Limnol. Oceanogr., 49(5), 2004, 1857–1870

Testing a selective tidal-stream transport model: Observations of female blue crab (*Callinectes sapidus*) vertical migration during the spawning season

James L. Hench[1]
Institute of Marine Sciences, University of North Carolina at Chapel Hill, 3431 Arendell Street, Morehead City, North Carolina 28557

Richard B. Forward, Jr.
Nicholas School of the Environment and Earth Sciences, Duke University, 135 Duke Marine Lab Road, Beaufort, North Carolina 28516

Sarah D. Carr
Institute of Marine Sciences, University of North Carolina at Chapel Hill, 3431 Arendell Street, Morehead City, North Carolina 28557

Daniel Rittschof
Nicholas School of the Environment and Earth Sciences, Duke University, 135 Duke Marine Lab Road, Beaufort, North Carolina 28516

Richard A. Luettich, Jr.
Institute of Marine Sciences, University of North Carolina at Chapel Hill, 3431 Arendell Street, Morehead City, North Carolina 28557

Abstract

Female blue crabs, *Callinectes sapidus*, mate in estuaries and undergo a seaward spawning migration to release larvae. According to the prevailing model, females with mature embryos use nocturnal ebb-tide transport (ETT) to move seaward, release larvae, and then reverse to nocturnal flood-tide transport (FTT) to move back into the estuary. We tested this model by examining the vertical migratory behavior of ovigerous and post-larval release female crabs. Simultaneous physical–biological data were collected for 38 d during Aug 2002–Sep 2002 in Bogue Sound, North Carolina. Crab water-column positions were determined with miniature internally recording pressure sensors. Local current and water properties were measured, and crab vertical migration times relative to observed currents were used to determine ETT and FTT behavior. Surface censuses of free-swimming crabs on 19 nocturnal ebbs were used to complement the intensive studies of individual crabs. The study found that (1) the pressure sensors had a measurable but small effect on swimming, (2) females migrated during day and night ebb tides, (3) females used ETT throughout embryo development, (4) ETT corresponded to the rate of decrease in water level (hydrostatic pressure), (5) larvae were released at high tide or when water level was falling, often within several hours of sunrise, and (6) post-larval release females continued ETT and did not switch to FTT. Thus, the data did not support the prevailing ETT-FTT reversal model. Rather, females continue ETT into coastal areas, releasing subsequent clutches farther seaward, which increases the potential for successful larval transport to favorable offshore developmental areas.

For many marine species, successful recruitment entails adult migration out of an estuary and subsequent larval transport into an estuary. While substantial literature is accumulating on the latter process (e.g., Weinstein 1988; Crowder and Werner 1999; Epifanio and Garvine 2001), comparatively little attention has been focused on the prerequisite egress of adult spawners, particularly for marine species with limited capability for long-distance migrations (e.g., invertebrates). Elucidating the physical processes that aid or direct spawning migrations for these less motile species is critical in developing a complete understanding of their life cycles.

One such species is the blue crab (*Callinectes sapidus*), which mates in upper portions of estuaries from spring to early fall. After mating, the females move to the higher-salinity areas of estuaries, where they oviposit and migrate seaward to coastal regions to release their larvae. If oceanic conditions are favorable, the larvae are transported offshore, where higher salinities and decreased predation are more conducive to development (e.g., Smyth 1980; McConaugha

[1] To whom correspondence should be addressed. Present address: Environmental Fluid Mechanics Laboratory, Stanford University, Stanford, California 94305-24-4020 (jhench@stanford.edu).

Acknowledgments

We thank Pia Moisander and Josh Osterberg for valuable assistance in the field. Sean Powers and two anonymous reviewers provided helpful suggestions on the manuscript. Funding was provided by NSF (OCE-0094938 and OCE-0095092) and NC Sea Grant (01-Bio-05 and 02-Bio-04).

et al. 1983; Epifanio et al. 1989). After a 4- to 7-week period as offshore meroplankton (Costlow and Bookhout 1959), blue crabs molt to a megalopae stage and return to estuarine nursery areas (e.g., Orth and van Montfrans 1987; Olmi et al. 1990). When blue crab larvae are first released, they are transported passively by currents. Thus, their initial survival depends on the successful seaward migration of adult female crabs and the timing of larval release relative to tidal phase. Perhaps the most important segment of the female spawning migration occurs during the period from when the female crab extrudes an egg mass and carries it on her lower abdomen (hereafter called an ovigerous crab) until the time of larval release. During this period, which lasts ca. 2 weeks, the egg mass changes color from orange to brown to black (ca. 2–3 d before release) as the embryos mature (Williams 1984).

Recently, Tankersley et al. (1998) proposed a migration model by which adult female blue crabs maximize their spawning migration movements using selective tidal-stream transport. The model was based on observations that ovigerous female crabs swim at the surface primarily on nocturnal ebb tides. In contrast, the crabs swimming at the surface on nocturnal flood tides were identified as post-larval release females. During the daytime, few crabs of any type were observed. On the basis of these observations, Tankersley et al. (1998) concluded that the ovigerous female crabs were migrating using nocturnal ebb-tide transport (ETT), a behavior in which an animal ascends into the water column during ebb to achieve horizontal seaward transport, whereas on flood tide, they remain on or near the seabed (Forward and Tankersley 2001). Post-release crabs were described as undergoing nocturnal flood-tide transport (FTT, similar to ETT but moving up-estuary on flood tides). The model proposed that the females would use repeated ETT-FTT round-trip migrations during the spawning season for each successive egg clutch.

In a subsequent laboratory study, Forward et al. (2003) found that the behavioral basis of ETT during the seaward phase of the spawning migration was an endogenous tidal rhythm (Palmer 1995) in vertical swimming. Under constant conditions in laboratory tanks, females with mature embryos (i.e., black egg mass) had a semidiurnal tidal rhythm, in which they repeatedly swam to the surface of the tank during the expected time of ebb tide at the location where they were captured and remained on the bottom during expected times of flood tide. The rhythm disappeared after larval release. When female crabs with young embryos (i.e., orange egg mass) were placed in laboratory tanks, they swam at the expected times of ebb tide for the first few tidal cycles, after which rhythmic swimming was not apparent. In all the experiments, the crabs swam on successive ebb tides, rather than on nocturnal ebbs only as observed by Tankersley et al. (1998). Also, none of the laboratory crabs switched to swimming at the times of flood tide after larval release.

A number of questions arise from these studies: (1) Does vertical migration during selective tidal-stream transport occur only at night? (2) What are the most active periods of swimming relative to tide phase, and what environmental cues (light : dark [LD] cycle, hydrostatic pressure, current speed and direction, salinity, and temperature) control the timing of vertical migration? (3) Does the ETT behavior of females change as the embryos develop? (4) What is the time of larval release relative to tidal phase and the LD cycle? and (5) After larval release, when do females cease ETT and begin FTT?

To address these questions, we coupled a novel technique for monitoring the vertical migration behavior of individual female crabs under field conditions with intensive physical observations. The approach was to attach miniature, internally recording pressure sensor tags to individual females that were tethered in a location where simultaneous measurements of local currents, water levels, and water properties were made. The pressure sensor data were used to determine crab positions in the water column. Relating the times of vertical migrations to observed local tides was used to determine whether ETT or FTT was occurring. Laboratory tests were conducted to determine the effect of the pressure tags on vertical migration behavior. This approach had the advantages that vertical migratory behavior relative to tides could be continuously followed in individual females, long-term records spanning embryo development and postlarval release periods could be obtained, and crabs could be continuously exposed to natural environmental conditions. To complement the detailed physical–biological data for individual crabs, surface censuses of crabs performing ETT on nocturnal ebbs were conducted to see if migration patterns of the tethered crabs were representative of the population.

Methods

Laboratory tests of tag effects—Laboratory experiments were conducted to evaluate the effect of the pressure tags (described below) on swimming behavior and to check for possible unrecorded vertical migrations due to the discrete sampling interval of the tags. Tagged and untagged ovigerous crabs (different individuals from those in the field experiments) were placed in separate 1.2-m-high, 0.44-m-diameter, translucent vertical tubes filled with local seawater. The crabs were maintained in constant conditions (aerated, low-level red light; temperature, 23°C; salinity, ~32), and swimming behavior was recorded on videotape (apparatus previously described in Forward et al. 2003). The laboratory test crabs were within 4 d of larval release as determined by egg stage (De Vries et al. 1983). Due to an endogenous rhythm, late egg stage crabs undergo vertical migration behavior under constant conditions (Forward et al. 2003). Continuous video recordings were analyzed to determine ascent and descent speeds, ascent durations, and number of ascents during the first 24 h (approximately two complete semidiurnal tidal cycles) that the crabs were in the tubes. Each vertical migration was analyzed for the time when the crab left the bottom of the tank until the time it reached the surface. The tank height was divided by the time interval to obtain ascent speed. Descent speeds were computed in a similar way. The total time of each vertical migration and the total number of vertical migrations were also determined. The mean of each of these values was computed for each crab, and combined means and 95% confidence intervals were computed for the set of untagged crabs and the set of tagged crabs.

Table 1. Description and deployment durations for individual crabs in field experiments. All dates are year 2002.

Crab	Carapace width (cm)	Observation dates	Observation duration (d)	Larval release date	Notes
1	15.7	16 Aug–12 Sep	27	18 Aug	Second set of eggs extruded on 26 Aug with second release on 4 Sep
2	13.0	16 Aug–26 Aug	10	19 Aug	Lost crab and tag after 10 d
3	15.0	18 Aug–5 Sep	18	21 Aug	Second set of eggs extruded (after removal from array), which were released on 20 Sep
4	15.5	4 Sep–23 Sep	19	9 Sep	
5	16.6	4 Sep–23 Sep	19	12 Sep	
6	16.0	5 Sep–9 Sep	4	9 Sep	
7	14.0	9 Sep–23 Sep	14	17 Sep	Lost crab to predation after 14 d
8	15.5	12 Sep–22 Sep	10	17 Sep	Lost crab to predation after 10 d

Field site—Simultaneous physical and biological data were collected continuously for 38 d (16 Aug 2002–23 Sep 2002) at a site (34°42.90′N, 76°46.30′W) in Bogue Sound, North Carolina. The main axis of the estuary runs roughly east–west and opens to the ocean at Beaufort Inlet, 12 km east of the field site. The site has a mean depth of 2.2 m, is dominated by tidal forcing, and is in a narrow fetch-limited part of the estuary where wind waves are minimal. Diver inspections of the immediate area around the field site found a flat, sandy bottom.

Physical measurements—Currents were measured using a bottom-mounted, upward-looking RD Instruments Workhorse 1.2-MHz acoustic Doppler current profiler (ADCP), sampling at 0.5 Hz and averaging over 6 min, which yielded a velocity precision of <0.02 m s^{-1}. Vertical bin size was 0.25 m, but given the shallow, generally well-mixed conditions in the system, we used only the first bin for analysis (centered 1.4 m above the bottom), which was roughly mid water column. The velocity data were rotated into along- and across-estuary components, which indicated that flow at this location was highly rectilinear with the along-channel velocity variance more than two orders of magnitude greater than the across-channel velocity variance. Therefore, the following analyses used the along-channel velocity component (hereafter called V) to determine ebb and flood. Temperature (T), salinity (S), and pressure were measured once per minute with a CTD (Sea-Bird SBE-37SM) attached to the ADCP frame. Pressure was converted to water-column height (H), and the mean was removed to obtain water surface elevation (η). The rates of change (or tendencies) of surface elevation, velocity, salinity, and temperature ($\partial\eta/\partial t$, $\partial S/\partial t$, and $\partial T/\partial t$, respectively) were approximated from the observed time series using finite differences. For example, $\partial\eta/\partial t$ at time t was computed as $(\eta^{t+1} - \eta^{t-1})/(2\Delta t)$, where η^{t+1} and η^{t-1} are the next and previous η values measured, respectively, and Δt was the time increment between measurements.

Tethering—Adjacent to the ADCP/CTD frame, an array of individual crabs was tethered to the bottom in a radial pattern out from the ADCP/CTD. Ovigerous females (mean carapace width, 15.3 cm range, 13.0–16.6 cm) were caught by dip net near the tethering location the night before deployment. Crabs were maintained in running seawater tables with ambient LD and salinity until the following day, when they were fitted with Vemco Minilog-TDX internally recording pressure tags (weight, 23 g in air and 10 g in water). A tag was strapped to the carapace of each crab with 18-gauge shielded copper wire. A small loop in the wire was attached to a 0.46-mm-diameter stainless steel tether anchored by 12-kg lead weights. The tethers were 5.0 m long, about twice the water-column height. Ground lines ran 10 m from the tether weights to a second set of weights that anchored surface floats that were used for recovery. Pressure tags recorded at 0.091 Hz, which permitted up to 25-h deployments. Crabs were checked from a boat every ca. 24 h (between 1100 h and 1400 h local time) by pulling up the weights and tethers. During each service, the crabs were photographed, their egg state was recorded, their tags were removed, and the data were downloaded to a laptop computer. The tags were then reprogrammed, and the crabs were retagged, retethered, and returned to the water. The average time each crab was held in the boat was 20 min, about 15 min of which was in an aerated, covered bucket of local seawater while the data were downloaded. Between two and four crabs were deployed at once, and a total of eight crabs were observed for periods ranging from 4 to 27 continuous days. All crabs started with external egg masses and were monitored until after larval release. Table 1 shows deployment details for each crab.

Pressure tag data processing—Physical measurements from the ADCP and CTD were interpolated to the times when the crab pressure sensors recorded data. The crab height above bottom, z (positive upward with $z = 0$ at the bottom), was computed by subtracting the pressure tag depth (d) from the total water-column height (H) as measured by the fixed bottom-mounted CTD, where $z = H - d$. The vertical resolution of the pressure tags and CTD was 0.20 and 0.001 m, respectively. Vertical crab movements of $z <$ 0.30 m above bottom were discarded as below resolution limits.

Censuses of free-swimming crabs at surface—To complement the intensive studies of individual crabs described above, we conducted surface censuses of free-swimming crabs. The censuses were conducted in the Port of Morehead

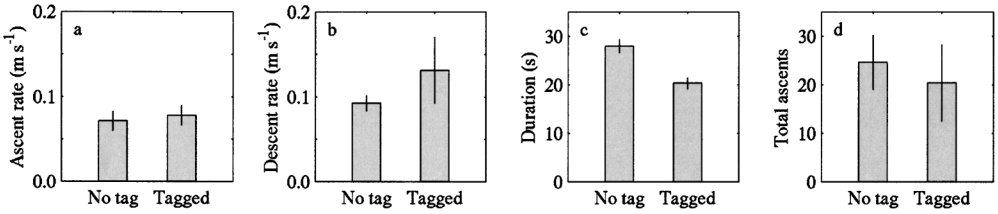

Fig. 1. Laboratory tests of tag effects obtained from video analysis of first 24 h in vertical tubes. Swimming behavior is compared for tagged crabs ($n = 3$) and untagged crabs ($n = 7$). Values are the means of the individual crab's mean values, and error bars indicate 95% confidence intervals.

City (34°43.10′N, 76°41.70′W), located between the tethering location and Beaufort Inlet. Local depths were 4–14 m. Censuses were conducted on 9 nocturnal ebbs in 2001 (Jul: 12, 18, 19, and 20; Aug: 2, 3, 14, 15, and 16) and on 10 nocturnal ebbs in 2002 (Jun: 24 and 25; Jul: 7, 8, 21, 22, and 23; Aug: 6, 7, and 8). Each night, a boat was driven at ca. 4.6 km hr^{-1} repeatedly throughout the 0.25-km^2 area of the port for 3 h. Every surface-swimming crab seen was captured by dip net (95% efficiency), and their egg status was recorded (orange, brown, black, or none). All crabs were swimming with the current when captured. For analysis, each night ebb was treated as an independent sample, and the percentage of each crab type (orange, brown, black, or none) was computed. The mean and 95% confidence interval for the combined set of night ebbs were computed for each year. This calculation preserves the relative proportions of crab types by normalizing for the absolute number of crabs caught each night.

Results

Laboratory tests of tag effects—The first goal of the laboratory experiments was to test if the tags affected crab vertical swimming speeds, duration, or frequency (Fig. 1). Mean ascent rates (Fig. 1a) for tagged and untagged crabs were both about 0.07 m s^{-1}. The mean descent rate (Fig. 1b) for untagged crabs was 0.10 m s^{-1} versus 0.12 m s^{-1} for tagged crabs. The mean swimming duration (the total time in the water column for a single vertical migration) was shorter for the tagged crabs (20 s) than for the untagged crabs (26 s) (Fig. 1c). Crabs with tags made 17% fewer ascents into the water column (Fig. 1d). However, of all the measures of swimming behavior, only swimming duration was statistically different between tagged and untagged crabs ($p < 0.05$, *t*-test). The second goal of these experiments was to determine whether pressure tags sampling at 0.091 Hz (i.e., 11-s intervals) would record all vertical migrations. Comparison between the total number of ascents obtained from continuous video and those obtained from the pressure tag records showed that the pressure tag data underestimated the mean number of ascents by <15% (data not shown). This difference arose from a number of very brief ascents that the 11-s sampling interval missed. The tests indicate that the tags have a measurable, but small, effect on crab vertical migration and that the discrete sampling interval of the pressure tags adequately captured ascent behavior.

Tethering issues—The laboratory tests described above may be a lower-bound test of experimental effects because they were performed in still water and did not include tethering effects. Tethers were necessary in the field experiments to keep crabs adjacent to a stationary CTD and current meter. The observed crab heights above bottom indicate that the crabs usually did not reach the water surface (Fig. 2), as observed in free-ranging crabs using ETT (Tankersley et al. 1998). The tethers were anchored to the bottom, and their downward force in a current most likely decreased the vertical positions in the water column that the crabs would have otherwise obtained. This tether effect is in addition to the weight of the tags studied in the laboratory tanks. Adult blue crabs are remarkably strong swimmers, capable of swimming speeds >1 m s^{-1} (Spirito 1972), and the field data indicate that they can overcome the weight of the tag and the tether force. However, the downward force of the tether increases the effort required for the crab to stay in the water column, and it therefore mostly likely decreases both the duration of each ascent and the crab height above the bottom. The limited precision and data storage of the tags did not permit observation of crab ascents <0.3 m above bottom or some crabs ascents <11 s in duration, so the data probably underestimate the actual number of vertical migrations. Thus, the observed swimming ascents in the field experiment are probably a lower bound of the vertical migratory activity of free-ranging crabs.

The tethers also prevented crabs from moving to other locations in the estuary (and ultimately to the ocean), as a free-ranging crab could. Changes in environmental cues that a free-ranging crab might experience (e.g., longitudinal salinity gradients) may affect crab behavior. The tethers also limited the foraging areas for the crabs; therefore, it is possible that feeding and energy levels were affected. However, the crabs remained alive and very active during the experiment, and two of the crabs extruded a second egg mass (had they been starving, they would have reabsorbed eggs). It is also likely that crab behavior was modified immediately following return to the water after handling each day. The data indicated that the crabs generally resumed vertical migratory behavior, within 1 h after being returned to the water if they were migrating beforehand.

Field data—The combined physical–biological data for the 38-d field experiment are shown in Fig. 2. The observed water level and velocity records show a dominant semidiurnal signal. Harmonic analysis of the water level and ve-

locity records indicated that the astronomical tides explained >92% and 97% of their respective variances. The largest constituent was the major semidiurnal tide, M_2 (period 12.42 h), which was nearly an order of magnitude larger than the largest diurnal constituent, K_1 (period 23.93 h). Water-level elevation and velocity were out of phase, with velocity leading elevation by about 1 h 40 min. Thus, flood flow at the study site continued even after water level began to drop. Similarly, flow continued to ebb after water level had begun to rise. A phase difference between water level and velocity is common in shallow estuaries and is indicative of a quasi-progressive tidal wave, where incident and reflected tide waves combine (*see* Dyer 1997, as well as Klavans 1983 and Luettich et al. 1999, for more detail on the circulation in this system).

It is clear from crab height above-bottom data in Fig. 2 that most vertical migrations were on ebb. The ascents occurred during both day and night. Crabs with early- (orange), mid- (brown), and late- (black) stage eggs ascended into the water column. Several of the crabs continued ebb-tide ascents after they had released their larvae. Most ascents did not reach the water surface, and as noted above, it is likely that the tethers combined with flow reduced the height above bottom that the crabs obtained. Nonetheless, the vertical migratory pattern was clearly evident.

Spectral analysis of ascent behavior—If crabs are using selective tidal-stream transport, then the cycles of their vertical migration activity should have a period similar to the local tides. The period length of the vertical migration cycles was estimated by computing power spectra from crab ascent time series (*see* Fig. 3). Peaks in the spectra indicate the periods at which the migratory activity is greatest. Each of the crabs showed consistent peaks at the dominant semidiurnal M_2 tidal period. However, the absolute power varied among the crabs, as some were more active than others. These spectra are consistent with vertical migration on consecutive (both day and night) ebbs. Two of the crabs (crabs 1 and 8) (Fig. 3a,h) also showed peaks near 2× the semidiurnal tidal period ($2M_2$), which is consistent with ascending on every other ebb tide. An examination of the ascent data for these two crabs indicated that there was weak activity during day ebbs (giving the narrow M_2 peak) and that they swam much more on night ebbs (giving the broad, energy-containing, $2M_2$ peaks). It was not obvious that this difference in ascents was due to a direct environmental cue because crabs 2 and 7 were in the water at the same time as crabs 1 and 8, respectively, but they swam on both day and night ebbs.

Environmental cues—Changes in observed pressure, velocity, salinity, and temperature were studied to determine if they were cues for vertical migratory behavior. Pressure tendency ($\partial\eta/\partial t$), the change in hydrostatic pressure with time, is positive with rising water level, negative on falling water level, and zero when water level is constant. Figure 4a shows the entire set of $\partial\eta/\partial t$ and velocity values that the crabs experienced, regardless of whether they were swimming in the water column or on the bottom. During the course of a tidal cycle, $\partial\eta/\partial t$ versus velocity values formed a clockwise, rotary pattern. Because of the phase difference between water surface elevation and velocity, at the beginning of ebb, water level was falling (Fig. 4a, lower left quadrant). At low tide, $\partial\eta/\partial t = 0$, but velocity was still ebbing at 0.2–0.4 m s^{-1}. For the remainder of ebb, water level was rising (Fig. 4a, upper left quadrant). Similar relative phase differences occurred during flood. Figure 4b shows a subset of the pressure tendency and velocity values in Fig. 4a; these are the values for times when ovigerous crabs were in the water column. The vast majority of ascents were during ebb, but there was a striking pattern showing that the crabs did not swim during the entire ebb. Rather, they swam only for the portion of the ebb when water level was falling (i.e., $\partial\eta/\partial t < 0$). Postrelease crabs showed a very similar behavior (Fig. 4c).

Velocity tendency ($\partial V/\partial t$) is the change in velocity with respect to time: positive when flow is increasing, negative when flow is decreasing, and zero when velocity is constant. The observed $\partial V/\partial t$ versus velocity relationship for all times the crabs were in the water is shown in Fig. 4d. During a tidal cycle, this relationship also formed a clockwise rotary pattern. The velocity tendencies for the times when the ovigerous and postrelease crabs were swimming in the water column are shown in Fig. 4e,f. For both crab types, most ascents occurred during the portion of ebb when $\partial V/\partial t = 0$, but not during the corresponding part of flood. This pattern indicates that most of the swimming was only during the middle part of the ebb, when currents were nearly constant.

A similar analysis was performed for the salinity and temperature tendencies ($\partial S/\partial t$ and $\partial T/\partial t$, data not shown), but no clear patterns emerged. The first 10 d of the experiment were characterized by nearly constant salinities, varying by only 0.5 during the entire period due to an extended drought in the watershed. Starting on 25 Aug 2002, a significant rain event drove salinities continuously downward (from 36 to 29), and the trend was much larger than any tidal fluctuations. Temperature variations during the experiment were dominated by diurnal heating and cooling. The mean temperature was 27.3°C and typically varied by 1–2°C during a diel cycle, which was also much greater than the semidiurnal tidal fluctuations. Therefore, the data did not support $\partial S/\partial t$ or $\partial T/\partial t$ as cues associated with selective tidal-stream transport.

Intratidal behavior—To study how crab migration patterns varied during the course of a tidal cycle, the multiday crab height time-series data were converted into a normalized single tidal cycle. This was done by dividing a single semidiurnal tidal cycle into ½-h time increments relative to slack-before-ebb (SBE, the time of the onset of ebb). The time of each crab ascent relative to SBE was computed, and the total number of ascents occurring in each ½-h time bin was determined. The number of ascents per ½-h time bin was normalized by the time the crab was in the water, which gave the average number of ascents per ½ h for each crab relative to SBE. This procedure was performed for each crab for the period when it was ovigerous as well as for the postrelease period. The number of ascents in each time bin was then expressed as the percentage of total ascents (which normalizes for the varying activity of each crab). Crabs ascended most often on the ebb portion of the tide (Fig. 5).

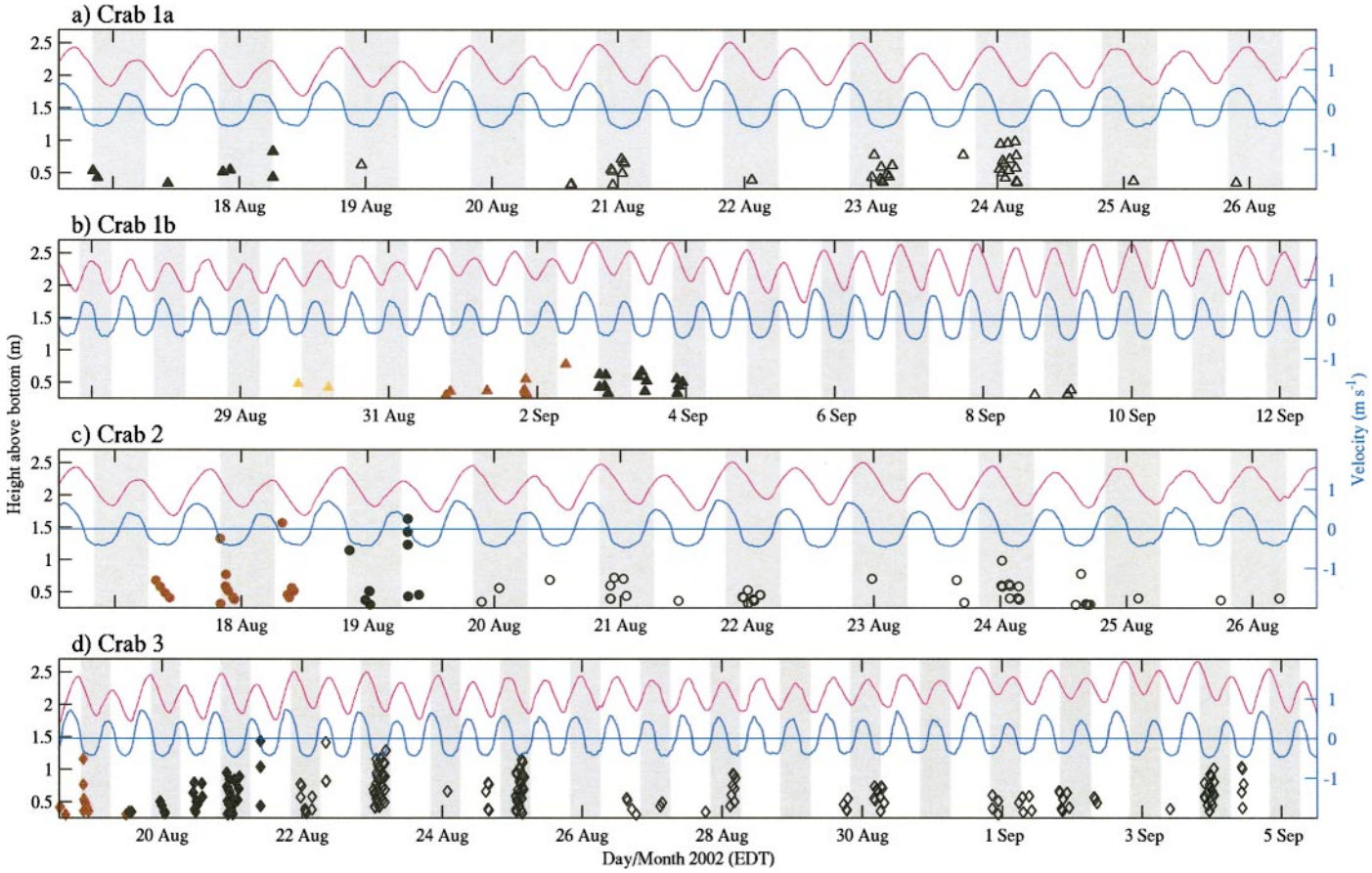

Fig. 2. Combined physical–biological data from the field experiment. The magenta line is the height of the sea surface above bottom (meters). The blue line is the along-estuary velocity (meters per second) with flood positive and ebb negative. Shading indicates the day : night cycle. The plot symbols are observed crab heights above bottom (meters). The symbol fill colors indicate the crab egg state (orange, brown, or black), with open symbols indicating postlarval release. The time series for crab 1 is split into two parts, corresponding to the first and second egg clutches.

The data did not support our initial hypothesis that the crabs would reverse their ascent times from ebb to flood after larval release. Rather, after larval release, the crabs continued ebb migratory behavior (crabs 1, 2, 3, and 7) or stopped swimming (e.g., crabs 5 and 6). Two crabs showed some indication of a reversal (crabs 4 and 8) to flood swimming, but these were some of the least active crabs, so the actual number of flood ascents observed was small.

We also computed the combined mean number of ascents per ½ h during the day and night for ovigerous and postrelease crabs (Fig. 6). For ovigerous crabs at night, ascent frequency progressively increased from about 1.5 h before SBE until about 2 h after SBE, and then progressively decreased to near zero about 5 h after SBE (Fig. 6a). During the day, ovigerous crabs showed a similar pattern (Fig. 6b), but the mean number of ascents was lower. For the postrelease crabs at night (Fig. 6c), the onset of swimming was very close to SBE, while the termination of ascents was around 4–5 h after SBE. The post-release crab ascents during the day did not show a clear pattern relative to tide phase (Fig. 6d).

Corresponding combined mean values for water level (η), current velocity (V), rate of change in water level ($\partial\eta/\partial t$), and rate of change in current velocity ($\partial V/\partial t$) relative to SBE are shown in Fig. 6e–h for comparison with the crab ascent data. The time of observed high tide was about 1.5 h prior to SBE (Fig. 6e) and coincided with the start of the active ascent period. The average low tide was about 5 h after SBE and coincided with the end of the active ascent period. Thus, most ascents occurred during the period of falling water level and decreasing hydrostatic pressure ($\partial\eta/\partial t < 0$) (Fig. 6g). The most active swimming period coincided with the period of the most rapid rate of decrease in water level (i.e., when $\partial\eta/\partial t$ ranged from 2 to 3 $\times$ 10^{-5} m s^{-1}). The relationship between vertical migration and rate of decrease in water level was most evident for the ovigerous crabs swimming at night. The timing of ascents coincided less well with velocity, as seen in Fig. 6f,h. The crabs started swimming prior to SBE and stopped swimming before the end of ebb. Although velocity has the same semidiurnal period as surface elevation, it is shifted out of phase from the vertical migration behavior. If the crabs were cueing to changes in velocity, one would expect the ascents to track $\partial V/\partial t$, but this was not the case.

Long-term behavior—The time series obtained for the crabs also permitted an evaluation of how vertical migration

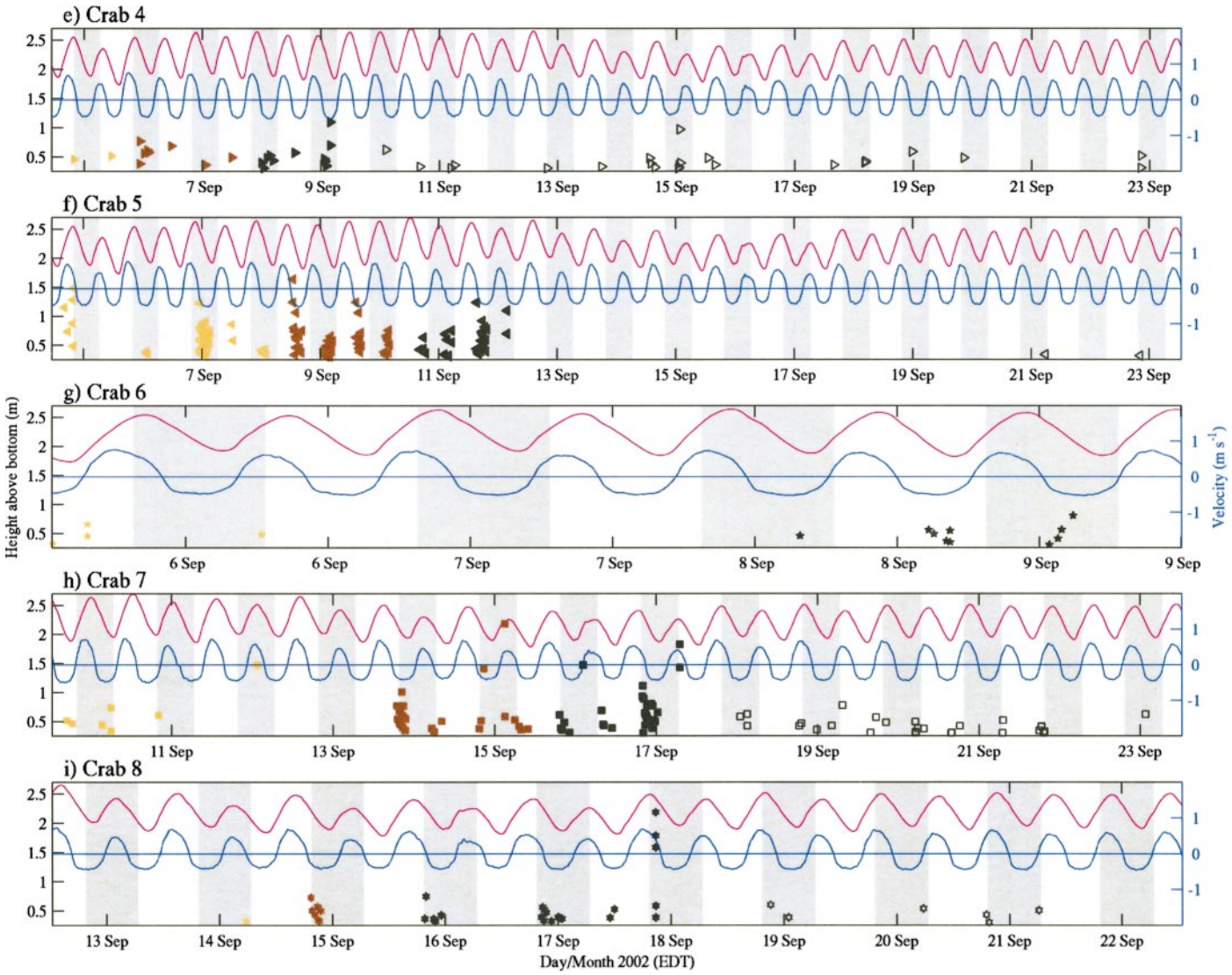

Fig. 2. Continued.

behavior changed during the period of embryo development and after larval release. The number of ascents per day (expressed as a percentage of total ascents) was computed for each crab and plotted relative to the day of release (Fig. 7). The crabs showed a general increasing trend in ascents as the release day approached. After release, there were large differences in ascent behavior. Some crabs stopped ascent behavior almost completely (crabs 1b, 5, and 6), while others continued migrating at levels similar to or even greater than before release (crabs 1a, 2, 3, and 4). Two crabs that continued ascent behavior after release (crabs 1 and 3) extruded a second egg mass (Table 1). Pooling all the daily ascent data (Fig. 7j) showed that, overall, the set of crabs ascended more during the period prior to release, showed an abrupt decrease immediately after release, and then displayed an increase in ascents during the week after release.

Timing of larval release—Blue crab larval release often occurs during a single ascent into the water column during which the female rapidly picks apart the egg mass with her walking legs and beats her abdomen (Forward unpubl.). In the present tethering study, the day of larval release for each crab was known from daily visual observations. The time of release was inferred from the pressure data records by identifying the time of the highest and longest-duration vertical ascent during the last day when each crab had eggs (this ascent was obvious in all but one of the releases). This may not have been the actual time of release, since crabs can release larvae on the bottom; however, the pressure sensor data provided a unique opportunity to estimate larval release times for individual crabs in the field. As shown in Fig. 8, all estimated releases occurred near the time of high tide or on a falling water level. Several crabs released their larvae very near sunrise, although in the case of crab 3, the tide was flooding and the water level was rising at sunrise, and it appears the crab waited until the falling tide to release. Half of the crabs released during late flood (crabs 1, 2, 7, and 8), which implies an initial landward transport for their larvae.

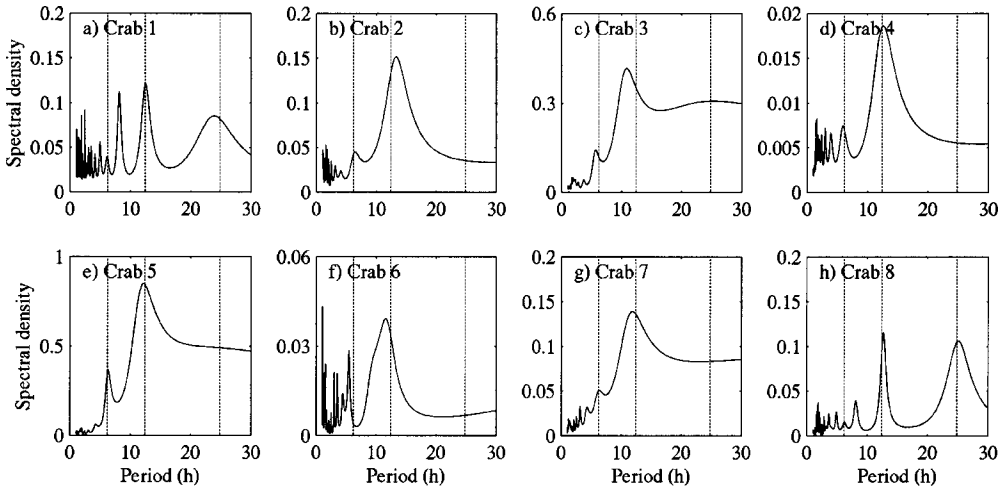

Fig. 3. Spectra computed from crab ascent time series. Vertical lines are at M_4 (6.21 h), M_2 (12.42 h), and $2M_2$ (24.84 h) tidal periods. Spectra for each crab were computed using an autoregressive spectral method (MESA; *see* Dowse and Ringo 1989; Percival and Walden 1993). Prior to computing the spectra, the raw crab height data were converted into number of ascents per ½-h time bin (effectively applying a weak low-pass filter to remove some of the intratidal ascent activity).

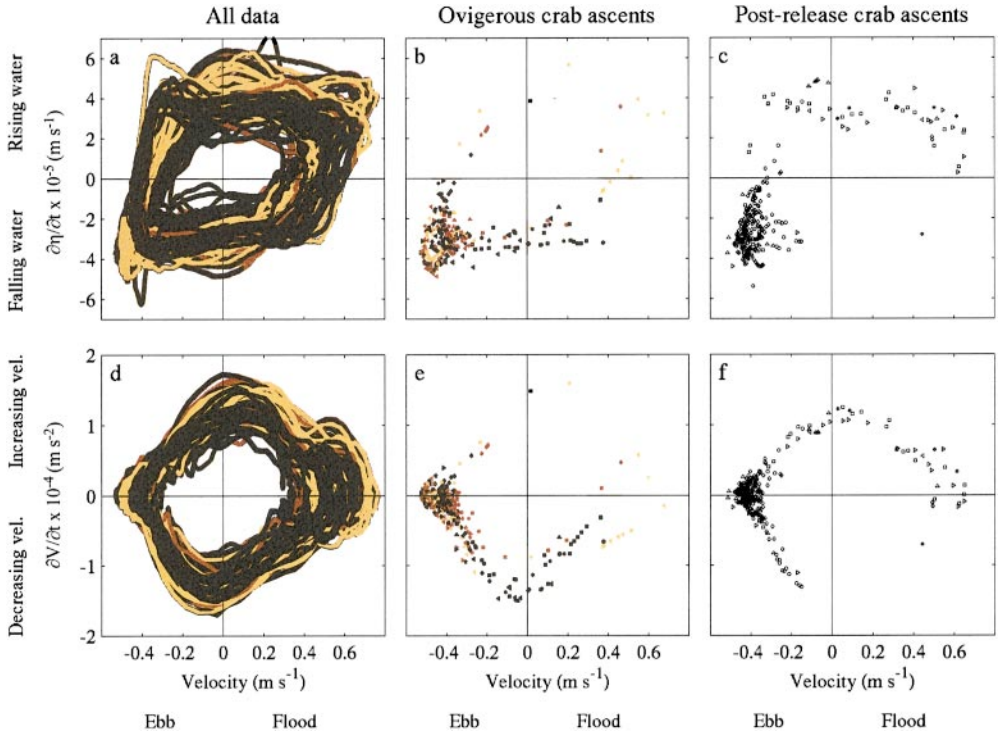

Fig. 4. Effect of observed pressure tendency ($\partial\eta/\partial t$) and velocity tendency ($\partial V/\partial t$) relative to flow velocity on vertical migration behavior. The left column of plots (a, d) shows all the flow conditions to which the crabs were exposed. The middle column of plots (b, e) shows a subset of these values for when ovigerous crabs were swimming in the water column, and the right column (c, f) shows values when postrelease crabs were swimming. Each plot is split into four quadrants, which reflect different tidal regimes. Positive velocity is flood, and negative velocity is ebb. Positive $\partial\eta/\partial t$ is rising water level, and negative is falling water level. Positive $\partial V/\partial t$ is increasing flow velocity, and negative is decreasing flow velocity. Plot symbols are the same as in Fig. 2.

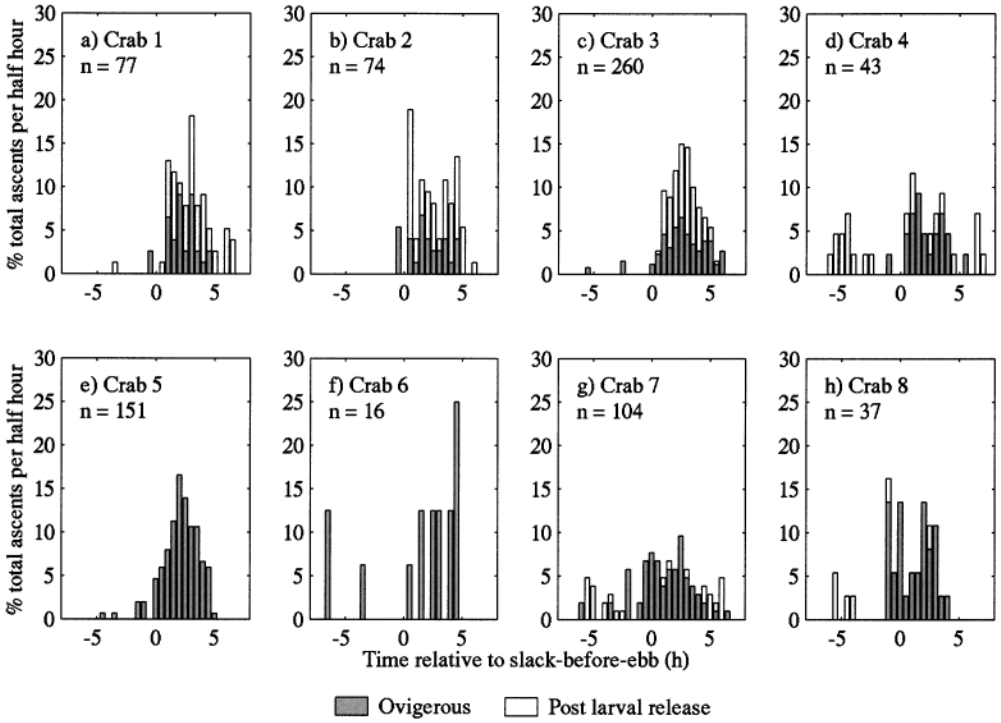

Fig. 5. Percentage of total crab ascents in ½-h time bins, relative to tide current phase. Time zero is slack-before-ebb (SBE). Positive times are ebb, and negative times are flood. The total number of ascents (*n*) for each crab is shown.

Censuses of free-swimming crabs at surface—In 2001 and 2002, we performed censuses of blue crabs migrating on the surface during 19 nocturnal ebb tides (Fig. 9). The goal of these surveys was to determine if the migration patterns relative to egg state in the intensively studied tethered crabs were representative of the ebb-migrating crab population. Of the 1,193 crabs captured, all were mature females except for one immature male crab. Females with late-stage (black) egg masses were the most common crab type observed (71% and 47% in 2001 and 2002, respectively). Early-stage crabs (orange and brown) were much less common—a total of $<10\%$ both years. There was also a significant number of nonovigerous female crabs migrating on ebb both years (21% and 46%, respectively).

Discussion

The life cycle of many marine species involves distinct periods of adult seaward migration for larval release and development, with subsequent ingress of postlarvae or juveniles back into the estuary. For large motile species (e.g., fish), adult migration is a relatively simple task. However, as mobility decreases, migration to spawning grounds becomes increasingly difficult and can rely heavily on the use of natural forcing. Consequently, a complete understanding of the life cycle and the capacity to predict recruitment success depends on coupling biophysical knowledge of adult migration, larval transport in offshore waters, and larval entry into the estuary. For marine decapod crustaceans, many of which have limited mobility (e.g., blue crabs, penaeid shrimp, dungeness crabs), adult seaward migration is a potentially perilous and energetically costly endeavor. We examined the migratory behavior of adult female blue crabs during their spawning migration and the relation to local physical forcing.

Returning to the initial five questions in the introductory section, the first considered whether selective tidal-stream transport occurs only at night. The current study found that ovigerous and post-release nonovigerous crabs migrated during both nocturnal and diurnal ebb tides (Figs. 2, 3). However, as a group, crabs made fewer ascents during the day (Fig. 6). These results appear to be inconsistent with those of Tankersley et al. (1998), but they do agree with laboratory studies of the endogenous rhythm in vertical migration by ovigerous crabs. When placed under constant low-level light in laboratory tanks, ovigerous crabs swam vertically at predicted times of consecutive ebb tides at the sites where they were captured (Forward et al. 2003). This rhythm was not affected by a LD cycle in the laboratory, as crabs continued to swim vertically during both the day and night phases (Forward and Cohen 2004). The light intensity during the day phase of these laboratory experiments should be visible to the crabs (Forward 1988) but was three orders of magnitude lower than that of sunlight. Collectively, these results suggest that crabs undergo ETT at depth during the day but that high sunlight intensity inhibits swimming near the surface.

The second question concerned the timing and environmental cues that may control vertical migration. The laboratory study of Forward et al. (2003) found that the endogenous rhythm in vertical migration associated with ETT was

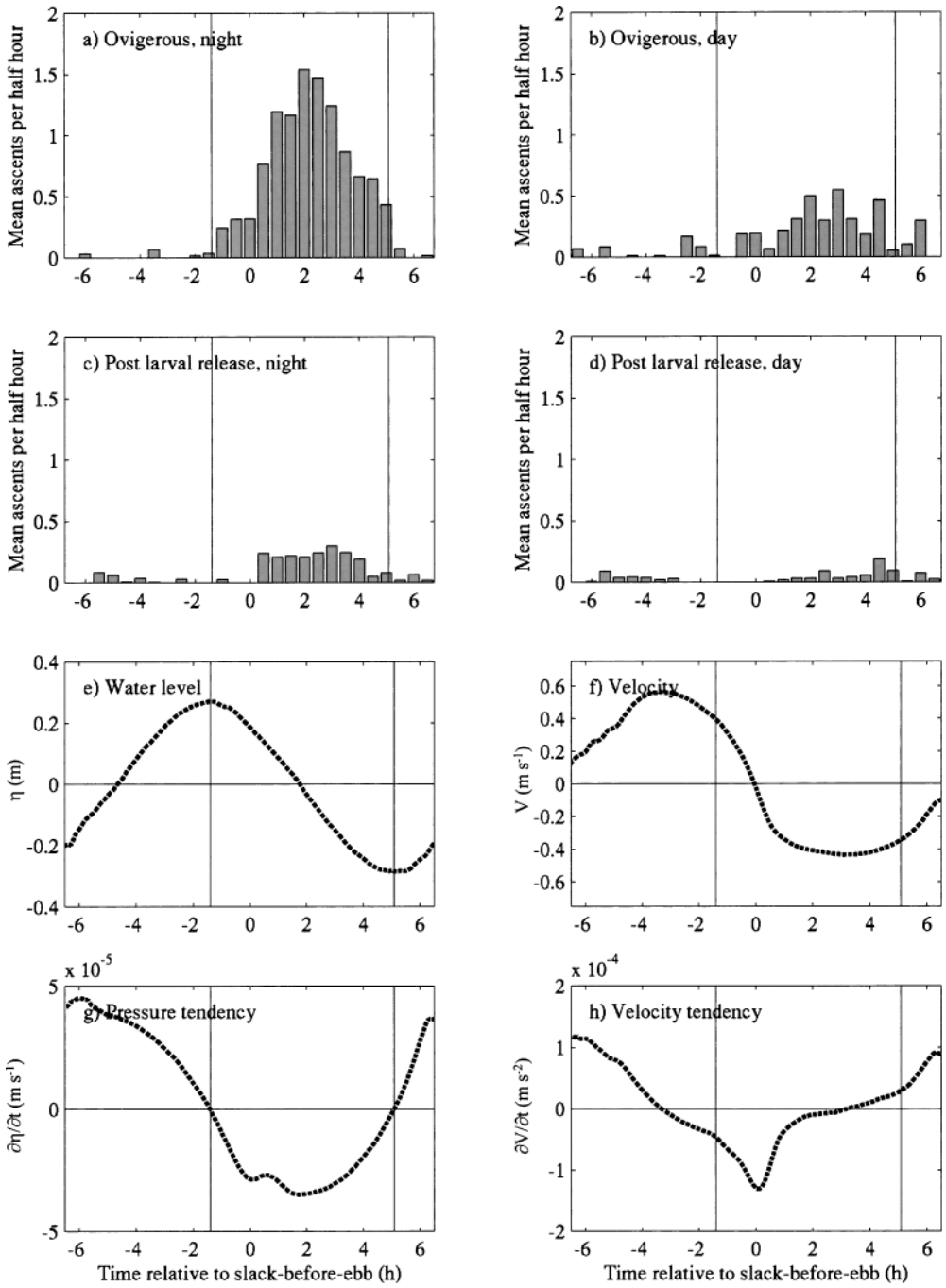

Fig. 6. Combined crab ascent and physical data (all crabs and all tidal cycles) for entire experiment relative to slack-before-ebb (SBE). Positive time is ebb, and negative time is flood. Vertical lines indicate high and low tide (the hydrostatic pressure maximum and minimum, respectively), which bracket the period of decreasing hydrostatic pressure ($\partial\eta/\partial t<0$). Crab vertical migrations correspond more closely to this period than to velocity or velocity tendency.

weak in females with young embryos, pronounced in females with mature embryos, and absent in postlarval release females. Since the present field study found that all three categories of crab swam vertically during ebb tides in the field, the timing of the ascents by females with young embryos and postlarval release females is probably regulated by environmental cues. Our detailed analysis of physical data indicated that changes in temperature, salinity, and current speed were not related to ETT. However, it appears that ETT is closely coupled to water level via changes in hydrostatic pressure (Figs. 4, 6). Early in their life cycle, blue crabs (as megalopae) ascend on an increase in hydrostatic pressure, using FTT to move up-estuary (Tankersley et al. 1995).

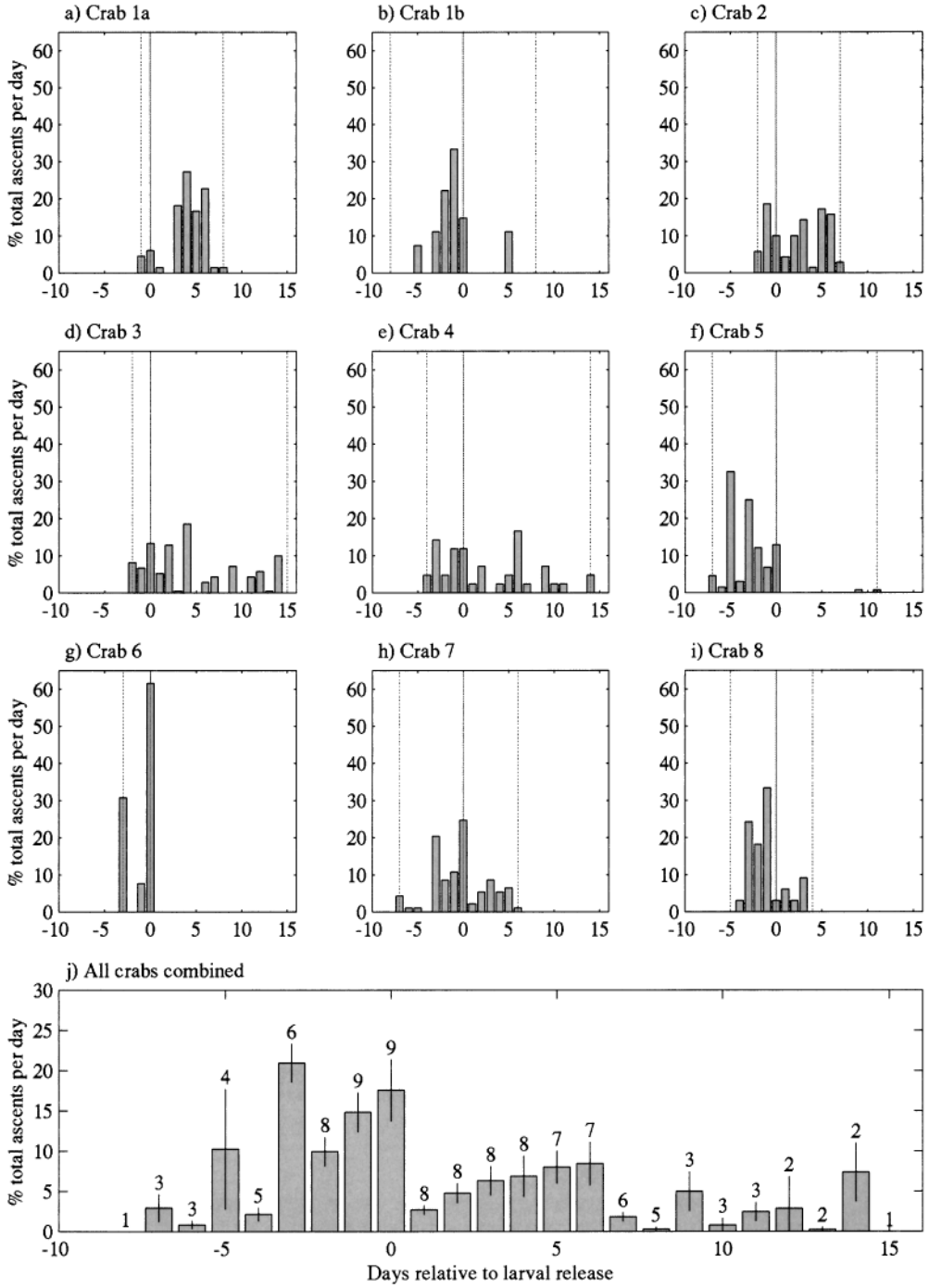

Fig. 7. Long-term ascent behavior as a function of time relative to release day. Day zero is the day of release, positive time is postrelease, and negative time is prerelease. Vertical dashed lines indicate beginning and end day of observation for each crab. The solid vertical lines indicate the day of larval release. For the combined data (plot j), *n* is the number of crabs observed, and error bars indicate 95% confidence intervals. The record for crab 1 was split into two parts (a and b), corresponding to when the crab had its first and second egg mass, respectively.

The crab ascents began at high tide (prior to SBE) and continued until low tide (Fig. 6), even though >1.5 h of ebb remained. Pressure and velocity did not coincide in time due to the quasi-progressive nature of the tide at the study site. Velocity elevation phase differences are common in many estuarine systems (Dyer 1997). Moreover, these phase differences can spatially vary within a single estuary over the length scales that the crabs migrate. It may be more advantageous to use pressure than velocity as a migration cue because the spatial structure of the pressure field tends to be

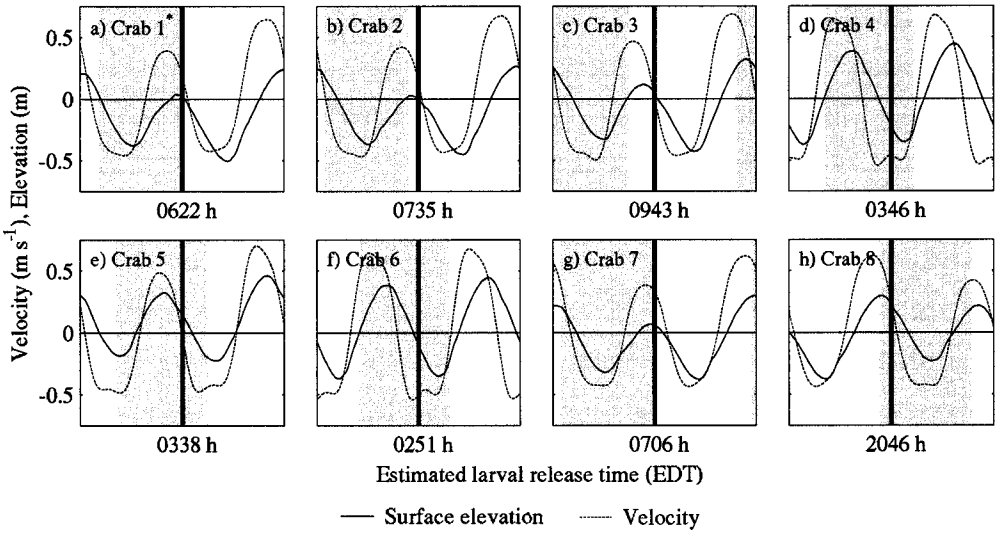

Fig. 8. Estimated larval release times relative to surface elevation, velocity, and day : night cycle (indicated by shading). The thick vertical line in each plot is the estimated time of larval release. Horizontal axis of each plot is two M_2 tidal cycles (24.84 h) long. Only the first release is shown for crab 1 because the second release was not readily discernible from the data.

more uniform and therefore more reliable for a migrating animal. For example, if a crab were in a small embayment or behind an obstacle that alters flow, the local velocities might be small or ambiguous in direction, but the water-level signal would still be clear.

The use of the rate of decrease in hydrostatic pressure as a cue for vertical migration may also help explain the discontinuous migratory behavior observed in the present study and a concurrent study of free-swimming crabs using biotelemetry (Carr et al. 2004). In both of these studies, crabs exhibited periods of active vertical swimming separated by periods on the bottom. The swimming durations did not appear to be limited by aerobic swimming capacity, which can exceed 1 h under laboratory conditions (Booth et al. 1982), nor did they appear to be associated with feeding in the water column, as blue crabs usually feed on benthic organisms, as indicated by gut content analysis (Tagatz 1968). Using hydrostatic pressure changes as a cue, a crab on the bottom would sense a negative $\partial\eta/\partial t$ associated with ebb tide. This cue would evoke an ascent into the water column. However, once in the water column and moving with the current in a Lagrangian fashion, the crab would be unable to sense large-scale hydrostatic pressure changes in the environment. Since behavioral responses to rates of change in environmental cues typically continue only a short time (e.g., minutes) after the rate of change ceases (e.g., Latz and Forward 1977; Forward and Tankersley 2001), crabs would need to return to the bottom to sense a new pressure cue in an Eulerian fashion. If the pressure tendency were favorable, the crab would undergo another brief ascent to travel seaward. If unfavorable, the crab would remain on the bottom until conditions indicated otherwise. Thus, sequential periods of vertical swimming are necessary if crabs use rate of decrease in pressure as a cue. In addition, previous work (Forward et al. 2003) showed that crabs with late-stage eggs have an endogenous tidal rhythm in vertical migration. Crab rhythm in the laboratory was somewhat noisy relative to the crabs studied here, which suggests that the crabs use external cues to time ETT precisely. Conversely, the endogenous rhythm may be essential to maintain effective ETT during the temporary absence or presence of ambiguous external cues.

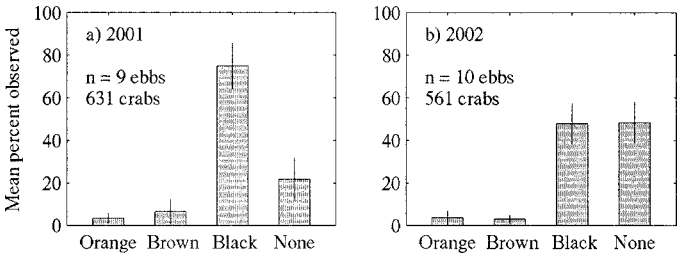

Fig. 9. Observed surface free-swimming female crabs near Beaufort Inlet on night ebbs in 2001 (Jul: 12, 18, 19, and 20; Aug: 2, 3, 14, 15, and 16) and 2002 (Jun: 24 and 25; Jul: 7, 8, 21, 22, and 23; Aug: 6, 7, and 8). For each night ebb, the percentage of each crab type (orange, brown, black, or no eggs) was computed. Then, the mean percentage of all nights was computed and plotted above. Error bars indicate 95% confidence interval.

The third question addressed the relationship between embryo development and ETT of ovigerous females. ETT was evident in females with embryos at all stages of development (Fig. 2). The only behavioral changes with embryo age were a general increase in the frequency of vertical migrations as the embryos matured, an abrupt decrease the day after release, and a general increase the week following larval release (Fig. 7). These results differ from laboratory studies of endogenous tidal rhythm in vertical migration. Forward et al. (2003) observed that females with early-stage embryos held in constant conditions swam at times corresponding to the first few predicted ebb tides in the field, after which the rhythm stopped. Laboratory females with late-stage embryos

had a pronounced but comparatively noisy endogenous tidal rhythm. These results suggest that exposure to ambient tidal conditions maintains ETT behavior of females with early-stage embryos in the field and reinforces the endogenous rhythm of the late-stage crabs.

The fourth question concerned the time of larval release relative to tidal phase and the LD cycle. In general, crabs released larvae at high tide or when water level was falling and often within several hours of sunrise (Fig. 8). We are unaware of any previous study that provides estimates of individual blue crab larval release times under field conditions. These results are consistent with previous observations of high concentrations of newly hatched blue crab larvae seen at high tide (Provenzano et al. 1983) as well as the laboratory study of Ziegler (2002). Releasing larvae at the start of ebb tide would maximize seaward transport and thereby increase the chance of larvae reaching a coastal or offshore developmental area. However, half of the tethered crabs actually released larvae during the end of flood, which implies an initial landward transport of larvae. Releasing on late flood is probably suboptimal, but release times coincided with the start of hydrostatic pressure decreases, suggesting that this is a cue involved in the release timing.

The final question considered the timing of the switch from ETT to FTT by post-larval release female crabs. Our results suggest that most females continue to use ETT throughout the spawning season and do not switch to FTT after the release of each larval clutch (Figs. 5, 7, 9) as proposed by Tankersley et al. (1998). Our observations are supported by the surveys of Dudley and Judy (1971), who found significant numbers of ovigerous female crabs with early-stage embryos offshore of Beaufort Inlet. It is possible that the switch from ETT to FTT occurs over a longer period than the duration of our experiment, however, and that some females reenter the estuary later in the year. Another possible reason that the reversal was not observed is that oceanic cues (or an absence of estuarine cues) are necessary to initiate FTT, and the tethered crabs were restrained from migrating offshore to receive appropriate cues. While some females may return to the estuary, female blue crabs mate only once, storing enough sperm for multiple clutches (Van Engel 1958; Milliken and Williams 1984), and there is no obvious reproductive necessity to return to the estuary. If females continue to migrate seaward between larval clutches, subsequent clutches will be released farther down the estuary or offshore and will have a higher probability of successful transport to offshore developmental areas (e.g., Epifanio et al. 1989).

Another consideration is that if a crab is transported seaward through the estuary inlet, local hydrodynamics may work against a return to the estuary, even using FTT. This is due to the asymmetry between inlet ebb and flood flow patterns (Hench and Luettich 2003). A crab leaving the estuary in the ebb jet would be swiftly carried offshore. If it settled out of the water column at the outer edge of the jet, the crab would find relatively weak flood currents to return to the estuary. Outside the jet, along-shelf currents may quickly transport crabs away from inlets such that return estuary migrations could be difficult or impossible. If female blue crabs do continue to use ETT throughout the spawning season, as our results suggest, a large proportion of the reproductive female population will be in oceanic habitats.

In summary, this study clearly demonstrates that the behavior involved in ETT occurs in ovigerous and post-larval release females. The new methodology using miniature internally logging pressure tags in conjunction with fixed oceanographic instruments was useful for monitoring vertical migration behavior of individual crabs under field conditions. Crabs used ETT on both day and night ebbs but were more active at night. Decreasing hydrostatic pressure appears to be a cue for migratory behavior, since swimming generally started at high water (prior to the start of ebb) and abruptly stopped at low water, even though ebb currents continued for some time. The period with the most active vertical migration behavior coincided with the tide phase with the greatest rates of decrease in hydrostatic pressure. Reproductive female crabs migrated almost exclusively on ebb tides during the spawning migration and used an ETT behavior during all stages of egg development. Larval release was close to high tide or when water level was falling. Immediately after larval release, some crabs continued ETT, while others stopped. The data did not support the prevailing model of ETT-FTT reversal after larval release, at least during the spawning season. Sustained ETT by females would transport them farther seaward and would progressively increase the probability of each sequential larval clutch reaching favorable offshore development areas. These results suggest that there is a net export of adult female crabs from the estuary during the spawning season, which may have significant implications for spawning success as well as management of the fishery.

References

Booth, C. E., B. R. McMahon, and A. W. Pinder. 1982. Oxygen uptake and the potentiating effects of increased hemolymph lactate on oxygen transport during exercise in the blue crab, *Callinectes sapidus.* J. Comp. Physiol. **148:** 111–121.

Carr, S. D., R. A. Tankersley, J. L. Hench, R. B. Forward, Jr., and R. A. Luettich, Jr. 2004. Movement patterns and trajectories of ovigerous blue crabs *Callinectes sapidus* during the spawning migration. Estuarine Coastal Shelf Sci. **60:** 567–579.

Costlow, J. D., and C. G. Bookhout. 1959. The larval development of *Callinectes sapidus* Rathbun reared in the laboratory. Biol. Bull. **116:** 373–396.

Crowder, L. B., and F. E. Werner [eds.]. 1999. Fisheries oceanography of the estuarine-dependent fishes of the South Atlantic Bight. Fish. Oceanogr. (suppl. 2) **8**.

De Vries, M. C., C. E. Epifanio, and A. I. Dittel. 1983. Lunar rhythms in the egg hatching of the subtidal crustacean *Callinectes arcuatus* Ordway (Decapoda: Brachyura). Estuarine Coastal Shelf Sci. **17:** 717–724.

Dowse, H. B., and J. M. Ringo. 1989. The search for hidden periodicities in biological time series revisited. J. Theor. Biol. **139:** 487–515.

Dudley, D. L., and M. H. Judy. 1971. Occurrence of larval, juvenile, and mature crabs in the vicinity of Beaufort Inlet, North Carolina. NOAA Tech. Rep., NMFS SSRF-637.

Dyer, K. R. 1997. Estuaries, a physical introduction, 2nd ed. Wiley.

Epifanio, C. E., and R. W. Garvine. 2001. Larval transport on the Atlantic continental shelf of North America: A review. Estuarine Coastal Shelf Sci. **52:** 51–77.

———, A. K. MASSE, AND R. W. GARVINE. 1989. Transport of blue crab larvae by surface currents of Delaware Bay, USA. Mar. Ecol. Prog. Ser. **54:** 35–41.

FORWARD, R. B., JR. 1988. Diel vertical migration: Zooplankton photobiology and behavior. Oceanogr. Mar. Biol. Annu. Rev. **26:** 361–393.

———, AND J. H. COHEN. 2004. Factors affecting circatidal rhythm in vertical swimming of ovigerous blue crabs, *Callinectes sapidus,* involved in the spawning migration. J. Exp. Mar. Biol. Ecol. **299:** 255–266.

———, AND R. A. TANKERSLEY. 2001. Selective tidal-stream transport of marine animals. Oceanogr. Mar. Biol. Annu. Rev. **39:** 305–353.

———, ———, AND P. N. POCHELON. 2003. Circatidal activity rhythms in ovigerous blue crabs, *Callinectes sapidus:* Implications for ebb-tide transport during the spawning migration. Mar. Biol. **142:** 67–76.

HENCH, J. L., AND R. A. LUETTICH, JR. 2003. Transient tidal circulation and momentum balances at a shallow tidal inlet. J. Phys. Oceanogr. **33:** 913–932.

KLAVANS, A. S. 1983. Tidal hydrodynamics and sediment transport in Beaufort Inlet, North Carolina. NOAA Tech. Rep. NOS 100.

LATZ, M. I., AND R. B. FORWARD, JR. 1977. The effect of salinity upon phototaxis and geotaxis in a larval crustacean. Biol. Bull. (Woods Hole) **153:** 163–179.

LUETTICH, R. A., JR., J. L. HENCH, C. W. FULCHER, F. E. WERNER, B. O. BLANTON, AND J. H. CHURCHILL. 1999. Barotropic tidal and wind-driven larval transport in the vicinity of a barrier island inlet. Fish. Oceanogr. (suppl. 2) **8:** 190–209.

MCCONAUGHA, J. R., D. F. PROVENZANO, AND R. C. MARIS. 1983. Seasonal distribution of larvae of *Callinectes sapidus* (Crustacea: Decopoda) in the waters adjacent to Chesapeake Bay. J. Crustac. Biol. **3:** 582–591.

MILLIKEN, M. R., AND A. B. WILLIAMS. 1984. Synopsis of biological data on the blue crab, *Callinectes sapidus* Rathbun. FAO Fisheries Synopsis 138, NOAA Tech. Rep. NMFS 1.

OLMI, E. F. III, J. VAN MONTFRANS, R. N. LIPCIUS, R. J. ORTH, AND P. W. SADDLER. 1990. Variation in planktonic availability and settlement of blue crab megalopae in the York River, Virginia. Bull. Mar. Sci. **46:** 230–243.

ORTH, R. J., AND J. VAN MONTFRANS. 1987. Utilization of seagrass meadow and tidal creek by blue crabs, *Callinectes sapidus.* I. Seasonal and annual variation in abundance with emphasis on post-settlement juveniles. Mar. Ecol. Prog. Ser. **41:** 283–294.

PALMER, J. D. 1995. The biological rhythms and clocks of intertidal animals. Oxford Univ. Press.

PERCIVAL, D. B., AND A. T. WALDEN. 1993. Spectral analysis for physical applications. Cambridge Univ. Press.

PROVENZANO, A. J., JR., J. R. MCCONAUGHA, K. B. PHILIPS, D. F. JOHNSON, AND J. CLARK. 1983. Vertical distribution of first stage larvae of the blue crab, Callinectes sapidus, at the mouth of Chesapeake Bay. Estuarine Coastal Shelf Sci. **16:** 489–499.

SMYTH, P. O. 1980. Callinectes (Decapoda: Portunidae) larvae in the Middle Atlantic Bight, 1975–1977. Fish. Bull. **78:** 251–265.

TANKERSLEY, R. A., L. M. MCKELVEY, AND R. B. FORWARD, JR. 1995. Responses of estuarine crab megalopae to pressure, salinity, and light: Implications for flood-tide transport. Mar. Biol. **122:** 391–400.

———, M. G. WIEBER, M. A. SIGALA, AND K. KACHURAK. 1998. Migratory movements of ovigerous blue crabs, *Callinectes sapidus:* Evidence for selective tidal-stream transport. Biol. Bull. **195:** 168–173.

VAN ENGEL, W. A. 1958. The blue crab and its fishery in Chesapeake Bay. Commer. Fish. Rev. **20:** 6–17.

WEINSTEIN, M. P. [ED.]. 1988. Larval fish and shellfish transport through inlets. Am. Fish. Symp. **3.**

WILLIAMS, A. B. 1984. Shrimps, lobsters and crabs of the Atlantic coast of the eastern United States, Maine to Florida. Smithsonian Institution.

ZIEGLER, T. A. 2002. Larval release behavior of the blue crab *Callinectes sapidus:* Entrainment cues and timing in different tidal regimes. M.S. thesis, Florida Institute of Technology. Melbourne, Florida.

Received: 19 July 2003
Accepted: 27 April 2004
Amended: 7 May 2004