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Factors affecting the circatidal rhythm in vertical swimming of ovigerous blue crabs, *Callinectes sapidus*, involved in the spawning migration

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Abstract

Ovigerous blue crabs, *Callinectes sapidus*, are observed to undergo nocturnal ebb-tide transport (ETT) during their seaward spawning migration. A previous study found that females undergoing the spawning migration have a circatidal rhythm in vertical swimming, which serves as the biological basis for ETT. The present study asked three questions about this endogenous rhythm. First, does the rhythm occur in females with mature embryos regardless of whether they are undergoing ETT? Second, when exposed to a light/dark cycle in the laboratory, do ovigerous females only swim vertically at the time of ebb tide during the dark phase? Third, do attachments to the backs of ovigerous crabs affect the circatidal rhythm? The circatidal rhythm occurred in all crabs with mid-stage embryos that were prevented from undergoing ETT. The rhythm was unaffected by the light/dark cycle, which implies that migration can occur at lower light levels at depth during the day. Finally, attachments did not affect the rhythm, which suggests that tags and transmitters will not affect the spawning migration.

Keywords: Blue crab; *Callinectes sapidus*; Circatidal rhythm; Vertical migration; Spawning migration

1. Introduction

Although adult blue crabs (*Callinectes sapidus*) occur in estuaries, ovigerous females migrate to the entrance of estuaries and coastal areas to release larvae. Tankersley et al.

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(1998) demonstrated that ovigerous females with mature embryos undergo ebb-tide transport (ETT; Forward and Tankersley, 2001) for movement seaward. They are observed to swim at the surface only during ebb tide at night but not at other times. Subsequently, post-spawning females undergo flood-tide transport for movement back into estuaries. They are observed to swim at the surface of the water column only during flood tide at night (Tankersley et al., 1998).

Forward et al. (2003) demonstrated that the behavioral basis of ETT during the spawning migration was a tidal rhythm in vertical swimming. Under constant conditions in vertical columns, females with mature embryos had a circatidal rhythm, in which they had frequent bouts of swimming to the surface of the column during the expected time of ebb tide in the field and remained on the bottom during times of flood tide in the field. This rhythm disappeared after larval release (Forward et al., 2003).

The present study addresses three questions associated with this circatidal rhythm. First, the initial study only tested ovigerous females with mature embryos that were collected as they underwent ETT. Thus, does the rhythm also occur in any female with mature embryos regardless of whether she is undergoing ETT?

Second, Tankersley et al. (1998) only observed females to undergo ebb-tide transport at night but the rhythm study found that females migrated vertically at times of consecutive ebb tides in the field (Forward et al., 2003). The implication is that the light phase in the field suppresses swimming during daytime ebb tides. Thus, when exposed to a light/dark cycle in the laboratory, do ovigerous females only vertically migrate during the time of ebb tide during the dark phase?

Third, a number of studies have been initiated in which attachments, such as transmitters, are attached to adult blue crabs to record and send data as they move throughout estuaries. A question is whether these attachments affect the circatidal rhythm that underlies ETT. Rhythms of ovigerous blue crabs were observed for crabs that were not undergoing ETT, in the presence of a light/dark cycle and with attachments. Circatidal rhythms were evident under all conditions.

2. Materials and methods

Ovigerous blue crabs, *C. sapidus* Rathbun, were collected at night during July through September 2002 from two sites in the Newport River Estuary, North Carolina, USA (34°43′ N; 76°40′ W). Crabs from the North River area of the estuary were obtained from commercial crab traps from an area with a large adult population. All other crabs were collected at night in shallow water on a tidal flat near the Duke University Marine Laboratory. Females were classified according to the developmental stage of their egg masses (De Vries et al., 1983) by examining small groups of eggs (100–200) under a dissecting microscope. Crab egg masses were grouped based upon yolk content and embryo eye development. Two crab categories were used in experiments. First, early-stage females contained eggs that were yellow/orange in color, contained embryos that lacked eyespots, and were >6 days from hatching (Stages 1–5 of De Vries et al., 1983). Second, mid-stage females possessed eggs that were dark orange/rust in color, contained <50% yolk, possessed embryos with newly formed eye

spots and well-developed structures, and were 3–4 days from hatching (Stages 6–7 of De Vries et al., 1983).

Endogenous rhythms in vertical swimming behavior were monitored under constant conditions (one exception, described below) in the laboratory. The experimental chambers were cylindrical tubes (1.23 m × 44 cm diameter), 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 crabs visual pigments (e.g., Cronin and Forward, 1988) predict they should have low visual sensitivity to red light. Crabs were placed individually in tubes and their behavior was monitored with a video camera (Cohu Model 4815-3000) and recorded with a time-lapse video recorder (Panasonic Model AGRT600A). Crabs were not fed during this time. Swimming behavior was recorded until either the crabs released their larvae or at least four tidal cycles had elapsed.

There were four sets of experiments. First, four females with mid-stage eggs were obtained from commercial crab traps in North River and placed under constant conditions in the tubes. Second, four females with early stage eggs were collected at night and placed individually in plastic baskets suspended at a depth of about 3 m (at high tide) from a dock at the Duke University Marine Laboratory (Beaufort, NC, USA). They remained in the baskets until the eggs matured to the mid-stage when they were placed under constant conditions in the tubes. Both the North River and Duke University Marine Laboratory estuarine areas have semi-diurnal tides. The intent of these two experiments was to determine whether crabs that were not caught while undergoing ebb-tide transport at night but had mid-stage eggs had a circatidal rhythm in vertical migration.

The third set of experiments tested the effect of a light/dark cycle on the circatidal rhythm. Eight females with early-stage eggs were maintained in the baskets until their eggs matured to mid-stage. They were then placed in the tubes and exposed to a light/dark cycle that had the same timing as the ambient cycle. Light was provided from cool-white florescent tubes at an intensity of 7.2×10^{14} photon cm^{-2} s^{-1}. This intensity is well above the visual threshold for crustaceans (about 10^7 photons cm^{-2} s^{-1}; e.g., Forward, 1988) but below that of full sunlight (about 10^{17} photons cm^{-2} s^{-1}). During the dark phase, crabs were exposed to the red florescent light for video recording.

The fourth set of experiments tested whether attachments to the backs of crabs affected their circatidal rhythms in vertical swimming. The two types of attachments were first a cylindrical transmitter (Sonotronic) measuring 67 mm long and 17 mm in diameter weighing 26.5 g in air. Smaller transmitters were used for tracking female blue crab migrations in estuaries (R. Tankersley, personal communication). Two crabs were tested having carapace diameters of 10.6 and 12.4 cm. The second attachment was rectangular (22 × 50 × 11 mm; weight in air = 18.3 g) and used by Dr. T. Wolcott for recording water parameters during blue crab movements. Four crabs were tested having carapace diameters of 8–12 cm. Both attachments were connected to the crab by wire around the lateral spines.

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 if a crab ascended to this level, it usually swam to the surface. Since 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 were analyzed for periodicity (Tankersley and Forward, 1994) using a combination of autocorrelation and maximum entropy spectral analysis (MESA) following

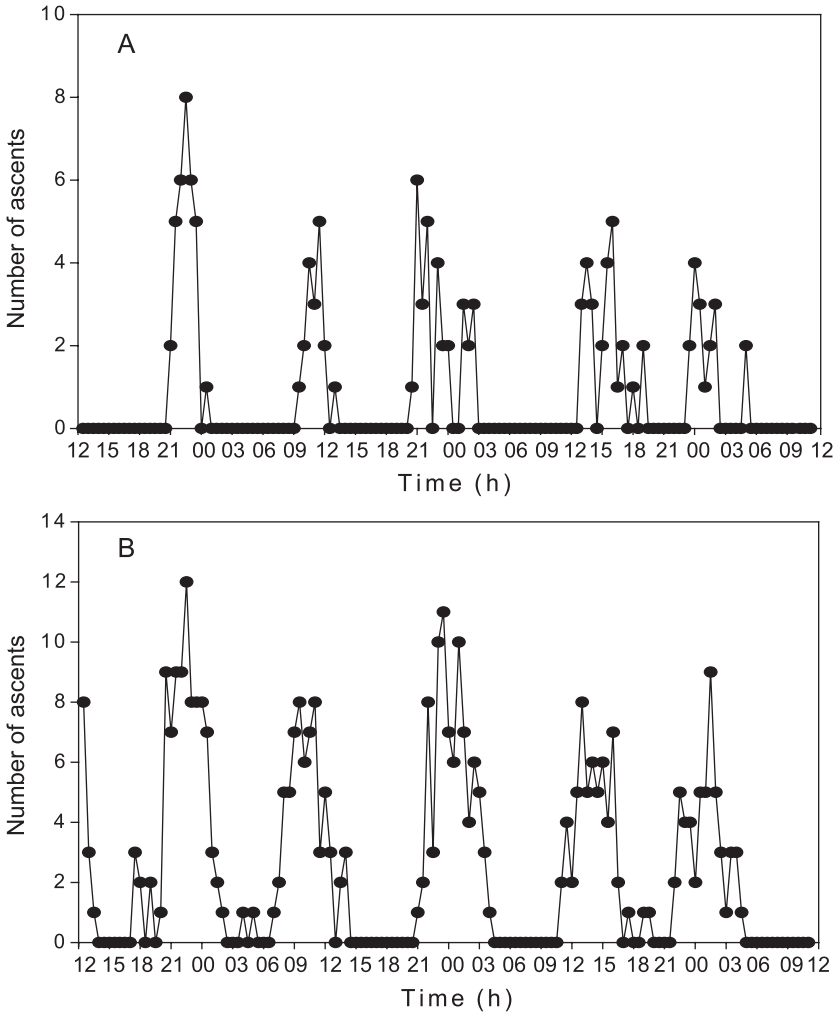

Fig. 1. *C. sapidus*. Actographs of the number of swimming bouts at 0.5 h intervals for two representative ovigerous blue crabs with mid-stage eggs (3–4 days from hatching) that were collected from crab traps on 7/22/03 in North River (North Carolina, USA) and maintained under constant conditions in the laboratory. The free running period length in A is 12.5 h and 12.6 h in B.

the procedures and algorithms described by Dowse and Ringo (1989). Rhythmicity was determined by plotting the autocorrelation coefficients calculated at 0.5-h lag intervals (= sampling interval) as a function of lag (correlogram; Broom, 1979). Peaks in the autocorrelation plots exceeding $\pm 2/\sqrt{N}$ (N = sample size) were considered to indicate statistically significant rhythmicity at $P < 0.05$ (i.e., 95% confidence intervals; Chatfield, 1989). Period lengths were confirmed with MESA, which fitted the data to an autoregressive (AR) stochastic model and used Fourier analysis of the AR coefficients to detect cyclical behavior and rhythmicity (Levine et al., 2002). 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. Plots of cross-correlations 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).

Table 1
Cross-correlation analysis for crabs from the North River, from baskets at the Duke University Marine Laboratory, exposed to a light/dark cycle and with attachments

Experimental condition	Maximum cross-correlation	Lag (h)
North River—crab 1	0.6810	1
North River—crab 2	0.6229	0
North River—crab 3	0.6418	1.5
North River—crab 4	0.8225	1.5
DUML—basket—crab 1	0.3830	0
DUML—basket—crab 2	0.5996	0
DUML—basket—crab 3	0.6850	1.5
DUML—basket—crab 4	0.5687	1
Light/dark cycle—crab 1	0.8879	1
Light/dark cycle—crab 2	0.8455	1
Light/dark cycle—crab 3	0.6250	1
Light/dark cycle—crab 4	0.7455	0.5
Light/dark cycle—crab 5	0.8215	0.5
Light/dark cycle—crab 6	0.8094	0
Light/dark cycle—crab 7	0.8379	0.5
Light/dark cycle—crab 8	0.8015	0.5
Rectangular attachment—crab 1	0.7440	1
Rectangular attachment—crab 2	0.6334	3
Rectangular attachment—crab 3	0.7755	− 0.5
Rectangular attachment—crab 4	0.5985	0.5
Cylindrical attachment—crab 1	0.7098	4
Cylindrical attachment—crab 2	0.6642	− 1

Lag is relative to time of high tide at 0 h.

3. Results

3.1. Rhythms by crabs from traps and baskets

All four crabs with mid-stage eggs that were obtained from crab traps in the North River had pronounced circatidal rhythms in vertical swimming (Fig. 1). The average free

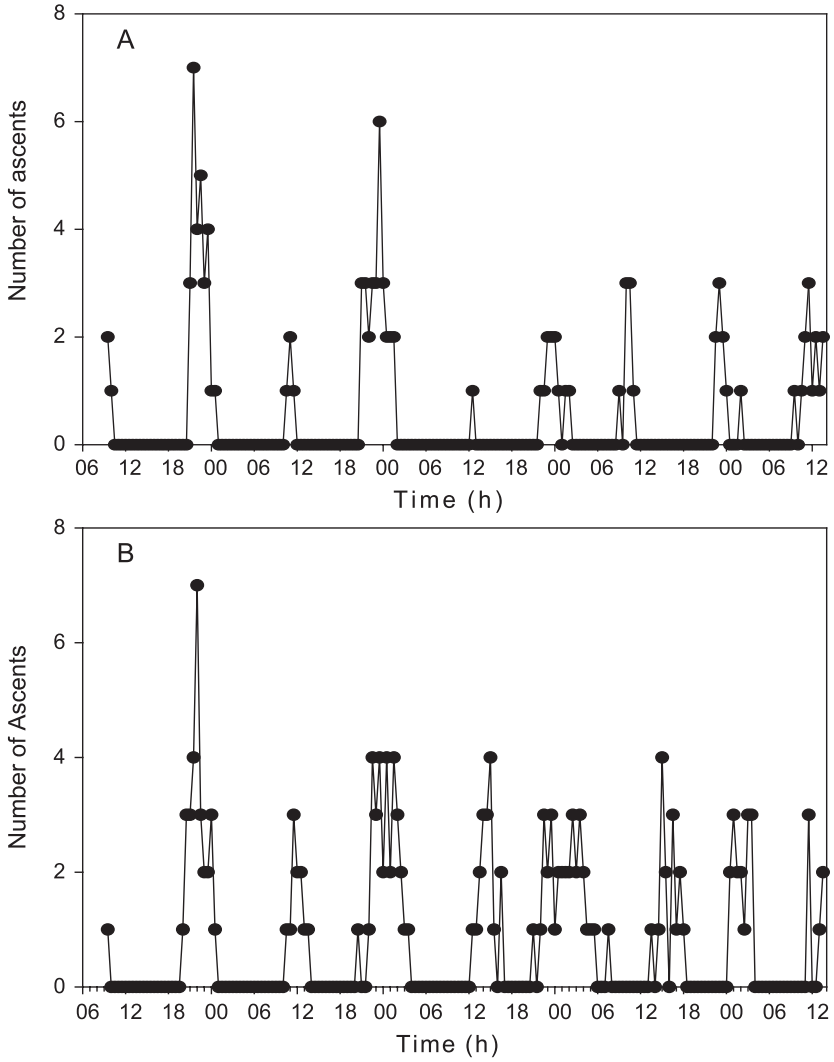

Fig. 2. *C. sapidus*. Actographs of the number of swimming bouts at 0.5 h intervals for two representative ovigerous blue crabs that had early embryos on 9/1/02. They were maintained in semi-diurnal tidal conditions in baskets until their eggs reached mid-stage on 9/8/02 when they were placed in constant conditions. The free running period length in A is 12.9 h and 12.6 h in B.

running period length was 12.5 h (S.D. = 0.65 h; $n = 4$). Swimming occurred during the time of ebb tide in the field with peaks occurring an average of 1 h (S.D. = 0.71 h; $n = 4$) after the time of high tide in the field (Table 1).

Four crabs with early-stage embryos were placed in baskets suspended from a dock at the Duke University Marine Laboratory and removed when the embryos matured to mid-stage. All of these crabs also had circatidal rhythms in swimming (Fig. 2) with an average

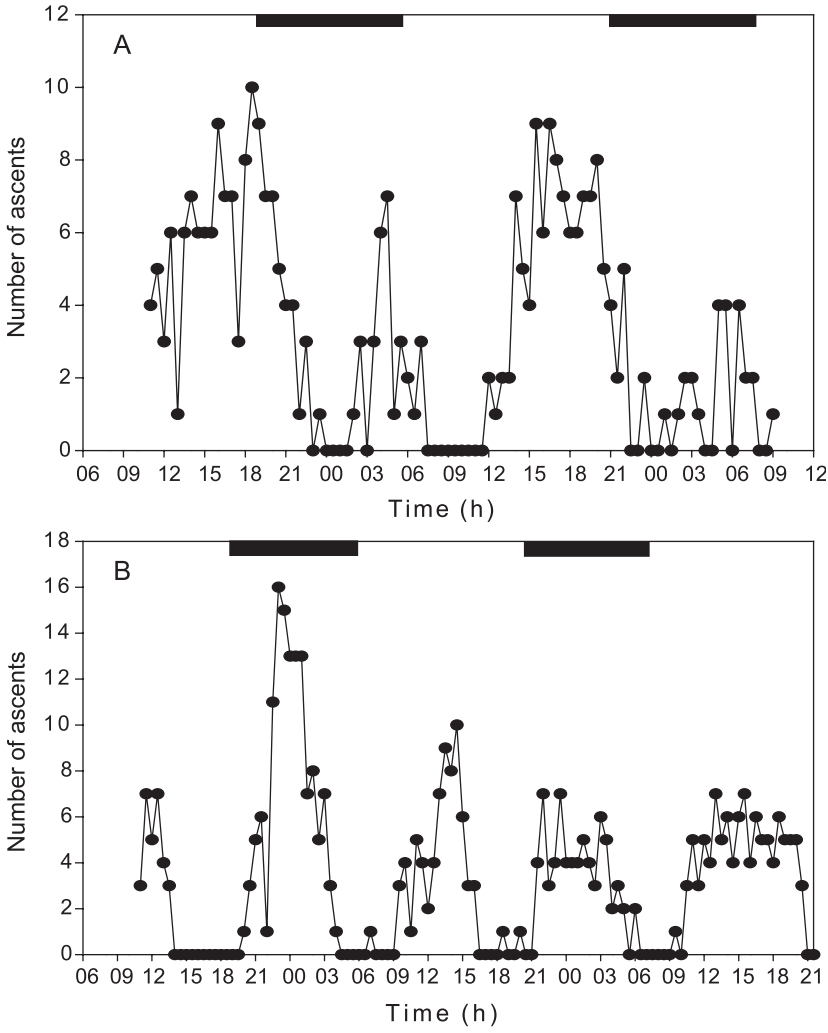

Fig. 3. *C. sapidus*. Actographs of the number of swimming bouts at 0.5 h intervals for two representative ovigerous blue crabs that had mid-stage eggs and were exposed to a light/dark cycle. The dark bars at the top of the figure show the time of darkness in the lighting cycle. Monitoring of crab A began on 8/02/02 and the period length was 11.9 h. Monitoring of crab B began on 8/11/02 and the period length was 12.0 h.

free running period length of 12.9 h (S.D. = 0.47 h; $n = 4$). The time of swimming coincided with the time of ebb tide in the field with peaks at an average of 0.63 h (S.D. = 0.75; $n = 4$) after the time of high tide (Table 1). Since both crabs from traps

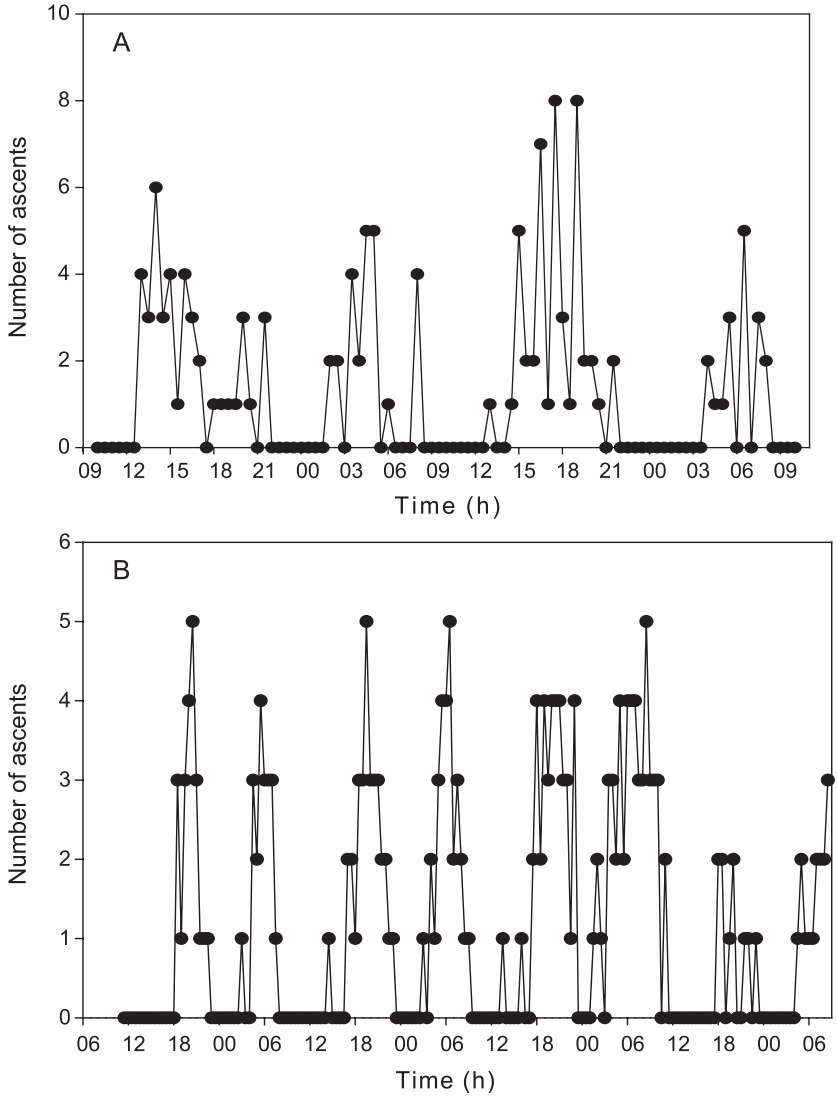

Fig. 4. *C. sapidus*. Actograph of the number of swimming bouts at 0.5 h intervals for two representative ovigerous blue crabs with mid-stage egg that had rectangular attachment to their backs. Crab A had a carapace width of 11.2 cm, went into constant conditions on 7/17/02 and had a free running period of 12.1 h. Crab B had a carapace width of 11.9 cm went into constant conditions on 9/18/02 and had a free-running period of 12.2 h.

and those that were maintained in baskets throughout embryo development had similar circatidal rhythms in swimming, the rhythm does not just occur in crabs collected while undergoing ebb-tide transport. Alternatively, the rhythm appears to be linked to exposure to tidal conditions and egg maturity.

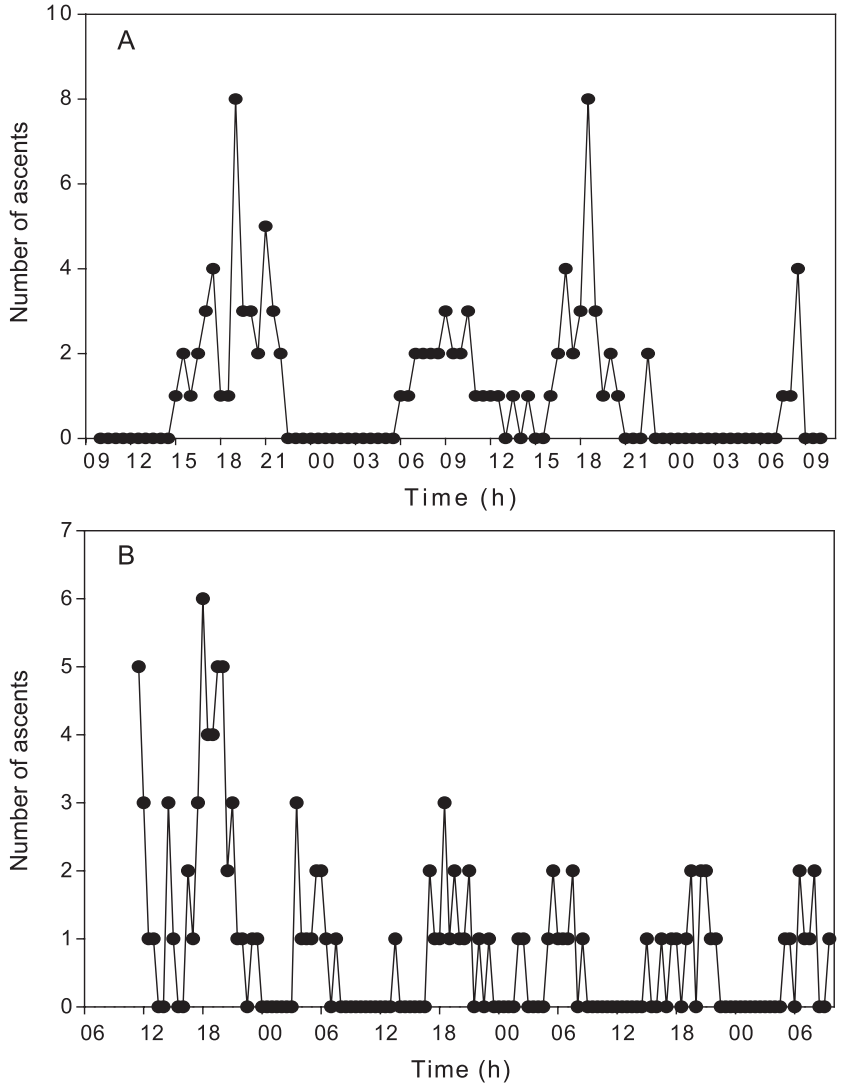

Fig. 5. *C. sapidus*. Actograph of the number of swimming bouts at 0.5 h intervals for two representative ovigerous blue crabs with mid-stage egg that had cylindrical transmitters attachment to their backs. Crab A had a carapace width of 10.6 cm, went into constant conditions on 7/17/02 and had a free running period of 12.2 h. Crab B had a carapace width of 12.4 cm, went into constant conditions on 9/18/02 and had a free-running period of 11.8 h.

3.2. Rhythms in the presence of a light/dark cycle

All eight of the crabs that were tested in the presence of a light/dark cycle retained the circatidal rhythm (Fig. 3). Peaks in swimming activity occurred during both the light and dark phases. The average free running period length was 12.1 h (S.D. = 0.33: $n = 8$). Swimming bouts were not suppressed by light and corresponded to the times of consecutive ebb tides in the field. The average time of peak swimming was 0.63 h (S.D. = 0.35; $n = 8$) after the time of high tide (Table 1).

3.3. Rhythms by crabs with attachments

Attachments to the backs of crabs did not affect the circatidal rhythms. Four crabs were tested with rectangular attachments. All crabs displayed circatidal rhythms (Fig. 4) with the average period length of 12.3 h (S.D. = 0.22 h; $n = 4$). The average time of peak swimming occurred 1.0 h (S.D. = 1.5 h; $n = 4$) after high tide during the time of ebb tide. Similarly, crabs with large cylindrical transmitters also had circatidal rhythms in swimming (Fig. 5). The average free running period length was 12.0 h (S.D. = 0.28 h; $n = 2$). The average time of peak swimming was 2.5 hr after the time of high tide (S.D. = 3.5; $n = 2$). Thus, crabs could swim vertically with the attachments and the rhythms persisted with swimming occurring during the time of consecutive ebb tides in the field (Figs. 4 and 5; Table 1).

4. Discussion

Female blue crabs, *C. sapidus*, undergo ebb-tide transport (ETT) during their spawning migration, in which they are observed swimming at the surface during ebb tide at night (Tankersley et al., 1998). Forward et al. (2003) demonstrated that ovigerous crabs with mid-stage eggs collected while undergoing their spawning migration have a circatidal rhythm in vertical swimming. They swam during the time of consecutive ebb tides in the field. The first question addressed in the present study was whether ovigerous females with mid-stage eggs have the rhythm regardless of whether they are undergoing ETT.

Crabs with mid-stage eggs collected from commercial crab traps clearly had the rhythm (Fig. 1). These crabs could be undergoing ETT but were caught during their benthic foraging activities. Alternatively, they were caught in an area with a large adult population that was about 10 km from an ocean inlet, which suggest they were just beginning their seaward migration.

The other group of test crabs was collected with very immature embryos and held in baskets in semi-diurnal tidal conditions until their embryos matured to mid-stage. Crabs with immature embryos lack a clear circatidal rhythm in vertical swimming (Forward et al., 2003). Crabs collected with early-stage embryos but held until embryos reached mid-stage also had the circatidal rhythm (Fig. 2), in which swimming occurred during the time of ebb tide in the field (Table 1). These results suggest that the circatidal rhythm in vertical migration develops as the embryos mature and is not restricted to crabs actually involved in ETT at night. Thus, females must be able to detect the developmental stage of

the embryos. Possible cues from the embryos are peptide pheromones, which are known to induce larval release behaviors in *C. sapidus* (Tankersley et al., 2002) and other brachyuran species (e.g., Forward et al., 1987; Rittschof et al., 1989; De Vries et al., 1991).

Tankersley et al. (1998) only observed females undergoing ETT during their spawning migration at night. However, under constant conditions, females vertically migrate during the time of consecutive ebb tides in the field (e.g., Figs. 1 and 2). Thus, the second question asked during this study was whether females exposed to a light/dark cycle in the laboratory only vertically migrate during the time of ebb tides during the dark phase. The circatidal rhythm was not affected by the light/dark cycle, as crabs swam vertically during both the day and night phases (Fig. 3). The intensity level of the light phase (7×10^{14} photons cm^{-2} s^{-1}) was above the visual threshold for crustaceans (e.g., Forward, 1988) but was three orders of magnitude below that of sunlight (about 10^{17} photons cm^{-2} s^{-1}). These results suggest that crabs may undergo ETT at depth during the day, but high sunlight intensities would likely inhibit swimming near the surface. Among other crustaceans, selective tidal stream transport is commonly observed only at night (Forward and Tankersley, 1994), but when placed under constant condition, crustaceans show a circatidal rhythm with swimming at the times of the appropriate tide during both the day and night (e.g., Tankersley and Forward, 1994). Alternatively, crabs from nontidal habitats can have circadian rhythms that are entrained by the light/dark cycle (e.g., Forward et al., 1982).

The final question was whether attachments to the backs of crabs affected the circatidal rhythm in vertical migration. An attachment could be heavy enough to prevent vertical swimming. Nevertheless, crabs with either rectangular attachments or a cylindrical transmitter displayed the circatidal rhythm (Figs. 4 and 5), which indicates that theses size attachments will not inhibit vertical swimming movements by large females. Thus, the circatidal rhythm in vertical migration is very robust. It occurs in crabs with mid-stage eggs regardless of previous ETT experience, and is unaffected by a light/dark cycle or by attachments.

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