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Aspects of Photoperiodic Time Measurement in the Crayfish *Orconectes immunis*

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Abstract

Experiments were conducted to determine if an hourglass model is a mechanism whereby photoperiodic time is measured by the crayfish *Orconectes immunis*. Two experiments were conducted in each of which there were 2 series of treatments. Series I and II were time (T) experiments in which T was the total length of the light–dark cycle. In Series I, the light phase of the cycle was held at 16 hours with varied lengths of darkness (LD 16;2, T 18: LD 16;8, T 24: LD16;20, T 36: LD16;32, T 48). In Series II, the dark phase was 8 hours and the hours of light were varied (LD 2;8, T 10: LD 16;8, T 24: LD 28;8, T 36: LD 40;8, T 48). No significant differences were found in the molting responses of the crayfish to the photoperiods in those experiments. The data would then indicate than an hourglass mechanism is not utilized to measure photoperiod under the conditions tested.

Introduction

Environmental factors such as temperature and photoperiod have been shown to affect molting in crayfish (Aiken 1969; Armitage et al. 1973; Mobberly 1963; Rice and Armitage 1974; Stephens 1955; Molley 1974, unpublished master's thesis, Western Kentucky University, Bowling Green, Kentucky; Sadewasser 1974, unpublished master's thesis, Western Kentucky University, Bowling Green, Kentucky; and Van Hoff 1976, unpublished master's thesis, Western Kentucky University, Bowling Green, Kentucky. Molley (unpublished thesis) and Sadewasser (unpublished thesis) have shown that crayfish respond linearly to temperature in that molting frequency increases with increases in temperatures, within limits. Temperatures fluctuate considerably during seasonal changes in temperate regions. Photoperiod progressively increases from a winter minimum daylength to a summer maximum daylength, thence cycling back to a winter minimum. Because of that predictability, photoperiod may be a more reliable environmental cue for the crayfish.

It has been demonstrated in plants and insects that a biological clock measures a time interval of the photoperiod (light or darkness) (Bowen and Skopik 1976, Hamner 1960, Lees 1966, Pittendrigh and Minis 1964, Went 1960). It is possible that crayfish (Crustacea) also use such a device. Various authors have reported that long-day photoperiods will produce higher molting frequencies than will short or normal day photoperiods (Aiken 1969, Armitage et al. 1973, Stephens 1955, Molley unpublished thesis, Sadewasser unpublished thesis, and Van Hoff unpublished thesis). Therefore, it would appear that crayfish use some type of mechanism to measure the duration of the light or dark period.

The mechanism of photoperiodic time measurement may be either an hourglass model or a circadian oscillator model (Pittendrigh 1972). It was the objective of this research to further define the role of photoperiod in the molt cycle of the crayfish *Orconectes immunis* by determining if an hourglass model is a mechanism for photoperiodic time measurement.

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MATERIALS AND METHODS

The crayfish *Orconectes immunis* used in these experiments were obtained from Wolf Lake Fish Hatchery, Kalamazoo County, Michigan, on 2 June and 11 September 1976 and transported immediately to Western Kentucky University. Those animals obtained in June were collected directly from a drained hatchery pond. In September, animals were obtained from the hatchery holding tanks where they had been held at 11 C for approximately 1 week.

The cephalothorax length of the crayfish collected in June ranged from 25.1 mm to 44.5 mm (mean = 35.9 mm) while those obtained in September ranged from 19.2 mm to 37.5 mm (mean = 25.6 mm).

The environmental units used in the experiments provided temperature and light control. Each unit contained 6 separate compartments. The light source in each compartment was a Westinghouse 15-watt coolwhite fluorescent light bulb, wrapped in opaque tape to reduce light to the appropriate intensity. Zinc coated screens with 6-mm mesh were used to cover the crayfish trays, thus allowing penetration of all wavelengths of light. Opaque dividers were placed in the trays since, at least in one instance, lack of privacy in the crab *Gecarcinus lateralis* inhibited molting (Bliss and Boyer 1964). Temperatures in the units were held constant at 22 C.

Two experiments, each containing 2 series were conducted. The first experiment was initiated on 4 June 1976 and the second on 14 September 1976. Experiment 1 was 80 days in duration while Experiment 2 was conducted for 160 days. Series I and II of each experiment were T experiments, in which T was the total length of the light–dark cycle. In Series I, the light phase of the cycle was held at 16 hours with varied lengths of darkness (LD 16;2, T 18: LD 16;8, T 24: LD 16;20, T 36: LD 16;32, T 48). In Series II, the dark phase was 8 hours and the hours of light were varied (LD 2;8, T 10: LD 16;8, T 24: LD 28;8, T 36: LD 40;8, T 48).

In Experiment 1, 2 light intensities were used, 223.56 lux and 413.64 lux. In Experiment 2, intensities of 32.4 lux were used in all treatments.

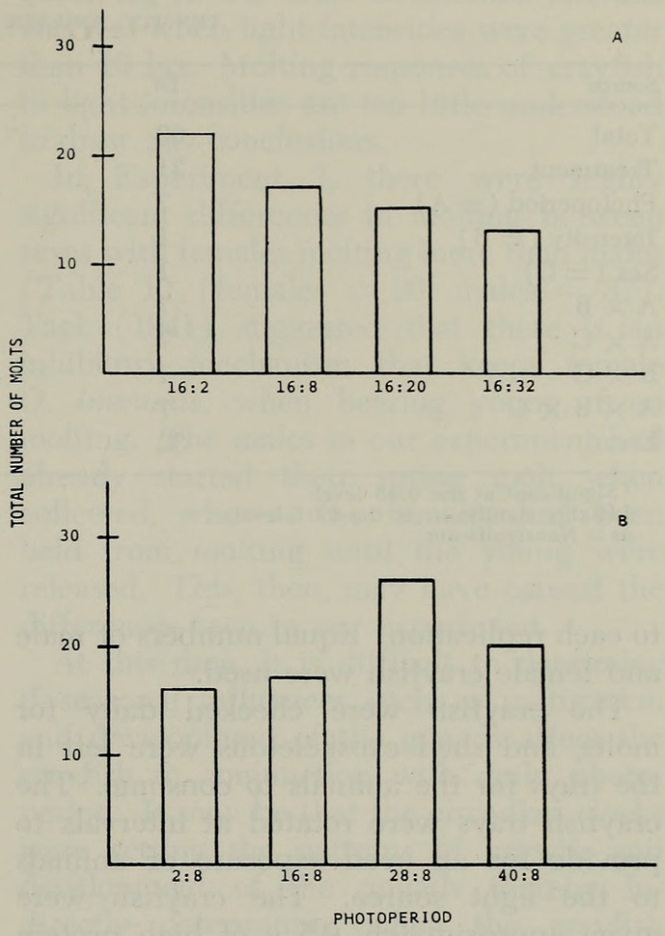

FIG. 1. Total molts occurring in response to 16L: varied dark and varied light:8D photoperiods in Experiment 1 (Series I and II). A. Molts in Series I (16L:Dark). B. Molts in Series II (Light: 8D).

Two hundred and twenty-four crayfish were used in each experiment. In Experiment 1, 7 photoperiod treatments (4 in Series I, and 3 in Series II) were further subdivided into 2 levels of intensity (223.56 lux and 413.64 lux). There was 1 replication of each treatment. Eight animals were assigned to each photoperiod intensity treatment and 8 to each replication. Equal numbers of male and female crayfish were used.

In Experiment 2, there were 7 photoperiod treatments (4 in Series I, and 3 in Series II). There were 3 replications of each treatment. Eight animals were assigned to each photoperiod treatment and

TABLE 1.—ANALYSIS OF VARIANCE OF THE TOTAL MOLTS OCCURRING IN RESPONSE TO PHOTOPERIOD, INTENSITY, AND SEX IN EXPERIMENT 1

Source	Df	SS	MS	F
Total	63	100.48		
Treatment	31	65.98	2.13	1.97[1]
Photoperiod (= A)	7	15.86	2.27	2.10 ns
Intensity (= B)	1	4.52	4.52	4.19[1]
Sex (= C)	1	19.14	19.14	17.75[2]
A × B	7	14.86	2.12	1.97 ns
A × C	7	6.73	0.96	0.89 ns
B × C	1	0.14	0.14	0.13 ns
A × B × C	7	4.73	0.68	0.63 ns
Error	32	34.50	1.08	

[1] Significant at the 0.05 level.
[2] Highly significant at the 0.01 level.
ns = Nonsignificant.

to each replication. Equal numbers of male and female crayfish were used.

The crayfish were checked daily for molts, and shed exoskeletons were left in the trays for the animals to consume. The crayfish trays were rotated at intervals to provide for an even exposure of animals to the light source. The crayfish were given approximately 0.5 g of high protein food every 5 days. Portions of the food not consumed were removed after 4 days.

An analysis of variance (ANOVA) using a completely random design with a factorial arrangement of treatments was used to analyze molting data.

RESULTS AND DISCUSSION

The numbers of molts in each photoperiod in Series I and II of Experiment 1 are presented in Fig. 1. From those data it appeared that the different photoperiods of constant light and varied dark caused similar molting responses in all the crayfish. The analysis of variance (Table 1) confirmed that there were no significant differences in the molting response of the crayfish to the various photoperiods of constant light and varied dark. In Experiment 2, the differences in the molts occurring for each photoperiod of constant dark and varied light were not statistically significant (Fig. 2, Table 2). Therefore, the data indicate that the crayfish were not using an hourglass mechanism for photoperiodic time measurement.

Bowen and Skopik (1976) indicated that the European corn borer *Ostrina nubialis* utilized an hourglass mechanism for measurement of photoperiod. In their experiments the 16L; varied dark conditions caused no termination of diapause except in 16L;8D, indicating that the amount of light or periods of darkness greater than 8 hours were not being measured by an hourglass clock. However, when the 8 hours of darkness were coupled with varying light periods, termination of diapause occurred in every instance. This would indicate that the system of time measurement in *O. nubialis* acted like an hourglass in which photoperiodic time measurement was determined by the length of the dark period. An hourglass model measuring a specific time interval and thereby inducing molts was not used by the crayfish *O. immunis* in those experiments because no constant time interval, when coupled with varying light or darkness, produced an increase in the number of molts.

Significantly greater numbers of molts were obtained in the 413.64 lux light intensity treatments than in the 223.56 lux light intensity treatments in Experiment 1 (Table 1). The reasons for this are not understood. There are few data available on the effects of light intensity on molt responses of crustaceans and the available

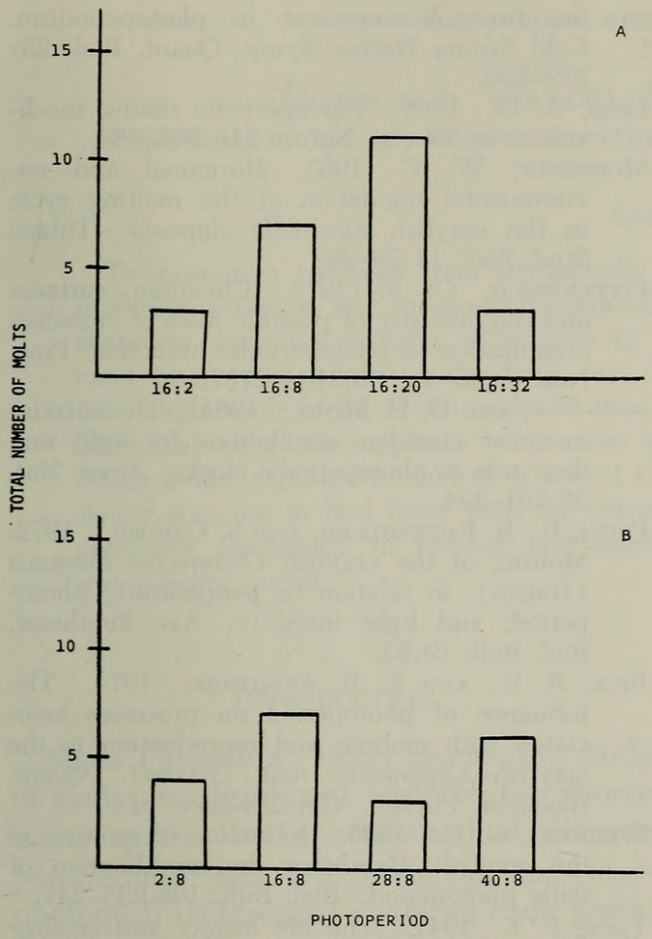

FIG. 2. Total molts occurring in response to 16L: varied dark and varied light:8D photoperiods in Experiment 2 (Series I and II). A. Molts in Series I (16L:Dark). B. Molts in Series II (Light:8D).

data indicate opposite responses from those obtained in these experiments. Prins et al. (1972), when using *O. immunis* from Kentucky, found that molting in the crayfish was less frequent in 120 ft-c (1,296.0 lux) intensities than in 15 ft-c (162.0 lux) intensities when the animals were kept at 22 C. Bliss (1954) found that the molting occurring in the crab *Gecarcinus lateralis* was less when light intensities were greater than 10 lux. Molting responses of crayfish to light intensities are too little understood to draw any conclusions.

In Experiment 1, there were highly significant differences in molting between sexes with females molting more than males (Table 1) (females = 90, males = 37). Tack (1941) suggested that there is an inhibitory mechanism that keeps female *O. immunis*, when bearing young, from molting. The males in our experiment had already started their spring molt when collected, whereas the females had been held from molting until the young were released. This, then, may have caused the differences seen in our experiment.

At this time, it is difficult to determine if seasonal influences such as maturation and development of the gonads affect the crayfish in conjunction with daily photoperiod. It may be that the circadian clocks were setting the patterns of growth and development of the gonads thereby indirectly determining when the crayfish molt. It is not certain if the stage of sexual development affects the measurement of photoperiodic time since no significant interactions between sex and photoperiod were detected.

SUMMARY

1. Experiments were conducted to determine if an hourglass model is a mechanism whereby photoperiodic time is measured by the crayfish *Orconectes immunis*. The effects of photoperiod, intensity, and sex on molting were measured in Experiment 1,

TABLE 2.—ANALYSIS OF VARIANCE OF THE TOTAL MOLTS OCCURRING IN RESPONSE TO PHOTOPERIOD AND SEX IN EXPERIMENT 2. ALL DIFFERENCES (F) WERE NONSIGNIFICANT

Source	Df	SS	MS	F
Total	71	65.99		
Treatment	17	19.24	1.13	1.30
Photoperiod (= A)	8	10.11	1.26	1.46
Sex (= B)	1	1.13	1.13	1.30
A × B	8	8.00	1.00	1.16
Error	54	46.75	0.87	

while the effects of photoperiod and sex were measured in Experiment 2.

2. The crayfish did not use an hourglass model for photoperiodic time measurement under the conditions tested.

3. The crayfish demonstrated significantly greater molts at higher light intensities than at lower light intensities in Experiment 1.

4. In the first experiment, females molted significantly more frequently than males.

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