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THERMAL EFFECTS ON EMBRYONIC DEVELOPMENT IN FOUR SUMMER SPECIES OF LIBELLULIDAE (ANISOPTERA)

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More than 32,000 fertilized eggs from *Erythemis simplicicollis* (Say), *Libellula cyanea* Fabr., *L. incesta* Hag. and *Perithemis tenera* (Say) were maintained at several regimes until eclosion. Between 20-35°C, development was progressively faster at each higher temperature; at this thermal range incidences of successful eclosions were also highest. At 15°C hatching was either greatly delayed or prevented, and at 40°C, no eclosions were noted; these temperatures mark the approximate lower and upper thermal thresholds for eclosion in these spp. Hatching peaks occurred during the first 1-3 days of the eclosion period. Numbers of eggs per clutch varied widely within and between spp.; clutch sizes ranged from a mean of 620 for *E. simplicicollis* to 1366 for *L. cyanea*.

INTRODUCTION

Egg development in the Odonata is a critical ontogenetic stage, and its rate is directly influenced by environmental conditions, especially temperature. However, the specific effects of temperature on egg development rates are not well understood due to the fragility of the eggs and the difficulties of capturing females between copulation and oviposition.

The effects of temperature on developmental rates in insects have been reviewed by DAVIDSON (1944), PRADHAN (1946), HOWE (1967), TAYLOR (1981) and others. CORBET (1980) summarized information on a number of aspects of the biology of the Odonata including oviposition, eggs and rate of development. He grouped species for which eggs either undergo delayed development, overwinter and hatch after 80-230 days (BOEHMS, 1971; SAWCHYN & CHURCH, 1973) or which develop directly and hatch within 5-40 days (LUTZ & PITTMAN, 1968; PILON, 1982; MASSEAU & PILON, 1982; HALVER-

SON, 1983; LEGRIS & PILON, 1985; DESFORGES & PILON, 1986; PILON et al., 1989).

The three libellulids that have been studied whose eggs have direct development include *Plathemis lydia* (HALVERSON, 1983, *Libellula julia* (DESFORGES & PILON, 1986) and *Leucorrhinia glacialis* (PILON et al., 1989). Egg development in all three species proceeded in similar overall fashion with rates being highest at experimental temperatures of 20-30°C. The lower thermal threshold for development was about 15°C while the upper thermal limits were at about 35°C. Mortality rates were found to be lowest for eggs maintained at about 25°C. However, taxonomic and latitudinal differences in populations make comparisons between studies difficult.

Clutch sizes in the Odonata have been the object of a number of studies, and the data are compared in CORBET (1962) and PITTMAN (1971). Numbers of eggs laid in a single clutch reportedly range from 150 to more than 5200, but these wide variations are attributable to a number of exogenous and endogenous factors.

It was the intent of this study to obtain massive numbers of eggs from a representative sample of females of four summer species of libellulid dragonflies. Eggs were then subjected to experimental temperatures in order to obtain reliable frequencies of successful eclosion and influences of temperature on rates of egg development and eclosion patterns. Additionally, we determined clutch sizes for these four species.

MATERIAL AND METHODS

Egg collections were made from mature adult females captured at three impoundments near Greensboro, Guilford County, North Carolina (USA) (latitude 36°05′ N; longitude 79°57′ W) in June-August, 1970. Pond A was located southeast of Greensboro near N.C. State Road No. 3000. Ponds B and C were located within 2 km of each other in northwest Greensboro near Westridge Road. The three ponds were similar to each other, more than 25 years old and typical of impoundments in this vicinity. Each had a surface area of about 0.5 ha, and each shoreline contained combinations of cleared areas and a climax oak-hickory forest. More than half of Pond B had a water depth of less than 1.5 m, while Ponds A and C had much smaller areas of 1.5 m or less in depth.

Eggs were obtained from 51 females representing four species of Libellulidae: *Erythemis simplicicollis* (Say), *Libellula cyanea* Fabricius, *L. incesta* Hagen, and *Perithemis tenera* (Say). Using an aerial net, 36 females were captured either while still in tandem or immediately after mating and before oviposition. The other 15 females were netted near the ponds, and though they were not observed mating prior to capture, they also supplied abundant clutches of fertile eggs.

Each female was held by both pairs of wings flexed dorsally while the posterior portion of her abdomen was rhythmically submerged in small vials of fresh pond water to simulate natural oviposition. Oviposition was considered complete after egg deposition had ceased for 2 min.

Eggs in each clutch were counted and divided, and groups of eggs were subjected to experimental temperatures and photoperiods in the laboratory. Egg subsets were cultured in pond water in finger bowls 10 cm in diameter and maintained in Precision Sci. Co. biological oxygen demand (BOD) boxes; each box supplied a different experimental regimen. Usually no more than about 100 eggs were

placed in each fingerbowl along with about 100 ml of pond water. The eggs were spread out in the fingerbowl so that each had ample access to oxygenated pond water. The incidences of eggs succumbing to water mold were small. Temperatures of 15°, 20°, 25 and 30°C were mainly used; at each temperature, photoperiods of 11 and 14 hr in a 24-hr cycle were employed. No differences were noted between eggs maintained on an 11- or 14-hr day; therefore, data at each temperature are grouped. In addition, a limited number of eggs of *L. incesta* and *E. simplicicollis* was placed at 35° and 40°C.

Experimental temperatures were constant to ± 1°C in each box. The photoperiod in an individual box was supplied by two, 15-watt, cool white, fluorescent lamps controlled by individual automatic G.E. time switches. Eggs were checked daily until seven days after last eclosion. Newly hatched prolarvae and larvae were removed by pipette.

Subsets of eggs from *L. incesta* (2 clutches, 131 eggs), *P. tenera* (3, 138) and *E. simplicicollis* (6, 538) were maintained at natural pond temperatures and photoperiods in 0.75 l plastic containers. The containers were submerged about 20-30 cm in Pond B at water temperatures between 28-32°C and at natural photoperiods of about 15-16 hr. These eggs were also checked daily to determine the onset of hatching.

Standard deviation (SD), standard error of the mean (SE) and analysis of variance were determined to assess levels of significance between means of the various groups. Curvilinear regression analyses were performed to obtain the best predictive curve based on the eclosion data.

RESULTS

Precisely 31,654 eggs from females of *Erythemis simplicicollis, Libellula cyanea, L. incesta* and *Perithemis tenera* were maintained at various experimental thermal regimes until all eclosions had occurred. For all four species, numbers of eggs subjected to experimental conditions, numbers hatched, percent mortality and statistical treatments of the data appear in Table I. Mean days to eclosion at all experimental temperatures are also shown graphically in Figure 1.

The average time from oviposition to eclosion for eggs at each experimental condition indicated that embryonic development was temperature-dependent. At progressively higher experimental temperatures between 20° and 35°C, uniformly faster egg development was noted in all species; however, few statistically significant differences (<0.001 level) were obtained between average times to eclosion at 5°C increments. Highly significant differences were observed in the mean responses of eggs to temperatures between 15° and 20°C. Eggs of *L. incesta* and *L. cyanea* developed in 13-15 days at 20°C but at 15°C required about 64 days or approximately 4-5 times longer for development. None of the eggs of *E. simplicicollis* or *P. tenera* hatched at 15°C. Very few significant interspecific differences were noted between responses of eggs at a given temperature. The highest incidences of successful eclosion were noted in the eggs of *L. cyanea* (80.2%) and at a temperature of 30°C (82.7% for all species combined). The lowest incidences of eclosion were noted for eggs of *L. incesta* (25.3%) and at 15° and 40°C for all species. The overall eclosion percentage for all eggs at all experimental conditions was 60.9%.

The 7-9 day average for eggs maintained at 30°C approximated the time for

Table I
Effects of temperature on rate of embryonic development and percent hatching in the four species

Species	Temp. °C	Nos started	Nos hatched	% hatched	Mean days to eclosion	SD	SE of the mean	% mortality
E. simplicicollis	15	3075	0	0.0	-	-	-	100.0
	20	3196	2517	78.8	14.00	2.00	0.04	21.2
	25	2919	2290	78.5	12.38	1.45	0.01	21.5
	30	2732	2079	76.1	8.14	1.44	0.03	23.9
	35	99	85	85.9	6.13	1.08	0.56	14.1
	40	34	0	0.0	-	-	-	100.0
	Totals	*12,055*	*6971*	*57.8*				
L. cyanea	15	987	324	32.8	63.42	3.32	0.18	67.2
	20	1834	1640	89.4	12.79	1.80	0.05	10.6
	25	1856	1603	86.4	12.66	0.82	0.02	13.6
	30	1753	1592	90.8	8.78	1.75	0.04	9.2
	Totals	*6430*	*5159*	*80.2*				
L. incesta	15	2852	20	0.7	64.40	3.93	0.88	99.3
	20	1117	638	57.1	14.83	1.14	0.05	42.9
	25	737	349	47.4	13.52	1.10	0.06	52.6
	30	630	306	48.6	8.32	1.91	0.11	51.4
	35	206	113	54.9	5.36	0.59	0.06	45.1
	40	100	0	0.0	-	-	-	100.0
	Totals	*5642*	*1426*	*25.3*				
P. tenera	15	775	0	0.0	-	-	-	100.0
	20	2187	1669	76.3	12.20	1.55	0.04	23.7
	25	2228	1980	88.9	10.61	1.02	0.02	11.1
	30	2337	2058	88.1	7.04	0.94	0.87	11.9
	Totals	*7527*	*5707*	*75.8*				

development of the eggs of *E. simplicicollis, L. incesta* and *P. tenera* maintained at natural conditions. The 807 eggs kept in Pond B required 6-8 days to hatch in each of the three species. These pond experiments were carried out during July and August when the water temperatures ranged between 28° and 32°C.

Figure 1 also shows the results of the curvilinear regression analyses in which the best predictive curve was statistically sought to represent the developmental rates as a function of temperature. It is very significant that the formula for the curvilinear regression analyses was identical for all species. That formula is:

$$y = a + b/x + c^2/x$$

where y = mean days to eclosion, x = temperature and a, b and c are constants of the curves for all four species. The statistical curve for each species was a second order hyperbola that closely approximated the actual data. Since the SD and SE ranges were so small (Tab. I), they are not shown for the means in Figure 1.

Once eclosion began, the rates at which hatching occurred proceeded at similar rates at most experimental temperatures. Figure 2 is a series of cumulative

percentage curves for eclosion illustrating the rates at which all eggs hatched at each experimental condition for each species during the first 10 days of hatching. No reference to eggs maintained at 40°C is made in this illustration since no hatching occurred. All four species show similar eclosion curves with most of the eggs hatching within four days after the onset of hatching.

Eggs of *E. simplicicollis* and *L. incesta* were cultured at 35°C, and these showed a somewhat faster eclosion rate than those at the lower temperatures with about 95% of all eclosions occurring in the first three days. These differences in response to 35°C were not statistically different than those to the lower temperatures of 20-30°C. At 15°C only a small percentage of eggs of *L. cyanea* (32.8%) and *L. incesta* (0.7%) hatched (Tab. I). The *L. cyanea* eggs hatched at a fairly uniform rate showing no obvious peak. This temperature of 15°C is undoubtedly near to the lower threshold for egg development and hatching in both species.

The percentages of eggs which hatched when cultured at each experimental

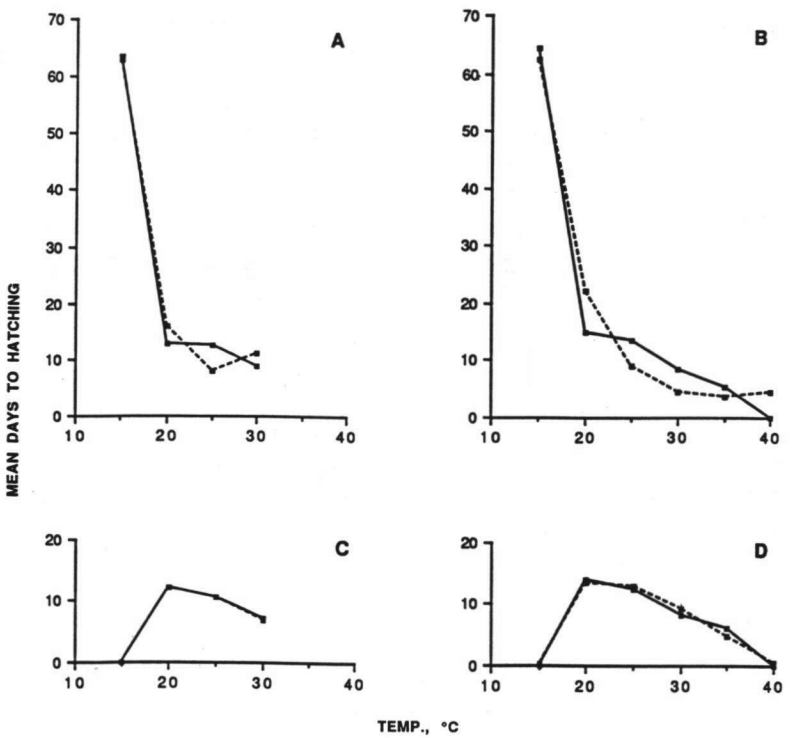

Fig. 1. Mean days to hatching of eggs at experimental temperatures. (Broken lines represent curvilinear predictions for the data): (A) *Libellula cyanea;* — (B) *L. incesta;* — (C) *Perithemis tenera;* — (D) *Erythemis simplicicollis.*

temperature for each species are illustrated in Figure 3; the mean eclosion percentages and standard error ranges (between separate subsets of eggs) are shown for each temperature. The optimal temperature for maximal eclosion was 20-35°C for all four species. Most eggs of the two species of *Libellula* failed to

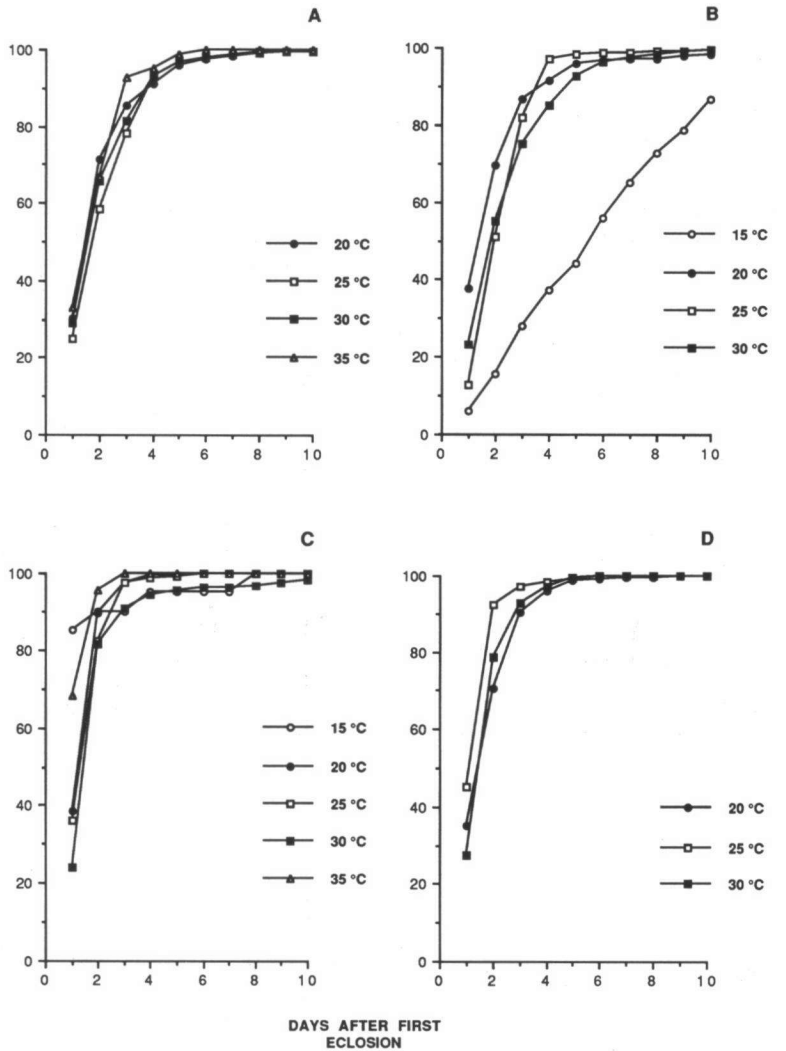

Fig. 2. Cumulative, 10-day, eclosion percentage curves for eggs cultured at various experimental temperatures: (A) *Erythemis simplicicollis;* — (B) *Libellula cyanea;* — (C) *L. incesta;* — (D) *Perithemis tenera.*

hatch at 15°C, and the highly significant differences (< 0.001 level) in percent hatched between 15° and 20°C indicate that the lower temperature threshold for eclosion was about 15°C. Eggs of *E. simplicicollis* and *P. tenera* developed and hatched normally at 20°C but failed to hatch at 15°C even though they did exhibit

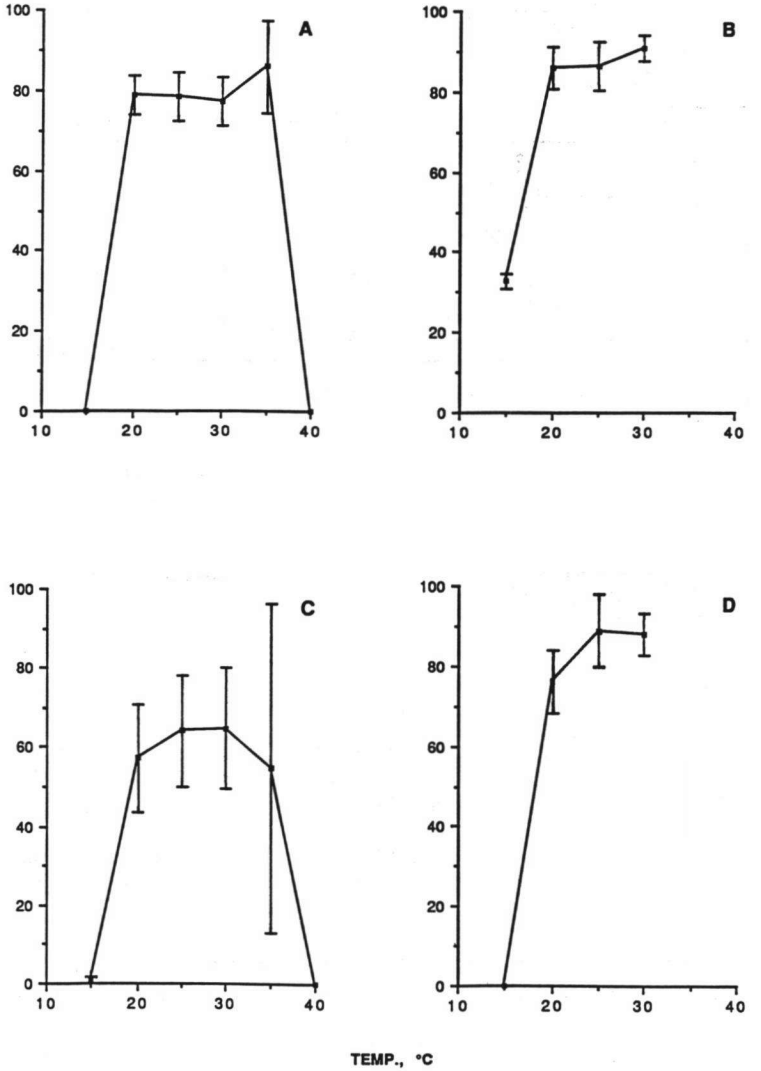

Fig. 3. Mean percentages of total eclosions in subsets of eggs maintained at various experimental temperatures. (SE ranges represented by vertical bars): (A) *Erythemis simplicicollis;* — (B) *Libellula cyanea;* — (C) *L. incesta;* — (D) *Perithemis tenera.*

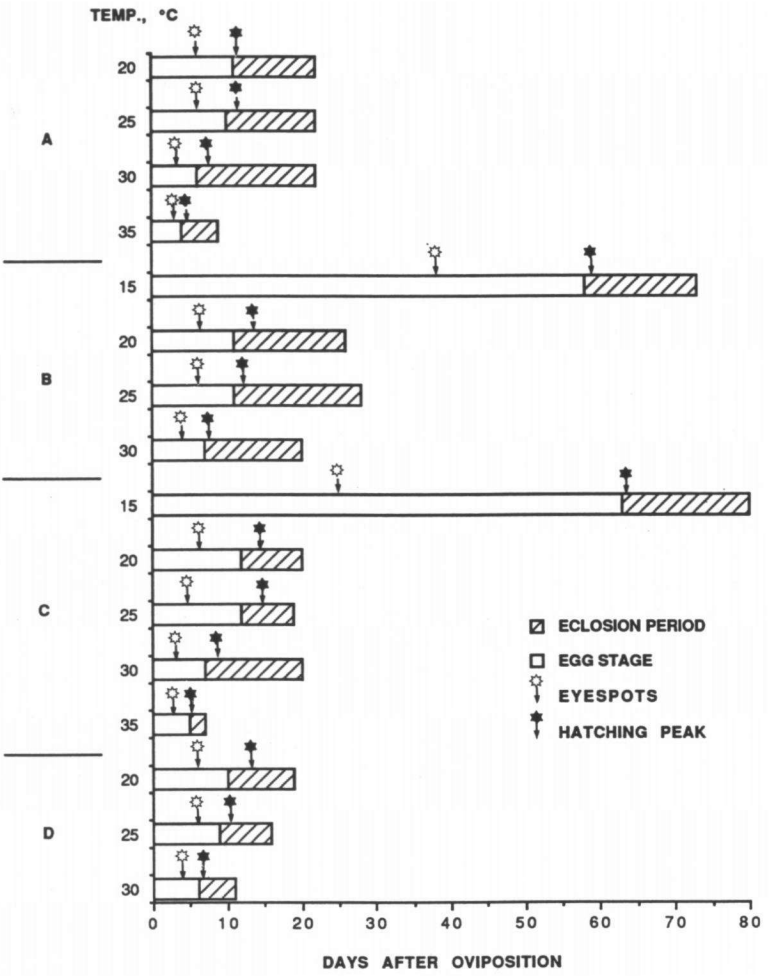

Fig. 4. Rate of embryonic development and hatching pattern as a function of temperature: (A) *Erythemis simplicicollis;* — (B) *Libellula cyanea;* — (C) *L. incesta;* — (D) *Perithemis tenera.*

some degree of development. A temperature of 15°C must be slightly below the lower temperature threshold for pre-eclosion development and hatching in these two species.

One hundred eggs of *L. incesta* and 34 eggs of *E. simplicicollis* maintained at 40°C failed to hatch even though one *L. incesta* embryo developed visible eye spots. Also, highly variable data were recorded for eggs of *L. incesta* at 35°C. These observations suggest that the upper temperature threshold is between 35°C

and 40°C for *L. incesta* and *E. simplicicollis.*

For a given species no statistically different results in eclosion rates in response to temperature were recorded at the thermal range of 20-35°C. The eggs of *L. incesta* had the lowest percentage of hatching at 25°C with only 47.4% successful eclosions, and those of *L. cyanea* had the highest incidence of hatching at 30°C (90.8%).

Figure 4 summarizes the rates of development and hatching patterns of eggs of the four species in response to the various experimental temperatures. This illustration also shows the mean time to appearance of larval eyespots and the average timing of the eclosion peaks. Generally, pre-eclosion developmental periods were shortened by progessively higher temperatures; however, once eclosion began, similar hatching patterns were observed. The appearance of larval eyespots occurred after about 50-65% of the total time of egg development had elapsed. Because of the relative shortage of data about the appearance of eyespots, we could not determine meaningful levels of significance between thermal groups.

Timing of the peak hatching day relative to the onset of hatching was not significantly different at various thermal conditions. The day of peak hatching invariably occurred during the first 1-3 days in the eclosion period. Eclosion peaks tended to occur earlier at progressively higher temperatures; for example, for eggs of *L. incesta* and *P. tenera* maintained at 20°C the peak was on Day 3, at 25°C the peak was on Day 2, and at 30°C the peak was on Day 1. The rates of egg development and hatching patterns were similar for all four species studied.

Clutch sizes for all four species are shown in Table II. A total of 40,636 eggs was obtained from females; of this number, 31,654 were cultured experimentally (see Tab. I). The smallest number of eggs from any one female was 52 (*E. simplicicollis)* and the largest clutch size was 2058 *(L. incesta).* For a given species, numbers of eggs per clutch were quite variable as the SE ranges indicate.

Table II
Clutch size data for four species of Libellulidae Odonata

Species	Range	n	$\bar{x}$	SE
E. simplicicollis	52-1822	21	620.3	110.3
L. cyanea	528-1759	6	1366.0	221.0
L. incesta	206-2058	10	935.0	182.6
P. tenera	63-1381	14	718.8	115.0

DISCUSSION

The effects of constant experimental temperatures on rates of embryonic development in libellulids with direct egg development fall into an overall predictable pattern. At 20-30°C, our results showed that egg development in all four species was linear and temperature dependent with faster developmental rates observed at progressively higher temperatures. Quite similar results have been

reported for *Libellula incesta* (PITTMAN, 1971), *L. julia* (DESFORGES & PILON, 1986), *Leucorrhinia glacialis* (PILON et al., 1989) and *Plathemis lydia* (HALVERSON, 1983). However, embryonic development for eggs maintained at 15°C in this study took 4-5 times longer than for those at 20°C; similar results were obtained for *Libellula incesta* (PITTMAN, 1971). At 15°C eggs of *L. julia* and *Leucorrhinia glacialis* took about twice as long to develop compared to those at 20°C (DESFORGES & PILON, 1986; PILON et al., 1989). At 35°C development was quite rapid in *Libellula incesta* (PITTMAN, 1971; this study), *Erythemis simplicicollis* (this study) and *Leucorrhinia glacialis* (PILON et al., 1989). At 40°C there were no reported successful eclosions in any of the libellulids.

The incidences of successful eclosions in this study were found to be generally high in eggs maintained at 20-30°C: *Erythemis simplicicollis* (76-86%), *Libellula cyanea* (89-91%), *L. incesta* (47-57%) and *Perithemis tenera* (76-89%). These results compare favorably with those obtained on *Libellula incesta* (55-65%) (PITTMAN, 1971), *L. julia* (85-90%) (DESFORGES & PILON, 1986), *Leucorrhinia glacialis* (78-100%) (PILON et al., 1989) and *Plathemis lydia* (80-90%) (HALVERSON, 1983). The atypically lower eclosion rates for *Libellula incesta* reported in this study and by PITTMAN (1971) cannot readily be explained. Mortality was highest at the extreme temperatures of 15° and 40°C.

Comparisons of data regarding the upper and lower thresholds for libellulid eggs with direct development appear in Table III. In general, the lower thresholds for development are at about 15°C (somewhat lower in *Libellula julia*). *L. incesta* eggs, initially held at 15°C for 30, 40 and 50 days, were then maintained at temperatures of 20-35°C until eclosion; about 10-30% of the eggs subsequently hatched (PITTMAN, 1971). Apparently, eggs can survive for 50 or more days at 15°C but cannot complete embryonic development in *L. incesta* (and most species) at 15°C. The upper threshold for embryonic development is about 40°C (Tab. III). At this temperature some critical proteins are apparently inactivated or denatured thus preventing any further development.

Table III
Upper and lower thresholds for egg development in libellulids with direct development

Species	Threshold (°C) for egg development		
	Lower	*Upper*	Source
Erythemis simplicicollis	15+	35-40	this study
Libellula cyanea	below 15	30+	this study
Libellula incesta	15	35-40	this study
Libellula incesta	15	40	PITTMAN 1971
Perithemis tenera	15+	30-35	this study
Libellula julia	10-12.5	30-35	DESFORGES & PILON 1986
Leucorrhinia glacialis	15-17.5	above 32.5	PILON et al. 1989
Plathemis lydia	below 18	above 30	HALVERSON 1983

Once eclosion began, we discovered eclosion peaks in groups of eggs for all four species at every temperature where hatching occurred. Eclosion peaks inevitably occurred early in the hatching period, usually in the first three days and often on Day 1. Eclosion peaks tended to occur earlier at progressively higher temperatures, a phenomenon also observed by PITTMAN (1971). In other investigations, it has not been clearly indicated where the eclosion peaks occurred thus making comparisons impossible. It is not surprising that early peaks in eclosion occur since presumably all eggs in a given clutch are physiologically similar, and developmental rates should also be correspondingly similar. In view of this statement, what is surprising is that the eclosion periods were as long as they were; in *Libellula cyanea* (at 20-30°C) and *Erythemis simplicicollis* at 30°C the eclosion period was 13-19 days.

Clutch sizes can be substantially different between individuals of a given species or between species due to a plethora of reasons. Typically females in our study were captured before oviposition began, and thus our data usually represent entire clutches, but still wide SE variations. In *E. simplicicollis* we noted a mean clutch size of 620 eggs or about twice the number reported by CURRI (1961) for the same species. For *Libellula incesta* our results of 935 eggs per clutch compare favorably with the 1060 value obtained for this same species by PITTMAN (1971). Mean number of eggs per clutch in our study for *Perithemis tenera* was 719, a value about five times greater than that reported by JACOBS (1955): his average of 150 was from "13 unusually productive females". We could not find comparable results for *Libellula cyanea.* From the results of our study it is clear that these four species have great reproductive potential values.

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