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# DIEL PERIODICITY IN HATCHING OF *EPITHECA CYNOSURA* (SAY) EGGS (ANISOPTERA: CORDULIIDAE)

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Eggs of *E. cynosura* were cultured under natural and controlled conditions to determine both the diel periodicity in hatching and whether photoperiod and fluctuating temperature act as environmental cues. 150 egg masses were examined under 10 environmental conditions: 3 pretreatment photoperiods, 3 posttreatment photoperiods, 2 temperature regimes, and a natural control. Hatchlings were enumerated and removed from the cultures 6 times each day. The data were transformed to percent hatch for 2- and 3-factor analyses of variance. A diel periodicity under both the natural and 12L:12D photoperiods was evidenced by hatching peaks (80-86 percent of the hatch) immediately after the onset of darkness. Under constant light and constant darkness no peak was obtained because of the large variations in hatching throughout the day. Eggs transferred to constant light or constant dark after 8 days of development under 12L:12D (eye spots present) exhibited no peak. This is evidence that the diel periodicity is not endogenously controlled. Exogenous control is evidenced by the difference in time of hatching peaks between natural and 12L:12D photoperiods (that is, a peak between 1800 and 1815 CDT for 12L:12D vs. a peak between 2045 and 2100 CDT for natural photoperiod). These times encompass the onset of darkness which triggered an immediate hatching response. Temperature fluctuation does not appear to cue hatching.

## INTRODUCTION

Hatching of insect eggs has been shown to be a rhythmic population phenomenon (SAUNDERS, 1977). ROBERT (1958) found that hatching of *Epitheca bimaculata* (Charpentier) was not well synchronized, but may be concentrated around sunset. Preliminary studies of hatching in *E. cynosura* (Say) (TENNESSEN, unpublished) indicated that eggs will hatch at various times of the day, but

that most hatch around dusk.

Therefore, we asked the following questions. Is there a diel periodicity in hatching of *E. cynosura* eggs? If so, is it endogenously or exogenously controlled? If control is endogenous, what is the control and when? If control is exogenous, what cues do the eggs respond to? We designed an experiment using large numbers of *E. cynosura* egg masses to answer these questions.

## MATERIAL AND METHODS

Freshly-laid egg masses of *Epitheca cynosura* were collected from April 30 to May 7, 1977, from an impoundment on Bear Creek, 6 km east of Lutts, Wayne County, Tennessee, United States. Water temperature ranged from 24 to 27°C. The eggs were transported to the laboratory in insulated coolers to prevent temperature changes.

The egg masses were separated and placed in individual containers: 22 masses were set outside to develop under natural conditions, and 128 masses were cultured in six refrigerator-size incubators. Photoperiods of 12L:12D (12 hr light and 12 hr dark, the light period being from 0600 to 1800 CDT), 0L:24D, and 24L:0D were combined with two temperature regimes – one constant (24°C) and the other fluctuating (24° from 0600 to 1800 CDT, 20° from 1800 to 0600 CDT) – to yield six controlled environmental conditions. Hatchlings were removed and counted six times each day (0700, 1100, 1500, 1700, 1900, and 2300 CDT). The data were transformed to hatching percentages for each period.

A transfer experiment was designed to determine whether hatching periodicity is controlled endogenously or exogenously. Eye spots are present by the eighth day of development. If the onset of darkness is the cue, a hatching peak should be obtained after the eggs with eye spots are transferred to constant light or constant dark if it is an endogenous rhythm. The absence of a definitive peak would indicate exogenous control. Therefore, egg masses were transferred from each experimental photoperiod on the eighth day of development. Five egg masses went to each of the other two photoperiods and five masses remained in the original photoperiod, giving three pretreatment and three posttreatment photoperiod conditions. No egg masses were transferred between different temperature regimes.

The diel periodicity data were analyzed by a 2-way analysis of variance with 16 replications for each treatment-time observation. Data used to distinguish endogenous vs. exogenous control were analyzed by a 3-factor analysis of variance with five replications for each treatment combination (SNEDECOR & COCHRAN, 1965).

## RESULTS AND DISCUSSION

The hatching distribution of *E. cynosura* eggs is shown in Figure 1. Hatching began on the tenth day after oviposition for some egg masses and required as long as 23 days for others. The peak around the sixteenth day agrees with KORMONDY's 1959 observation, but the apparent multimodal distribution of hatching has never before been reported for Odonata. The greatest number of hatchlings from one egg mass was 1,438; the average was over 600. Most eggs within an egg mass hatched within two to four days although hatching was observed for as long as ten days. Hatching in some egg masses was reduced or totally inhibited; these masses had the blue-green alga *Lyngbya* sp. (probably *majuscula*) growing on them. Several blue-green algae have been shown to be toxic to mosquito larvae (GERHARDT, 1955).

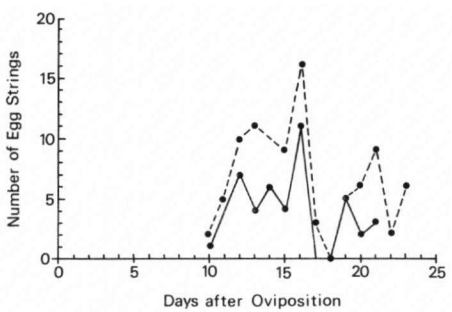


Fig. 1. Frequency distribution of hatching onset (number of days after oviposition until hatching began) for 93 egg strings of *Epitheca cynosura*. Solid line represents egg strings cultured only at natural or 12L:12D photoperiod; dashed line represents all treatments.

Results of hatching at different times of the day for natural and controlled photoperiod and temperature are summarized in Table I. An average of nearly 81 percent of the eggs hatched within 15 min after the onset of darkness, whereas only 2 to 3 percent of the hatching occurred in the mornings and afternoons and 6 to 8 percent just before dark and during nights. These differences are significant (Tab. II). Diel periodicity in hatching is evidenced by the peak after the onset of darkness. There was nog significant interaction between the different environmental conditions and time of hatching (Tab. II).

Because there was no significant difference in hatching between natural conditions and the 12L:12D laboratory photoperiod at the two temperatures, these conditions were used as controls in the transfer experiment to determine whether hatching periodicity is controlled endogenously or exogenously. Results are summarized in Table III. Significant differences were observed for pretreatment condition, posttreatment condition, temperature, and the interaction between posttreatment and temperature (Tab. IV). Egg masses transferred from 12L:12D to constant light and constant dark were extremely variable in hatching percentages over the six time periods (Tab. V); no definite hatching peaks were obtained. These results indicate that hatching periodicity is not endogenously controlled and does not constitute a circadian rhythm as defined by CORBET

Table I
Summary of hatching results at different times of the day for natural and controlled photoperiod and temperatures. Means and standard errors are expressed as percent hatch, based on 16 replications

| Condition | Mean percent hatch ± standard error | | | | | |
|---|---|---|---|---|---|---|
| | *Morning* | *Afternoon* | *Before dark* | *Dark* | *Night* | *Average* |
| Natural | 0.59 ± 0.35 | 5.44 ± 1.71 | 3.74 ± 0.97 | 83.30 ± 2.76 | 7.62 ± 1.78 | *20.14 ± 0.79* |
| 12L:12D 24°:20° | 3.71 ± 0.87 | 2.20 ± 0.78 | 5.73 ± 1.12 | 81.85 ± 2.46 | 6.54 ± 1.65 | *20.01 ± 0.79* |
| 12L:12D 24°:24° | 1.88 ± 0.60 | 1.26 ± 0.58 | 8.17 ± 1.92 | 77.49 ± 3.46 | 10.27 ± 2.01 | *19.81 ± 0.79* |
| *Average* | *2.06 ± 1.02* | *2.97 ± 1.02* | *5.88 ± 1.02* | *80.88 ± 1.02* | *8.14 ± 1.02* | |

Analysis of variance table for natural vs. controlled photoperiod and temperature on hatching

| Variance source | df | MS | F | 95% L.S.D. |
|---|---|---|---|---|
| Time of day (T) | 4 | 55,899.54 | 1126.41** | 1.44 |
| Environmental condition (E) | 2 | 2.13 | 0.04NS | |
| TxE interaction | 8 | 99.71 | 2.01NS | |
| Error | 225 | 49.63 | | |

(1966). The immediate hatching response within 15 min after the onset of darkness (whether natural at 2045-2100 CDT or controlled photoperiod at 1800-1815 CDT) is evidence of exogenous control of hatching periodicity. The 12L:12D photoperiod yielded the highest average percentage – roughly 40 percent for the pretreatment photoperiod and 74 percent for the posttreatment photoperiod (Tab. III). There was virtually no difference in hatching between the constant light and constant dark pretreatments or posttreatments, but the pretreatment averages were about 2.5 times greater than those for posttreatment under constant conditions. Fluctuating temperature accounted for about 7 percent more hatching on the average than constant temperature (36 percent vs. 29 percent).

Temperature does not appear to act as an environmental cue for hatching. For the 12L:12D photoperiod, no difference in peak percentage of hatching was observed between constant and fluctuating temperature regimes. It is noteworthy that eggs pretreated under constant light or constant dark conditions and then transferred to 12L:12D yielded markedly lower peak percentages of hatching (about 31-33 \percent lower) for constant temperature than for fluctuating temperatures (Tab. III). This result seemingly indicates that temperature fluctu-

Table III

Summary of hatching results for different environmental treatments
(C = constant temperature of 24°C; F = fluctuating temperature of 24°:20°C)

| Pretreatment | Posttreatment | Temp. | % Hatched after dark | Pretreat. average | Posttreat. average | Temp. average |
|---|---|---|---|---|---|---|
| 0L:24D | 0L:24D | C | 15.60 | | | |
| | | F | 13.88 | | | |
| | 12L:12D | C | 52.68 | | | |
| | | F | 83.39 | | | |
| | 24L:0D | C | 9.51 | | | |
| | | F | 2.30 | | | |
| | | | | 29.56 | 12.32 | |
| 12L:12D | 0L:24D | C | 11.10 | | | |
| | | F | 11.95 | | | |
| | 12L:12D | C | 84.34 | | | |
| | | F | 84.89 | | | |
| | 24L:0D | C | 22.24 | | | |
| | | F | 23.79 | | | |
| | | | | 39.72 | 74.19 | |
| 24L:0D | 0L:24D | C | 9.72 | | | |
| | | F | 11.64 | | | |
| | 12L:12D | C | 53.50 | | | |
| | | F | 86.35 | | | |
| | 24L:0D | C | 1.39 | | | |
| | | F | 4.01 | | | |
| | | | | 27.77 | 10.54 | |
| | | C | | | | 28.90 |
| | | F | | | | 35.80 |
| 95% L.S.D. | | | 16.5 | 4.87 | 4.87 | 3.98 |

Table IV

Analysis of variance table for different environmental treatments

| Variance source | df | MS | F | Significance |
|---|---|---|---|---|
| Replications | 4 | 8.89 | 0.05 | NS |
| Pretreatment (Pr) | 2 | 1245.97 | 7.00 | ** |
| Posttreatment (Po) | 2 | 39418.29 | 221.33 | ** |
| Temperature (T) | 1 | 1071.78 | 6.02 | * |
| Pr x Po | 4 | 408.24 | 2.29 | NS |
| Pr x T | 2 | 248.03 | 1.39 | NS |
| Po x T | 2 | 1180.32 | 6.63 | ** |
| Pr x Po x T | 4 | 324.38 | 1.82 | NS |
| Error | 68 | 178.10 | | |

Table V

Average percent hatch (rounded to nearest 0.1%) for different environmental treatments at different times of the day (C = constant temperature of 24°C; F = fluctuating temperature of 24°:20°C)

| Pretreatment | Posttreatment | Temp. | Clock Time (CDT) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | *0700* | *1100* | *1500* | *1700* | *1900* | *2300* |
| 0L:24D | 0L:24D | C | 29.1 | 11.2 | 21.0 | 6.7 | 6.3 | 25.7 |
| | 12L:12D | C | 6.2 | 1.5 | 4.9 | 25.4 | 54.3 | 7.7 |
| | 24L:0D | C | 28.8 | 20.8 | 19.6 | 6.3 | 11.3 | 13.2 |
| 12L:12D | 0L:24D | C | 6.0 | 54.0 | 10.1 | 1.4 | 19.2 | 9.3 |
| | 12L:12D | C | 3.7 | 1.5 | 3.9 | 4.5 | 78.0 | 8.4 |
| | 24L:0D | C | 25.8 | 19.3 | 10.7 | 5.0 | 19.2 | 20.0 |
| 24L:0D | 0L:24D | C | 10.6 | 4.3 | 4.8 | 59.8 | 7.3 | 13.2 |
| | 12L:12D | C | 4.7 | 1.3 | 16.0 | 6.7 | 63.0 | 8.3 |
| | 24L:0D | C | 31.7 | 13.3 | 32.4 | 0.5 | 1.9 | 20.2 |
| 0L:24D | 0L:24D | F | 27.9 | 19.2 | 3.4 | 10.4 | 5.7 | 33.4 |
| | 12L:12D | F | 3.2 | 0.6 | 0 | 11.7 | 78.7 | 5.8 |
| | 24L:0D | F | 11.2 | 9.8 | 20.6 | 25.8 | 1.5 | 31.1 |
| 12L:12D | 0L:24D | F | 21.6 | 34.1 | 6.3 | 13.4 | 12.7 | 11.9 |
| | 12L:12D | F | 1.6 | 0.5 | 0.2 | 5.2 | 85.0 | 7.5 |
| | 24L:0D | F | 16.0 | 9.0 | 0 | 33.4 | 30.7 | 10.9 |
| 24L:0D | 0L:24D | F | 39.9 | 13.4 | 9.4 | 3.0 | 11.0 | 23.3 |
| | 12L:12D | F | 1.7 | 0.6 | 0 | 4.5 | 86.3 | 6.9 |
| | 24L:0D | F | 14.7 | 15.8 | 12.4 | 28.6 | 3.8 | 24.7 |

ation acts as a secondary cue; however, since there was no difference in peak percentages of hatching between the two temperature regimes under total 12L: 12D photoperiod, the above differences must be attributable to artificial conditioning.

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