

A TIDAL RHYTHM IN PHOTOTAXIS OF LARVAL GRASS SHRIMP (*PALAEEMONETES PUGIO*).

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An endogenous phototaxis rhythm was measured in newly hatched larvae from the estuarine shrimp *Palaemonetes pugio*. Shrimp were maintained in constant conditions and phototaxis tested using a 500-nm light stimulus. Larvae exhibited large temporal variations in both positive and negative phototaxis, with peaks in responsiveness at 12–14h intervals. A constant phase relationship was observed between these peaks and tidal cycles in the field, regardless of the diel timing of the tides. Least squares fits of the data to a sine wave demonstrate a strong circatidal cycle in photore sponsiveness. The circatidal pattern persists for 2–3 cycles in constant conditions, and is still expressed in larvae after 3 tidal cycles of embryonic development in the laboratory. We conclude that an endogenous circatidal rhythm affects the photobehavior of *P. pugio* larvae. If coupled with a tidal cycle in vertical migration in the field, the negative phototaxis rhythm could help the larvae avoid predators such as ctenophores.

KEY WORDS: Tidal rhythm, crustacean, larvae, phototaxis, time series analysis.

INTRODUCTION

The behavior and physiology of estuarine organisms is often timed according to the strong tidal and daily changes that typify their physical environment (Palmer, 1973; Enright, 1975; DeCoursey, 1976a; Naylor, 1982). Among planktonic organisms, endogenous diel rhythms are well-known in freshwater and marine environments (Harris, 1963; Enright and Hamner, 1967; Duval and Geen, 1976), and have also been described in some estuarine plankton (e.g. Saint-Jean and Pagano, 1983; Marcus, 1985; Stearns, 1986). Tidal rhythms, however, are known in only two estuarine plankters (DeCoursey, 1976b; Cronin and Forward, 1979). Larvae of the estuarine crab *Rhithropanopeus harrisi* exhibit tidal rhythms of vertical migration, activity and phototaxis (Cronin and Forward, 1979, 1983; Forward and Cronin, 1980). The vertical migration rhythm probably helps *R. harrisi* larvae avoid seaward transport during development, and could also reduce predation (Cronin and Forward, 1983; 1986).

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The present study was undertaken to determine whether larval grass shrimp also have rhythms in phototaxis. *Palaemonetes pugio* (Palaemonidae) is a dominant estuarine decapod whose known range extends from southern Maine (Knowlton, 1971) to Florida and the Gulf of Mexico (Holthuis, 1952). Larvae hatch as zoeae and pass through approximately ten zoeal stages before the metamorphic molt to the first postlarva (Broad, 1957). In common with most other estuarine crustaceans, but unlike *R. harrisi*, *P. pugio* larvae appear to be exported from tidal creeks to offshore waters soon after hatching (Allen and Barker, 1985).

The results of this study demonstrate remarkably large, yet predictable temporal variations in phototactic responsiveness of newly hatched grass shrimp zoeae. In addition, a least squares curve-fitting technique is presented which offers several advantages over more traditional methods of time series analysis. As Enright (1965) discusses, simple "form estimates" that examine only one or two hypothesized periods can be misleading. Periodogram and Fourier methods perform more comprehensive analyses, but still suffer from a number of limitations. These methods assume data that span a large number of cycles, yet cyclical behaviours can be short-lived and rapidly damped under constant laboratory conditions. No phase information is provided, and it is difficult to test periods that are not multiples of the time interval between measurements. Moreover, since periodogram and Fourier techniques are essentially forms of harmonic analysis, data that contain only a circatidal (ca. 12.4h) period can easily produce a spurious "peak" at a circadian period (ca. 24h). Finally, the unavoidable discontinuity at each end of a time series can distort the results of harmonic analyses, particularly when applied to small numbers of cycles. In this study, a least squares method was used to find best fits of the data to a sinusoidal function. This technique provides a conceptually simple alternative to periodogram and Fourier analysis, and is particularly useful for data that span only a few cycles of the period(s) in question.

MATERIALS AND METHODS

Ovigerous *Palaemonetes pugio* Holthuis were collected near the time of low tide at Calico Creek, Newport River Estuary, Morehead City, North Carolina, USA. Semidiurnal tides occur at the collection site, where the salinity varied between about 32 and 13 ppt near the time of this study (JKD, pers. observation). Shrimp were taken immediately to the Duke University Marine Laboratory (Beaufort, North Carolina). In order to prevent possible rephasing of rhythms by sudden environmental changes, the initially low salinity was increased gradually to a final value of 20 ppt around the time of the next high tide. (Maximum survival of *P. pugio* larvae in the laboratory is obtained near 20 ppt; e.g. McKenney and Neff, 1979). During this initial adjustment period, room temperature was maintained near that in the field at 25°C. After salinity adjustment, the animals were transferred into 20 ppt seawater filtered to remove particles larger than 5 μm . The shrimp were maintained in fingerbowls at ca. 25°C, in constant dim light provided by a 15-watt incandescent bulb (ca. 1×10^{17} quanta $\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Room lights were turned off at the time of sunset

in the field on the day of collection, leaving only the constant dim light. Light intensities were measured just under the water surface with a Biospherical QSL-100 quantum irradiance meter (San Diego CA, sensitivity range 400–700 nm).

Phototactic responses of newly hatched zoea larvae were tested using procedures similar to those of Forward (1980). Larvae were stimulated with a horizontal light beam from a slide projector that was located in a light-tight room adjacent to the room in which shrimp were maintained. The projector was fitted with a 300-W incandescent bulb, diffuser, Schott heat filters, and a 500-nm interference filter (Ditric Optics, 8 nm half band pass). Phototactic responsiveness of *P. pugio* larvae is maximal near 500 nm (Douglass, 1986). Stimulus intensity was controlled with neutral density filters (Ditric Optics) calibrated at 500nm. The projector was operated via a voltage stabilizer (Sola Electric, Elk Grove IL) to minimize any short-term fluctuations in stimulus intensity that might arise from power surges. The projector and filters were in a light-tight enclosure that permitted light to escape only through the filters themselves. Stimulus light intensity was measured with a photometer (EG and G model 550–1, Princeton NJ).

A 150×30×30 mm lucite testing chamber was positioned 37 cm from the filter holder at the center of the light beam, with a rectangular aperture (100mm wide by 40mm high) immediately in front of the chamber to block stray light. The chamber was filled to a depth of ca. 25mm with filtered seawater, and could be divided into 5 equal sections with a set of lucite partitions removable as a unit.

Groups of ca. 20 larvae were kept in small plastic vials in the constant dim light. Phototactic responses of each group were tested at 2-hour intervals as follows: A vial of larvae was brought into the projector room and the larvae were transferred to the middle section of the testing chamber, where they were allowed to acclimate to total darkness for 60s. The lucite partitions were then gently raised while simultaneously turning on the light stimulus; 20s later the partitions were lowered and the number of shrimp in each section was counted. The level of negative phototaxis was recorded as the proportion of larvae found in either of the two sections farthest from the light stimulus; positive responses were recorded for larvae in the 2 sections closest to the light. Time was kept in the dark with an electric metronome. The control procedure was identical to that just described, except that no light stimulus was provided while the partitions were up.

Upon the completion of a test, larvae were returned to their original vial with a pipette, and were tested again at successive 2-h intervals. The chamber was filled with previously unused seawater prior to each test. Occasionally, a larva was injured as the partitions were lowered. Animals that appeared unable to swim normally or were known to be injured were removed before subsequent tests. Consequently, later tests tended to employ fewer individuals per group. Newly hatched *Artemia* spp. nauplii (Greatwall Brand, Peoples Republic of China) were added to each vial at random times immediately following a test. Feeding and testing are unlikely to have acted as inadvertent entraining cues, since food was continuously available in the vials and the time interval between tests (2h) is unlikely to induce 12–25h rhythmicities (c.f. Harris and Morgan, 1984.)

Like all brachyuran crustacean larvae (Forward, 1977, 1987a), *P. pugio* larvae

generally exhibit negative phototaxis in response to very low light intensities, and change to positive phototaxis at higher intensities (Figure 1; Wilson *et al.*, 1985; Douglass, 1986). If a rhythm in photoresponsiveness is present, it should be detectable with a constant stimulus intensity that produces intermediate levels of either positive or negative phototaxis. An appropriate stimulus intensity was chosen by testing responses to a series of light intensities (Figure 1).

Next, the chosen stimulus intensity (4.6×10^{13} quanta·m⁻²·s⁻¹) was used to test for temporal changes in phototaxis. A control group was tested after each experimental group. Two experiments were performed. Experiment 1 began on August 29, 1984 when falling tides in the field occurred near noon and midnight, and Experiment 2 began on September 19, 1984 when falling tides were near dawn and dusk. Females carrying late-stage eggs had been collected during the previous day and placed in constant dim light. Larvae hatched at night near the time of high tide. Testing was begun shortly after hatching, using groups of ca. 20 zoeae from each brood. Experiment 1 employed 5 broods that all hatched during a 4-h interval on the first

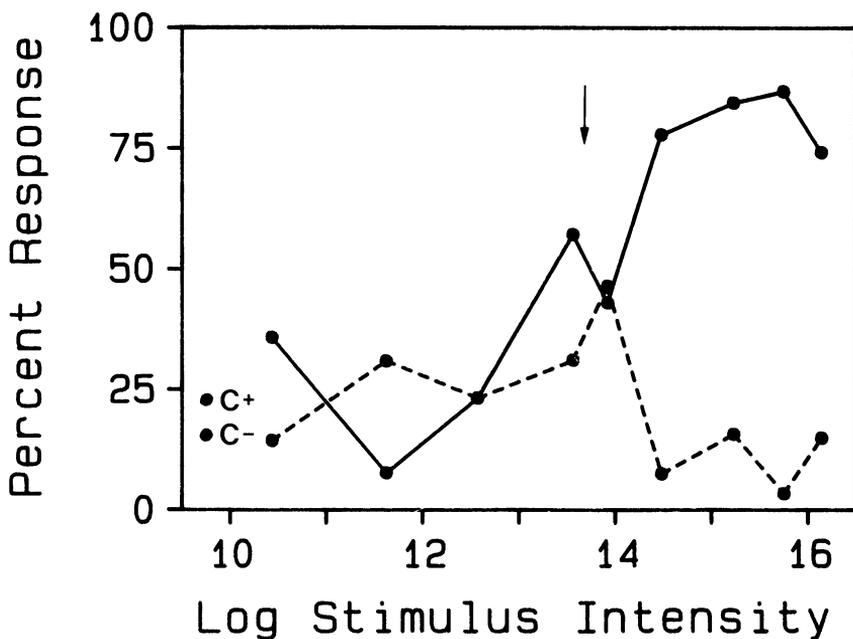


Figure 1 Phototactic responses (ordinate) of newly hatched zoeae as a function of log stimulus intensity (abscissa, in quanta·m⁻²·s⁻¹). Solid lines show positive phototaxis, dotted lines negative phototaxis, and C's denote control responses. The stimulus intensity selected for subsequent rhythm experiments is marked with an arrow. Responses were tested between 1530 and 1800h on August 23, 1984, and 13 to 42 larvae were tested at each intensity.

night since collection. Experiment 2 employed 5 broods that hatched within a ca. 30-h interval.

Time series analyses of data from Experiment 2 employed only the first 21 data points (40 hours), since the amplitude of response variations had often greatly diminished beyond this point. Experiment 1 covered a shorter period of time (see below), and all points were analyzed. For comparisons with control responses, ten independently-seeded random datasets were generated by a computerized random number generator (Turbo Pascal 4.0; Borland International, Scotts Valley, CA). Each 21-point random dataset represents "responses" from a single "brood." Raw data from each brood were first arcsine transformed (Sokal and Rohlf, 1981) and zeroed to the average response level. Least squares fits were then calculated between response data and a sine function

$$y = A \cdot \sin [F \cdot (x + ph)] \quad (1)$$

In Equation 1, y = the response level, A = amplitude, F = frequency (or period^{-1}), Ph = phase, and x = the time of a test, expressed relative to either midnight or the last high tide before the start of the experiment (see below). Various combinations of period, phase and amplitude were tested; the combination giving the minimum sum of squared differences between experimental (\hat{y}) and theoretical (y) values represents the minimum least squares fit for N data points:

$$\text{best fit} = \text{minimum} \sum_{i=1}^N (\hat{y}_i - y_i)^2 / N \quad (2)$$

Initial best fit parameters were obtained by testing periods from 5 to 35 hours at 1h intervals, phases from 0 to 360° at 7.5° intervals, and amplitudes from 0 to 20 at intervals of 1. These results were then "fine-tuned" using 0.5h period intervals and amplitude increments of 0.25. Periods less than 5 hours were not tested, because phototaxis data were collected at a frequency (f) of $0.5 \cdot \text{h}^{-1}$, and frequencies greater than $f/2$ (the Nyquist frequency) can not be reliably detected (e.g. Kendall, 1973).

Preliminary analyses of the phototaxis data all yielded periods close to 13h, suggesting an endogenous circatidal pacemaker that free-runs at a slightly longer period than the natural tidal zeitgeber (12.4h). Testing of all broods began soon after hatching, but since hatching times varied, so did the duration of "free-running" conditions prior to testing. In order to compare the extent of phase synchrony in time series from different groups, the following adjustment for free-running was made. Testing times were expressed relative to the "expected" time of the last high tide in the field before the start of an experiment, assuming a 13.0h free-running period.

Since control response amplitudes (" A " in Equation 1) were generally lower than for phototaxis, best fit values tend to be lower for controls. To permit unbiased comparisons between control, experimental and random data, each dataset was normalized to its own best fit amplitude before final least squares computations. Dunnett's T test was used to compare more than one treatment with a single control (Dunnett, 1955, 1964).

In order to calculate a reliable periodogram (Harris and Morgan, 1983, Dörrscheidt and Beck, 1975), at least several cycles of interest should be covered in the data. Since the few tidal (and fewer diel) cycles represented in this study do not

meet this condition, no periodogram results are presented. Least squares analysis is amenable to smaller numbers of cycles, but clearly this method also has limits. In most cases it would be difficult to argue for a true "periodicity" if the data represent fewer than 2 complete cycles.

Times of high and low tide in the field were obtained from water level measurements that are made continuously at the Duke University Marine Laboratory docks in the Newport River estuary (NOAA records, 1984). Under the weather conditions encountered during this work, these times were applicable to the collection site with no correction.

RESULTS

Phototaxis varies dramatically as a function of time (Figures 2a, c; 3a, c, e). During the first 30 to 40 hours, both negative and positive percent responses are maximal (and minimal) about every 12 to 14 hours. Such a pattern of two maxima per day could arise from either a circatidal (ca. 12.4h) or a bimodal circadian (ca. 24h) cycle. Negative phototaxis, however, is always maximal before or during low tide, and positive phototaxis always peaks just before high tide. This pattern persists regardless of whether the tides begin to ebb near midnight and noon (Figure 2) or near dawn and dusk (Figure 3). Therefore, the major fluctuations in phototactic responsiveness are clearly circatidal. Nevertheless, a weaker diel cycle may also be present. In both experiments, there is a slight tendency for negative phototaxis to be greater at night, and the overall level of positive phototaxis is usually higher during the day.

Temporal changes in control responses levels (Figures 2b, d; 3b, d, f) generally exhibit smaller amplitudes than changes in phototaxis, and comparisons among hatches show no consistent temporal pattern. For example, positive responses of one group (Figure 2b) suggest that control maxima might occur near the time of high tide, but no other control responses fit this trend. Moreover, there appears to be little or no correlation between control and experimental data. These conclusions hold whether the data represent a single brood (Figures 2c, d and 3a, b) or averaged responses from 2 to 4 broods (Figures 2a, b and c-f).

In larvae that hatched only hours after removal from field conditions (Figures 2, 3a), strong circatidal patterns of both positive and negative phototaxis continue for 2-3 cycles. These results suggest the presence of an endogenous tidal rhythm in photoresponsiveness. The endogenous nature of the clock (or clocks) that control this rhythm is confirmed by the expression of cycles in phototaxis following embryonic development in the laboratory. Larvae that hatched after ca. 3 tidal cycles in constant conditions (Figure 3c, e) express a vigorous circatidal phototaxis pattern, which persists for 2-3 cycles of negative responses and up to 4-5 cycles of positive responses.

Least squares analyses strongly confirm the circatidal timing of the cycles in phototaxis (Table 1). The best periods are consistently near the mean (from Table 1, \bar{X} = 12.9h for both positive and negative responses, weighted for numbers of

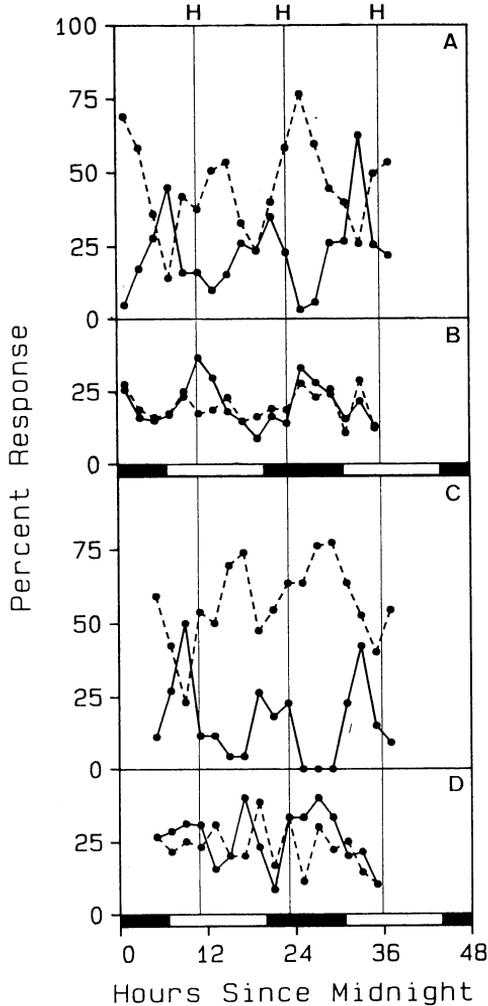


Figure 2 Temporal variations in phototactic behavior during constant conditions (Experiment 1). High tides in the field occurred just before midnight and noon. Solid lines show positive phototaxis, dotted lines negative phototaxis. Larvae hatched at night near the time of high tide, from ovigerous females collected during the previous day. Responses (ordinate, untransformed percentages) are plotted vs. the time since midnight (abscissa). Phototactic responses (A, C) appear above control responses (B, D) from the same brood(s). A & B show averaged responses from 4 broods (hatches 1-4 in Table 1); C & D represent one brood (hatch 5 in Table 1) that hatched soon after testing of the others had begun. Thin vertical lines (H) show measured times of high tide in the field, and dark horizontal bars, predicted periods between sunset and sunrise in the field. The stimulus intensity was 4.6×10^{13} quanta $\cdot m^{-2} \cdot s^{-1}$.

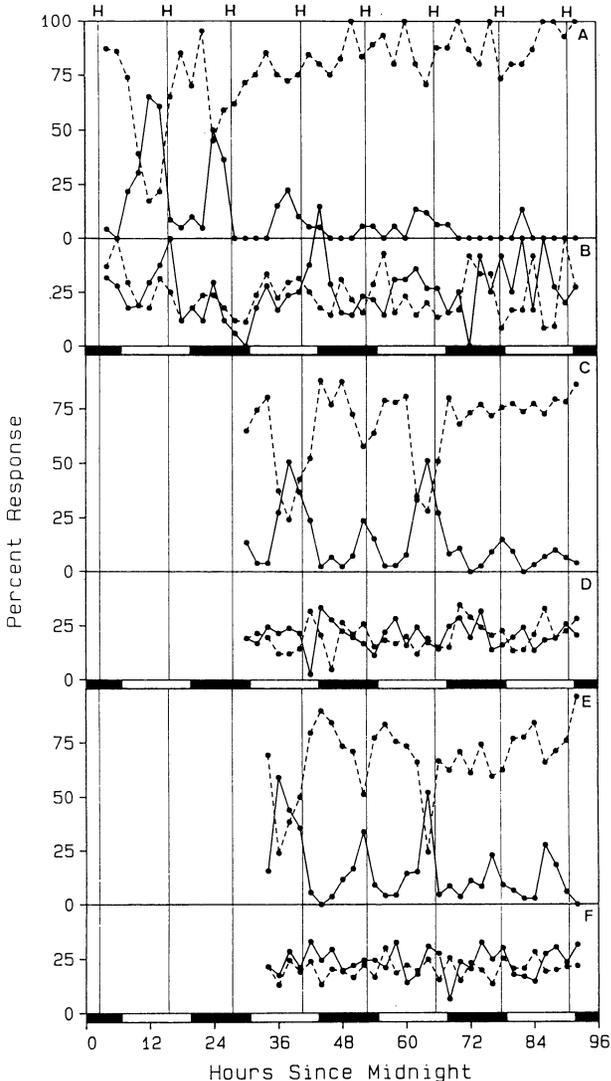


Figure 3 Temporal variations in phototactic behaviour during constant conditions (Experiment 2), plotted as in Figure 2. High tides in the field occurred a few hours before dawn and dusk and ca. 4.1 hours later than in Experiment 1. As in Experiment 1, ovigerous females had been collected near the time of low tide on the day before the first brood hatched. A & B show respective phototactic and control responses of one brood (hatch 1, Table 1); similarly, C & D and E & F represent 2 broods each that hatched during the second night of the experiment (hatches 2 & 3 and 4 & 5 respectively, Table 1).

Table 1: Minimal least squares fits between a sine wave and the initial phototactic responses of newly hatched *P. pugio* zoeae. Responses from hatches sharing the same initial testing time were arcsine-transformed, zeroed and averaged prior to calculation of fits. **Pd**=period (in hours), **Ph**=phase (in degrees) from Equation 1. **N** is the number of points analyzed, and **Fit** the best least squares fit from Equation 2. The raw data used for these calculations appear in the indicated figures. As explained in the Methods, testing times were normalized to the previous "expected" high tide in the field assuming a 13.0h "free-running" circatidal period. Mean best fit parameters for the random data (\pm s.e.) appear at the bottom of the table.

Sign of taxis	Hatch Numbers	Pd	Best fit parameters			Figure
			Ph	N	Fit	
Experiment 1						
Experimentals						
-	1-4	12.5	22.5	19	0.229	2a
-	5	13.0	345.0	17	0.357	2c
+	1-4	13.0	225.0	19	0.175	2a
+	5	12.5	165.0	17	0.188	2c
Controls						
-	1-4	6.5	292.5	18	2.10	2b
-	5	9.0	285.0	16	2.15	2d
+	1-4	14.5	112.5	18	0.253	2b
+	5	17.5	232.5	16	1.35	2d
Experiment 2						
Experimentals						
-	1	13.5	7.5	21	0.710	3a
-	2 and 3	12.5	330.0	21	0.395	3c
-	4 and 5	13.5	30.0	21	0.510	3e
+	1	13.0	187.5	21	0.412	3a
+	2 and 3	12.5	142.5	21	0.300	3c
+	4 and 5	13.0	195.0	21	0.333	3e
Controls						
-	1	9.0	330.0	21	0.848	3b
-	2 and 3	9.5	315.0	21	0.867	3d
-	4 and 5	5.0	172.5	21	2.33	3f
+	1	9.5	345.0	21	0.905	3b
+	2 and 3	11.5	232.5	21	0.771	3d
+	4 and 5	6.5	255.0	21	2.55	3f
Random Data						
n.a.	1-10	10.6 \pm 2.8	n.a.	21	1.42 \pm 0.25	n.a.

hatches). The phase also remains fairly constant, both in August (Experiment 1) and September (Experiment 2). Considering negative and positive phototaxis separately, nearly all phases lie within 30° of their respective averages. The only exception to this pattern is in Experiment 2, hatches 2 and 3, where the phase (142.5°) differs by some 50° from the average for positive responses (193°). Meanwhile, negative and positive phototaxis are expected to be about 180° out of phase, due to the experimental design. The observed average phase difference is 175.5°, very close to the expected value.

Best fits to control responses fail to indicate any true periodicity (Table 1). Control "periods" vary widely between 5.0 and 17.5 hours, and there is no significant difference between the periods of random data and either positive or negative controls ($n=10$, Dunnett's T test, control data serving as the "treatments"). Control phases also vary widely, and there is no apparent relationship between positive and negative phases.

The phototaxis data yield far better fits to Equation 1 than either control or random data (Table 1 and Figure 4). Best fits for negative phototaxis differ significantly from negative control fits ($p<0.01$), while random fits are indistinguishable from negative controls ($p>>0.05$; Dunnett's T test). For positive responses, the corresponding Dunnett's T test shows no significant difference. If the random fits are excluded from the analysis, however, positive phototaxis and positive control fits do show a significant difference ($p<0.05$, standard T test).

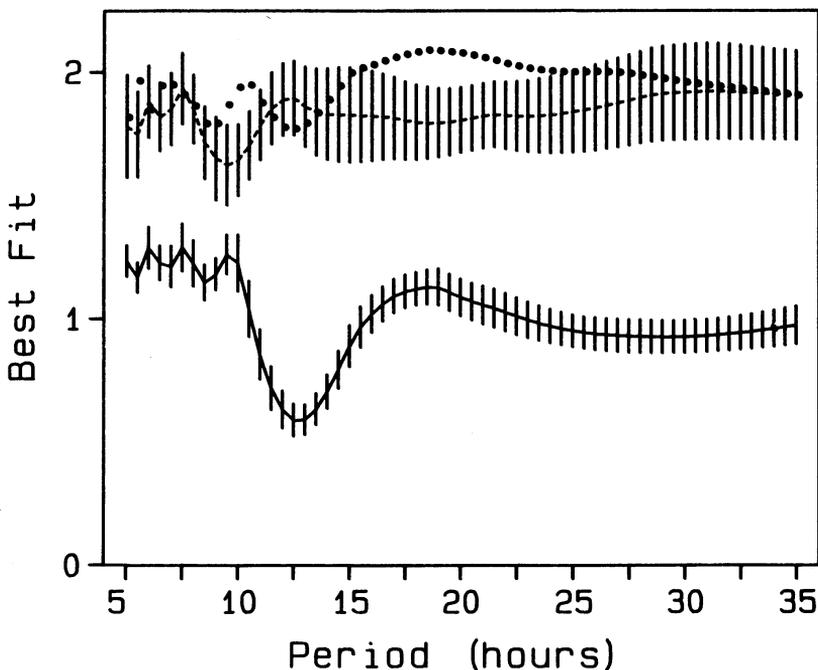


Figure 4 Best fits (ordinate) of normalized data to Equation 1, tested between periods of 5 and 35 hours (abscissa). Lower values represent better fits. The phase was varied at 7.5° intervals; amplitude was kept constant at 1. The lower solid trace (\pm s.e.) is calculated from negative phototactic responses of the 10 broods used in Experiments 1 and 2. The upper traces show the corresponding results from negative control responses (solid trace) and random "responses" (dots). $N=10$ broods for each trace. For clarity of presentation, the fits to random data are plotted with a horizontal offset and without error bars. The standard errors for random data are all slightly larger than in the controls.

Finally, comparing the quality of fits to various periods (Figure 4), the best periods for phototaxis (near 12.5–13h) are far superior to other periods, while control and random “best periods” differ little from the worst. There is no evidence of a circadian periodicity.

Taken as a whole, the least squares analysis confirms the presence of a circatidal cycle in phototaxis. The close agreement among phases after adjusting for the 13-h free-running period permits the calculation (Figure 5) of an overall average time course from the first 17 to 21 points in Figures 2 and 3. Only negative responses are shown, but positive responses give similar results. The combined data yield a very good fit to a sine wave (period=13.5h, phase=0°, best fit value=0.325), and there is good agreement between Experiments 1 and 2. Possible reasons for the disappearance of the rhythm after 2–4 tidal periods are considered in the Discussion.

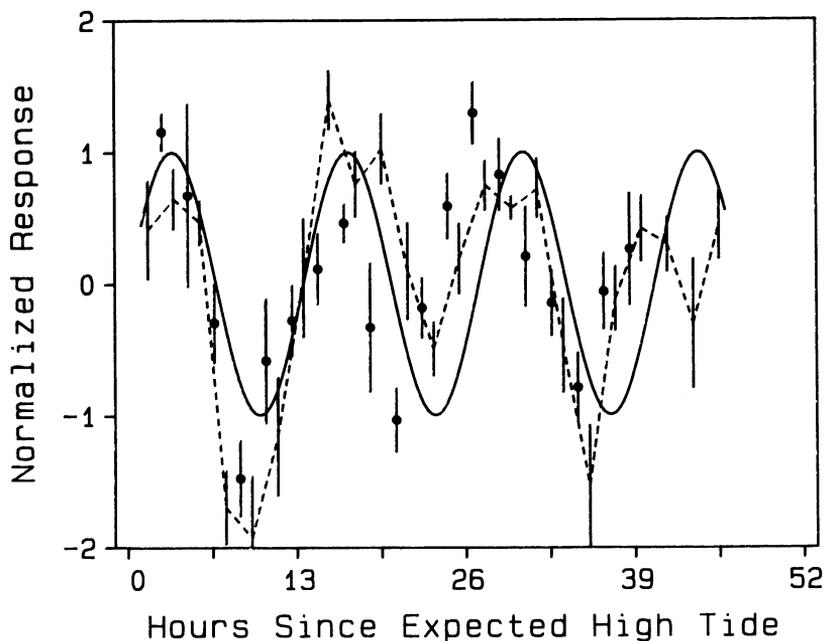


Figure 5 Comparison of normalized negative phototactic responses of ten broods with the best fit sine wave (solid curve, amplitude held constant at 1). Responses (ordinate) are displayed relative to the “expected” time of high tide (see text for details). Unconnected circles show averages \pm s.e. from the 5 broods in Experiment 1, except $n=4$ for the first 2 points. Connected points also represent 5 broods (Experiment 2) except for the first 2 ($n=3$) and last 2 points ($n=2$). Period=13.5h, phase=0°, sum of least squares=0.325.

DISCUSSION

This investigation measured phototaxis of newly hatched *Palaemonetes pugio* zoeae maintained in constant dim light. The results demonstrate a strong circatidal rhythm in phototaxis. The raw data also suggest a diel component, but subsequent analyses fail to confirm this. Although the circatidal rhythm is rapidly damped following hatching in the laboratory, embryos maintained in constant conditions for up to 3 tidal cycles prior to larval release are still able to express a strong rhythm. It is unknown whether the embryo, the ovigerous female or both control the entrainment of the biological clock that underlies the phototaxis rhythm.

Cycles in phototaxis could result partly from an activity rhythm that is unrelated to photoreception. If so, one might expect a cyclical variation in the distance that larvae swim from the starting point in control tests. If larvae swim far enough, these variations should be reflected in the control "response" data. Furthermore, assuming no directional bias in locomotor activity, positive and negative control responses should be correlated. The control percent responses do seem large enough for such variations to be detectable, but fail to suggest either a consistent periodicity or a correlation between positive and negative responses. The observed temporal variations in phototaxis therefore appear directly linked to changes in the visual system.

Currently there are several hypotheses regarding the physiological basis (or bases) for tidal rhythms, as Reid and Naylor (1989) have summarized. Circatidal rhythmicity could be controlled by a single bimodal circadian (ca. 24h) oscillator, two circalunadian (lunar-day, ca. 24.9h) clocks operating in antiphase, or a truly circatidal (ca. 12.4h) clock. As noted in the results, the circatidal phototaxis rhythm in *P. pugio* larvae is inconsistent with a bimodal circadian clock, because peaks in phototaxis maintain a constant phase relationship with the tides, not with time of day. The results of the present investigation do not permit a distinction between circatidal and circalunadian clocks.

Individual animals express rhythms in constant conditions for varying lengths of time. Some circatidal rhythms may persist for months (Enright, 1972; Palmer, 1973), while the overt expression of circatidal rhythms in larval release, discernible in groups of brachyuran crabs, usually lasts for only one "cycle" in individual adult females (Forward, 1987b). In the present study, up to 3 tidal cycles of embryonic development in the absence of tidal entraining cues failed to abolish the expression of a phototaxis rhythm in newly hatched *P. pugio*. The rhythm, however, seems to lose strength from 2 to 4 tidal cycles after larval release.

This apparent damping of the circatidal rhythm could easily be dismissed as a "laboratory artifact," but some more specific explanations are intriguing to consider. First, individual animals could become desynchronized relative to the group. In this case, however, negative and positive phototactic response levels should both approach 50%. Instead, negative responses stabilize at high values, while positive responses tend toward zero (Figure 3). Developmental changes could also affect the rhythm. The time when temporal variations in phototaxis diminish corresponds roughly to the expected time of the first molt, when the previously sessile compound

eyes become stalked (Broad, 1957). A change in visual behavior related to development and/or ecdysis could therefore alter the *expression* of the rhythm without abolishing it. If so, larvae stimulated at a different light intensity might continue to show a rhythm. Alternatively, continued expression of the rhythm may require re-entrainment after molting. In field conditions where tidal zeitgebern are available to the larvae, tidal behavioral cycles may persist long after the first molt.

The functional significance of the circatidal phototaxis rhythm in *P. pugio* is unknown. Larvae of the estuarine crab *Rhithropanopeus harrisi* exhibit circatidal rhythms in phototaxis, swimming speed and vertical migration (Cronin and Forward, 1979, 1983; Forward and Cronin, 1980). The circatidal phototaxis rhythm does not appear to augment the expression of the migration rhythm, as negative phototaxis is maximal at the time of ascent, not descent. Forward (1976, 1986) has argued that the negative phototaxis of *R. harrisi* can function during a shadow response used to avoid predation by ctenophores and cnidarian medusae. In this case, an increase in negative phototaxis upon ascending should be advantageous, since these predators are more abundant at the surface (Cronin *et al.*, 1962; Burrell & Van Engel, 1976). In *P. pugio*, the circatidal maxima in negative phototaxis could function in a similar manner. Circatidal changes in the vertical distribution of *P. pugio* larvae have been reported (Allen and Barker, 1985), but details of the timing of these changes are not available. If the timing of the ascent in *P. pugio* coincides with maximal negative phototaxis, the circatidal phototaxis rhythm may indeed enhance predator avoidance.

Acknowledgements

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