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Title: ENDOGENOUS ACTIVITY RHYTHMS OF THE VERTICALLY MIGRATING OCEAN SHRIMP, PANDALUS JORDANI

Abstract approved: Dr. W. G. Pearcy

This study is an investigation of the endogenous, or internal, rhythms of activity in the ocean shrimp, Pandalus jordani. Groups of shrimp were kept in constant light and temperature conditions in the laboratory, and activity was monitored visually and with infrared photography. Other groups of animals were exposed to 24-hour light-dark cycles of blue-green light; the activity of these animals was recorded with infrared photography after the light cycle was terminated.

It was found that shrimp not exposed to a light-dark cycle did not display any rhythms of activity in constant conditions in the laboratory. After exposure to the light cycle, some shrimp swam significantly more during the "night" hours in constant conditions. These shrimp "entrained" to the light cycle; they synchronized their activity with the periodicity of the light cycle, and retained the periodicity in
the absence of the rhythmical light cues.

The relationship of these results to the nocturnal vertical migration of ocean shrimp in the field is discussed. It is suggested that shrimp possess a biological clock which synchronizes with rhythmical light cues and mediates vertical swimming, and that the rhythmicity of the vertical migration in the field is endogenous, with internal and external factors interacting with the internal rhythm. Conclusions are summarized and areas for further research are suggested.
Endogenous Activity Rhythms of the Vertically Migrating Ocean Shrimp, *Pandalus jordani*

by

John Richard Frey

A THESIS

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APPROVED:

[Redacted for privacy]

Professor of Oceanography in charge of major

[Redacted for privacy]

Dean of the School of Oceanography

[Redacted for privacy]

Dean of Graduate School

Date thesis is presented 11 May 1973

Typed by Opal Grossnicklaus for John Richard Frey
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INTRODUCTION

Diel vertical migration is a behavior pattern of pelagic animals that still has not been completely explained. Many planktonic animals rise toward the surface at night and sink or swim away from it during the day. Shrimp are no exception, and several species are known to perform extensive vertical migrations (Bainbridge, 1961; Foxton, 1970; Pearcy and Forss, 1966; Pearcy, 1970; Waterman et al., 1939).

Most investigations of vertical migration have concentrated on the effects of light, with emphasis on times of rapid change of light intensity. The daily rhythmicity of migration has generally been considered to be exogenous, that is, due solely to concurrent environmental stimuli. The idea of endogenous or internal rhythmicity as a contributing factor, although suggested by Esterly in 1917, has received little attention. "Biological clocks" are known to be an important factor in the cyclic activity of many terrestrial and marine animals (Aschoff, 1965; Bünning, 1967). Therefore, their involvement in a manifestation of daily rhythmicity as widespread as diel vertical migration is likely. A few studies do, in fact, suggest the importance of endogenous rhythms to the vertical migrations of several crustacean species (see Section C).
The present study was undertaken to investigate the possible contribution of endogenous rhythms to the vertical migration of the ocean shrimp, *Pandalus jordani* Rathbun. The background for this study will be presented in four parts: A) Laboratory and field investigations of vertical migration; B) Evidence for the importance of endogenous rhythms to vertical migration; C) Evidence for the vertical migration of *Pandalus jordani* and *Pandalus borealis*; and D) Endogenous rhythms in shrimp.

**Laboratory and Field Investigations of Vertical Migration**

Although no laboratory experiments on vertical migration have been done with shrimp, the techniques and results are relevant to the present study. The laboratory approach allows the experimenter to manipulate such factors as light and temperature, and observe how animals respond. One drawback is the artificiality of the laboratory environment. It is impossible to duplicate natural habitats, and some unnatural mortality and aberrant behavior are inevitable. Of particular importance to vertical migration studies are the spatial restrictions which a small experimental tank imposes. The collection procedure is another weakness. Animals are generally collected in a net, and the stress involved may have subtle and long-lasting physiological and behavioral consequences. Nevertheless, laboratory
studies of vertical migration can provide useful information if the results are interpreted in light of these shortcomings.

Most of the studies have investigated the role of environmental factors in vertical migration, particularly light. Bainbridge (1961) expresses the prevalent view when he states, "The primary dependence of diurnal migrations upon changes in light intensity is beyond doubt" (p. 440). Similarly, Cushing (1951) believes that, "... the vertical migration of planktonic Crustacea is mediated by the change in light penetration throughout the day" (p. 187). Three important studies that demonstrate the emphasis on light effects are: Hardy and Bainbridge (1954), Harris and Wolfe (1955), and Ringelberg (1961).

Hardy and Bainbridge avoided the problem of vertical spatial restrictions with their "plankton wheel", a cylindrical observation chamber which could be rotated to simulate an infinite water column. They observed the swimming behavior and speeds of copepods in their "plankton wheel" and verified that these animals are capable of the speed and duration required for extensive vertical migrations. They studied the effect of light intensity on copepod swimming behavior, but their results were inconsistent. Light reduction in the evening induced upward migration, for example, but during the day did not. They suggest an endogenous rhythm of light sensitivity to explain their results.

Harris and Wolfe designed their experiments to test the
importance of gradients of light intensity to vertical migration. They simulated a natural light range in a tank of water with an India ink suspension. *Daphnia magna* in this tank could be made to undergo a complete cycle of "vertical migration" by varying the intensity of the overhead light source. They assign to light a "primary role" in the vertical migration of *Daphnia magna*, and describe the entire cycle of migration in terms of light effects.

Ringelberg proposed that the stimulus for diel migrations is the relative change in illumination. His field observations and the results of experiments with *Daphnia magna* seem to support this idea.

Many field studies emphasize the importance of light to vertical migration. McNaught (1966) concluded that the vertical migration of planktonic cladocerans in Lake Michigan depends on the rate of change of light intensity and the spectral composition of light at depth. Boden and Kampa (1967) followed a vertically migrating community of animals with an echo-sounder, and simultaneously followed the ascent and descent of a constant light level in the sea. They concluded that "... the diurnal vertical migrations of the animals comprising this sonic-scattering layer are controlled primarily by changes in the photoenvironment". Other studies which support this conclusion are those of: Clarke and Backus (1956), Kampa (1970), and Kampa and Boden (1954).
Evidence for the Importance of Endogenous Rhythms to Vertical Migration

It is clear that light can influence swimming direction, swimming intensity, and the vertical position of planktonic crustaceans. There are reasons to believe, however, that light is not the only operational factor in vertical migration. Harris (1963) points out that some vertical migrations include a "midnight sinking" and a "dawn rise" at time of negligible light change. Since changes in the photoenvironment apparently do not provide the stimulus for these phases of the migration cycle, he suggests that an endogenous rhythm of activity is responsible.

There have been three laboratory studies of the involvement of endogenous rhythms in vertical migration. Esterly (1917) kept three species of copepods (Acartia tonsa, A. clausi, and Calanus finmarchicus) in constant darkness. He found that, on the average, there were more copepods in the top portion of the cylinder in the evening. Most of the experimental animals died after one day, but the survivors showed an evening ascent on the second night also. Esterly postulated that these animals possess internal timing in their swimming behavior.

Harris (1963) investigated the role of internal timing in the vertical migration of Calanus finmarchicus and Daphnia magna. The animals were kept in a small tank in constant darkness and their distribution was recorded photographically every hour. Harris'
results provide evidence for a persistent cycle of locomotor activity in both organisms. He suggests that light intensity governs the depth of the animals and overshadows the "intrinsic rhythm of activity" except during hours of darkness. At this time the rhythm manifests itself and provides the stimulus for the "dawn rise" well before true dawn.

Enright and Hamner (1967) questioned the results of Esterly and of Harris. They felt that the behavior of the experimental animals in these studies may have been affected by: 1) the stress of net capture, 2) the small size of the experimental tanks, and 3) the light used to record distribution. In their study of the importance of internal rhythmicity to the vertical migration of nearshore zooplankton they used techniques which they felt eliminated or minimized these problems. By obtaining zooplankton from an unfiltered seawater system they avoided the problem of net capture. A large (11×5×2.5 meter deep) tank minimized spatial restrictions and decreased mortality. Nets were drawn across the surface of the tank at regular intervals to record distribution. In this way, the problem of using light to record distributions was avoided. Enright and Hamner's techniques are not without drawbacks, however. Animals obtained from a seawater system must be drawn through a pump. This process may be almost as stressful as net capture. Also, the nets used to record distribution may disturb behavior, and they only provided
information on surface distribution. Enright and Hamner performed two experiments. Zooplankton in the experimental tank were exposed to a light-dark cycle for several days, then to constant dim light. Surface abundance was sampled during part of the former period, and all of the latter. Enright and Hamner found that,

. . . internal rhythms, synchronized by a light-dark cycle, are of dominant importance for the vertical migration of several species of crustaceans. For certain other organisms, the vertical migrations observed in the experiments can be accounted for as direct responses to light intensity only. Perform-ances intermediate between these extremes were also observed, as well as behavior based on biological timing mechanisms that are not rhythms in the usual sense.

Evidence for the Vertical Migration of
Pandalus jordani and Pandalus borealis

The diel vertical migration of these pandalid shrimp is well documented by field studies. The evidence is in the form of mid-water and bottom trawl, and surface-to-bottom pot data. Larger night than day midwater catches and larger day than night bottom catches strongly support the idea of nocturnal ascent and diurnal descent to the bottom. Barr (1970) studied the distribution of Pandalus borealis in Alaskan waters and found vertical migrations of up to 100 meters. Haynes and Wigley (1969) found that Pandalus borealis in the Gulf of Maine perform similar migrations.

In a study of the distribution and biology of Pandalus jordani
off the Washington coast, Tegelberg and Smith (1957) caught few shrimp on the bottom in areas with good daytime catches. These results seem to reflect a nocturnal migration off the bottom. Robinson (in press), working off the coast of Oregon, corroborated the findings of Tegelberg and Smith. Pearcy (1972) found that daytime bottom trawl catches of ocean shrimp always exceeded nighttime catches at the same station, and that midwater trawls only caught shrimp at night. These results and similar findings by Alverson, McNeely, and Johnson (1960), and Schaefers and Powell (1958) provide good evidence for nocturnal migrations off the bottom.

**Endogenous Rhythms in Shrimp**

No studies like those described in Section B have been done with shrimp. The activity rhythms of the shrimp *Penaeus duorarum* have been investigated, however. This penaeid shrimp is nocturnally active on the substrate surface, and remains burrowed during the day. Fuss and Ogren (1966) and Wickham (1967) found that the shrimp show a persistent rhythm of nocturnal activity when kept in constant light conditions. They attribute this to an endogenous component of the activity patterns.
The Purpose of the Present Study

The vertical migration of the ocean shrimp *Pandalus jordani* in the field is well documented (Alverson et al., 1960; Robinson, in press; Pearcy, 1972; Schaefers and Fowell, 1958; and Tegelberg and Smith, 1957. No previous workers have investigated the possible contribution of endogenous activity rhythms to this migration. The present study attempts to do this by investigating in the laboratory any endogeneity in the activity patterns of ocean shrimp, and determining whether the activity patterns can be synchronized to a light-dark cycle. Rhythms of activity that will synchronize with cycles of external conditions, and persist with the same periodicity when the external cycles are absent, are endogenous. The process of synchronization is known as "entrainment" (Marler and Hamilton, 1967).
MATERIALS AND METHODS

Collection and Maintenance of Experimental Animals

_Pandalus jordani_ were collected from the R/V Cayuse with otter and beam trawls 23 miles off the Oregon coast at depths of about 200 meters. Tows were short to minimize injury to the animals. The beam trawl injured the animals less than the otter trawl. The liveliest shrimp from each catch were placed in styrofoam containers filled with sea water. Ice packs in the containers maintained a low temperature. Shrimp were sensitive to temperature changes, and extreme fluctuations at times caused heavy mortality. The shrimp were maintained in a constant temperature room in eight-gallon, seasoned, plastic holding tanks. A pump and filter system continuously recirculated the water in each tank through activated charcoal. The water and charcoal were changed every two weeks. "Instant Ocean Marine Filter Mix" on the bottom of the holding tanks helped to buffer the water and maintain a high pH. The temperature of the room was kept at 8.0±0.1 °C to approximate that of the habitat of ocean shrimp. There were no cyclical temperature changes.

Pearcy (1970) found the euphausiids _Euphausia pacifica_ and _Thysanoessa spinifera_ to be the most common identifiable food in the stomachs of ocean shrimp caught in midwater trawls. Shrimp in the laboratory readily ate these euphausiids, which were kept frozen.
Experiments were conducted in a 10"×2'×3' deep clear plexiglass tank which held ~ 35 gallons of sea water. The substrate was "Marine Filter Mix" gravel. Sub-gravel filters made it possible to clean the water between experiments.

Experimental Techniques

It is difficult to follow the rhythmic activities of marine animals in the laboratory without disturbing their behavior. Photographing distribution at regular intervals is the simplest method, but even very short pulses of light may disturb endogenous rhythms (Bünning, 1967; Marler and Hamilton, 1967). Infrared photography is ideal if the animals are insensitive to infrared light, and there is reason to believe that this is true of ocean shrimp. There is an approximate correspondence between the absorption bands of the pigments in the eyes of deep-living crustaceans and the predominant wavelengths of light that reach their habitat (Munz, 1965). Since infrared light is attenuated very rapidly in sea water (Neumann and Pierson, 1966) it is reasonable that ocean shrimp would be insensitive to it. Preliminary experiments were performed to verify this. Shrimp were kept in dim red light for two weeks and then exposed to flashed white and infrared light of similar duration and intensity. Observations of the responses were made visually. Flashes of infrared light caused
no detectable response, while shrimp exposed to flashes of white light began swimming immediately.

Klima (1968) compared the effects of flashed white and infrared light on the nocturnal behavior of the shrimp *Penaeus duorarum*. He found that periodic ten-second flashes of infrared light suppressed activity more than three-second flashes of white light. His results are inconclusive, however, because he used a filter (Corning 2-64) which passes visible light as part of his infrared light source. The infrared light source in the present study consisted of a Mecablitz 202 electronic flash unit in a lighttight box with a circular opening in one end. Over the opening were six Wratten No. 87 visible-absorbing gelatinous filters in tandem. Each filter transmits less than 0.1% in the visible range, so that six filters in tandem reduce the transmitted visible light to a negligible level. The flash duration was 1/500 second.

The camera used was a motor-driven Nikon F250. An enlarged back on this camera held enough high-speed infrared film for 250 exposures. This film was chosen because its maximum sensitivity range corresponded with the maximum transmission band of the Wratten No. 87 filters.

The experimental arrangement is depicted in Figure 1. The bounce-lighting arrangement shown provided the best pictures of the experimental tank. A movie projection screen set at a 45° angle
to the tank diffused infrared light from the light source and reflected it through the tank to the camera. The resulting photographs showed the shrimp in silhouette, as in Figure 2.

A timer connected to the motor drive of the camera triggered the shutter and advanced the film every 32 minutes. The distribution of shrimp in the experimental tank could be recorded in this way until the film supply was exhausted, about five days.

The light cycle apparatus used in the entrainment experiments had two parts, a light source and a control unit. The light source consisted of a 40 Watt incandescent bulb in a lighttight box with a filter-covered opening at one end. The filter was a Corning No. 4-67 with maximum transmission at 480-490 m\(\mu\) (blue-green). The light box stood two feet above the top of the experimental tank on ring stands with the opening down. A translucent sheet of plexiglass covering the top of the experimental tank diffused the light entering the water. Blue-green light was used to simulate the light conditions of the ocean shrimp's habitat. Also, Gordon and Brown (1971) provide evidence that the blue-green is the most effective region of the spectrum for entraining most invertebrates. Since high-speed infrared film is insensitive to blue-green light, the light regime did not interfere with the infrared photography. The incandescent bulb in the light box was regulated by a control unit in the adjacent room. This unit, depicted schematically in Figure 3, consisted basically
of a time switch and a rheostat. The cams on the time switch were set so that the light cycle included 1/2 hour "sunrises" and "sunsets". Artificial twilights may enhance entrainment in laboratory experiments (Marler and Hamilton, 1967). Times of "sunrise" and "sunset" could be set on the time switch.

There was little data on irradiance at depth off the coast of Oregon to use in determining a reasonable light intensity for the light regime. A photometer equipped with a Corning No. 4-67 filter was used to measure irradiance with depth 55 miles off Newport, Oregon, and 15 miles off Depoe Bay, Oregon (Pearcy, personal communication). The former cast was made at 3:00 PM on April 24, 1971, with clear skies. Irradiance at 150 meters was 0.13 µw/cm², and at 200 meters was 0.015 µw/cm². The latter cast was made at 3:00 PM on April 27, 1971, with bright, overcast skies. Irradiance at 150 meters was 0.057 µw/cm², and no measurements were made at 200 meters. Irradiance at the top of the experimental tank measured about 0.1 µw/cm² with the rheostat completely on ("day" phase). A cosine receptor and a photometer equipped with a Corning No. 4-67 filter were used to make this measurement.
Experiment 1

Purpose

The purpose of the first experiment was to determine whether any endogenous rhythms of activity present in the field persist in constant light and temperature conditions in the laboratory.

Experimental Design

The experimental tank was partitioned vertically with clear plexiglass sheets, and one shrimp occupied each compartment. The distribution of the shrimp in the tank was observed visually every hour for the first 24 hours, followed by 48 hours of infrared photography. Two 7 Watt red bulbs on the ceiling provided enough light for visual observations. These lights were on continuously for this and all subsequent experiments. Three of these experiments were performed, with four shrimp in two experiments and five shrimp in one experiment. A different group of animals was used in each repetition. The animals were not fed during this or subsequent experiments.

Experimental Animals

The capture dates of the three groups of animals were April 8, April 21, and May 23, 1972. The animals were held in constant dim
red light for 23, 6, and 3 days, respectively, before being observed. The shrimp were 1 year old males and 1 1/2 year old females with carapace lengths ranging from 14 to 21 mm.

Experiment 2

Purpose

The purpose of this experiment was like that of Experiment 1.

Experimental Design

The experimental tank was not partitioned, and 15 shrimp were used. The activity under constant temperature and light conditions was recorded photographically for three days. Three repetitions of this experiment were performed.

Experimental Animals

The capture dates of the three groups of shrimp were March 4, March 16, and June 29, 1972. The shrimp were held in constant dim red light for 1, 10, and 4 days, respectively, prior to observation. The ages, sexes, and the range of carapace lengths were like those of the shrimp in Experiment 1.

Data Analysis

The vertical swimming data for each group of shrimp was
arranged in a 2x2 contingency table, and \( \chi^2 \) values were calculated for each table. The cross-classifications of the contingency tables were time of day and position of a shrimp in the experimental tank. The subdivisions of "time of day" were "night" and "day", and of "position in the tank" were "up" and "down", that is, in the water column or on the bottom. The table, then, looked like this:

<table>
<thead>
<tr>
<th></th>
<th>Up</th>
<th>Down</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Night</td>
<td>A</td>
<td>B</td>
<td>A+B</td>
</tr>
<tr>
<td>Day</td>
<td>C</td>
<td>D</td>
<td>C+D</td>
</tr>
<tr>
<td>Totals</td>
<td>A+C</td>
<td>B+D</td>
<td>A+B+C+D</td>
</tr>
</tbody>
</table>

with numbers of shrimp over the three-day observation period filling the appropriate cells. In order to set up the tables, the constant conditions of the experiment had to be broken down into "night" and "day". This was done in two ways. In one, the times of day and night on the capture date of the animals being observed were used; in the other, times of "day" and "night" were the same as in the entrainment schedule of experiment 3. The activity data of each group, then, were arranged in two tables, and \( \chi^2 \) values were calculated for each. The \( \chi^2 \) values were calculated with a continuity correction according to Tate and Clelland (1957).

A runs test was performed to determine if the vertical swimming was distributed randomly throughout the time series. The two
different events for this test were: 1) number of shrimp up in a frame larger than the median, and 2) number of shrimp up smaller than or equal to the median, for the swimming activity of the first group of shrimp; and 1) presence of at least one shrimp in the water column in a frame, and 2) absence of above, for the swimming data of the second and third groups. Since the numbers of the two different events were greater than 20, values of $z$ were calculated from the formula in Tate and Clelland (1957).

Experiment 3

Purpose

The purpose of this experiment was to investigate the possibility of entraining ocean shrimp to a 24-hour light-dark cycle, and to determine if a rhythm of activity with the periodicity of the entraining cycle will persist in the absence of this cycle.

Experimental Design

Shrimp in a partitioned tank (one animal in each compartment) were exposed to a 24-hour light cycle for four days, with "sunset" from 8:30 to 9:00 PM, and "sunrise" from 6:30 to 7:00 AM. The light cycle was then discontinued, and the activity of the shrimp in constant conditions was recorded photographically for four days. Seven of
these experiments were performed with four shrimp, and two were performed with 15 shrimp in a non-partitioned tank. One of the latter was discontinued when several of the animals died during the post-entrainment observation period.

**Experimental Animals**

The following are the capture dates in 1972 and number of days in captivity prior to entrainment for the groups of shrimp in Experiment 3: group 1, March 16, 41 days; group 2, March 16, 50 days; group 3, May 23, 27 days; group 4, experiment discontinued; group 5, August 19, 13 days; group 6, August 19, 26 days; group 7, October 23, 4 days; group 8, October 23, 15 days; and group 9, October 23, 47 days. All of these animals were females with carapace lengths ranging from 17 to 22 mm.

**Data Analysis**

The data were arranged in contingency tables and $\chi^2$ values were computed for each table. The contingency tables of all but the group 5 data differed from those of Experiment 2 in that each table represented the activity of only one shrimp. The numbers in the cells of each table were the numbers of times that particular shrimp had been "up" or "down" during the "day" and "night" for the four days of observation. The activity data for each shrimp were arranged in
two contingency tables. The times of "day" and "night" in one were taken as those of the entrainment schedule. Activity during the two 32-minute "twilight" periods was not included. In the second arrangement, "sunset" and the three preceding 32-minute periods were added to the "night".
RESULTS

Experiment 1

The shrimp in the first experiment displayed very little vertical swimming. None of the shrimp in the first group (held 23 days prior to observation) swam off the bottom during the first 24-hour period (visual observation). One shrimp was recorded in the water column during the following three days of photographic observation (Figure 4A).

The second group of animals (held three days before observation) was considerably more active during the first 24-hour period. Most of this activity occurred from late "morning" to middle "afternoon". The film record of the following three days did not turn out.

The third group (held 6 days before observation) was not recorded off the bottom during the four days of observation.

The bottom activity of the shrimp in this experiment was monitored by noting whether location on the substrate changed from frame to frame. Bottom activity did not appear to depend on time of day. Over all three groups of shrimp, animals changed bottom position in 78% of the frames during "daytime" hours, and in 81% of the frames during "nighttime" hours.

These results suggest that the swimming behavior of the shrimp declined rapidly in constant conditions. The only group of animals
that swam actively had been in captivity only three days prior to observation. The activity of this group seemed to be 12 hours out of phase with field behavior (swimming during the "day" rather than the "night"). These animals were exposed to bright lights during the evening hours of the day they were caught, and this may account for the phase shift.

**Experiment 2**

The shrimp in Experiment 2 showed more vertical swimming activity than those in Experiment 1. The swimming activity of these three groups of animals is depicted in Figure 5. Between groups, there is less activity with longer captivity times. Factors other than captivity time probably influence differences in amounts of activity within experiments. The shrimp of group 1 were more active on the third day of observation than the shrimp of group 2 were on the first day of observation, although captivity times were the same. These other factors may include physiological condition and season of capture. It was not possible to follow the bottom activity of individual shrimp with no partitions in the experimental tank.

The vertical swimming for all three groups appeared to occur with equal intensity throughout the 24-hour periods; that is, diel rhythms of vertical swimming were not apparent. Since none of the \( \chi^2 \) values for Experiment 2 (Table 1) is significant at the 5% level,
Table 1. \( \chi^2 \) values for Experiments 2 and 3. "Arrangements" 1 and 2 are explained in the text. Experiment 2 \( \chi^2 \) values are for groups of shrimp, while Experiment 3 values are for individual shrimp (except Group 5). The asterisks denote significant \( \chi^2 \) values (P < .05) and indicate shrimp which swam significantly more during "night" than "day" hours.

### Experiment 2

<table>
<thead>
<tr>
<th>Arrangement 1</th>
<th>Arrangement 2</th>
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<tbody>
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</tr>
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</tr>
<tr>
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<td>0.0022</td>
</tr>
<tr>
<td>1.5167</td>
<td>0.9808</td>
</tr>
<tr>
<td>Group 1</td>
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<tr>
<td>Group 3</td>
<td>Total</td>
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### Experiment 3

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there is no reason to discard the null hypothesis of no association between time of day and vertical swimming (position in the tank). Therefore, we can conclude that the shrimp possessed no persistent rhythms of vertical swimming in phase with either field conditions or with the entrainment schedule.

The calculated values of $z$ for the three groups of shrimp were -1.946, -1.938, and -4.920. These large negative values of $z$ indicate a low probability of obtaining such few runs by chance (a probability of 0.025 corresponds to $z = 1.960$, and 0.005 to $z = 2.576$), hence, a lack of randomness. This may reflect social interstimulation or remnants of a field activity rhythm disturbed by capture and constant conditions.

**Experiment 3**

The post-entrainment activity of the shrimp in this experiment is depicted in Figures 6-13. This is the "free-running" activity or activity in the absence of rhythmic cues. Figures 6-13 show considerable variability in the extent and time of vertical swimming both between and within experiments. Usually one or two of the animals in a group contributed most to the vertical swimming activity. Bottom activity was not dependent on time of day. Over all groups of shrimp in this experiment, animals changed bottom position in 54% of the frames during "daytime" hours, and in 55% of the frames during
"nighttime" hours. Some shrimp appeared to swim off the bottom on some days, but not others. This may be an artifact of the observation technique, since we have only one observation every 32 minutes. Swimming activity in general did not decrease from day 1 to day 4 of the observation period. The total number of times shrimp in all groups were recorded off the bottom during the first 24 hours of observation was 68, during the second 24 hours was 75, 84 during the third, and 85 during the fifth. The average number of times a shrimp was recorded off the bottom during each of the four days was 2.8, 3.1, 3.5, and 3.5.

The total number of times each group with four shrimp was recorded off the bottom during the four days following entrainment was: group 1, 47 times; group 2, 52 times; group 3, 29 times; group 6, 61 times; group 7, 29 times; group 8, 44 times; group 9, 50 times. For groups with the same capture date (groups 1 and 2, and groups 7, 8, and 9), there appears to be more post-entrainment swimming activity with longer captivity times. One group with only 29 times off the bottom was captured in May, during which month Pearcy (1970) caught no ocean shrimp in midwater trawls. Seasonality is probable in the vertical movements of ocean shrimp (Alver son et al., 1960; Pearcy, 1970), but more experiments are necessary to clarify the relationship between season, length of captivity, and vertical swimming activity.
The calculated $\chi^2$ values for data of Experiment 3 are given in Table 1. In four instances "daytime" swimming exceeded "nighttime" swimming, but for none of these were the $\chi^2$ values significant at the 5% level (greater than 3.84). The total value and 8 of the 24 individual $\chi^2$ values computed for the first arrangement (left column in Table 1) were significant at the 5% level. Eleven individual values were significant at the 5% level for the second arrangement, and the total value was larger than that for the first arrangement. There were significant and non-significant $\chi^2$ values for each extreme of season of capture, length of captivity, and reproductive state.

The significant $\chi^2$ values provide evidence for entrainment. Entrained shrimp swim significantly more often during the "night" than during the "day" under constant conditions, demonstrating endogenous rhythms of swimming that can be entrained to a 24-hour light-dark cycle. In view of the stress of capture and maintenance in the laboratory, it is not surprising that not all shrimp entrained significantly. Many of the shrimp began swimming about two hours before "sunset". This explains the increased significance of the $\chi^2$ values when this activity is included in the "night" (the second set of contingency tables), and implies an "anticipation" of "sunset". Again, more work is needed to determine the relationship between entrainability and season of capture, length of captivity, and reproductive state.
DISCUSSION

Ocean shrimp brought into constant conditions in the laboratory do not show any rhythmical patterns of swimming activity. Any rhythms the shrimp possess in the field seem to be desynchronized by the process of capture. Also, swimming activity declines with captivity time, although the activity level differences between groups may be partly due to other factors such as physiological state and season of capture. After exposure to a 24-hour light-dark cycle, some shrimp continue to swim more at "night" than during the day in constant conditions. On the other hand, shrimp were as active on the bottom during the "day" as the "night" in both the entrainment and non-entrainment experiments. These results indicate that ocean shrimp possess biological clocks that mediate vertical swimming activity, but not bottom activity. Without repeating these experiments in a tank which simulates the actual vertical extent of the migration of ocean shrimp, we cannot conclusively state that the rhythmicity of the vertical migration of these shrimp is endogenous. The evidence that shrimp in the field perform extensive migrations off the bottom at night (Tegelberg and Smith, 1957; Robinson, in press; Pearcy, 1970, 1972), and that some shrimp entrained to a cycle of blue-green light in the laboratory swim significantly more often at "night" than during the "day" in the absence of rhythmical light cues,
make it reasonable to hypothesize that this is the case. It is likely that both internal rhythmicity and concurrent light conditions are important to the normal vertical migration of ocean shrimp. The importance of rhythmical light cues to the activity of shrimp in the laboratory has been shown in the present study.

The value of endogenous rhythmicity is that it allows an animal to perform its activities at an optimal time in terms of energy expenditure, efficiency, and risk involved (Bünning, 1967). Pearcy (1970) reports that ocean shrimp feed on euphausiids and copepods which also perform nocturnal vertical migrations. Presumably, the shrimp would be less vulnerable to predation at night, and the endogenous rhythms would function in concentrating vertical swimming (and feeding) behavior within this optimal time. On the other hand, there is probably no optimal time for feeding on the bottom, so bottom activity would not be under the control of the clock.

The most widely evoked evolutionary explanation of the existence of internal timing is readiness for onset of activity (Bünning, 1967). This may explain why the shrimp in post-entrainment, constant conditions began swimming off the bottom several hours before "sunset".

Because of their large size, ocean shrimp are not ideal experimental animals for vertical migration studies in the laboratory. Previous studies have used small animals whose movement could be
followed in a number of ways. Photography is the only feasible way of recording the activity of animals in a tank as large as the one used in this study, but interval photography has the disadvantage of recording only a small portion of the total activity. Despite the difficulties, ocean shrimp are interesting and economically important, and merit further study in the laboratory. A continuous record of activity would be invaluable in any such studies. A movie camera might be considered in this regard. Longer time series of observations would also be helpful in such studies, as data could be analyzed by time series analysis.

To verify the conclusions of the present study, entrainment experiments should be carried out with a reversed photoperiod (light during night hours, dark during day hours). If shrimp can entrain to this unnatural light-dark cycle and maintain its periodicity in the absence of light cues, then endogenous activity rhythms in shrimp are certain.

Many aspects of the vertical migration of pink shrimp could be investigated in the laboratory. An example is the apparent non-daily nature of this migration. Several of the shrimp which entrained in the present study apparently did not leave the bottom every day. The vertical migration of shrimp in the field may also be irregular. Pearcy (1970) caught ocean shrimp on the bottom in night trawls. An analysis of the stomach contents of shrimp caught on the bottom
and in the water column also suggests that shrimp may not migrate vertically every day. The stomachs of animals caught in midwater contained mainly pelagic animals, while the stomachs of animals caught on the bottom contained benthic food and little or no pelagic food. A continuous post-entrainment activity record of shrimp in the laboratory would be helpful in determining whether some shrimp perform non-daily migrations. Non-daily migrations do not preclude the importance of internal rhythms, but imply the involvement of other factors. It may be, for example, that the biological clock alerts the shrimp each day to the optimal time for vertical migration, but the stimulus of hunger must be present to initiate an actual migration. This idea could be investigated by comparing the post-entrainment activity of hungry and satiated shrimp. Zusser (1959) suggests that evening light extinction is a signal to migrate for hungry organisms, but that satiation inhibits the response to this signal.

There is reason to believe that vertical migration varies with season and sex, but any such effects in the present study were masked by individual variability. Pearcy (1970) caught shrimp in midwater at night in all months except May and June. The greater tendency of male shrimp to perform vertical migrations is suggested by the consistently smaller average sizes of shrimp caught on the bottom during the day (Pearcy, 1972). Further entrainment studies in the laboratory could shed light on the effect of sex and season on vertical
migration. To detect differences due to these factors, it would be necessary to have continuous recording of activity and longer time series of observations than in the present study.

The effect of some external factors on the endogenous rhythm of shrimp could be investigated further in the laboratory. Robinson (1971) reports that daytime shrimp fishing is poorer in turbid water than in clear water. The light conditions in this case may be affecting the activity of the shrimp directly (exogenous control), or affecting the activity rhythms. This could be investigated by determining how shrimp activity and entrainment vary with wavelength and intensity of light. Although endogenous rhythms are largely unaffected by temperature changes (Bünning, 1967; Marler and Hamilton, 1967), the manifestations of the rhythms may not be. Thermoclines, for example, are thought to restrict the vertical migrations of ocean shrimp (Robinson, in press). If an artificial thermocline could be devised, this question could be studied in the laboratory. It was suggested previously that the migration of ocean shrimp may be closely associated with that of euphausiids and copepods. Food may be an external stimulus that acts with the internal rhythmicity in determining activity patterns. It would be interesting to determine how the activity of entrained shrimp would vary in the presence of benthic food organisms or live pelagic food animals.
CONCLUSIONS

1. The vertical swimming of the ocean shrimp, *Pandalus jordani* appears to decline rapidly in the laboratory in constant light and temperature conditions. The observed differences between groups, however, may be partly due to other factors such as sex and season of capture.

2. No persistent rhythms of activity in phase with field conditions are shown by shrimp in constant conditions. It is likely that any field rhythms are desynchronized by the capture process.

3. Bottom activity does not decline, and is fairly continuous throughout the day.

4. The vertical swimming activity of ocean shrimp can be entrained to a light-dark cycle of blue-green light. Rhythmic activity persists in the absence of the entraining light cycle.

5. No clear association was found between amount of activity or entrainability and season of capture or sex. More experiments are needed in this area.

6. Ocean shrimp possess a biological clock which can synchronize with rhythmical light cues, and which is important to vertical swimming activity. It is suggested that the rhythmicity of the vertical migration of ocean shrimp is endogenous, with internal and external factors interacting with the internal rhythm. The
rhythm may function in concentrating vertical swimming at night, when food species (euphausiids and copepods) perform vertical migrations, and when predation is presumably minimal.
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Robinson, J. G. In press. The vertical distribution and diel migration of pink shrimp (Pandalus jordani) off Oregon. Oregon Fish Commission


APPENDIX
Figure 1. The experimental arrangement.
Figure 2. An example of the infrared photographs recording shrimp activity (non-partitioned tank).
Figure 3. Schematic for light-cycler control unit.

Plexiglass disc mounted on shaft of rheostat

Electrical connection to light bulb in light box (from rheostat)

Reversible motor; shaft turned plexiglass disc

Capacitor

Time switch

Cams on the time switch operated these opening-closing switches at the appropriate times

Outlet
Figure 4. Experiment 1 A) Four-day activity of one shrimp in group 1 in constant conditions (held 23 days prior to observation). Column indicates swimming off the bottom at time of photograph. B) 24-hour activity (visual observation) of the four shrimp in group 2 (held 3 days before observation).
Figure 5. Experiment 2. 3-day activity of 15 shrimp in constant conditions. Shrimp were held 2, 4, and 10 days prior to observation, respectively. Columns indicate number of shrimp swimming at time of photograph.
Figure 6. Experiment 3. 4-day activity of group 1 shrimp (held 41 days prior to observation) in constant conditions following 4 days of exposure to a light-dark cycle. "Twilight" periods (according to the entrainment schedule) are indicated, and the dotted line represents onset of "day". Columns indicate times at which shrimp were recorded swimming in the water column.
Figure 7. Experiment 3. 4-day post-entrainment activity of group 2 shrimp (held 50 days prior to observation).
Figure 8. Experiment 3. 4-day post-entrainment activity of group 3 shrimp (held 27 days prior to observation).
Figure 9. Experiment 3. Post-entrainment activity of group 5 shrimp (held 13 days prior to observation) in constant conditions. Columns denote number of shrimp swimming at the indicated time.
Figure 10. Experiment 3. 4-day post-entrainment activity of group 6 shrimp (held 26 days prior to observation).
Figure 11. Experiment 3. 4-day post-entrainment activity of group 7 shrimp (held 4 days prior to observation).
Figure 12. Experiment 3. 4-day post-entrainment activity of group 8 shrimp (held 15 days prior to observation).
Figure 13. Experiment 3. 4-day post-entrainment activity of group 9 shrimp (held 47 days prior to observation).