DAILY "PREDAWN" PEAK OF LOCOMOTION IN FISH*

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The relation between daily rhythms in the locomotion of fish and corresponding daily changes in environmental illumination has been studied by numerous authors (Harder & Hempel, 1954; Jones, 1955, 1956; Kleerekoper, Taylor & Wilton, 1961; Spencer, 1939; Spoor, 1946; Steven, 1959; Wikgren, 1955). This paper deals with the "predawn peak" of locomotion found in bluegill and largemouth bass subjected to 24-hour periodicities of illumination and feeding. Experiments were undertaken seeking the relation between the co-ordination of the predawn peak and the periodicities of light and feeding.

Methods

Material and Treatments

Six adult bluegill (*Lepomis m. macrochirus*) and six adult largemouth bass (*Micropterus salmoides*) were confined by species in groups of 3 to separate light-tight chambers. The fish were caught several weeks before the experiments, and subjected in storage to an irregular daily schedule of light and feeding. In the chambers they were given alternating daily periods of bright light (400 lux at the water surface) and very dim light (not measured; light from a 6 v. panel lamp operated continuously at 4 v.), and they were fed at regular times. The daily cycle of bright-dim changes will be referred to as a light-"dark" cycle. Water temperature was held between 23·0 and 26·0°C. from day to day, and it increased gradually 2°C or less during the light period and decreased a corresponding amount in the "dark" period. The periodicities of illumination and feeding were altered in a sequence of experiments to determine their role in co-ordinating the predawn peak of locomotion. These treatments are summarized in Table I.

Feeding

The chambers were opened for 20 to 30 minutes at feeding time. Each fish ate from 1 to 4 earthworms daily. Following feeding the aquaria

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were cleaned and then replenished with fresh water. For feeding at the onset of the light period the chambers were in fact opened several minutes later. During this time the fish recovered from the sudden exposure to bright light ("light-shock reaction", Davis, 1962). It was found earlier that if the chambers were opened before the fish recovered they often became excited and would sometimes fail to eat.

Measurement of Locomotion

The locomotion detector was a network of rubber bands on a rectangular frame of aluminium wire which was suspended in the water by two rubber bands from a rigid support (Fig. 1). The detector fit closely to the sides and bottom of the tank so that a fish changing ends of the tank had to push the frame aside or pass through the network where it usually collided with one of the bands. Two silver wire contacts formed an electrical switch between the wire frame and the rigid support above the tank. The contact on the frame vibrated against the contact on the rigid support when the fish pushed the detector or disturbed the nearby water with forceful

Table I. Synopsis of the experimental alterations in the 24-hour Periodicities of Light and Feeding.

DAY	↑— FEEDING TIME ▬▬ – 'DARK' PERIOD ▭–LIGHT PERIOD
1-45	▬▬▬▬▬▬↑▭
46-56	▬▬▬▬▬↑▭
57-61	▭▬▬▬▬▬▬▭
62-67	▬▬▬▬▬↑▭
68-76	▬▬▬↑▭▭
77-89	▬▬▬↑▭▭
90-98	▬▬↑▭▭▭
99-107	▬▬▬↑▬▭
108-124	▬▬↑▭▬▭
125-128	▬▬↑▬▬▭
129-139	▬▬↑▭▬▭
140-142	▭▭▭▭
148-160	▭▬▬▬↑▭

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swimming movements. Two detectors were placed parallel to one another several centimetres apart across the middle of the experimental tank.
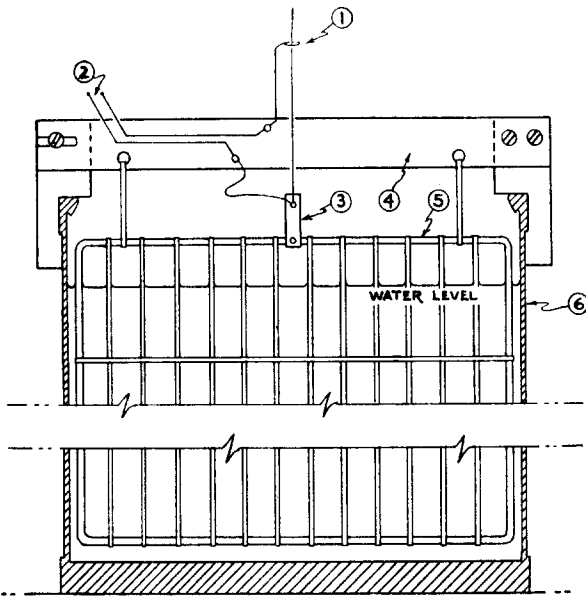

Fig. 1. Diagram of one locomotion detector suspended in the water by 2 rubber bands; 2 such detectors were placed close together across the middle of the experimental tank. (1) detector switch contacts, (2) wires connecting the switch in series with the second detector (not shown) to the thyratron circuit outside the chamber, (3) contact insulator, (4) rigid support, (5) aluminium wire frame with a taut network of rubber bands.

Movements of the detectors were transformed for recording with a thyratron circuit (Fig. 2). The switches of the 2 detectors in the tank were "normally closed" and connected in series, to work as a unit, in the thyratron cricuit. Disturbances of a detector switch which initiated the anode current and worked the thyratron relay (5000 ohm coil) were integrated over a 1-minute interval. At the beginning of an interval, the condenser (0·5 microfarad (mfd), non-electrolytic) in series with the 2 detector switches and the control electrode of the triode (RCA 2050) was discharged. The time required to load the condenser could be regulated by varying the voltage on the control electrode, and for any voltage the loading time decreased as the number of disturbances of the detector switches increased. The voltage was adjusted so that several movements of the detectors charged the condenser. Once the condenser became charged, the anode current was sustained hold-

ing closed the contacts of the thyratron relay.

An Esterline-Angus operation recorder registered from minute to minute the output of the individual thyratron circuits. The "normally open" contacts of the thyratron relay were connected with the recorder through a cam-actuated microswitch (Fig. 2). Once every 60 seconds the microswitch was closed for about 1 second. If the thyratron relay was closed at that time, the pen-magnet of the recorder was actuated deflecting the pen from the baseline on the chart. If the thyratron relay was open, the pen was not deflected and the record showed an absence of locomotion for that minute. An instant after the first microswitch was opened, a second microswitch, wired in parallel with the integrating condenser, was closed momentarily. This shortcircuiting of the condenser dissipated the charge accumulated during the previous minute initiating the next 1-minute interval of recording.

The registrations from the individual thyratrons, representing the swimming activity of the groups of fish in different chambers, were recorded at a chart speed of 1·9 cm./hour. Continuous records were kept for 160 days from January through June of 1960. The daily records were mounted one beneath the other on sheets of cardboard and photographically reduced for analysis.
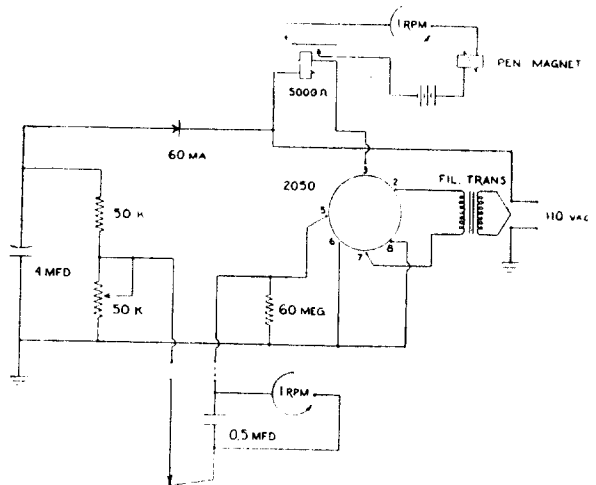

Fig. 2. Schematic diagram of the thyratron, integrator, and recording circuits (explained in the text).

Results
The Predawn Peak of Locomotion (Days 1 to 45)
The fish received daily 12 hours of light from

1200 (noon) to 2400 (midnight), and 12 hours of "darkness" from 2400 to 1200 and they were fed at the onset of the daily light period. The blue-gills and the bass (Fig. 4) were both most active in light but the bluegills also showed prolonged bursts of locomotion in "darkness" on some days (e.g. days 17 to 24, Fig. 3). The predawn peak of locomotion appeared between the tenth and twentieth days when the fish became active during the last 1 to 3 hours of the "dark" period. There was an indication of a predawn peak on days 3, 4, 5 and 8 for the bluegills (Fig. 3) and on days 3, 5 and 7 for the bass (Fig. 4) but a peak became consistent in the record between day 10 and 20. The bass and the bluegill showed similar predawn peaks and their responses in the following experiments were the same.

It is only assumed that the predawn peak reflected an increase in locomotion. The possibility that the fish were engaged in some other activity is temporarily rejected. Direct observations during the light period showed that the fish seldom rested in touch with a detector, when slight body movements might be registered, and they seldom poked or bit the detectors. Within the first week the fish showed social hierarchies and for some fish the detectors seemed to be territorial boundaries. A large part of the activity in light, if not in "darkness", was probably in the defence of boundaries.

Effect of Delaying the Onset of Light and Feeding (Days 46 to 56)

In response to a 6-hour delay in the onset of light and feeding time on day 46 the predawn peak delayed a small amount a day for 4 to 6 days until its original relation to the onset of light was restored (Fig. 5). The records for one group of bluegills were obscured by long intervals of nearly continuous locomotion in the "dark" period on some days before and after the light-"dark" cycle was altered (days 40 to 44 and 49 to 52, Fig. 6). There was ample evidence that the irregular locomotion in "darnkess" on those days was caused by fighting. At feeding time these fish were excited, fed furtively, and the skin of one fish was torn and bleeding. To reduce the fighting, the aggressor was separated from the other two fish by a sheet of perforated, transparent plexiglas inserted between the two locomotion detectors. The detectors continued to operate as a unit summing the locomotion of the 3 fish. Fig. 6 shows that this change was accompanied by a reduction of irregular locomotion in "darkness".

Effect of Delaying and Advancing the Onset of "Darkness" (Days 57 to 67)

The onset of the "dark" period was delayed 6 hours on day 57, and then it was advanced 6 hours on day 62 to restore the previous light schedule. These shifts in the onset of "darkness" were not followed by corresponding shifts in the start of the predawn peak which persisted in the last 1 to 2 hours of the "dark" period (Figs. 5 and 6).

Effect of Advancing the Onset of Light and Feeding Time (Days 68 to 76)

When the onset of the light period and feeding time were advanced 6 hours on day 68, the "dark" period ended 3 to 4 hours before the time at which the predawn peak would have been expected to begin had "darkness" continued. The fish were intermittently active in the light period and there was no peak corresponding to the predawn peak. The predawn peak reappeared in the "dark" period after 2 days for one group of bluegill (day 70), Fig. 6) and one of bass, and after 3 days for the other group of bluegill and of bass (day 71, Fig. 5).

Effect of Delaying and Advancing Feeding Time (Days 77 to 98)

Feeding time was delayed 6 hours to the middle of the light period beginning on day 77 and then advanced 6 hours to coincide again with the onset of light 13 days later. The predawn peak continued to occur in the last 1 to 2 hours of the "dark" period indicating that the timing of the peak was not affected by these changes (Figs. 7 and 8).

Effect of Altering the Phase of the Light-"Dark" Cycle (Days 99 to 124)

The phase of the light-"dark" cycle was advanced 6 hours, in relation to the unaltered periodicity of feeding, so that feeding occurred in the middle of the light period 9 days (days 99 to 107, Figs. 9 and 10). The predawn peak reappeared in the "dark" period in 2 days (day 101, Figs. 9 and 10); it was as though the onset of light and feeding time had been advanced together without shifting the onset of "darkness" (days 68 to 70), Fig. 6).

The phase of the light-"dark" cycle was delayed 6 hours for one group of bluegill and one of bass on day 108 restoring the previous relation between light and feeding. It took 5 days for the predawn peak to regain its position in the last 1 to 3 hours of the "dark" period (days 108 to 113, Fig. 9). To control the effect of
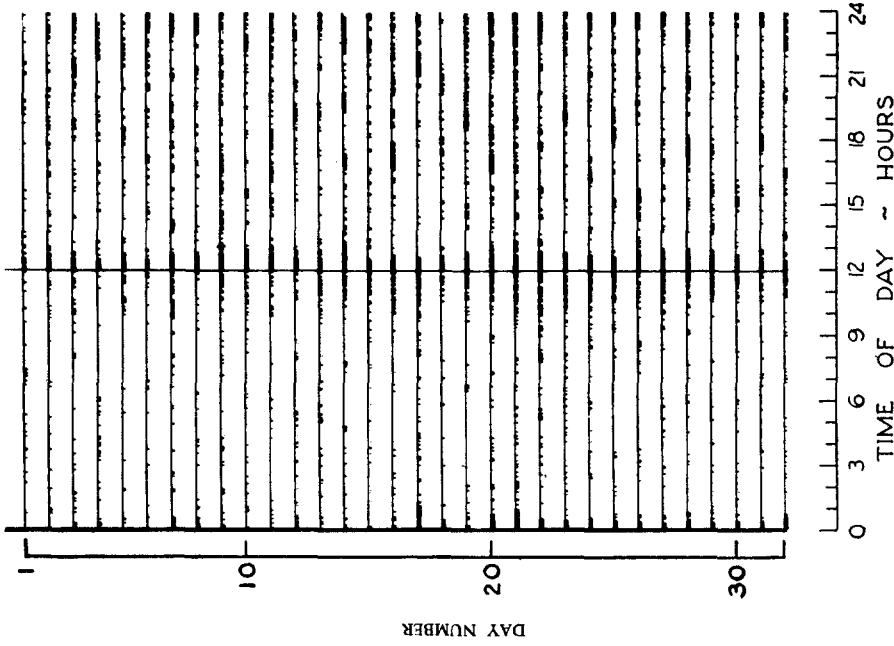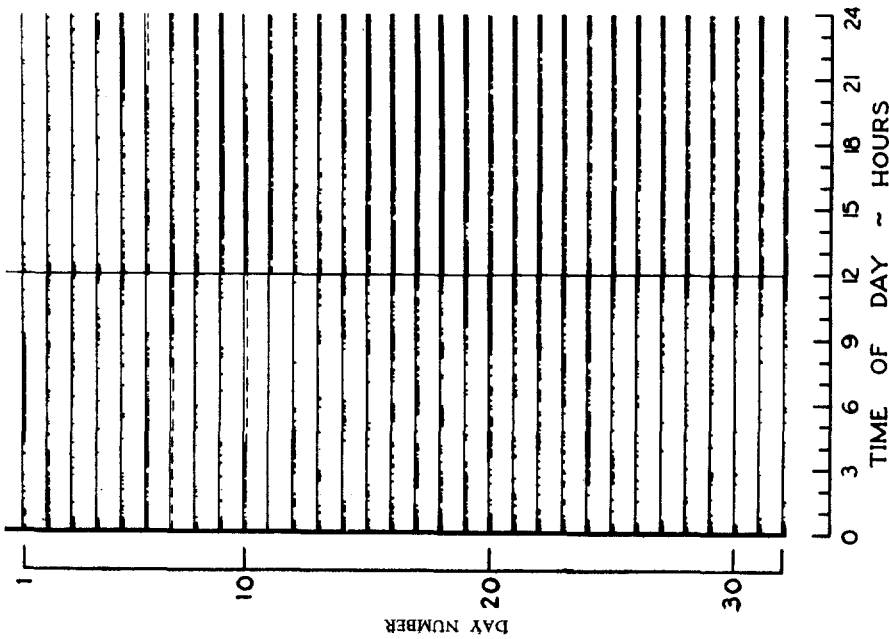
Fig. 4. Establishment of the predawn peak of locomotion of 3 bass (group 3) under a daily schedule of 12 hours of "darkness" (2400-1200) and 12 hours of light (1200-2400) with feeding at the onset of light. The downward deflections from the baseline of the daily record indicate locomotion. The dashed, horizontal lines beneath the baseline denote intervals of apparatus failure. The thick vertical line at 2400 marks the onset of "darkness", and the thin vertical line at 1200 the onset of light and feeding time.

Fig. 3. Establishment of the predawn peak of locomotion of 3 bluegill (group 1) under a daily schedule of 12 hours of "darkness" (2400-12-00) and 12 hours of light (1200-2400) with feeding at the onset of light. The downward deflections from the baseline of the daily record indicate locomotion. The thick vertical line at 2400 marks on onset of "darkness", and the thin vertical line at 1200 the onset of light and feeding time.
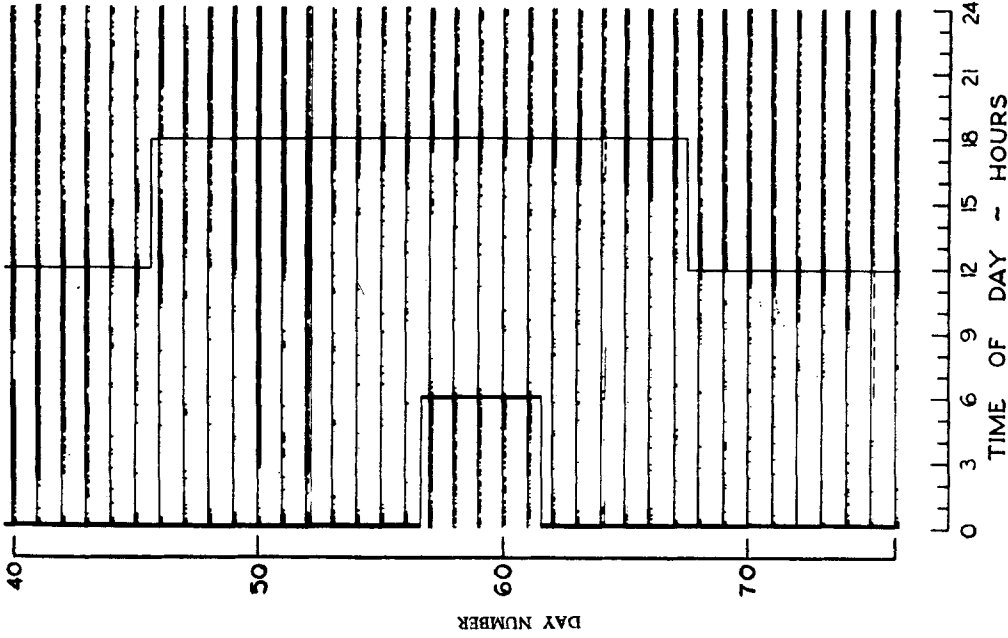
Fig. 6. Effect on the predawn peak of 3 bluegills (group 1) of changes in the 24-hour periodicities of illumination and feeding. Day 46: the onset of the light period and feeding time (thin vertical line) delayed 6 hours. Day 57: the onset of the "dark" period (thick vertical line) delayed 6 hours. Day 62: the onset of the "dark" period advanced 6 hours.
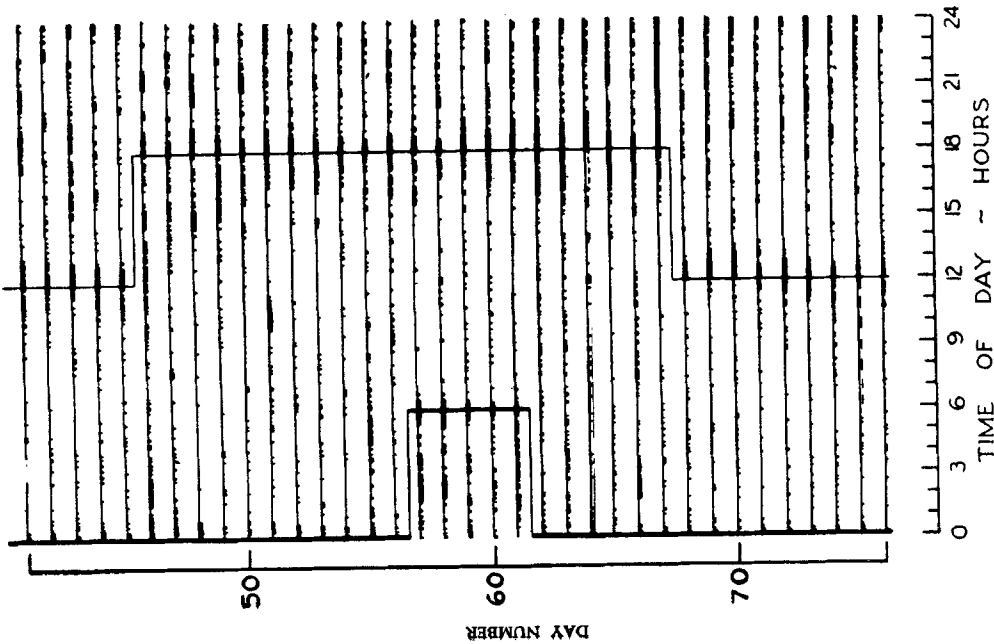

Fig. 5. Effect on the predawn peak of 3 bass (group 3) of changes in the 24-hour periodicities of illumination and feeding. Day 46: the onset of the light period and feeding time (thin vertical line) delayed 6 hours. Day 57: the onset of the "dark" period (thick vertical line) delayed 6 hours. Day 62: the onset of the "dark" period advanced 6 hours.
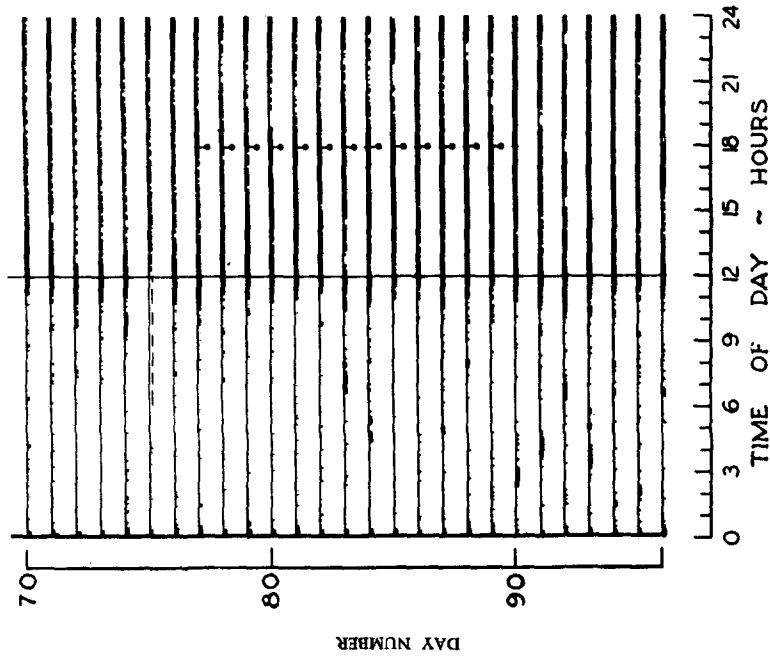
Fig. 8. Effect on the predawn peak of 3 bluegill (group 1) of changes in feeding time. Prior to day 77 feeding time coincided with the onset of the light period (thin vertical line). On day 77 feeding time was delayed 6 hours and the new schedule was kept for 13 days (see solid dots beneath the daily records). On day 90 feeding time was advanced 6 hours restoring the previous schedule.
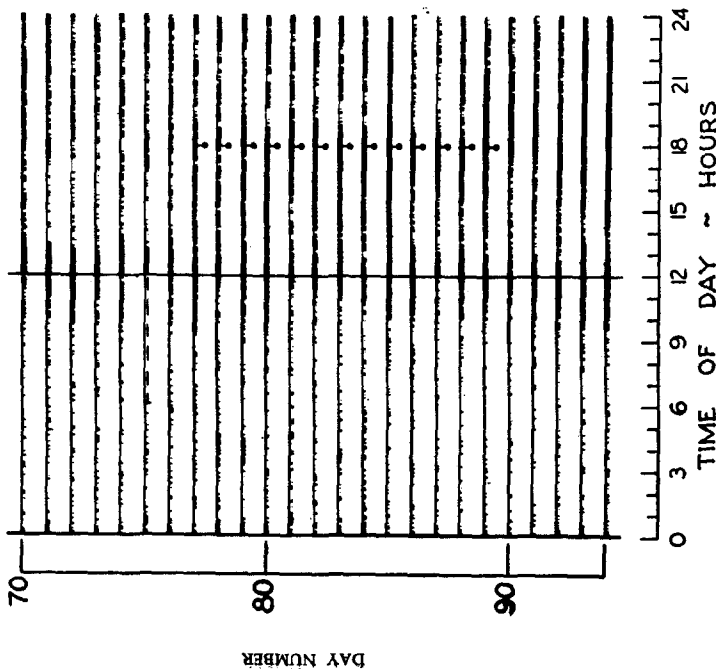

Fig. 7. Effect on the predawn peak of 3 bass (group 4) of changes in feeding time. Prior to day 77 feeding coincided with the onset of the light period (thin vertical line). On day 77 feeding time was delayed 6 hours and the new schedule was kept for 13 days (see solid dots beneath the daily records). On day 90 feeding time was advanced 6 hours restoring the previous schedule.
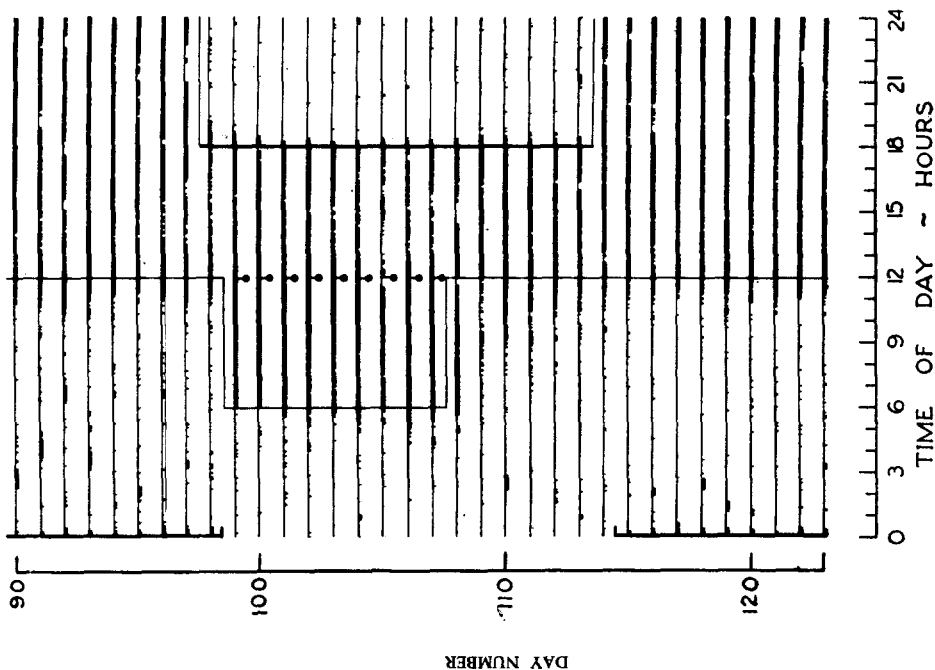
Fig. 10. Effect on the predawn peak of 3 bluegill (group 2) of changes in the phase of the 24-hour light-"dark" cycle. The schedule of 12 hours of light and 12 hours of "darkness," with feeding at the onset of light (thin vertical line) was altered on day 98 by advancing the phase of the light-"dark" cycle 6 hours, so that feeding occurred in the middle of the light period (see solid dots beneath the daily records). A predawn peak subsequently reappeared on day 101. On day 108, the onset of light was delayed 6 hours to coincide with feeding time at 1200; during the next 5 days the predawn peak delayed approximately 6 hours by shifting a small amount a day. The 6-hour delay in the onset of "darkness," on day 113, which restored the schedule of 12 hours of light and 12 hours of "darkness", did not affect the timing of the predawn peak.
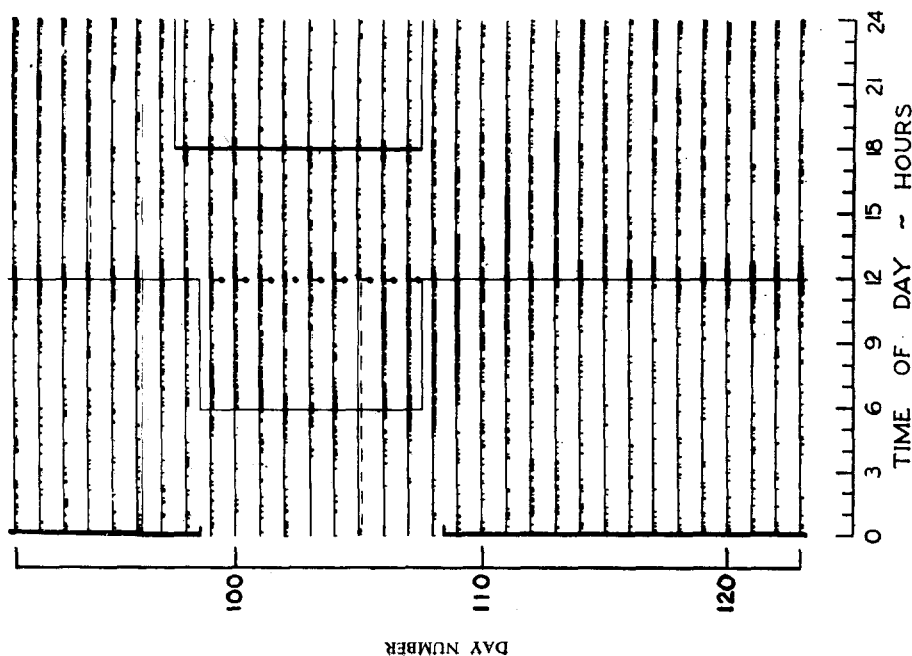

Fig. 9. Effect on the predawn peak of 3 bass (group 4) of changes in the phase of the 24-hour light-"dark" cycle. The schedule of 12 hours of light and 2 hours of "darkness," with feeding at the onset of light (thin vertical line) was altered on day 98 by advancing the phase of the light-"dark" cycle 6 hours, so that feeding occurred in the middle of the light period (see solid dots beneath the daily records). A predawn peak reappeared on day 101. The previous phase-relation between light and feeding was restored on day 108 by delaying the light-"dark" cycle of 6 hours.

shifting the onset of light and of "darkness" on the same day, the other two groups of fish, one of bluegill and one of bass, experienced these changes on different days. The onset of light was delayed 6 hours on day 108 and the result resembled that achieved by shifting the onset of light and "darkness" on the same day (day 108 to 113, Fig. 10). The onset of the "dark" period was delayed 6 hours on day 113 without noticeable change in the timing of the predawn peak (Fig. 10).

Effect of Constant Illumination Without Feeding (Days 125 to 160)

The room was left undisturbed during these experiments. Before and after each experiment the fish received daily 12 hours of light and 12 hours of "darkness" with feeding at the onset of the light period.

Constant "darkness" without feeding was maintained for 4 days by omitting the light period on days 125 to 128. On day 126 and 127 both the bluegill and the bass showed a peak of locomotion corresponding to the predawn peak, and on day 128 the fish were only irregularly active. The peak on days 126 and 127 seemed to start later than the predawn peak on previous days under the light-"dark" cycle (Figs. 11 and 12). Following the resumption of the light-"dark" cycle and daily feeding at the onset of light, a predawn peak reappeared in the "dark" period on the first day for one group of bass (day 130, Fig. 11) and on the second day for the bluegill (day 131, Fig. 11) and the other group of bass.

Constant light without feeding was given for 3 days by omitting the "dark" period on days 140 to 142. The fish were only irregularly active on these days (Figs. 11 and 12). When the light-"dark" cycle and daily feeding were resumed a predawn peak reappeared in the "dark" period in 4 to 6 days (day 143 to 160), Figs. 11 and 12) concurrent with a reduction or irregular activity in the daily "dark" period. There was a high level of locomotion in constant light and in the following 3 to 5 daily, "dark" periods which would have obscured the predawn peak.

Discussion

The co-ordination of the predawn peak of swimming suggests regulation by an inner time sense, or internal rhythm, which itself is co-ordinated by the daily light-"dark" cycle. The predawn peak seems to be a learned response with a training time of 10 to 20 days (Figs. 3 and

4). According to Bull (1957) the number of presentations to effect conditioning in fishes is rarely less than 10 or more than 80, and is usually about 30. That the predawn peak disappeared after 2 days in constant "darkness" without feeding and then reappeared in several days—instead of 10 to 20 days—when the periodicities of light and feeding were resumed suggests that part of the previous learning endured through the days of constant "darkness". The existence of an internal rhythm and its dependence on the light-"dark" cycle was indicated by the stepwise delay in the predawn peak which compensated for a 6-hour delay in the onset of the light period (Fig. 5). For 4 to 6 days following the one on which light was delayed, the peak recurred at intervals slightly longer than 24-hours. The deviation in cycle-length from 24 hours suggests that the rhythm in locomotion is not the direct result of corresponding changes in the physical environment, but that the interval between successive onsets of the peak represents the cycle-length of an internal rhythm which deviates from 24 hours to respond to the change in the light-"dark" cycle. In addition, the reappearance of the predawn peak 2 to 3 days after the onset of light was advanced 6 hours would imply that the cycle-length of the internal rhythm was shorter than 24 hours on those days to compensate for the advance in light. The experimental changes in light and feeding indicated that the phase of the internal rhythm was regulated daily by the transition from "dark" to light, or"dawn"; the onset of the daily "dark" period ("dusk") and feeding time were not noticeably involved in phasing the internal rhythm. The persistence of the peak under constant "darkness" without feeding also suggests that it was regulated by an internal rhythm. The predawn peak seemed to shift to a later time of day in constant "darkness" and it reappeared only after 1 or 2 days in the resumed light-"dark" cycle (Figs. 11 and 12). One explanation of these changes is that the cycle-length of the internal rhythm was longer than 24 hours in constant "darkness" and shorter than 24 hours for a time under the resumed light-"dark" cycle. Alternatively, there is the possibility that a reduction in "amplitude", or a "damping out", of the peak produced the apparent delay in its starting time and not an increase in the period of the internal rhythm.

There is correspondence between the rhythm of the predawn peak of locomotion and the sun-orientation rhythm of bluegills (Hasler &
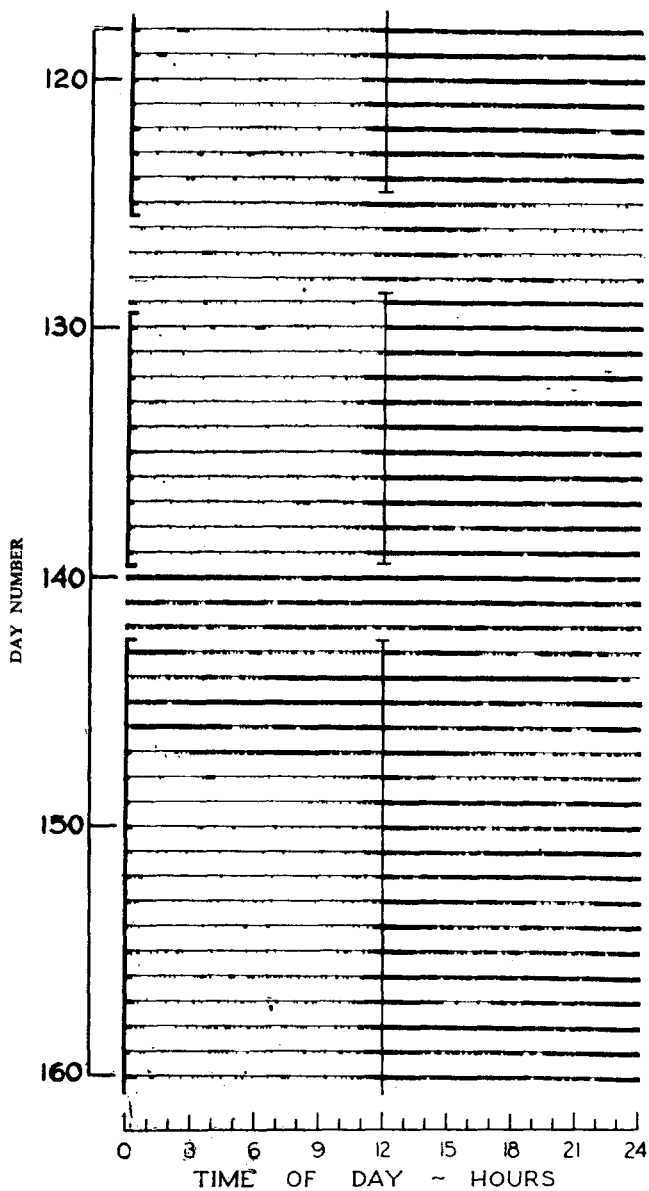
Fig. 11. Effect on the predawn peak of 3 bluegill (group 1) of constant "darkness" without feeding (days 125 to 128) and constant light without feeding (days 140 to 142). On days before and after the intervals of "constant conditions" there was 12 hours of "darkness" (2400-1200) and 12 hours of light (1200-2400) with feeding at the onset of light (thin vertical line).
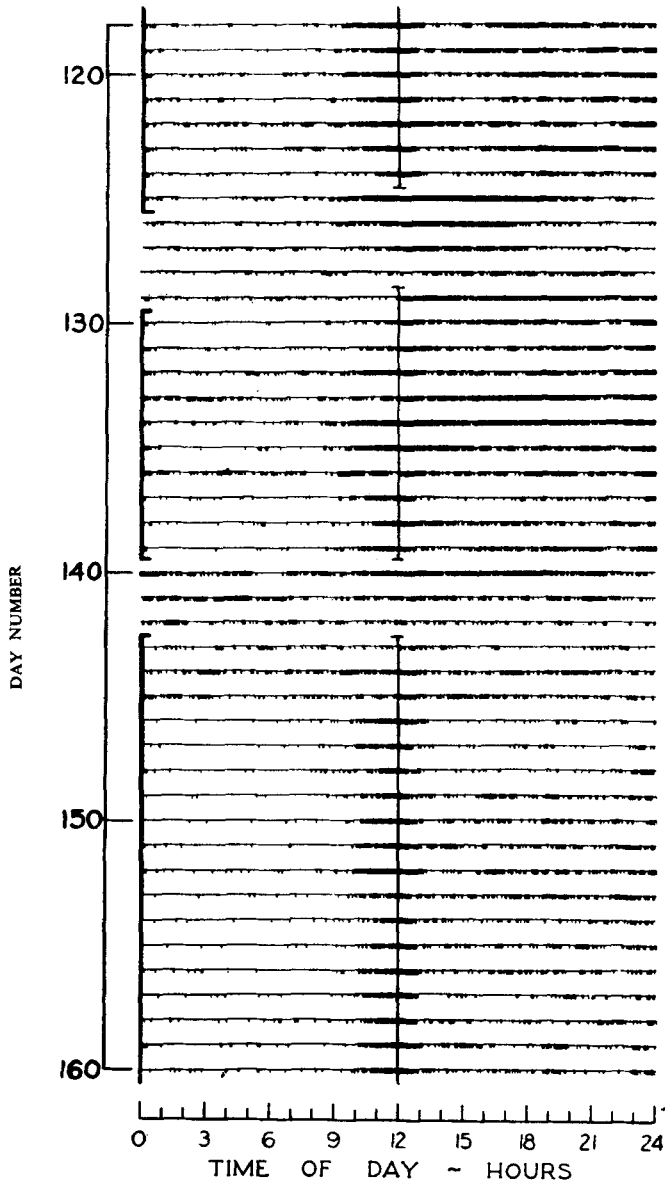
Fig. 12. Effect on the predawn peak of 3 bass (group 3) of constant "darkness" without feeding (days 125 to 128) and constant light without feeding (days 140 to 142). On days before and after the intervals, of "constant conditions" there were 12 hours of "darkness" (2400 1200) and 12 hours of light (1200-2400) with feeding at the onset of light (thin vertical line).

Schwassmann, 1960). The internal rhythm, or inner time sense, enabling bluegills to alter their angle of orientation to the sun throughout the day can be shifted in relation to local time by a 24-hour light-dark cycle. Following a 6-hour delay in the phase of the light-dark cycle, the sun-orientation rhythm shifts a corresponding amount in 4 days (Braemer, 1960). The phase-shifting characteristics of the sun-orientation rhythm compares closely with that of the pre-dawn peak suggesting that the sun-orientation rhythm is also co-ordinated by the daily change from dark to light.

The stepwise shifting of the predawn peak in response to changes in the light-dark" cycle relates it to endogenous daily rhythms found in numerous biological functions of many animals (general references: Ashcoff, 1958; Cloudsley-Thompson, 1961; Harker, 1958). An endogenous daily rhythm is caused in the animal, in contrast to an exogenous daily rhythm which is caused by periodic external environment (Aschoff, 1960). Endogenous rhythms are kept in phase with local time by such agents as daily changes in illumin-ation and temperature. Under constant environ-mental conditions the cycle-length of the endo-genous rhythm deviates from 24 hours and the phase of the rhythm changes in relation to local time. Regulation of the phase of endogenous daily rhythms by light has been demonstrated by shifting the phase of a 24-hour light-dark cycle with which an animal is synchronised. When the phase of the light-"dark" cycle is shifted 6 hours, the endogenous rhythm shifts a small amount a day for several days to restore the original phase-relation. In contrast, the phase of an exogenous rhythm always coincides with the light-"dark" cycle. The rhythm of the pre-dawn peak in the locomotion of bluegills and largemouth bass fails to fit the definition of an endogenous rhythm, because it seems to be a learned response to the periodic environment. Nevertheless, the co-ordination of the predawn peak clearly resembles the co-ordination of an endogenous rhythm. It is proposed therefore that the predawn peak of locomotion, as learned response, depends on the presence of an internal rhythm allied to that which is implicated in endogenous daily rhythms.

Summary

Bluegill and largemouth bass held under alternating, 12-hour periods of light and "dark-ness" (very dim light) and fed daily at the onset of light show a predawn peak of locomotion. The peak appeared between the tenth and twen-tieth days when the fish became active daily during the last 1 to 3 hours of the "dark" period. When the onset of light and feeding were shifted 6 hours to a later time of day, the pre-dawn peak delayed a small amount a day for 4 to 6 days to restore its original relation to the onset of light. When the onset of light and feed-ing time were advanced 6 hours to an earlier time of day, the predawn peak reappeared in 2 to 3 days. Six-hour shifts in the onset of "dark-ness" or in feeding time failed to cause cor-responding shifts in the predawn peak. When the onset of light and "darkness" were both shifted 6 hours, the predawn peak shifted as though the onset of light alone had been altered. A peak of locomotion corresponding to the predawn peak persisted in constant "darkness" without feeding for 2 days, then disappeared and reappeared in 1 to 2 days when the periodicities of illumination and feeding were resumed. The conclusion is discussed that the predawn peak is regulated from within the fish by an internal rhythm which itself is co-ordinated by the daily change from "dark" to light.

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ABSTRACT

Bluegills (*Lepomis m. macrochirus*) and largemouth bass (*Micropterus salmoides*) held under alternating, 12-hour periods of light and "darkness" (very dim light) and fed daily at the onset of of light showed a predawn peak of locomotion. The peak appeared between the tenth and twentieth days when the fish became active daily during the last 1 to 3 hours of the "dark" period. The predawn peak of locomotion was coordinated by the daily change from "dark" to light and not by the light-to-"dark" change or feeding time. A peak of locomotion corresponding to the predawn peak persisted for 2 days in constant "darkness" without feeding. It is proposed that the predawn peak is regulated from within the fish by an internal rhythm which itself is co-ordinated by the light-"dark" cycle.