release of subsequent clutches of larvae would occur in coastal areas where the probability of larval transport to offshore development areas increases and transport back into the estuary is less likely. These results predict that the proportion of reproducing females in coastal areas will increase throughout the spawning season and that larval release will continue after the observed seasonal peak in the number of ovigerous females migrating seaward from estuaries.

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