Mar Biol (2010) 157:2415–2425
DOI 10.1007/s00227-010-1506-5

ORIGINAL PAPER

Endogenous swimming rhythms underlying the spawning migration of the blue crab, *Callinectes sapidus*: ontogeny and variation with ambient tidal regime

M. Zachary Darnell · Dan Rittschof · Richard B. Forward Jr.

Received: 1 April 2010 / Accepted: 23 June 2010 / Published online: 13 July 2010

Abstract Spawning female blue crabs, *Callinectes sapidus*, use ebb-tide transport (ETT) to migrate seaward. In estuaries with semi-diurnal tides, ETT in ovigerous blue crabs is driven by a circatidal rhythm in vertical swimming in which crabs ascend into the water column during ebb tide. The ontogeny of this rhythm was examined by monitoring swimming behavior of females before the pubertal molt, females that had recently undergone the molt but had not yet produced a clutch of eggs, and ovigerous females from an estuary with strong semi-diurnal tides. To assess variation in swimming rhythms with ambient tidal regime, swimming rhythms of ovigerous females from semi-diurnal (Beaufort, North Carolina), diurnal (St. Andrew Bay, Florida), and non-tidal (South River, North Carolina) estuaries were compared. Experiments were conducted during the summers of 2006–2008. Female crabs prior to oviposition had variable endogenous swimming rhythms (circadian, circatidal, or circalunidian). Ovigerous females from estuaries with semi-diurnal and diurnal tides had pronounced circatidal or circalunidian rhythms with swimming during the time of ambient ebb tide. Swimming rhythms of several ovigerous crabs switched between circatidal and circalunidian during the ~5-day observation period. Ovigerous crabs from a non-tidal estuary had a circadian rhythm with vertical swimming around the time of sunset. These results suggest that, while endogenous swimming rhythms are present in some female blue crabs prior to oviposition, rapid seaward movement via ETT in tidal estuaries begins following oviposition of the first clutch of eggs.

Communicated by J. P. Grassle.

Electronic supplementary material The online version of this article (doi:10.1007/s00227-010-1506-5) contains supplementary material, which is available to authorized users.

M. Z. Darnell · D. Rittschof · R. B. Forward Jr.
Duke University Marine Laboratory,
Nicholas School of the Environment,
135 Duke Marine Lab. Rd., Beaufort, NC 28516, USA

Present Address:
M. Z. Darnell (✉)
Marine Science Institute,
The University of Texas at Austin,
750 Channel View Drive, Port Aransas, TX 78373, USA
e-mail: mzd@mail.utexas.edu

Introduction

Estuarine organisms often use tidal currents to enhance migratory ability or decrease the energetic cost of migrating. Selective tidal-stream transport (STST) is a common mechanism and occurs when an organism ascends into the water column during one phase of the tide and is transported by tidal currents. During the opposite tidal phase, the organism remains on or near the bottom (Forward and Tankersley 2001). The net result is transport in or out of an estuary. Using STST, small organisms such as larvae are able to use tidal currents for long-distance migrations, while larger organisms such as crabs and fish save substantial amounts of energy compared to active migration against tidal currents (Metcalfe et al. 1990).

The behavior underlying STST is a tidal vertical migration into the water column during one phase of the tide. Selective tidal-stream transport can be described as either ebb-tide transport (ETT) or flood-tide transport (FTT), depending on the phase of the tide for vertical migration into the water column. While vertical migration can result from responses to exogenous environmental cues (e.g. Welch et al. 1999), it is often driven by an endogenous

circatidal rhythm (Cronin and Forward 1979; Zeng and Naylor 1996; Forward and Tankersley 2001; Lopez-Duarte and Tankersley 2007). Species inhabiting different habitats at different life-history stages may undergo ontogenic changes in the direction or underlying mechanism of STST (Forward and Tankersley 2001).

Blue crabs, *Callinectes sapidus*, have a complex, migratory life history. Most adults mate in the low-salinity waters of upper estuaries (Millikin and Williams 1984; Ramach et al. 2009). Some time after mating, females undertake a spawning migration to high-salinity waters of the inlets and coastal ocean (Millikin and Williams 1984; Tankersley et al. 1998; Carr et al. 2004), during which multiple clutches of larvae are released (Hines et al. 2003; Dickinson et al. 2006; Darnell et al. 2009). Ovigerous females (Tankersley et al. 1998; Forward et al. 2003) and females between clutches of eggs (Hench et al. 2004; Forward et al. 2005) use ETT to migrate seaward. In spawning female blue crabs, ETT is driven by an endogenous rhythm in which females swim upward into the water column during ebb tide and remain on the bottom during flood tide (Forward et al. 2003). This rhythm is present in ovigerous blue crabs during all stages of egg development, in many crabs between clutches of eggs and continues as crabs cycle through successive clutches (Hench et al. 2004; Forward et al. 2005). The effects (if any) of the semi-lunar spring/neap cycle on this swimming rhythm, and thus on migratory behavior, have yet to be investigated.

In estuaries with semi-diurnal tides, the endogenous swimming rhythm takes one of two forms: a circatidal rhythm with a period of ~12.4 h or a circalunidian rhythm with a period of ~24.8 h (Forward et al. 2003, 2005). Crabs with a circatidal rhythm have one peak in swimming each tidal cycle (12.4 h), during ebb tide. Crabs with a circalunidian rhythm have one peak in swimming every lunar day (24.8 h), corresponding to every other ebb tide. While the movements and behaviors of ovigerous crabs have been examined extensively (e.g., Forward et al. 2003, 2005; Carr et al. 2004; Hench et al. 2004), little is known about the movements and rhythmic behaviors of adult crabs prior to oviposition or the stage at which the circatidal rhythm is first expressed for ETT.

Because blue crabs use ETT for the spawning migration, vertical swimming behavior is synchronized to the tidal cycle in the home estuary. Tidal regimes, however, are vastly different over much of the blue crab's range, with semi-diurnal tides along the majority of the east coast of the United States and South America, diurnal tides in the Gulf of Mexico (e.g., Morgan 1996), mixed tides in the Caribbean, and some estuaries that are essentially non-tidal (e.g., Roelofs and Bumpus 1953; Luettich et al. 2002). To date, all studies of the circatidal swimming rhythm in ovigerous blue crabs have been conducted in an estuary with strong semi-diurnal tides (Forward et al. 2003, 2005; Forward and Cohen 2004), and the behavior of ovigerous crabs from other tidal regimes, including estuaries with negligible tidal cycles, remains unknown.

The purpose of this study was to examine (1) the ontogeny of the endogenous rhythms in vertical swimming of female blue crabs that underlie ebb-tide transport and (2) variation in rhythms among ovigerous females from non-tidal estuaries and estuaries with semi-diurnal and diurnal tides.

Methods

To examine the ontogeny of the circatidal rhythm in vertical swimming that underlies ebb-tide transport during the *Callinectes sapidus* female spawning migration, vertical swimming behavior was monitored under constant conditions for: (1) immature females >50 mm carapace width prior to initiation of physical changes related to the terminal molt, (2) females that had recently undergone the terminal molt but were not yet showing visible mature ovaries, (3) females showing visible mature ovaries but not yet ovigerous, and (4) ovigerous females. Because swimming behavior of ovigerous crabs tethered in the field is highly variable based on tethering site (Darnell 2009), we hypothesized that food availability would alter swimming behavior and fewer ascents into the water column would occur with high food availability. To test this hypothesis, ovigerous females were tested both with and without food. Crabs that had recently undergone their terminal molt (hereafter referred to as recently molted) were identified by incomplete calcification of the cuticle such that manual depression of the carapace below the large lateral spines was possible. Crabs in this stage are typically within 14 days after the terminal molt (unpubl. data). Crabs showing visible mature ovaries were identified by the internal orange crescent visible at the base of the lateral spines. Ovigerous crabs were identified by the large, external egg mass.

Crabs were collected from the Rachel Carson National Estuarine Research Reserve, Beaufort, North Carolina (34°42.65′N, 76°40.40′W), which has a semi-diurnal tidal regime with a tidal range of ~1 m. Crabs were collected at night by hand with dip nets and transported to the Duke University Marine Laboratory in individual buckets containing ~1.5 L of ambient estuarine water. Individuals that had mated but not yet produced a clutch of eggs were obtained by collecting mating pairs from the same location. Pairs in the pre- or post-copulatory embrace were separated for transport, while pairs actively copulating were transported together. After mating was complete, females were confined individually in minnow traps buried half way into the sediment on their long axis in a shallow subtidal area

using established procedures (Dickinson et al. 2006; Darnell et al. 2009) until mature ovaries were visible through the carapace. Ovigerous crabs were collected using the same method in the Reserve.

To analyze endogenous rhythms in spawning female crabs from tidal regimes other than semi-diurnal, vertical swimming behavior was observed for ovigerous females collected from: (1) St. Andrew Bay, Florida (30°8.95′N, 85°42.79′W), and (2) South River, North Carolina (34°57.37′N, 76°34.48′W). St. Andrew Bay is located near Panama City, Florida and has a diurnal tidal cycle with a tidal range of ~0.5 m. South River is a subestuary of the Albemarle-Pamlico Estuarine System and is a primarily wind-driven system with a very weak lunar tidal cycle (Roelofs and Bumpus 1953).

Ovigerous crabs with early-stage eggs (Stages 1–4 of DeVries et al. 1983) from St. Andrew Bay, Florida were collected by hand with dip nets during nocturnal low tides. Individual crabs were confined subtidally in plastic minnow traps near the collection site for 1–3 days until transport. Crabs were transported to the Duke University Marine Laboratory by truck in individual perforated, plastic containers within large coolers of aerated seawater from the collection site. Transport time was 15–18 h, and water in the coolers was changed after 7–9 h. Water temperature in the coolers was maintained near the ambient temperature (25–30°C) at the collection site using sealed containers of ice as necessary.

Females with mid- to late-stage eggs (Stages 5–9 of DeVries et al. 1983) from the wind-driven South River were collected during the day in crab pots with the assistance of local commercial crabbers. After being removed from the pots, crabs were held in a tank with flow-through seawater for 1–4 h while on the boat. Crabs were transported to the Duke University Marine Laboratory in individual perforated, plastic containers within a large cooler of aerated seawater from the collection site. Transport time was ~0.75 h, and water was not changed during transport.

Crabs were collected in multiple batches. The number of test columns for monitoring rhythmic behavior was limited, which necessitated multiple collections to increase sample sizes. When possible, collections were timed such that the phasing between the tidal cycle and the diel cycle varied. This procedure ensured that circadian and circatidal rhythms could be differentiated.

Endogenous rhythms in vertical swimming were analyzed for at least 10 females from each life-history stage and collection site using the methods described by Forward et al. (2003). Crabs were placed individually into transparent vertical columns (1.22 m × 30.5 cm diameter, Aquatic Eco-Systems, Inc. model T4), filled with estuarine water diluted to the salinity of the collection site. Salinity in the Beaufort, North Carolina collection site is relatively constant at 34–35, except after large tropical and subtropical storms. Salinity in St. Andrew Bay, Florida during the study period ranged from 33 to 35. Salinity in South River, North Carolina ranged from 15.5 to 18.5 (Online resource 1). The water was filtered to remove particles >5 μm and aerated for the duration of the experiment. Water temperature was maintained at room temperature, 24–25°C. Columns were continuously illuminated with dim red light from fluorescent lamps covered with red cellophane (e.g., Forward et al. 2003). Blue crabs are insensitive to long-wavelength light, so red light approximates constant darkness (Cronin and Forward 1988). Vertical swimming behavior was monitored for 5–6 days and recorded with a video camera (Panasonic WV-BP330) and time-lapse video recorder (Panasonic AG-RT850). With the exception of six ovigerous crabs from Beaufort, North Carolina, crabs were not fed for the duration of the experiment. The six ovigerous females tested with food were fed 10 fiddler crabs *Uca pugilator* at the initiation of monitoring and 6 *U. pugilator* daily thereafter, at haphazard times. Vertical swimming behavior was quantified by counting the number of ascents in each 30-min interval. A swimming bout was considered an ascent if the crab ascended at least 40 cm above the bottom of the column. If larval release occurred before the end of the monitoring period, monitoring was ceased, as Forward et al. (2003, 2005) found that female blue crabs become arrhythmic following larval release unless re-entrained to the tidal cycle.

Swimming behavior was analyzed for periodicity using autocorrelation and maximum entropy spectral analysis (MESA). Rhythmicity was assessed using autocorrelation, which plots autocorrelation coefficients as a function of lag at 0.5-h intervals. Peaks with autocorrelation coefficients exceeding $\pm 2/\sqrt{N}$, where N is number of 0.5-h intervals, indicate statistical significance at $P < 0.05$ (Dowse and Ringo 1989). Period estimates were obtained using MESA, which fits an autoregressive model to the data and uses Fourier analysis to construct a power spectrum, from which period estimates can be obtained (Levine et al. 2002). Peaks in the MESA spectrum were validated by comparison with peaks in the correlogram.

Rhythmic activity was compared to the expected tidal cycle at the collection site using cross-correlation analysis, by plotting cross-correlation as a function of lag at 0.5-h intervals. Peaks exceeding $\pm 2/\sqrt{N}$ are statistically significant ($P < 0.05$) and indicate the relationship between maximum activity and the time of high tide. Predicted tidal data for the different sites were obtained from Tides & Currents Pro Version 3.3 (Nobeltec Corp., Portland, OR). For analyses of crabs from Beaufort, North Carolina, predictions were obtained for Tides & Currents station 2802 (“Beaufort, Duke Marine Lab, North Carolina”, 34°43.12′N, 76°40.12′W). For analyses of crabs from St. Andrew Bay,

Florida, predictions were obtained for Tides & Currents station 4505 ("St. Andrew Bay, Panama City", 30°09.06′N, 85°40.00′W). Rhythmic activity was compared to the diel cycle using standard circular statistical techniques to determine mean times of activity for each crab. Only the first 2 days of data from each crab were used for cross-correlation and circular statistical analyses to account for free-running period lengths that would gradually shift the time of activity relative to the tidal and diel cycles over the course of the experiment.

Because the ranges of free-running period lengths for circadian (~24 h) and circalunidian (~24.8 h) rhythms often overlap, circular statistics were used to differentiate between the two types of rhythms when MESA produced period estimates near 24 h. To test for a circadian rhythm, the mean time of activity for each crab was converted to an angle, with 0° representing 00:00 hrs (midnight) and 180° representing 12:00 hrs (noon). Rao's spacing test was used to test the uniformity of the distribution of mean times of activity for each group of crabs (Batschelet 1981). A significant deviation from a uniform distribution suggests a circadian activity rhythm, while a non-significant result indicates that activity was uniformly distributed throughout the diel cycle. To test for a circalunidian rhythm, the cross-correlation lag for each crab was converted to an angle with 0° representing the time of high tide and 180° representing 12.4 h after high tide. Rao's spacing test was used to test the uniformity of the distribution of mean cross-correlation lags for each group of crabs. In this case, a significant deviation from a uniform distribution suggests a circalunidian activity rhythm. A non-significant result indicates that activity was uniformly distributed throughout the tidal cycle, and thus a circalunidian rhythm is unlikely.

Mean swimming frequency for each crab was determined by dividing the total number of ascents by the length of the observation period. Data were log-transformed to meet assumptions of normality and homoscedasticity. A *t*-test was used to compare swimming frequency of ovigerous crabs with and without food. Swimming frequency was compared among life-history stages, and among collection sites for ovigerous crabs, using analyses of variance (ANOVA), followed by Holm-Sidak multiple comparison tests when ANOVA indicated a significant difference at $P < 0.05$.

Results

Juvenile female crabs

Eleven intermolt juvenile female *Callinectes sapidus* from Beaufort, North Carolina were tested for rhythmicity. Swimming frequency averaged 5.59 ± 1.36 ascents h^{-1} (mean ± SE). Seven crabs displayed significant rhythmicity (Table 1, Online resource 1). The remaining 4 crabs were arrhythmic. Distribution of period estimates for rhythmic crabs was bimodal. Two crabs had period estimates in the circatidal range (~12.4 h). Period estimates for these crabs were 12.4 and 13.0 h. Cross-correlation lags indicated that peak swimming took place 0 and 5.5 h after high tide, respectively.

Five crabs had period estimates in the circadian/circalunidian range (~24–24.8 h, Fig. 1). Period estimates for these crabs averaged 24.44 ± 0.39 hrs. Cross-correlation analysis indicated that peak swimming occurred 1.24 ± 0.90 h after high tide. Time of day at peak swimming averaged $20{:}39 \pm 1.67$ h. Both cross-correlation lags and times of peak activity were significantly different from a uniform distribution (Rao's spacing test, $P < 0.05$).

Table 1 Summary of swimming rhythms observed for crabs collected in Beaufort, North Carolina, St. Andrew Bay, Florida, and South River, North Carolina

Stage	Collection site	Tidal regime	*n*	Circatidal (~12.4 h)	Circalunidian (~24.8 h)	Circadian (~24 h)	Arrhythmic[a]
Juvenile	Beaufort	Semi-diurnal	11	2, ebb tide	5[b], ebb tide	5[b], sunset	4
Recently molted	Beaufort	Semi-diurnal	14	4, ebb tide	0	9, sunset	1
Mature ovaries	Beaufort	Semi-diurnal	12	5, high tide	2, high tide	0	5
Ovigerous	Beaufort	Semi-diurnal	12	4, ebb tide	6, ebb tide	0	2
Ovigerous + food	Beaufort	Semi-diurnal	6	4, ebb tide	2, ebb tide	0	0
Ovigerous	St. Andrew Bay	Diurnal	16	1, high tide	12, ebb tide	0	3
Ovigerous	South River	Non-tidal	11	0	0	9, sunset	2

n is the number of female crabs tested. Numbers in each rhythm column indicate the number of crabs with that type of rhythm, followed by the phase of the tidal or diel cycle for peak swimming

[a] Includes crabs that did not swim at all during the observation period

[b] Ambiguous results. Rhythms in these 5 crabs may be circalunidian with swimming during the time of ebb tide or circadian with swimming around the time of sunset

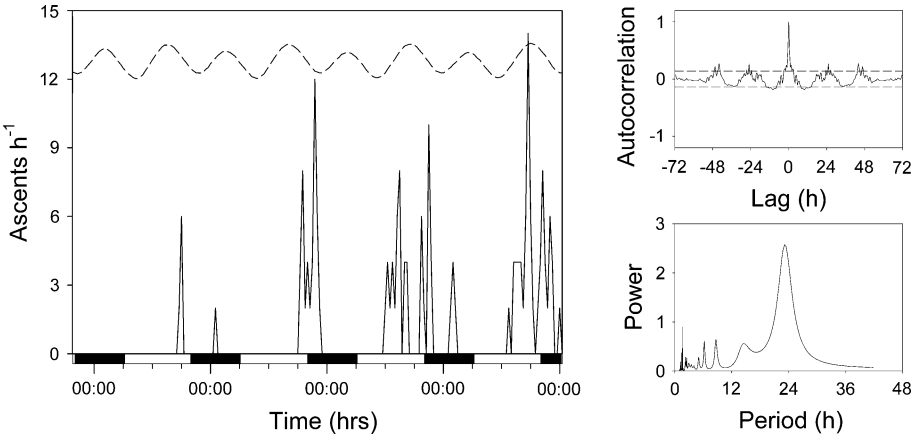

Fig. 1 Actogram of vertical swimming for representative juvenile female with circalunidian rhythm of swimming during the time of ebb tide. Ambient light:dark cycle is shown at bottom. Tidal cycle at the collection site is represented by the broken line. *Top right panel* is autocorrelation output, *bottom right panel* is MESA spectrum. Only the first 48 h of observations were used for cross-correlation and circular statistical analyses

Recently molted female crabs

Fourteen recently molted and mated female blue crabs from Beaufort, North Carolina were tested for rhythmicity. Swimming frequency averaged 1.61 ± 0.43 ascents h^{-1}. Thirteen crabs displayed significant rhythmicity and one was arrhythmic (Table 1). Distribution of period estimates was bimodal. Period estimates for 4 of the crabs were in the circatidal range and averaged 12.00 ± 0.48 h. Three of these crabs had cross-correlation lags of 0.5–1.5 h after the time of high tide. The fourth crab did not swim during the first 2 days. Thus, a cross-correlation lag could not be calculated.

Period estimates for the remaining 9 crabs were in the circalunidian or circadian range and averaged 24.11 ± 1.06 h (Fig. 2). Two of these did not swim during the first 2 days in the columns and were excluded from cross-correlation and circular statistical analyses. The distribution of peak swimming times, determined by cross-correlation analysis, was uniform with respect to the tidal cycle (Rao's spacing test, $P > 0.05$). Time of day at peak swimming averaged 20:00 ± 1.42 hrs, and the distribution of times of peak swimming with respect to the diel light:dark cycle was significantly different from uniform (Rao's spacing test, $P < 0.05$).

Female crabs with visible mature ovaries

Twelve female blue crabs with visible mature ovaries from Beaufort, North Carolina were tested for rhythmicity. Swimming frequency averaged 1.67 ± 0.37 ascents h^{-1}. Seven exhibited significant rhythmicity, 3 were arrhythmic, and 2 did not swim during the observation period (Table 1). Distribution of period estimates was bimodal. Period estimates for 5 of the crabs were in the circatidal range and averaged 13.72 ± 0.74 h (Fig. 3). Cross-correlation analysis indicated that peak swimming occurred 0.18 ± 0.68 h after high tide.

Period estimates for the remaining 2 crabs were in the circalunidian or circadian range and averaged 26.30 ± 1.30 h. Cross-correlation analysis indicated that peak swimming for both crabs occurred at the time of high tide (lag = 0 h). In relation to the diel cycle, peak swimming occurred at 17:03 and 20:20 hrs.

Ovigerous female crabs from Beaufort, North Carolina

Twelve ovigerous blue crabs collected from Beaufort, North Carolina were tested for rhythmicity. This estuary experiences semi-diurnal tides. Swimming frequency

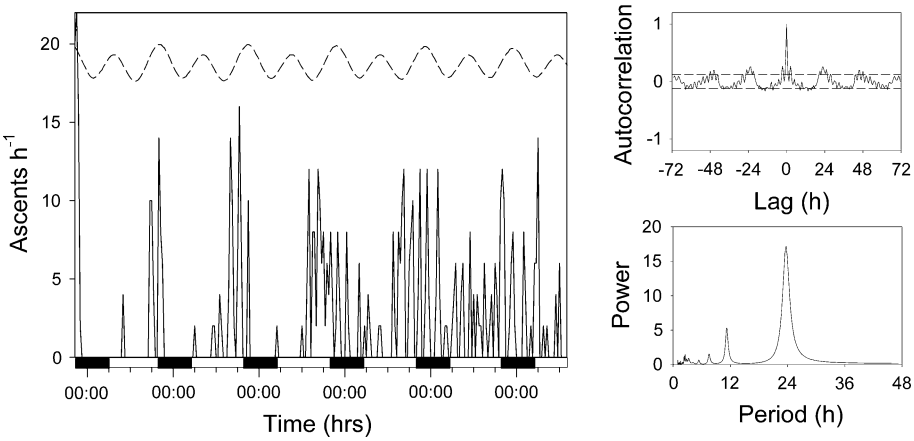

Fig. 2 Actogram of vertical swimming for representative recently molted female with a circadian rhythm of swimming around the time of sunset. Ambient light:dark cycle is shown at bottom. Tidal cycle at the collection site is represented by the *broken line*. *Top right panel* is autocorrelation output, *bottom right panel* is MESA spectrum. Only the first 48 h of observations were used for cross-correlation and circular statistical analyses

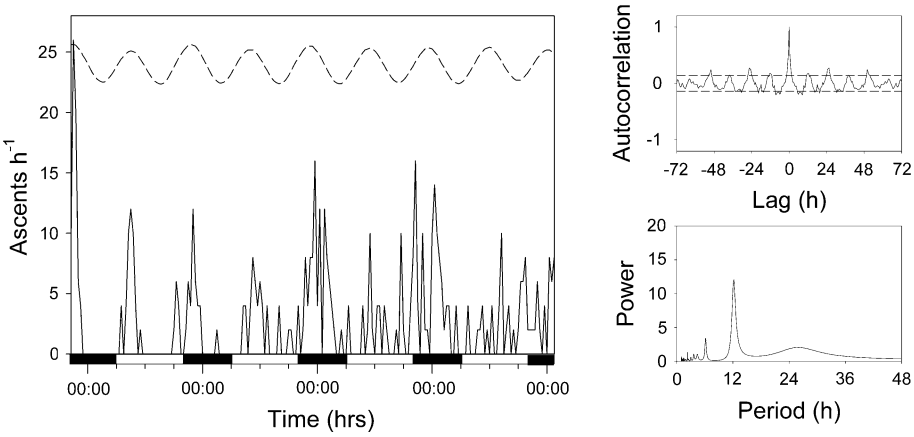

Fig. 3 Actogram of vertical swimming for representative female with visible mature ovaries with a circatidal swimming rhythm of swimming around the time of high tide. Ambient light:dark cycle is shown at *bottom*. Tidal cycle at the collection site is represented by the *broken line*. *Top right panel* is autocorrelation output, *bottom right panel* is MESA spectrum. Only the first 48 h of observations were used for cross-correlation and circular statistical analyses

averaged 2.84 ± 1.21 ascents h^{-1}. Ten exhibited significant rhythmicity while 2 were arrhythmic (Table 1). Distribution of period estimates was bimodal. Period estimates for 4 of the crabs were in the circatidal range and averaged 12.40 ± 0.24 h. Cross-correlation analysis indicated that peak swimming occurred 0.75 ± 0.20 h after high tide.

Period estimates for the remaining 6 rhythmic crabs were in the circalunidian or circadian range and averaged 23.58 ± 0.60 h (Fig. 4). Two of these crabs appeared to switch from a circatidal rhythm to a circalunidian/circadian rhythm. Cross-correlation analysis indicated that peak swimming occurred 1.48 ± 0.77 h after high tide, and the distribution of cross-correlation lags was significantly different from uniform (Rao's spacing test, $P < 0.05$). Time of day at peak swimming averaged 20:26 ± 1.42 hrs, and the distribution of times of peak swimming was significantly different from uniform (Rao's spacing test, $P < 0.05$), indicating that swimming takes place on nocturnal ebb tides.

Ovigerous female crabs from Beaufort, North Carolina with food

A further six ovigerous blue crabs collected from the Rachel Carson National Estuarine Research Reserve were tested for rhythmicity with food present. Swimming frequency averaged 3.51 ± 0.68 ascents h^{-1}, which was not significantly different from the swimming frequency of ovigerous crabs tested without food. All 6 displayed significant rhythmicity (Table 1). Distribution of period estimates was bimodal. Period estimates for 4 of the crabs were in the circatidal range and averaged 12.63 ± 0.06 h (Online Resource 2). One of these crabs appeared to switch from a circatidal rhythm to a circalunidian/circadian rhythm. Cross-correlation analysis indicated that peak swimming occurred 0.49 ± 0.03 h after high tide.

Period estimates for the remaining 2 crabs were in the circalunidian or circadian range and averaged 25.30 ± 0.40 h. Cross-correlation analysis indicated that peak

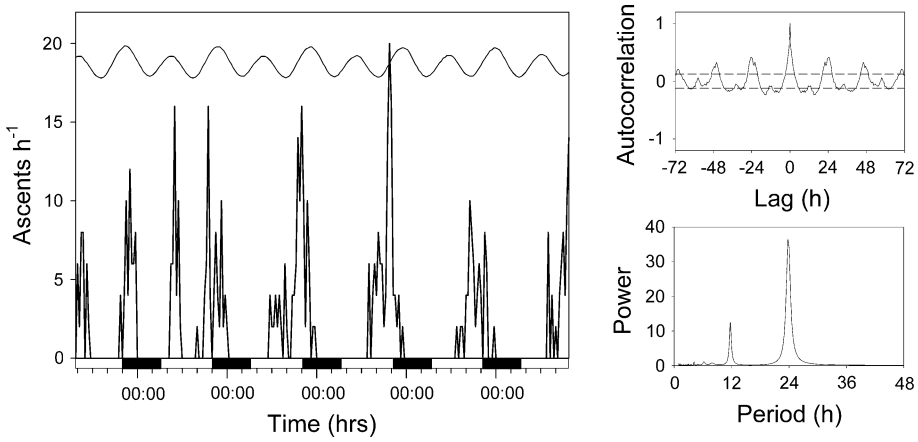

Fig. 4 Actogram of vertical swimming for representative ovigerous female from Beaufort, North Carolina. This crab switched from a circatidal rhythm to a circalunidian rhythm, with peak swimming during the time of ebb tide. Ambient light:dark cycle is shown at *bottom*. Tidal cycle at the collection site is represented by the *broken line*. *Top right panel* is autocorrelation output, *bottom right panel* is MESA spectrum. Only the first 48 h of observations were used for cross-correlation and circular statistical analyses

swimming for both crabs occurred 0.5 h after high tide. In relation to the diel cycle, peak swimming occurred at 22:00 and 15:52 hrs.

Ovigerous female crabs from St. Andrew Bay, Florida

Sixteen ovigerous blue crabs from St. Andrew Bay, Florida were tested for rhythmicity. This estuary experiences diurnal tides. Swimming frequency averaged 3.44 ± 0.86 ascents h^{-1}. Thirteen crabs exhibited significant rhythmicity, while 3 were arrhythmic (Table 1). One of the rhythmic crabs had a period of 11.9 h, though this crab ceased swimming after 2 days. Thus, the accuracy of the period estimate for this crab is low, as accuracy increases with increasing time-series length. Period estimates for the remaining 12 crabs were in the circalunidian or circadian range and averaged 25.82 ± 0.54 h (Fig. 5). One of these did not swim during the first 2 days in the columns and was excluded from cross-correlation and circular statistical analyses. Cross-correlation analysis indicated that peak swimming occurred 9.94 ± 1.01 h after high tide, during the ambient ebb tide, and the distribution of cross-correlation lags was significantly different from uniform (Rao's spacing test, $P < 0.05$). Time of day at peak swimming averaged $18{:}23 \pm 0.93$ hrs, and the distribution of times of peak swimming was significantly different from uniform (Rao's spacing test, $P < 0.05$).

Ovigerous female crabs from South River, North Carolina

Eleven ovigerous crabs from South River, North Carolina were tested for rhythmicity. This estuary does not experience lunar tides. Swimming frequency averaged 2.52 ± 0.66 ascents h^{-1}. Nine crabs exhibited significant rhythmicity, while 2 were arrhythmic (Table 1). Period estimates were in the circadian range and averaged 24.90 ± 0.35 h (Fig. 6). Time of day at peak swimming averaged $19{:}59 \pm 0.78$ hrs, and the distribution of times of peak swimming was significantly different from uniform (Rao's spacing test, $P < 0.05$).

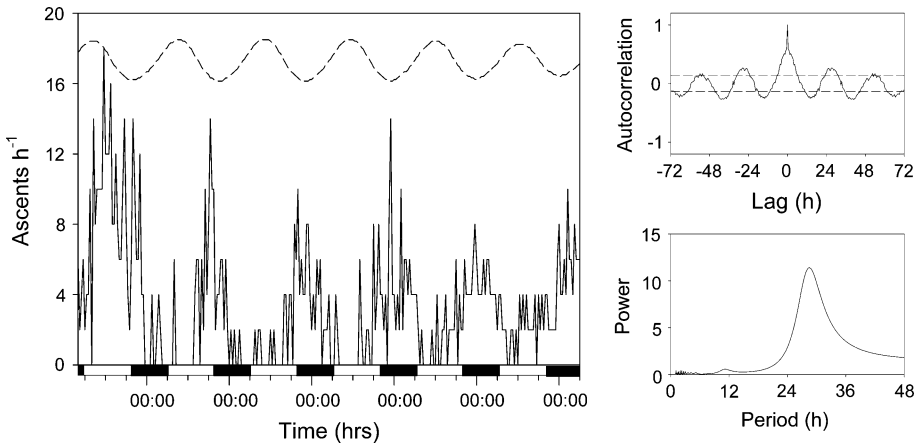

Fig. 5 Actogram of vertical swimming for representative ovigerous female from St. Andrew Bay, Florida. This crab had a circalunidian rhythm with peak swimming during the time of ebb tide at the collection site. Ambient light:dark cycle is shown at bottom. Tidal cycle at the collection site is represented by the broken line. *Top right panel* is autocorrelation output, *bottom right panel* is MESA spectrum. Only the first 48 h of observations were used for cross-correlation and circular statistical analyses

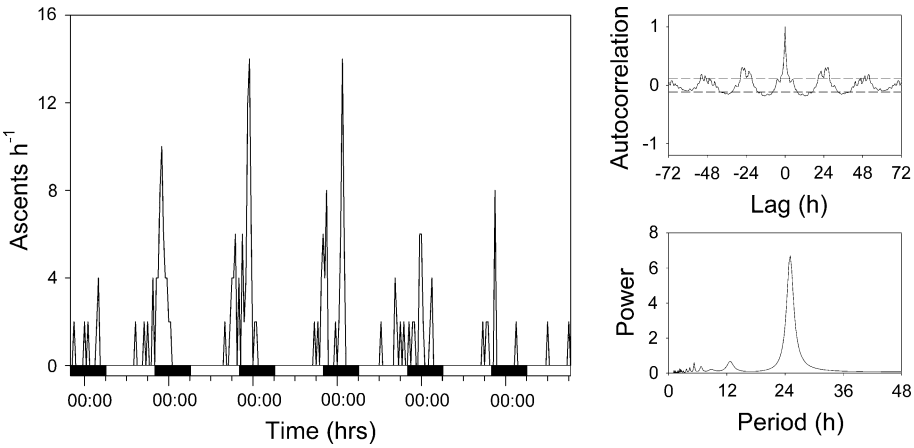

Fig. 6 Actogram of vertical swimming for representative ovigerous female from South River, North Carolina. This crab had a circadian rhythm with peak swimming around the time of sunset. Ambient light:dark cycle is shown at *bottom*. *Top right panel* is autocorrelation output, *bottom right panel* is MESA spectrum. Only the first 48 h of observations were used for circular statistical analyses

Comparisons of swimming frequency

Swimming frequency (ascents h^{-1}) was similar between ovigerous females from Beaufort, North Carolina both with and without food (*t*-test, $P = 0.322$). Thus, all ovigerous crabs from Beaufort, North Carolina were grouped for all subsequent comparisons. Swimming frequency varied significantly among life-history stages (ANOVA, $P = 0.021$) (Fig. 7). Swimming frequency was significantly higher for juvenile females than for recently molted females or females with mature ovaries (Holm-Sidak, $P < 0.05$). Swimming frequency of ovigerous females was intermediate and not significantly different from the 3 other stages (Holm-Sidak, $P > 0.05$). Swimming frequency did not vary significantly among collection locations for ovigerous females (ANOVA, $P = 0.683$) (Online resource 3).

Discussion

Endogenous rhythms in vertical swimming were present in all life-history stages tested, as well as in ovigerous blue crabs from each of the different tidal regimes. Female blue crabs from an area having semi-diurnal tides experience ontogenic changes in vertical swimming rhythms over the range of life-history stages tested. There were also variations in rhythm type and period within stages (Fig. 8). These ontogenic changes in endogenous swimming rhythms have consequences for the distribution and movements of each life-history stage.

Intermolt juvenile female blue crabs had the highest swimming frequency and exhibited a variety of swimming patterns (Fig. 8). Arrhythmicity was observed in 36% of the crabs. Several crabs had a clear circatidal rhythm with peak swimming during the time of ebb tide. The rhythm in the remaining crabs was either circadian or circalunidian. Peak swimming occurred during the time of high or ebb tide in the field, and the average time of peak swimming (20:39 hrs) was around the time of sunset (~20:30 hrs) at the collection site. Further study testing the rhythm at times with different timing relationships between tides and the light:dark cycle is necessary to determine whether this rhythm is actually circadian or circalunidian. A circalunidian rhythm with swimming on nocturnal ebb tides would suggest that these crabs may be undergoing ebb-tide transport and moving seaward. A circadian rhythm of swimming around the time of sunset would not result in net movement with the tides, but would allow movement within foraging areas and would reduce the risk of predation by diurnal visual predators.

Recently molted females, tested within ~14 days after the terminal molt, displayed one of two rhythms. About 70% (9 of 13) of rhythmic individuals exhibited a circadian

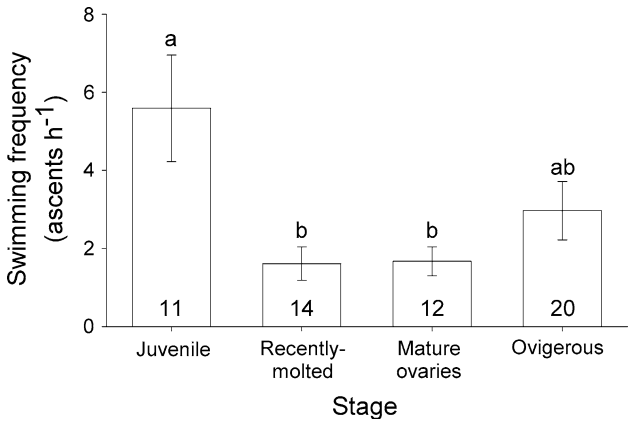

Fig. 7 Swimming frequency of female crabs at different life-history stages from Beaufort, North Carolina. Ovigerous crabs tested with and without food have been grouped together. Different letters indicate statistically significant differences at $P < 0.05$. *Error bars* indicate ± 1 SE, and numbers inside bars indicates sample size

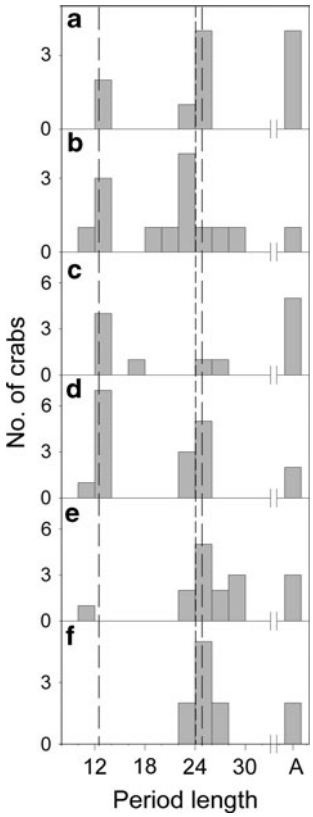

Fig. 8 Histograms of period lengths for each group of female blue crabs tested: **a** intermolt juvenile females; **b** recently mated females; **c** females with visible mature ovaries; **d** ovigerous females from Beaufort, North Carolina; **e** ovigerous females from St. Andrew Bay, Florida; **f** Ovigerous females from South River, North Carolina. Ovigerous crabs tested with and without food have been grouped together in *panel d*. *Long-dashed vertical lines* indicate 12.4 and 24.8 h, *short-dashed vertical line* indicates 24.0 h. "A" on the *x*-axis represents arrhythmic crabs or crabs that did not swim at all

rhythm with peak swimming occurring at ~20:00 hrs, approximately the time of sunset. Period lengths for these rhythms, however, were quite variable (Fig. 8). The remaining 4 individuals exhibited a circatidal rhythm with peak swimming occurring during ebb tide. Thus, similar to juvenile females, recently molted females have rhythms that are suggestive of either non-migratory movement within foraging areas or, to a lesser extent, ebb-tide transport. Swimming frequencies were relatively low compared to juvenile females and ovigerous females. Mark-recapture studies of recently molted female blue crabs indicate that following mating, females typically forage for a period of weeks to months before beginning the seaward spawning migration (Turner et al. 2003; Aguilar et al. 2005; Darnell 2009).

Female blue crabs with visible mature ovaries typically displayed a circatidal or circalunidian swimming rhythm with peak swimming occurring around the time of high tide, although a high proportion of crabs were either arrhythmic or did not swim at all (Fig. 8). There was no evidence of a circadian rhythm. Swimming frequencies remained low during this stage. Swimming centered around the time of high tide would result in no net movement with the tides, but could represent foraging movements.

Ovigerous female blue crabs from an estuary with semi-diurnal tides exhibited very clear circatidal or circalunidian rhythms in vertical swimming with and without food, having peak swimming occurring during the time of ebb tide. This well-established rhythm of swimming on ebb tides is the basis for the spawning migration (Forward et al. 2003, 2005), in which ovigerous female blue crabs migrate seaward from estuaries to coastal areas where larvae are released. Qualitatively, the circatidal/circalunidian rhythm of ovigerous blue crabs was much more clearly defined that any circatidal or circalunidian rhythms observed in earlier life-history stages. Mean swimming frequency of ovigerous females was 78% higher than females with mature ovaries and 84% higher than recently molted females. The lack of a statistical difference in swimming frequency between ovigerous crabs and these two prior stages may be due to a relatively low sample size (11–14 crabs for each stage). These results suggest that the circatidal or circalunidian rhythm underlying the seaward spawning migration is manifested in most females when they become ovigerous and thus rapid seaward movement via ETT begins following oviposition of the first clutch of eggs.

Ovigerous female blue crabs were tested both with and without food to determine whether food availability affects swimming behavior. This experiment was conducted because ovigerous blue crabs tethered in the field in high-salinity areas display highly variable amounts of vertical swimming, depending on the tethering site (Darnell 2009). Additionally, ovigerous blue crabs are regularly observed swimming at the surface in certain areas (Tankersley et al. 1998; Carr et al. 2004; Hench et al. 2004) but not in others, despite extensive monitoring (Rittschof et al. unpubl data). We hypothesized that in high salinity (≥22, Rittschof et al. In Review), some areas may serve as foraging habitat while others may serve as migratory corridors and that food availability and physical factors may influence swimming frequency. No obvious differences were apparent in the form, period, or amplitude of the circatidal rhythm between ovigerous crabs tested with food and those tested without food. Additionally, swimming frequencies were similar, indicating that food availability does not affect the circatidal swimming rhythm. Further tests of more females with a range of food concentrations are necessary to confirm this observation.

Ovigerous blue crabs possess an endogenous rhythm in vertical swimming, though the type of rhythm present, and the period length of the rhythm, depends on the tidal regime at the collection site. Ovigerous female crabs collected from Beaufort, North Carolina, where a semi-diurnal tidal cycle is present, exhibited circatidal or circalunidian rhythms in vertical swimming, with peak swimming occurring during ebb tides. Periods were variable, with individuals swimming either every ebb tide (circatidal rhythm) or every other ebb tide (circalunidian rhythm) (Fig. 8). Either swimming rhythm would move crabs seaward. Swimming on every ebb tide would result in a faster rate of movement, though crabs would be in the water column during daytime ebb tides as well as nighttime ebb tides, subjecting them to predation risk from diurnal visual predators. Swimming only on nocturnal ebb tides would reduce the predation risk by diurnal visual predators, but would result in slower seaward movement. Circalunidian rhythms would also be more easily synchronized to the tidal cycle, as deviations from the ideal 12.4-h period of a semi-diurnal tidal cycle are much greater that deviations from the 24.8-h period of every other tide in nature (Palmer 1995).

Ovigerous blue crabs from St. Andrew Bay, Florida displayed a circalunidian rhythm, with peak swimming occurring at the time of ebb tides. Tides in St. Andrew Bay are diurnal, with a single ebb tide and single flood tide per lunar day. Thus, a circalunidian rhythm corresponds to one peak in swimming each tidal cycle, during ebb tide. Periods for all but one of the rhythmic crabs averaged 25.8 h, indicating that the period of the circalunidian rhythm approximates that of the tidal cycle in St. Andrew Bay. There was some evidence of a circadian rhythm in ovigerous crabs from St. Andrew Bay. The time of day of peak swimming averaged 18:23 hrs, and the distribution of times of peak swimming was significantly different from a uniform distribution. We hypothesize that this is not due to a circadian swimming rhythm, but is instead an artifact of the relationship between the tidal and diel cycle at the collection site.

Crabs from St. Andrew Bay were collected on 13 June, 25 June, and 25 July, 2007. On those dates, the phasing between the tidal and diel light:dark cycles was similar, as high tide occurred at 08:26, 06:49, and 06:45 hrs, respectively. It is also possible, though unlikely, that these crabs possess a circadian rhythm in swimming that results in seaward movement. During the months of June and July 2007, the times of high tide in St. Andrew Bay ranged from 04:44 to 14:39 hrs and averaged 09:39 $\pm$ 0.35 hrs. High tide generally occurs in the morning in St. Andrew Bay. A circadian rhythm of peak swimming in the late afternoon would, in general result in seaward movement, as that time of day generally corresponds to ebb tide.

Ovigerous blue crabs from the non-tidal South River exhibited circadian rhythms in vertical swimming with peak swimming occurring around the time of sunset. The average time of day of peak swimming was 19:59 hrs. The time of sunset during this component of the study was ~20:00 hrs. While the period of the circadian rhythm seen in South River crabs is similar to the period of the circalunidian rhythm seen in crabs from Florida, peak swimming for South River crabs consistently occurred around sunset rather than during a specific tidal phase. Any seaward movement resulting from such a circadian rhythm would be due only to wind-driven currents and residual flow seaward (Forward and Tankersley 2001). Thus, we hypothesize that seaward migration in non-tidal estuaries is not a consequence of ETT, but perhaps of seaward-directed walking. Such directed walking has been observed in ovigerous blue crabs in a strongly tidal estuary during flood tides (Carr et al. 2004).

Differences in endogenous rhythms in crabs from different tidal regimes could result from either genetic differences among the areas, or more likely, from phenotypic plasticity in the rhythm and clock mechanism. Genetic differences of crabs from different tidal regimes are unlikely. Blue crabs exhibit extremely high levels of genetic diversity (McMillen-Jackson and Bert 2004). Although a latitudinal cline in haplotype diversity and significant range-wide patchiness exist (McMillen-Jackson et al. 1994; McMillen-Jackson and Bert 2004), geographic structuring is absent in both allozyme allele frequencies (McMillen-Jackson et al. 1994) and mitochondrial DNA haplotype distribution (McMillen-Jackson and Bert 2004). Additionally, estimates of interpopulation gene flow are high enough to suggest panmixia among US populations (McMillen-Jackson et al. 1994). We hypothesize that the clock mechanism in blue crabs is phenotypically plastic, such that it can be entrained to either semi-diurnal tidal, diurnal tidal, or 24-h light:dark cycles. Phenotypic plasticity in rhythm form has been demonstrated in crab egg hatching rhythms (Forward et al. 1982; Weaver and Salmon 2002; Christopher et al. 2008) and activity rhythms (Barnwell 1968). Translocation experiments of blue crabs between different tidal regimes would provide further clarity on this hypothesis. We also hypothesize that the circadian rhythm seen in crabs from South River may be based on the same clock mechanism as the circatidal and circalunidian rhythms in crabs from tidal areas. Due to the lack of a tidal cycle, the rhythm is not entrained to tides, and the diel light:dark cycle or temperature cycle takes over as the primary entrainment cue. While no direct evidence of single-clock control of both types of rhythms is available for blue crabs, circumstantial evidence from a variety of other marine organisms supports the single-clock hypothesis (Palmer 1990, 1995).

Female blue crabs at each life-history stage and from each collection site had endogenous rhythms in vertical swimming when monitored under constant conditions in the laboratory. The type of rhythm present and the period length of the rhythm varied based on life-history stage and tidal regime at the collection site. Based on the results of this study, we now know the range of rhythms present in female blue crabs at each stage. The swimming rhythm of ovigerous blue crabs has been confirmed by field observations of tethered crabs (Hench et al. 2004; Darnell 2009) as well as observations of crabs swimming at the surface during ebb tides (Tankersley et al. 1998; Carr et al. 2004; Hench et al. 2004). Similar field studies are needed to verify that vertical swimming rhythms observed in crabs at the life-history stages preceding oviposition occur outside of the laboratory and to provide information on the functional significance of these rhythms.

Acknowledgments We are grateful to Gary and William Cannon for their assistance collecting crabs from South River. We also thank NMFS Panama City Laboratory for the use of their facilities while collecting crabs from St. Andrew Bay as well as for assistance with salinity measurements. Kelly Darnell provided much-appreciated assistance with crab collection and video analysis. Larry Crowder, Anson Hines, and Bill Kirby-Smith provided valuable comments on an earlier version of this manuscript. Funding for this study was provided by the North Carolina Sea Grant Blue Crab Research Program (grants 06-Biol-04 and 07-Biol-03).

References

Aguilar R, Hines AH, Wolcott TG, Wolcott DL, Kramer MA, Lipcius RN (2005) The timing and route of movement and migration of post-copulatory female blue crabs, *Callinectes sapidus* Rathbun, from the upper Chesapeake Bay. J Exp Mar Biol Ecol 319:117–128

Barnwell FH (1968) Role of rhythmic systems in adaptation of fiddler crabs to intertidal zone. Am Zool 8:569–583

Batschelet E (1981) Circular statistics in biology. Academic Press, London

Carr SD, Tankersley RA, Hench JL, Forward RB, Luettich RA (2004) Movement patterns and trajectories of ovigerous blue crabs *Callinectes sapidus* during the spawning migration. Est Coast Shelf Sci 60:567–579

Christopher CE, Salmon M, Forward RB (2008) Is the hatching clock of fiddler crab larvae (*Uca thayeri*) phenotypically plastic? J Crustac Biol 28:328–333

Cronin TW, Forward RB (1979) Tidal vertical migration: an endogenous rhythm in estuarine crab larvae. Science 205:1020–1022

Cronin TW, Forward RB (1988) The visual pigments of crabs. 1. Spectral characteristics. J Comp Physiol A Sens Neural Behav Physiol 162:463–478

Darnell MZ (2009) Spawning biology of female blue crabs, *Callinectes sapidus*. Dissertation, Duke University, Durham

Darnell MZ, Rittschof D, Darnell KM, McDowell RE (2009) Lifetime reproductive potential of female blue crabs *Callinectes sapidus* in North Carolina, USA. Mar Ecol Prog Ser 394:153–163

DeVries MC, Epifanio CE, Dittel AI (1983) Lunar rhythms in the egg hatching of the subtidal crustacean: *Callinectes arcuatus* Ordway (Decapoda: Brachyura). East Coast Shelf Sci 17:717–724

Dickinson GH, Rittschof D, Latanich C (2006) Spawning biology of the blue crab, *Callinectes sapidus*, in North Carolina. Bull Mar Sci 79:273–285

Dowse HB, Ringo JM (1989) The search for hidden periodicities in biological time-series revisited. J Theor Biol 139:487–515

Forward RB, Cohen JH (2004) Factors affecting the circatidal rhythm in vertical swimming of ovigerous blue crabs, *Callinectes sapidus*, involved in the spawning migration. J Exp Mar Biol Ecol 299:255–266

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

Forward RB, Lohmann K, Cronin TW (1982) Rhythms in larval release by an estuarine crab (*Rhithropanopeus harrisii*). Biol Bull 163:287–300

Forward RB, Tankersley RA, Pochelon PN (2003) Circatidal activity rhythms in ovigerous blue crabs, *Callinectes sapidus*: implications for ebb-tide transport during the spawning migration. Mar Biol 142:67–76

Forward RB, Cohen JH, Darnell MZ, Saal A (2005) The circatidal rhythm in vertical swimming of female blue crabs, *Callinectes sapidus*, during their spawning migration: a reconsideration. J Shellfish Res 24:587–590

Hench JL, Forward RB, Carr SD, Rittschof D, Luettich RA (2004) Testing a selective tidal-stream transport model: observations of female blue crab (*Callinectes sapidus*) vertical migration during the spawning season. Limnol Oceanogr 49:1857–1870

Hines AH, Jivoff PR, Bushmann PJ, van Montfrans J, Reed SA, Wolcott DL, Wolcott TG (2003) Evidence for sperm limitation in the blue crab, *Callinectes sapidus*. Bull Mar Sci 72:287–310

Levine JD, Funes P, Dowse HB, Hall JC (2002) Signal analysis of behavioral and molecular cycles. BMC Neurosci 3:1

Lopez-Duarte PC, Tankersley RA (2007) Circatidal swimming behavior of brachyuran crab zoea larvae: implications for ebb-tide transport. Mar Biol 151:2037–2051

Luettich RA, Carr SD, Reynolds-Fleming JV, Fulcher CW, McNinch JE (2002) Semi-diurnal seiching in a shallow, micro-tidal lagoonal estuary. Cont Shelf Res 22:1669–1681

McMillen-Jackson AL, Bert TM (2004) Mitochondrial DNA variation and population genetic structure of the blue crab *Callinectes sapidus* in the eastern United States. Mar Biol 145:769–777

McMillen-Jackson AL, Bert TM, Steele P (1994) Population genetics of the blue crab *Callinectes sapidus*: modest population structuring in a background of high gene flow. Mar Biol 118:53–65

Metcalfe JD, Arnold GP, Webb PW (1990) The energetics of migration by selective tidal stream transport: an analysis for plaice tracked in the southern North Sea. J Mar Biol Assoc UK 70:149–162

Millikin MR, Williams AB (1984) Synopsis of biological data on the blue crab *Callinectes sapidus* Rathbun. FAO Fisheries Technical Paper 138, 43 pp

Morgan SG (1996) Plasticity in reproductive timing by crabs in adjacent tidal regimes. Mar Ecol Prog Ser 139:105–118

Palmer JD (1990) The rhythmic lives of crabs: the same biological clock that controls circadian rhythms may also drive crustacean tidal rhythms. Bioscience 40:352–357

Palmer JD (1995) Review of the dual-clock control of tidal rhythms and the hypothesis that the same clock governs both circatidal and circadian rhythms. Chronobiol Int 12:299–310

Ramach SM, Darnell MZ, Avissar NG, Rittschof D (2009) Habitat use and population dynamics of blue crabs, *Callinectes sapidus*, in a high-salinity embayment. J Shellfish Res 28:635–640

Roelofs EW, Bumpus DF (1953) The hydrography of Pamlico Sound. Bull Mar Sci Gulf Caribb 3:181–205

Tankersley RA, Wieber MG, Sigala MA, Kachurak KA (1998) Migratory behavior of ovigerous blue crabs *Callinectes sapidus*: evidence for selective tidal-stream transport. Biol Bull 195:168–173

Turner HV, Wolcott DL, Wolcott TG, Hines AH (2003) Post-mating behavior, intramolt growth, and onset of migration to Chesapeake Bay spawning grounds by adult female blue crabs, *Callinectes sapidus* Rathbun. J Exp Mar Biol Ecol 295:107–130

Weaver A, Salmon M (2002) Hatching rhythms of *Uca thayeri*: evidence for phenotypic plasticity. J Crustac Biol 22:429–438

Welch JM, Forward RB, 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

Zeng CS, Naylor E (1996) Endogenous tidal rhythms of vertical migration in field collected zoea-1 larvae of the shore crab *Carcinus maenas*: implications for ebb tide offshore dispersal. Mar Ecol Prog Ser 132:71–82