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

R. B. Forward, Jr., J. K. Douglass and B. E. Kenney

Duke University Marine Laboratory; Beaufort, North Carolina 28516, USA and Zoology Department, Duke University; Durham, North Carolina 27706, USA

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), diel related rhythm (Ennis, 1973; Branford, 1978; Moller and Branford, 1979; Forward et al., 1982), semilunar related rhythm (Saigusa, 1980; Christy, 1982). The Zeitgeber 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 Zeitgeber 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 Zeitgeber 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 $15 \pm 0.5\%$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 $0.7 \text{ W/m}^2$ 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 2001 liters 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 entrance 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