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Entrainment of the larval release rhythm of the crab *Rhithropanopeus harrisii* (Brachyura: Xanthidae) by cycles in salinity change

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Abstract

Ovigerous females of the crab *Rhithropanopeus harrisii* (Gould) were collected from an estuary having irregular tides and exposed to only a semi-diurnal tidal cycle in salinity change in the laboratory. A circatidal rhythm developed, in which larval release occurred around the predicted time of highest salinity (high tide). Thus, salinity cycles can entrain a tidal rhythm. After exposure to only a diel light/dark (LD) cycle, releases occurred mainly in the first 2 h of the dark phase. When exposed to both a tidal salinity cycle and an LD cycle, larval release depended upon the time of nocturnal high tide. Crabs only released at night. If high tide was near the beginning or within about 3 h of the end of the dark phase, releases occurred at the beginning of the dark phase. As high tide moved toward the middle of the night, an increasing proportion of crabs released around the predicted time of high tide. These data support the suggestions that (1) the LD and tidal salinity cycles control the specific time of hatching and (2) there is a hierarchy in the functional advantages for the timing of larval release, in which reduced vulnerability to visual predators is more important than avoidance of stressful salinities or initial seaward transport.

Introduction

Rhythms in larval release are common among crustaceans. The timing may be related to lunar phase (Christy, 1978, 1982; Saigusa and Hidaka, 1978; Wheeler 1978; Saigusa, 1981; Salmon and Hyatt, 1983), time of day (Ennis, 1973, 1975; Branford, 1978; Moller and Branford, 1978; Forward *et al.*, 1982) or phase of the tide (De Coursey, 1979; Bergin, 1981; Forward *et al.*, 1982; Saigusa, 1982). In some cases these rhythms persist in the laboratory without the presence of the environmental cycle which corresponded to the larval release rhythm [tidal related rhythm (Bergin, 1981; Forward *et al.*, 1982), diel related rhythm (Ennis, 1973; Branford, 1978; Moller and Branford, 1979; Forward *et al.*, 1982), semilunar related rhythm (Saigusa, 1980; Christy, 1982)]. The Zeitgebern for entraining each type of rhythm are probably different. Semilunar rhythms may be entrained by cycles in tidal amplitude (Christy, 1982) and moonlight (Saigusa, 1980), while the Zeitgeber for solar day rhythms seems to be the LD cycle (e.g. Branford, 1978; Forward *et al.*, 1982). Aspects of the tides which serve as Zeitgebern for entrainment of tidal rhythms in hatching are unknown.

When collected from the Newport River estuary, which has pronounced semi-diurnal tides, the crab *Rhithropanopeus harrisii* has a circatidal rhythm under constant conditions in the laboratory in which larval release occurs around the time of high tide in the field (Forward *et al.*, 1982). Tidal cycles in hydrostatic pressure and salinity are the most probable Zeitgebern for entrainment in this estuary because, over a tidal cycle, water depth changes up to about 1 m and salinity can vary over a 20‰ range. Light intensity varies little over the solar day at the depths where females are found (Cronin, 1982). Cycles in hydrostatic pressure are well known to entrain tidal rhythms in marine animals (reviewed by Naylor, 1982), but entrainment by salinity cycles has only been demonstrated by Taylor and Naylor (1977).

The present study considers whether a tidal cycle in salinity can entrain a tidal rhythm of larval release in *Rhithropanopeus harrisii*. Crabs from a non-tidal estuary, which normally show a circadian rhythm in larval release (Forward *et al.*, 1982), were subjected to a tidal cycle in salinity to determine whether the rhythm in larval release would change to a circatidal rhythm. The results indicate that a salinity cycle does entrain a tidal rhythm. In addition, the study considers the larval release pattern when the crabs are exposed to both a tidal cycle in salinity and a diel LD cycle in the laboratory. These results are compared to release patterns of crabs which are naturally exposed to these two cycles.

Materials and methods

Rhithropanopeus harrisii (Gould) were collected from the Neuse River estuary (North Carolina). Although crabs are exposed to the natural LD cycle at this site, tides are aperiodic (Roelofs and Bumpus, 1953). Physical factors, such as salinity, water depth and wave turbulence, which usually co-vary with the tide, vary instead with wind direction or rain. Thus, crabs from this area are not exposed to semi-diurnal tides and have a solar day rhythm in egg hatching. Females release larvae in the interval beginning at the end of the light phase and concluding about 2 to 3 h later (Forward *et al.*, 1982).

The eggs of ovigerous females were staged embryologically according to eye development and amount of remaining yolk. Crabs with eggs which were expected to hatch in 7 to 10 d were used in all experiments. Crabs were not fed in the laboratory.

The first experiment was designed to determine whether crabs from this estuary would develop a rhythm in larval release related to tides if exposed in the laboratory to a salinity cycle having a tidal periodicity. Ovigerous females were randomly divided into control and experimental groups, each placed in Plexiglas aquaria (10 cm high; 20 cm wide; 45 cm long) containing water and clean oyster shells.

For controls, the water (constant salinity 15 ± 0.5 ‰S) was changed every 3 d at a random time during the day. The aquarium was placed in an enclosure surrounded by black plastic. Low level illumination within the enclosure was provided by a 6-W incandescent lamp, which remained on continuously. The intensity at the top of the aquarium directly under the bulb was 0.7 W/m^2 as measured with a YSI radiometer. Since it was not possible to make the enclosure entirely light-tight, room lights from the adjacent area remained on continuously.

The experimental crabs were placed in the enclosure in a similar aquarium connected to a system that provided a variation in salinity with a tidal periodicity. The design of the system was similar to that used by Davenport *et al.* (1975). It consists of reservoirs containing either about 200 l of fresh or sea water (Fig. 1). The fresh water was unchlorinated, aerated tap water; the sea water was provided by the sea water system at the Duke University Marine Laboratory. Salinity varied from about 32 to 34‰ depending on rainfall. Float valves controlled the flow of water into the reservoirs to maintain a constant head pressure. Flow from the reservoirs to the mixing chamber (volume 1.28 l) was regulated by pinch valves (Gorman Rupp Industries; Model V-395). After mixing, the water flowed through a conductivity cell (Beckman, Model RA-5 Direct Reading conductivity meter) into the bottom of the aquarium. An air stone was placed near the entrance tube. The flow rate of about 180 ml min^{-1} was regulated by the size of the entrance and exit port of the mixing chamber. Since the water passed through the aquarium in 38 min, there was a gradient in salinity between the extrance and exit port. The magnitude of the gradient varied with phase of the salinity cycle. Temperature was measured (YSI, Model 44TD) in both the experimental and control tanks and varied less than 0.8 C° between the tanks. The average temperature for all experiments was 24.6 °C ± 1 C°.

The salinity cycle was regulated by a Commodore 64 computer through the available user-addressable I/O port. The pinch valves were controlled so that, in a given 30-s interval, the salt water valve was open for a specified time and the fresh water valve for the remaining time. Each open/closed ratio was maintained for 5 min, and then was changed to a new value. The ratios followed a sine wave with a period of 12.42 h to match a natural tidal cycle. The computer also monitored conductivity through an A/D converter and printed out salinity at 15-min intervals. A representative recorded salinity cycle is seen in Fig. 2. The computer was programmed to provide a salinity cycle from 5 to 25‰ for all experiments. However, due to small variations in the input salinity, the measured average low and high salinities were 5.5 and 24.3‰, respectively. This range of salinities is representative of the natural range (Cronin, 1982).

After 7 d all crabs were restaged for embryo development. All the crabs (experimental and control) that were expected to release larvae within 2 d were removed at the

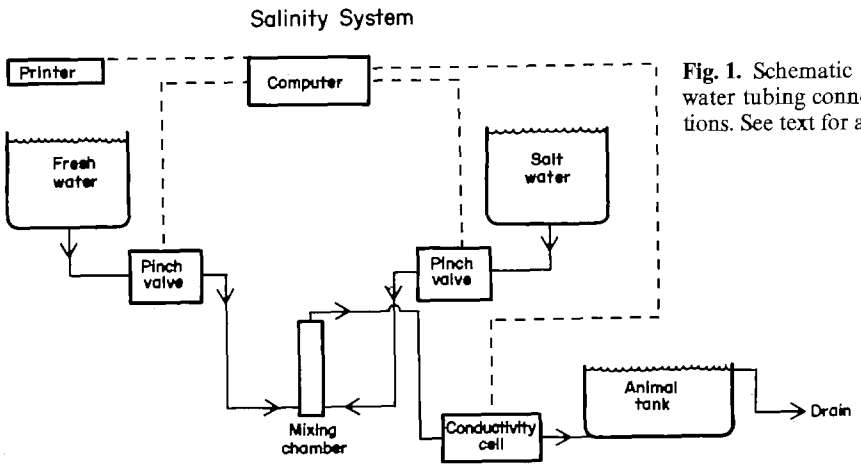

Fig. 1. Schematic diagram of salinity system. Solid lines indicate water tubing connections while dashed lines show electrical connections. See text for a discussion of the system

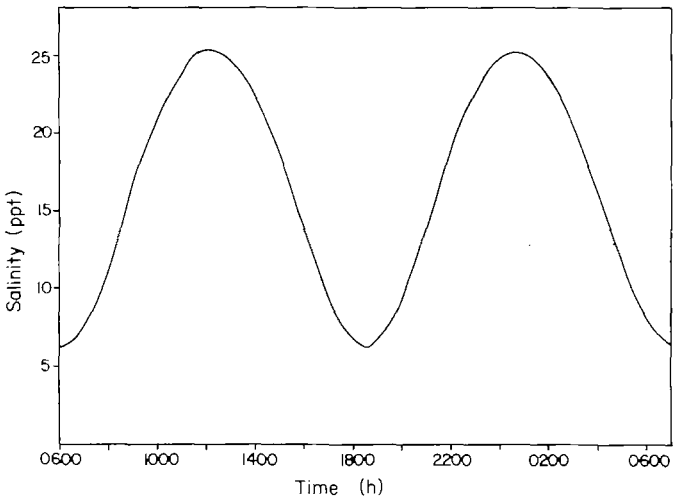

Fig. 2. Representative salinity cycle as measured by the conductivity cell. A continuous cycle is shown even though salinity changed in steps every 5 min and the data were recorded at 15-min intervals

time of highest salinity in the experimental tank. The remainder of the crabs was returned to the aquaria. Removal of crabs with mature embryos was repeated each subsequent day. Using this procedure, the experimental crabs were exposed to the salinity cycle for a minimum of 7 and maximum of 10 d. There was no obvious variation in the relationship of hatching times to salinity due to length of exposure (7–10 d) to salinity cycles. Once removed the crabs were placed in constant conditions. The temperature was 24 °C and again a 6-W incandescent bulb was used for constant illumination. Since the salinity for the control crabs was 15‰, the constant conditions were essentially identical to those under which the control crabs had been previously maintained for 7–10 d. The salinity for the experimental group was the level found at the time of simulated high tide (24‰). For timing purposes, we considered high tide in the salinity system to occur when the rising salinity reached a value that was within 1‰ of the maximum. This value was selected because during the cycle the salinity rose very rapidly to this level (Fig. 2) and then remained within 1‰ of the highest salinity for about 2 h.

Larval release was monitored by a sequential transfer technique. Crabs were initially placed in 7.9-cm finger bowls containing about 50 ml of water. At 1-h intervals they were transferred to new bowls. For each crab, transfer continued until its eggs hatched. The number of larvae released in each hourly interval was recorded. Since most of a crab's larvae were usually released in a single 1-h interval, this was considered the time of egg hatching for that crab. In those few cases where a crab released many larvae over several intervals, the mean time interval of release was computed (Forward and Lohmann, 1983). Thus, after removal from the enclosure, a crab was maintained in constant conditions until its time of larval release was determined, which in most cases was a single event.

This first experiment was performed twice. The first time, the salinity cycle was timed so that on the seventh day of exposure the highest salinity occurred at about 02.00 hrs. In order to determine whether hatching times were associated with tides and not specific times in the solar day, the second run had high tide displaced 5 h to 21.00 hrs on the seventh day. The two data sets were combined and, for the experimental crabs, hatching times were related to the time of high tide.

In a second experiment the effects of both salinity and LD cycles upon hatching were tested. Crabs with eggs that were expected to hatch in 7 to 10 d were simultaneously placed in either the experimental or control (constant salinity) aquarium. All crabs were exposed to a 13 hL: 11 hD cycle timed so that beginning of the dark phase occurred at 14.00 hrs. The length of the light and dark periods of the LD cycle were similar to those in the field. Previous studies have shown that the timing of the circadian rhythm in larval release can be reset within 5 d (Forward *et al.*, 1982). Lights were provided by cool white fluorescent lamps. The light intensity at the surface of the aquaria was 4.3 W/m^2. The salinity cycle for experimental crabs was similar to that described above. This experiment was also repeated twice in consecutive 2-week intervals with each replicate having a different timing of simulated high tide (high salinity) in the dark phase. In the first run, high tide occurred during the first 6 h of the dark phase on the days egg hatching was monitored (7 to 11 d after beginning the experiment), and for the second run, during the last 5 h of the dark phase.

After 7 d of exposure to these cycles crabs were restaged for embryo development and hatching times determined for those crabs with mature embryos. Experimental crabs were removed from the salinity cycle at the time of high salinity during the light phase and maintained in the enclosure in finger bowls containing 24‰ S sea water. The control crabs were similarly removed from their aquarium and maintained in constant conditions in 15‰ S sea water. Preliminary observations indicated that all hatching occurred during the dark phase. Therefore hatching was subsequently monitored for 12-h periods beginning 1 h before the commencement of the dark phase. At the beginning of monitoring, crabs were kept for 1 h in the lights. They were then removed from the LD cycle and maintained under constant conditions which included dim light provided by a 6-W red incandescent lamp. Hatching was monitored daily during the predicted time of the entrained dark phase until larval release occurred. Between monitoring periods crabs were maintained in 10.6-cm diameter finger bowls. Even though in most cases a crab released larvae only once, the length of time a crab was maintained under constant conditions before this occurred could vary between crabs.

The third experiment was designed to test whether hatching times of the control crabs were affected by the presence of flowing water. Crabs were maintained for 7 to 10 d in the experimental aquarium under a 13 hL:11 hD cycle timed so the dark phase began at 14.00 hrs. Water at

15‰ S (± 1‰) was made up daily in the 200-l holding tanks and flowed through the mixing chamber into the experimental tank. In this way the crabs were exposed to the same flow rates as the experimental crabs in the previous experiments. After 7 d in these conditions, crabs with eggs that were expected to hatch in 2 d were removed 1 h before the end of the light phase and placed in 15‰ S water. Hatching was monitored over the next 5 h. Crabs were placed in constant conditions at the beginning of the dark phase. If a crab did not release larvae during the first monitoring period, she was maintained for the next 19 h in a 10.6-cm diameter finger bowl and then monitored again for hatching.

A Kolmogorov-Smirnov goodness of fit test was used to determine if releasing was nonuniform throughout a given monitoring period or over a 24-h day.

Results

Hatching rhythm upon exposure to a tidal cycle in salinity

After 7 to 10 d in constant conditions, larval release by control crabs was not well defined over the 24-h day (Fig. 3). Even though the hatching pattern differed from that expected for a uniform distribution on Day 7 ($P < 0.01$) and Day 10 ($P < 0.02$), hatching did not occur in a consistent narrow time interval.

In contrast, crabs which had been exposed to a salinity cycle and then transferred to constant conditions, released their larvae around the time of predicted high tide (Fig. 4). Larval release patterns were not uniform over a 12-h time interval centered on the predicted time of high tide for Tides 2, 3 and 4 ($P < 0.02$). The pattern for Tide 1 has the same general distribution but is not significant, probably because of the low sample size. The timing of larval release was not identical for each high tide, as the mean time of release occurred progressively earlier with each succeeding tide.

It is possible that the apparent rhythm spuriously results from relating hatching time to the predicted time of high tide. This seems unlikely because the data were combined from two runs in which tidal times differed by 5 h. Another method for eliminating this possibility is to compare composite graphs of hatching time relative to the predicted time of high tide for both the experimentals and the controls. Hatching by the experimental crabs is related to high tide. Within 2 h of high tide, 82% of the crabs released larvae, and the distribution is not uniform ($P < 0.01$) over the 12 h interval (Fig. 5 A). in contrast, the hatching pattern of control crabs (Fig. 5 B) does not differ from a uniform distribution.

Hatching rhythm upon exposure to both tidal and LD cycles

When crabs were maintained under an LD cycle alone, hatching occurred almost exclusively in the 2-h interval

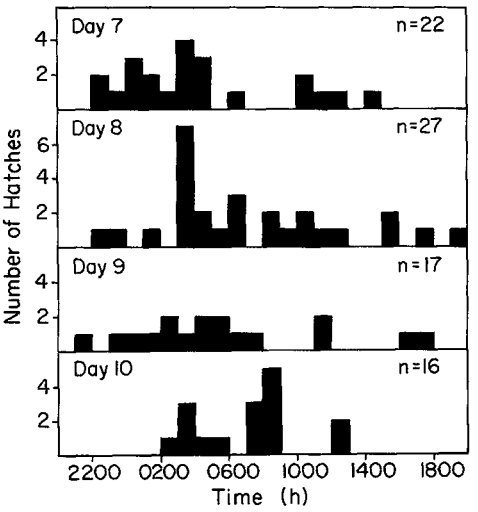

Fig. 3. *Rhithropanopeus harrisii.* Distribution of larval release of crabs held under constant low level light, salinity and temperature for at least 7 to 10 d. Number of crabs releasing larvae (ordinate) is shown relative to time of day (abscissa). Time starts at 20.00 hrs because this was about the beginning of the night in the field. *n* is the number of crabs that released larvae each day

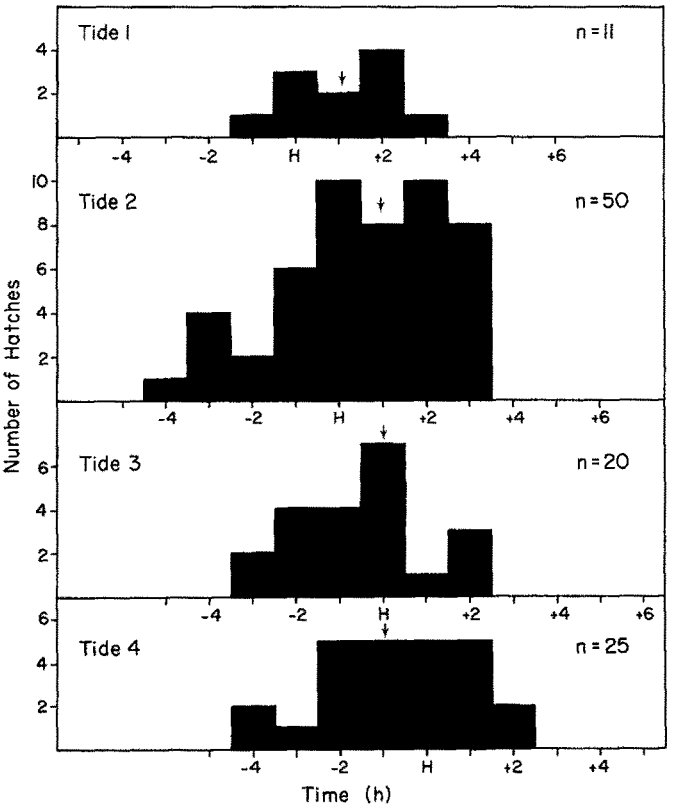

Fig. 4. *Rhithropanopeus harrisii.* Distribution of larval releases of crabs held in the salinity system for at least 7 to 10 d. Number of crabs releasing larvae (ordinate) is plotted at times (abscissa) relative to the time of predicted high tide (H) in the salinity system. The arrow indicates the mean time of larval release for each high tide. Hatching was monitored continuously in constant conditions over a time interval which covered 4 tidal cycles by the salinity system. *n* is the sample size for each tide

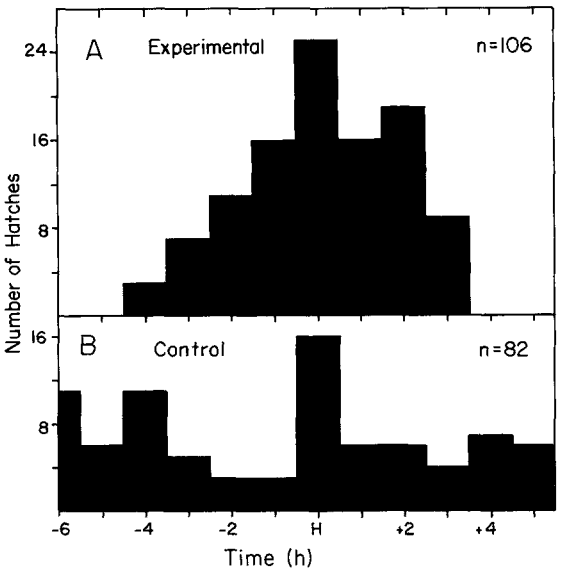

Fig. 5. *Rhithropanopeus harrisii.* Distribution of larval releases (ordinate) for all of the experimental (A) and control (B) crabs relative to the predicted time of high tide (abscissa) in the salinity system. *n* is the total sample size

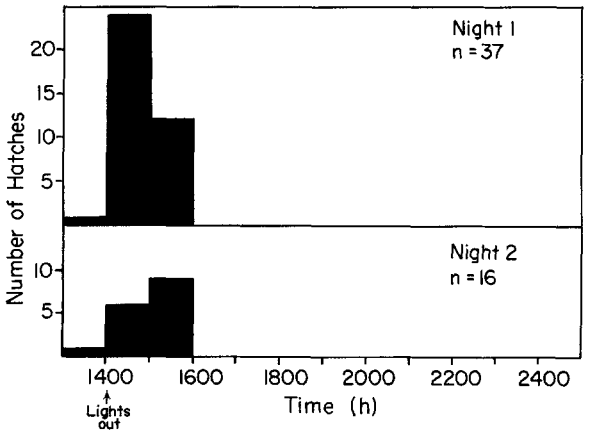

Fig. 6. *Rhithropanopeus harrisii.* Distribution of larval releases (ordinate) for crabs exposed to an LD cycle for at least 7 d and then placed in constant conditions. Hatching time is plotted relative to the beginning of the dark phase (lights out) of the entrainment LD cycle (abscissa) for crabs maintained 2 nights in constant conditions. *n* is the sample size for each night

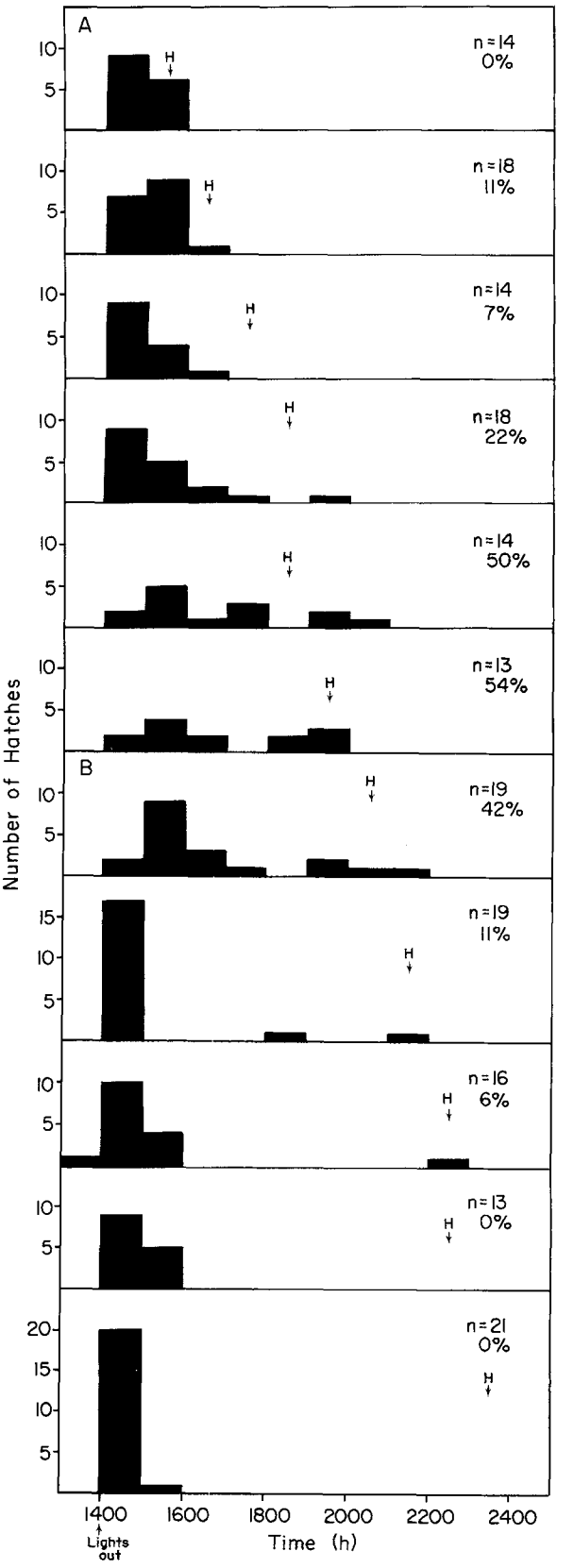

Fig. 7. *Rhithropanopeus harrisii.* Distribution of larval releases (ordinate) for crabs exposed to both an LD and a salinity cycle for at least 7 d and then placed under constant conditions. Hatching is plotted relative to the beginning of the dark phase (lights out) of the entrainment LD cycle (abscissa). The dark phase ended at 01.00 hrs. H shows the time of high tide for the salinity cycle on each monitoring night and *n* is the sample size. The percent value is the percentage of crabs that released their larvae at times other than the 3-h time interval (1 h before the beginning of the dark phase to 2 h later) in which the LD-only controls released larvae (Fig. 6). A and B are the beginnings of the first and second runs, respectively

after the beginning of the dark phase (Fig. 6). This relationship is maintained for two cycles in constant conditions and the hatching pattern during the time of predicted night is not uniform ($P < 0.01$). Thus, the predicted time of hatching, if it is only related to the LD cycle, is the first 2 h after the beginning of the dark phase. For crabs exposed to both salinity and LD cycles, a deviation from this 2-h interval would indicate that the salinity cycle is also affecting hatching. The predicted time of larval release for crabs entrained to the salinity cycle is around high tide (Fig. 4).

For individuals exposed to both cycles, all hatching times are plotted relative to the predicted times of high salinity and the dark phase (Fig. 7). Hatching is not separated according to the length of time the crabs were in constant conditions as in Fig. 4. Combining the data seems justified because: (1) Fig. 4 clearly indicates that a rhythm in hatching will persist in constant conditions, (2) all hatching took place within 48 h of placement in constant conditions and (3) the amount of variation in the hatching times due to the difference between the free running period length (at about 28 °C; Forward *et al.*, 1982) and a salinity period is about 24 min between consecutive night time high tides. This is much less than the time resolution for determining hatching times (1 h).

With the exception of one crab, egg hatching only occurred during the predicted night phase (Fig. 7). However, whether hatching was related to the salinity and/or the LD cycle depends upon when high tide occurred in the dark phase. The effect of the salinity cycle is indicated by the relationship of hatching to the time of high tide and the percentage of the hatches which occurred at times other than the interval predicted for the LD cycle alone (1 h before the beginning of the dark phase to 2 h later). When high tide occurred at the beginning of the dark phase, hatching occurred at the time predicted relative to the LD cycle alone. As high tide moved through the night, some crabs released near the time of high tide while others persisted in releasing near the beginning of the dark phase. The greatest proportions of crabs released larvae relative to the salinity cycle when high tide occurred from 5 to 7 h after the beginning of the dark phase. After this time, tide had a diminishing influence. Hatching completely reverted to the beginning of the dark phase when high tide was within about 3 h of the beginning of the light phase.

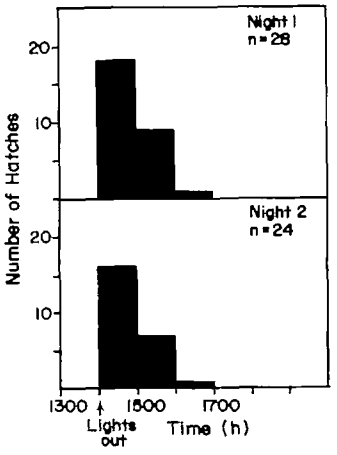

Fig. 8. *Rhithropanopeus harrisii.* Distribution of larval releases (ordinate) for crabs exposed to a LD cycle and constant current flow. Hatching is plotted relative to the beginning of the dark phase (lights out) of the entrainment LD cycle (abscissa). Hatching was monitored over 2 nights in constant conditions. *n* is the sample size on each night

Hatching upon exposure to LD cycle and current flow

Nearly all hatching (96%) of crabs maintained in a LD cycle and constant current flow of 15‰ S water occurred in the 2-h interval after the predicted beginning of the dark phase (Fig. 8). This pattern is almost identical to that for crabs exposed to the LD cycle without current flow (Fig. 6), which indicates that constant current does not affect hatch times and that the salinity cycle did cause the previously observed shifts in hatching time between experimental and control crabs (Figs. 4, 7).

Discussion

Ovigerous *Rhithropanopeus harrisii* removed from an estuary lacking regular tides and exposed to only a tidal cycle in salinity in the laboratory displayed a rhythm in larval release when subsequently placed in constant conditions in which egg hatching occurs around the predicted time of highest salinity (high tide). The relationship of hatching times to high tide suggests the presence of a circatidal rhythm. This type of rhythm is normally considered an endogenous rhythm which persists for at least 4 to 5 cycles in a single individual under constant conditions and which has a free running period close to but not exactly 12.4 h. For each crab, larval release occurs either as a single event or at most two events at the time of consecutive high tides (Forward *et al.*, 1982). The site of rhythmicity in *R. harrisii* is the developing embryo and not the female (Forward and Lohmann, 1983). Thus the persistence of the rhythm in a single individual for 4 to 5 cycles cannot occur since an egg hatches only once. Nevertheless, the rhythmic release of larvae by a population of crabs does persist under constant conditions in the laboratory for 4 (Fig. 3) to 7 cycles (Forward *et al.*, 1982). These considerations suggest that there is a circatidal rhythm in egg hatching which is observed as rhythmic larval releases by a crab population at the time of successive high tides.

The present study indicates that a rhythm in hatching can be entrained by a tidal cycle in salinity. This result agrees with that of Taylor and Naylor (1977), who found that salinity cycles can entrain a circatidal locomotor rhythm in the crab *Carcinus maenas*. In the present study, egg hatching occurred around the predicted time of highest salinity, which simulated the time of high tide.

This pattern in larval release is identical to that observed in *Rhithropanopeus harrisii* from a habitat having regular semi-diurnal tidal cycles in salinity and probably no LD cycle (Forward *et al.*, 1982). We conclude that salinity change is a natural Zeitgeber for entraining the tidal rhythm in *R. harrisii*. In addition, hatching around the time of high tide has frequently been observed in other species of crabs when they have a tidal rhythm in larval release (e.g. Bergin, 1981; Saigusa, 1982). However, in the case of *Sesarma* sp. (Saigusa and Hidaka, 1978) and *Uca pugillator* (Bergin, 1981) it is unlikely that the developing eggs were exposed to tidal cycles in salinity. Thus, tidal rhythms in hatching can probably also be entrained by cycles in other environmental factors.

Rhithropanopeus harrisii displays a solar day or circadian rhythm in larval release after exposure to a diel LD cycle (Forward *et al.*, 1982; Fig. 6), in which hatching mainly occurs in the initial 2 h of the dark phase. However when exposed to both a tidal cycle in salinity and an LD cycle, the crabs showed a complex release pattern dependent upon the time of high tide at night. Crabs only released larvae during the predicted time of night. Studies of other crab species, in which larval release times have been accurately determined relative to daily and tidal cycles, have also found hatching only at night (Saigusa and Hidaka, 1978; Saigusa, 1981, 1982; Bergin 1981).

When high tides were near the beginning or end of the dark phase, hatching by *Rhithropanopeus harrisii* occurred at the beginning of the dark phase, as if the crabs had only been exposed to an LD cycle. As high tide moved to the middle of the night, hatching occurred over a broad range of times with an increasing proportion of hatches occurring around the time of high tide. This pattern is very similar to those observed in other crab species in the field (*Sesarma* species: Saigusa and Hidaka, 1978; Saigusa, 1981, 1982) and laboratory (*Uca pugilator:* Bergin, 1981). Individuals in the field were exposed to simultaneous, natural tidal and LD cycles while those in the laboratory were removed from an environment having both cycles. The general pattern for these species was that, when high tide was before and at sunset, hatching occurred at the beginning of night. When high tide was about 2 h after sunset and later, hatching occurred around high tide. For *Sesarma* sp., once high tide was within 3 to 5 h of sunrise, hatching reverted to the several-hour interval at the beginning of the dark phase (sunset). Since very different patterns are observed when *R. harrisii* is exposed to either tidal or LD cycles alone, the consistent (between species) but complex hatching pattern observed when these cycles are presented together seems to result from the interplay of the time of high tide and of night.

Most often estuarine crabs have a semi-lunar rhythm (Christy, 1978; Saigusa and Hidaka, 1978; Saigusa, 1981, 1982; Christy, 1982; Christy and Stancyk, 1982), in which the number of females releasing larvae varies with the phase of the moon. Saigusa (1982) suggested that the number of females with mature embryos is related to the lunar cycle, but the actual time of hatching is regulated by the combination of solar day and local tidal cycles. *Rhithropanopeus harrisii* does not display a semi-lunar rhythm as the number of females with mature embryos does not vary over the lunar month (Forward *et al.*, 1982). Nevertheless, the results of the present study support the importance of daily and tidal cycles in controlling the specific hatching time.

The present study also adds information about the hierarchy of functional advantages for the various times of hatching. By entering the water column near the time of high tide, the larvae are potentially exposed to high salinity, and initial horizontal movements are seaward as the tide recedes. Thus, it has been argued that release at high tide is a function to avoid stressful salinity conditions (Forward *et al.*, 1982) and larval dispersal (Christy, 1978, 1982; Saigusa and Hidaka, 1978; Wheeler, 1978; Bergin, 1981; Saigusa, 1981; Christy and Stancyk, 1982). Considering the LD cycle, egg hatching almost always occurs at night (reviewed by Forward *et al.*, 1982). The primary functional advantage for nocturnal larval release may be avoidance by larvae and adults of predators which visually sight and actively pursue their prey Ennis, 1975; Branford, 1978; DeCoursey, 1979; Bergin, 1981; Christy, 1982). For larvae this entails reduced vulnerability to visual predators: (1) immediately after hatching when larvae are concentrated in a local area, and (2) throughout the larval phase, since a potentially greater amount of development occurs in darkness. In addition, nocturnal release may also function as an initial escape from lethal high temperature (Dollard, 1978).

The present study indicates that hatching times are related to high tide only when it occurs between about 2 h after the beginning of the dark phase and 3 to 5 h before the beginning of the light phase. When high tide occurs at other times, larvae are released relative to the LD cycles at the beginning of night, even when low tide occurs near sunset. Since release only occurs near high tides which fall in a narrow nocturnal interval, factors which influence hatching relative to the LD cycle must be of the greatest functional importance. Reduced vulnerability to visual predators would then be more important than avoidance of extreme salinities or initial seaward transport.

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