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In order to investigate the respective roles of photoperiod and temperature in seasonal regulation of the life cycle, experiments were undertaken to measure the rate of growth of dragonfly larvae when maintained to emergence under constant conditions of light and temperature. Eight experimental conditions were used: two photoperiods (11 and 14 hours) at each of four temperatures (15, 20, 25, and 30 °C). The effects of light could thus be determined when photoperiod was the variable and temperature was held constant; the effects of temperature could be analyzed where it was the variable and photoperiod was constant. The final-instar larvae were collected from September, 1969, to May, 1970, and were subjected to the experimental conditions in order to observe the effect of seasonal variations in relation to photoperiod and temperature.

Rate of growth was quantitatively measured by the number of days intervening from the time of collection to emergence. Comparative statistics indicated that the longer photoperiod stimulated rate of growth, whereas the shorter photoperiod was inhibitory to growth. Likewise, higher temperatures were stimulatory and lower temperatures were

inhibitory to rates of growth. The degree of these effects varied with the season of the year. Larvae collected later in the study period emerged more rapidly than larvae collected earlier and maintained under identical experimental conditions.

It is concluded that photoperiodic effect may be a primary stimulus for metamorphosis of larvae at the outset of the natural reproductive season in the early spring. Temperature exerted a marked influence on growth rates at 20 °C when contrasted with rates at 15 °C. This effect may also constitute one factor in seasonal regulation of the life cycle.

GROWTH OF LARVAE OF *PLATHEMIS LYDIA* DRURY AS INFLUENCED BY CONTROLLED PHOTOPERIOD AND TEMPERATURE (ODONATA: ANISOPTERA)

by

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INTRODUCTION

Throughout the course of evolution all species have been subjected to diverse extrinsic physical factors in the environment. Of all these factors, however, only the daily period of sunlight repeats itself with unfailing accuracy year after year at any latitude or longitude on the surface of the globe. Thus it is not surprising that many species of plants and animals, particularly in temperate zones, have evolved seasonal or annual cycles based largely on inherent responses to predictable photoperiods. Biologically such cycles are vital. Seasons unfavorable for growth or reproduction must be avoided if the species is to survive. Most efficient utilization of a given habitat results from temporal distribution of competitive species living out their life spans on different cycles.

The exact biochemical mechanisms of photoperiodic response have not yet been delineated. Withrow (1959) has pointed out that research on any complicated physiological phenomenon such as this must necessarily first begin with quantitative measurements of the biological effects of the phenomenon. This has been done only recently in regard to photoperiodism in insects, inasmuch as relatively few species are suitable for such quantitative measurements.

Among those species which can adapt to laboratory conditions, the processes of internal growth and maturation are not always manifested externally with any degree of measurable accuracy.

Lees (in Withrow, 1959) reviewed prior measurements of photoinduced diapause in many Lepidoptera and in the Colorado beetle Leptinotarsa. Beck (1964) identified physiological rhythms which responded to photoperiodic stimuli in the European corn borer Ostrinia nubilalis. Morris (1958) measured photoperiodic response in the Tanganyika desert locust, a species inhabiting low-latitude areas where the annual absolute daylength variation is only one hour.

The order Odonata includes species well-suited to experimental measurement of photoperiodic response. The life span is relatively short, large numbers can be accommodated within a confined space, and survival rate is high under most conditions. Further, the process of emergence into the adult form provides a definitive measure of growth response. Indeed, it is a measure of growth over and above normal metabolic rates inasmuch as it involves first the histolysis of larval tissue and then the ensuing morphogenesis of adult structures. As in all hemimetabolous insects, metamorphosis takes place within the exoskeleton of the final instar without a pupal case, and the larvae can be observed throughout the process.

Jenner (1959) reported results of preliminary experiments on six odonate species in which larval development was promoted by a long photoperiod. The six species varied, however, in the degree of response. Lutz and Jenner (1960, 1964), working with Tetragoneuria cynosura, found seasonal variation in response of larvae to long-day and short-day photoperiods. In fall experiments those raised under longer photoperiods developed much more rapidly than larvae under short-day conditions, but the difference in rate of development tended to become obscured in spring experiments. Lutz (1968b) maintained larvae of Lestes eurinus under 11-hr and 14-hr photoperiods at four experimental temperatures as well as under natural conditions. The longer light period induced more rapid larval development at any of the temperatures until the effect diminished in the spring. Corbet (1962) correlated photoperiodic responses with seasonal emergence patterns of odonates.

The experiments herein described utilized the locally abundant species Plathemis lydia Drury, one of the libellulid dragonflies. Its precise life history has not as yet been documented but it fits Corbet's (1958b, 1964) general definition of a "summer species"; i.e., one in which the emergence of adults is temporally dispersed over a long period of time. Needham and Westfall (1955) recorded the extremes of the adult flying season as ranging from April 18

in Mississippi to October 16 in Tennessee. Observations confirm that the flying season began April 21 at the collection site, but no data were recorded on the terminal flying date. Jacobs (1955) observed that emergence occurred through August in Indiana.

Using P. lydia larvae in the final instar, experiments were undertaken to determine the separate and interacting effects of two variables, photoperiod and temperature. Inevitably, a third variable influenced the results: season of collection. In the discussion an attempt is made to correlate this third factor with the first two but in no case were the experiments designed to compare the artificial conditions with natural conditions of the life cycle.

MATERIALS AND METHODS

Collection of Specimens. Nine collections were made between 28 September 1969 and 3 May 1970, at intervals of approximately three weeks or one month. The minimum number of larvae in any one collection was 88, while the maximum number was 116. Total larvae subjected to experimental conditions numbered 851.

All collections were made at two ponds belonging to Ashland Chemical Company, Route 109, Thomasville, Davidson County, North Carolina. These impoundments, approximately 25 years old, supported a rich and diverse aquatic community. Maximum depth was about 2.5 meters but most of the shoreline had extensive shallow areas with submerged vegetation. Since the ponds were connected by a short culvert, the total pond area could be considered one unit of approximately 0.8 hectares. Surrounding vegetation ranged from mature deciduous woodland to open areas of grasses and reeds.

Distribution of larvae in the ponds varied with the season. Beneath the ice cover of winter months the larvae were scattered in random distribution at depths of one to two meters. In warmer weather of fall and spring they were found concentrated in shallow water beneath submerged vegetation. With the natural onset of metamorphosis in the

spring, larvae in the final instar moved closer to the edge where they could climb upward on vegetation for their emergence into the adult form.

Only final-instar larvae were collected for the experiments. A hardy species, they survived very well the process of being scooped up and sifted out of the conglomerate mixture of mud, decaying leaves, and algae.

Installation. Before installation in experimental conditions, all larvae were measured on a Bogusch measuring slide with a binocular microscope in order to verify that all specimens were in the final instar. Preliminary work had established that final-instar larvae ranged in total length from about 17.8 mm to 23.2 mm.

Experimental conditions included two different photoperiods (11 and 14 hours of light in a 24-hour cycle) at each of four different temperatures (15, 20, 25 and 30 °C). This range of conditions had yielded meaningful results in previous research on odonates by other workers (Lutz and Jenner, 1960, 1964; Lutz, 1968b). Light-proof plywood cabinets in an air-conditioned laboratory, with temperature continuously monitored, provided accurate conditions for the 20 °C experiments. All other specified temperatures were maintained in modified Biological Oxygen Demand boxes (Precision Scientific Company). At each of the four temperatures, one box or cabinet was equipped to

provide 11 hours of light; the second box was equipped to provide 14 hours of light. Cool white fluorescent bulbs totalling 30 watts in each compartment supplied ample light intensity, well above the minimum level (less than 0.002 lux) found to induce a growth response in odonates (Lutz and Jenner, 1964). Automatic timing switches (General Electric Company) insured accuracy of the photoperiods.

Maintenance. Larvae were maintained in individual eight-ounce jars filled with pond water. In keeping with their predaceous habits, the larvae were fed enchytraeid worms twice weekly. A short length of dowel, one centimeter in diameter and 12 centimeters long, was placed in each jar to facilitate the normal process of emergence.

Prior to installation in the two highest temperatures, larvae were placed in the 20-degree room for at least one day as conditioning for the elevated temperatures. Larvae destined for the 30-degree boxes were maintained for 24 to 36 hours each at temperatures of 20 °C and 25 °C before final installation at 30 °C. This procedure appeared to decrease the mortality rate at high temperatures.

Criteria for Recording Data. All specimens were checked three or four times per week. Records were kept to indicate condition of each larva and the number of days ensuing between installation in the experimental condition and the date of emergence or death.

Any larva which at least started the process of emergence with the splitting of the exoskeleton was listed as having emerged. The conclusion of metamorphosis in such cases was obvious even if the larva did not successfully attain a viable adult form. In certain other cases dead larvae were found within the boxes, away from their jars. Presumably these had responded to the inherent stimulus to leave the water at the onset of emergence. They had fallen from the dowels and died before the dorsal thorax split open in emergence. Such larvae were dissected and if their adult wings were fully formed within the wing pads, they were deemed ready to emerge and were recorded as having completed metamorphosis. Those larvae which were too desiccated for such dissection were simply omitted from the analysis of emergence data.

One comprehensive sample of the total population was taken on 29 January 1970, in order to establish the size range of the overwintering larvae. Those which had not yet entered the final instar were returned to the ponds after measurement.

RESULTS

After calculation of mean days-to-emergence under all conditions in all collections, it was apparent that growth rates were generally stimulated by the longer photoperiod and by increased temperature. There was a wide range in the degree of stimulation, however, with the greatest effects developing at the two lowest temperatures. Particular effects of the longer light period or increased temperature were obscured most often at the two highest temperatures. Statistical methods were therefore used to identify significant differences in the computed averages. Within each collection all data were subjected to the t-test, which allowed comparison between any two conditions and which took into account the sample size and standard error. Results of the t-tests are included in the account of each collection in the form of a list of significant differences at the 5% level.

Collection #1. 28 September 1969 (Figure 1A)

At 20 °C and at 25 °C the average emergence time of the 14-hr larvae was less than half that of the 11-hr larvae. No larvae emerged under the 11-hr photoperiod at either 15 °C or 30 °C, although larvae survived to a maximum of 270 and 103 days respectively in those boxes. Deaths were recorded

throughout these periods without undue mortality at any one time. The greatest effect of temperature differential was noted in the averages at 15 and 20 °C under 14 hours of light.

Significant differences:
a. Photoperiod as variable
11-hr/14-hr at 20 °C
" at 25 °C

b. Temperature as variable
At 11-hr: 20°/25° At 14-hr: 15°/20°
15°/25°
15°/30°
20°/30°

Collection #2. 2 November 1969 (Figure 1B)

The second collection produced results similar to those in the first collection, in that the 11-hr larvae required a longer average time to complete metamorphosis than did those larvae maintained under the longer photoperiod. These results were evidenced at temperatures of 15, 20, and 25 °C but the difference was not significant at 25 °C. No larvae emerged at 30 °C. The effect of temperature differential was most obvious under the 11-hr photoperiod where highly significant differences were found. A highly significant difference also resulted under the 14-hr photoperiod in the contrast between the two lowest temperatures.

Significant differences:
a. Photoperiod as variable
11-hr/14-hr at 15 °C
" at 20 °C

b. Temperature as variable

At 11-hr:		At 14-hr:	
	15°/20°		15°/20°
	15°/25°		15°/25°
	20°/25°		

Collection #3. 28 November 1969 (Figure 2A)

Emergence times generally followed the pattern set in the first two collections, the significant differences being designated below. Again, no larvae emerged at 30 °C under the shorter photoperiod, a fact discussed later under seasonal variation.

Significant differences:

a. Photoperiod as variable

11-hr/14-hr at 15 °C
" at 25 °C

b. Temperature as variable

At 11-hr:		At 14-hr:	
	15°/20°		15°/20°
	15°/25°		15°/25°
			15°/30°
			20°/25°
			20°/30°
			25°/30°

The next three collections, all mid-winter, might well be considered together due to close similarity of results. Four pertinent trends should be noted. First, average time to emergence was shorter at all temperatures under the 14-hr photoperiod, with only one minor exception (collection of 29 January 1970 at 30 °C). Second, an increase in temperature resulted in shorter average times to emergence under similar photoperiods. This is particularly evident in contrasting results at 15 and 20 °C in each collection. Larvae at 20 °C emerged in approximately

Figure 1. Average days to emergence of larvae under controlled conditions of photoperiod and temperature. Standard deviations shown.

A: Collection of 28 September 1969

B: Collection of 2 November 1969

Broken line = responses of larvae under 11-hr photoperiod

Solid line = responses of larvae under 14-hr photoperiod

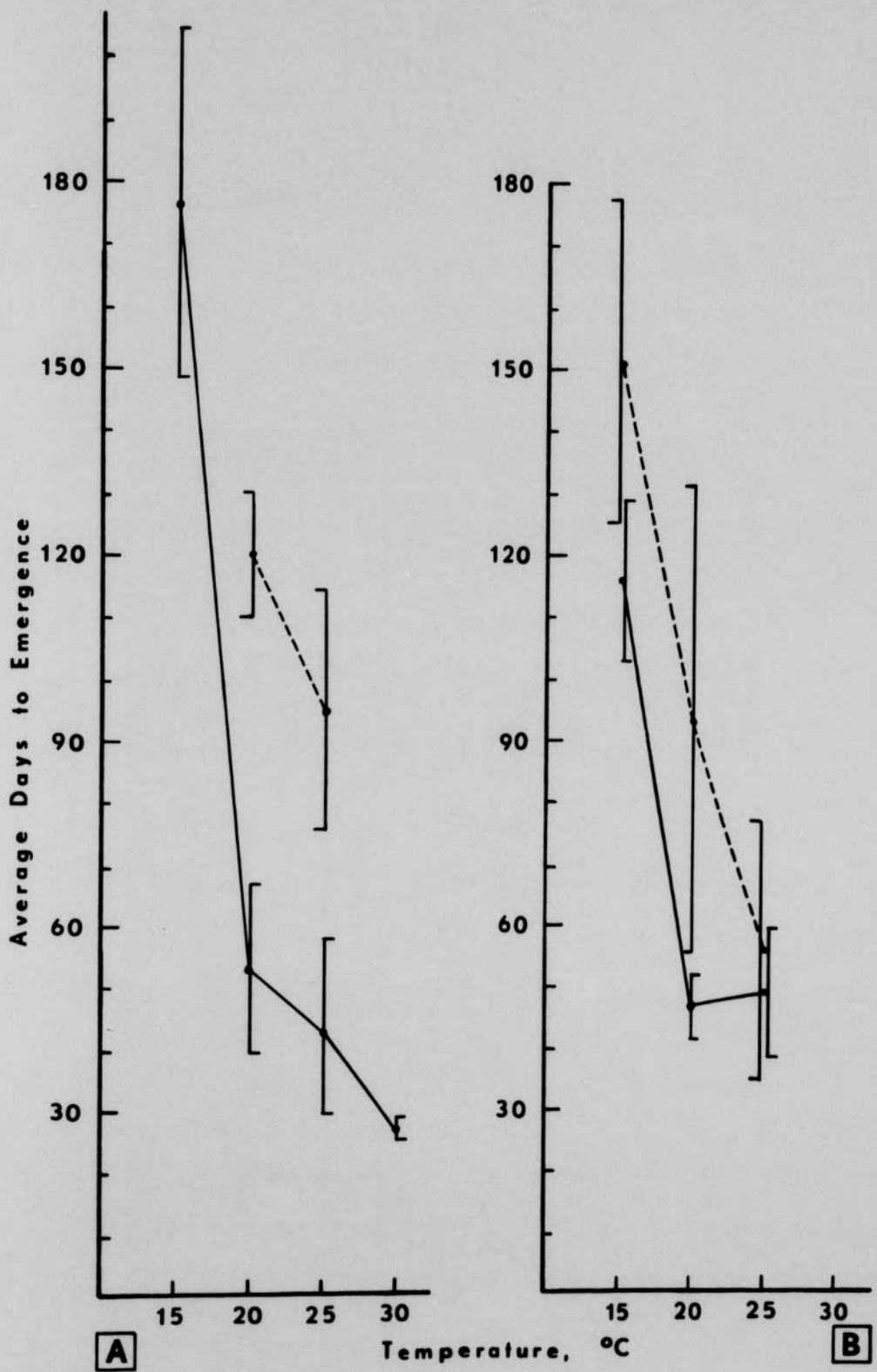

Average Days to Emergence
180
150
120
90
60
30
15
20
25
30
A
B
Temperature, °C

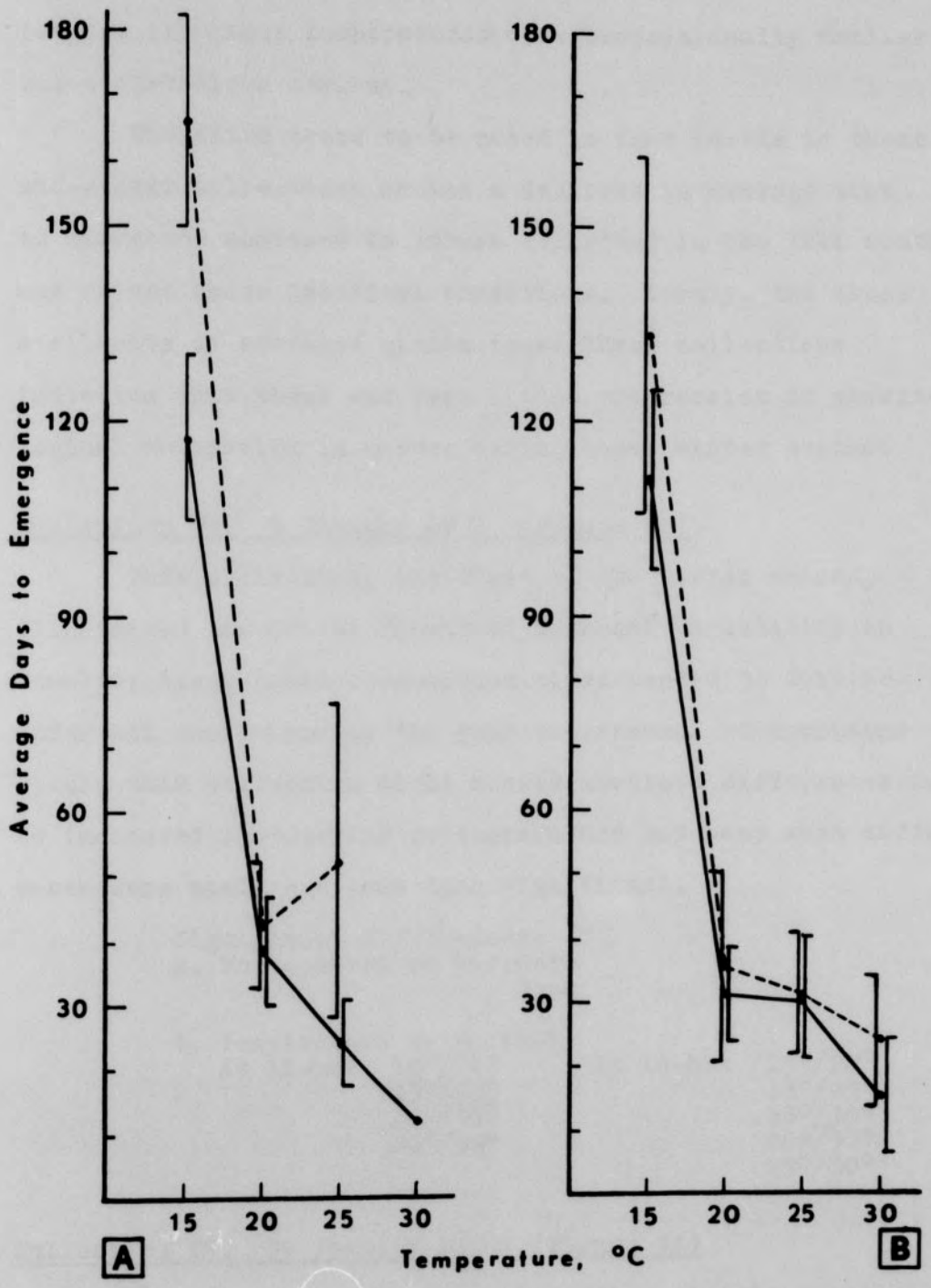
Figure 2. Average days to emergence of larvae under controlled conditions of photoperiod and temperature. Standard deviations shown.

A: Collection of 28 November 1969

B: Collection of 1 January 1970

Broken line = responses of larvae under 11-hr photoperiod

Solid line = responses of larvae under 14-hr photoperiod


180
150
120
90
60
30
Average Days to Emergence
15
20
25
30
A
Temperature, °C
B

one-third the average time required at 15 °C. Differences between the other temperatures were proportionally smaller but nevertheless obvious.

The third trend to be noted is that larvae in these mid-winter collections showed a decrease in average time to emergence compared to larvae collected in the fall months and raised under identical conditions. Lastly, the close similarity of averages within these three collections indicates that there was very little progression in physiological maturation in nature during these winter months.

Collection #4. 1 January 1970 (Figure 2B)

This collection, the first of the winter season, illustrated one of the facets of seasonal variability in results; i.e., average emergence times tended to decline under all conditions as the year progressed. Comparisons within this collection alone showed absolute differences due to increased photoperiod or temperature but many such differences were small and less than significant.

Significant differences:

a. Photoperiod as variable
None

b. Temperature as variable

At 11-hr:		At 14-hr:	
	15°/20°		15°/20°
	15°/25°		15°/25°
	15°/30°		15°/30°
	20°/30°		20°/30°
			25°/30°

Collection #5. 29 January 1970 (Figure 3A)

Absolute values in this collection closely paralleled

those of the preceding collection but more significant differences were found. Increased photoperiod and temperature were clearly stimulatory in nearly all conditions.

Significant differences:
a. Photoperiod as variable
11-hr/14-hr at 15 °C
" at 20 °C
" at 25 °C

b. Temperature as variable

At 11-hr:		At 14-hr:	
	15°/20°		15°/20°
	15°/25°		15°/25°
	15°/30°		15°/30°
	20°/25°		20°/25°
	20°/30°		
	25°/30°		

Collection #6. 28 February 1970 (Figure 3B)

The continued close similarity of results in this and the two preceding collections indicated that little progression in physiological maturation occurred during the winter months. Absolute differences in averages were once again recorded in the usual pattern but significant differences were calculated primarily at the differential between the two lowest temperatures. There were no significant differences attributable to photoperiod.

Significant differences:
a. Photoperiod as variable
None

b. Temperature as variable

At 11-hr:		At 14-hr:	
	15°/20°		15°/20°
	15°/25°		15°/25°
	15°/30°		15°/30°
	20°/30°		20°/30°
			25°/30°

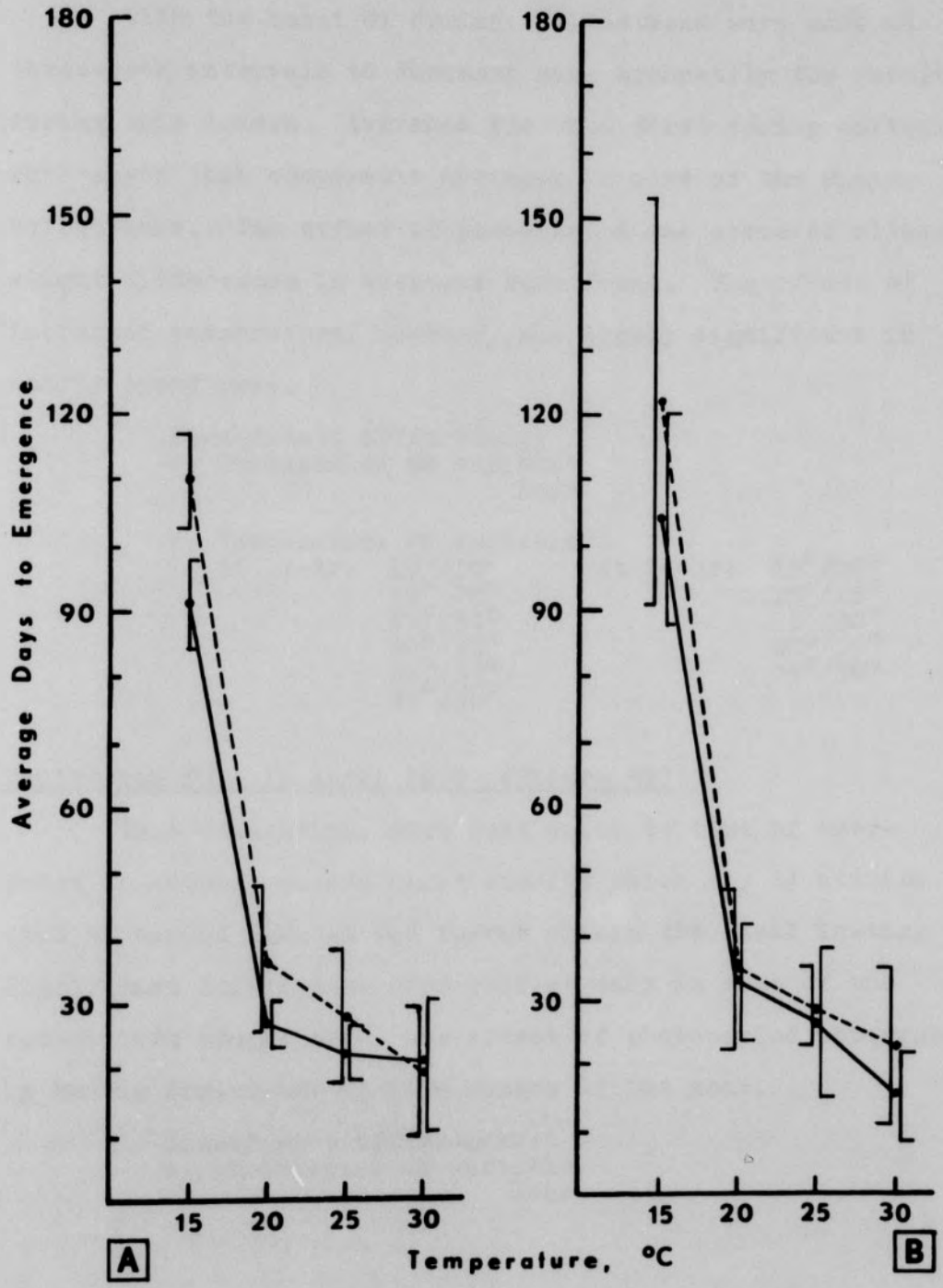
Figure 3. Average days to emergence of larvae under controlled conditions of photoperiod and temperature. Standard deviations shown.

A: Collection of 29 January 1970

B: Collection of 28 February 1970

Broken line = responses of larvae under 11-hr photoperiod

Solid line = responses of larvae under 14-hr photoperiod


180
150
120
90
60
30
Average Days to Emergence
15
20
25
30
A
Temperature, °C
B

Collection #7. 21 March 1970 (Figure 4A)

With the onset of spring, collections were made at three-week intervals to document more accurately the results during this season. Averages for this first spring collection were lower than comparable averages in most of the winter collections. The effect of photoperiod was obscured although slight differences in averages were found. The effect of increased temperature, however, was highly significant in nearly every case.

Significant differences:
a. Photoperiod as variable
None

b. Temperature as variable

At 11-hr:		At 14-hr:	
	15°/20°		15°/20°
	15°/25°		15°/25°
	15°/30°		15°/30°
	20°/25°		20°/25°
	20°/30°		20°/30°
	25°/30°		

Collection #8. 11 April 1970 (Figure 4B)

This collection, made just prior to time of emergence in nature, showed mixed results which may be attributed to varied ages of the larvae within the final instar. Significant differences were evident only in some of the temperature comparisons, the effect of photoperiod apparently having diminished at this season of the year.

Significant differences:
a. Photoperiod as variable
None

b. Temperature as variable

At 11-hr:		At 14-hr:	
	15°/20°		15°/20°
	15°/25°		15°/25°
	15°/30°		15°/30°
	20°/30°		20°/30°
	25°/30°		25°/30°

Collection #9. 3 May 1970 (Figure 5)

Even the inconsistent results in this collection were valuable since they demonstrated that the effect of photoperiod and temperature may vary with the time of year and the physiological maturity of the larvae at time of collection. The results at 15 °C are technically incomplete since larvae were still surviving when final comparisons were made on 22 July 1970, due to time limitations. It should be noted that these larvae at 15 °C showed a significant difference that was diametrically opposite to the normal pattern. The 11-hr larvae emerged in approximately half the time required by the 14-hr larvae, but this ratio may well be reversed when all emerge.

Significant differences:

a. Photoperiod as variable

11-hr/14-hr at 15 °C (Reversal)

b. Temperature as variable

At 11-hr:		At 14-hr:	
	20°/30°		15°/20°
	25°/30°		15°/25°
			15°/30°
			25°/30°

While all the t-tests listed above designate the 5% level of significance (equal to 95% level of confidence), it is pertinent to note that all those tests comparing

Figure 4. Average days to emergence of larvae under controlled conditions of photoperiod and temperature. Standard deviations shown.

A: Collection of 21 March 1970

B: Collection of 11 April 1970

Broken line = responses of larvae under 11-hr photoperiod

Solid line = responses of larvae under 14-hr photoperiod

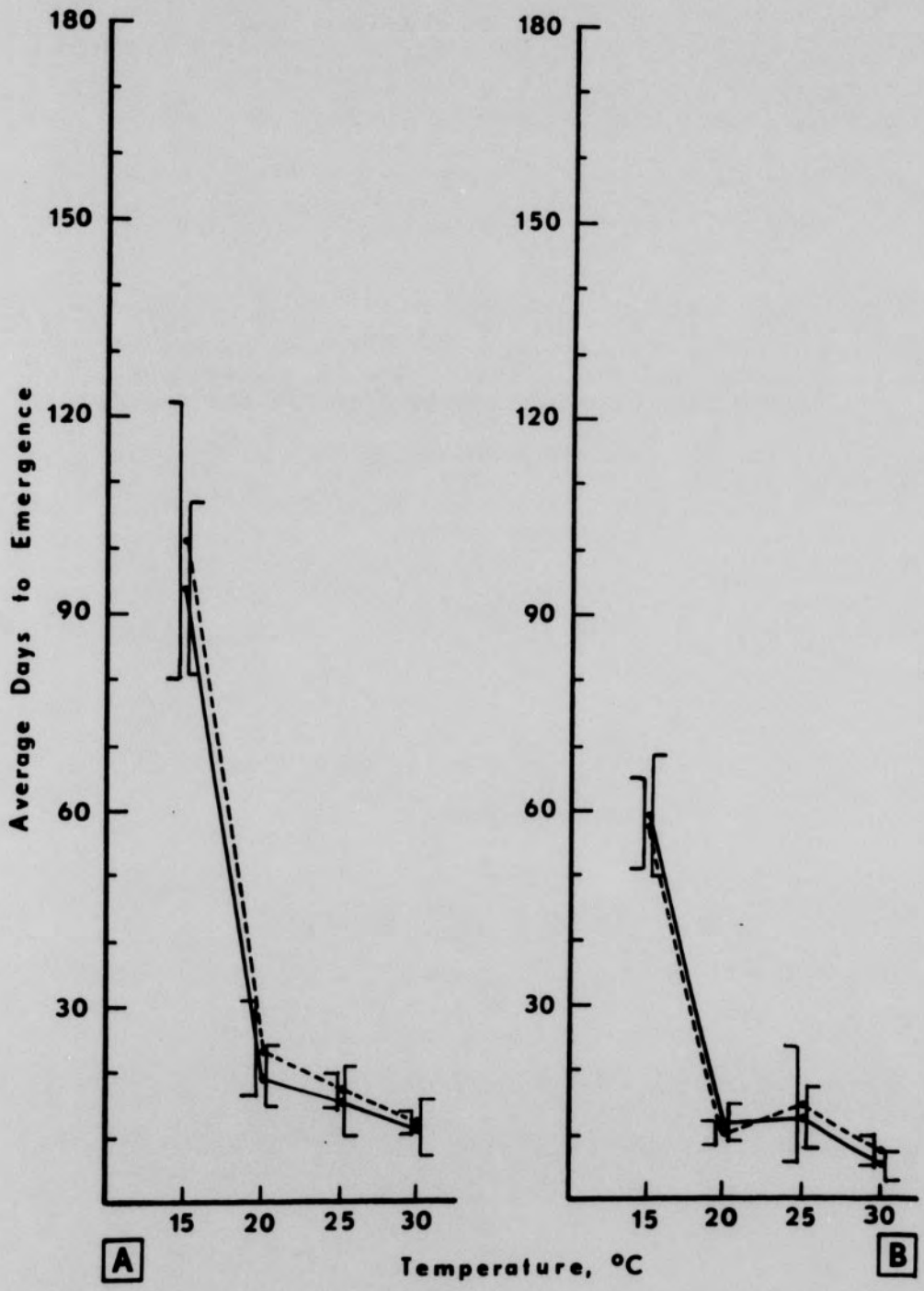

180
150
120
90
60
30
Average Days to Emergence
15
20
25
30
A
B
Temperature, °C

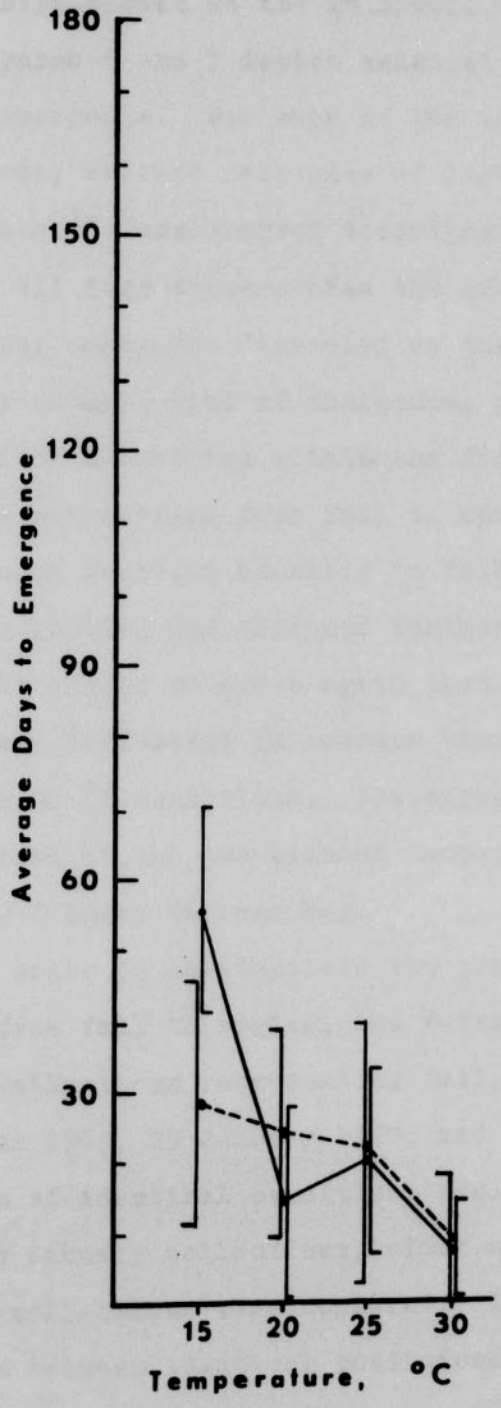
Figure 5. Average days to emergence of larvae under controlled conditions of photoperiod and temperature. Standard deviations shown.

Collection of 3 May 1970

Broken line = responses of larvae under 11-hr photoperiod

Solid line = responses of larvae under 14-hr photoperiod

Note: 15 °C results recorded as of 22 July 1970. Seven larvae still surviving under 11-hr photoperiod; four larvae surviving under 14-hr photoperiod.


180
150
120
90
60
30
Average Days to Emergence
15
20
25
30
Temperature, °C

averages due to the thermal influence of 15 and 20 °C are actually significant at the 1% level.

Figures 6 and 7 depict seasonal variation in average times to emergence. For each of the constant experimental temperatures, average responses of larvae under each of the two photoperiods are plotted according to the date of collection. At all four temperatures the average number of days required for emergence decreased as the season progressed toward the natural time of emergence, presumably due to physiological maturation within the final instar. The decrease was not uniform from fall to spring, however. Rather, most averages declined steadily in fall collections, leveled off in the winter, and declined further with the onset of spring. It should be noted again that at all seasons there was a marked difference in average time to emergence under the 15 and 20 °C conditions. The effect of thermal influence was minimized at the two highest temperatures in the collections from January through May.

In order to substantiate the progressive decrease in averages from fall to spring, the t-test was applied to three selected collections representing fall, winter, and spring (2 November 1969, 29 January 1970, and 11 April 1970). The comparison of identical conditions was made between the November and January collections, after which the January and April collections were compared. In every instance the difference between identical conditions was significant.

Figure 6. Seasonal variation in responses of larvae to constant temperatures of 15 and 20 °C.

Open circles = average days to emergence at 15 °C

Closed circles = average days to emergence at 20 °C

Broken line = responses of larvae under 11-hr photoperiod

Solid line = responses of larvae under 14-hr photoperiod

Note: Results at 15 °C in collection of 3 May 1970 recorded as of 22 July 1970. Seven larvae still surviving under 11-hr photoperiod; four larvae surviving under 14-hr photoperiod.

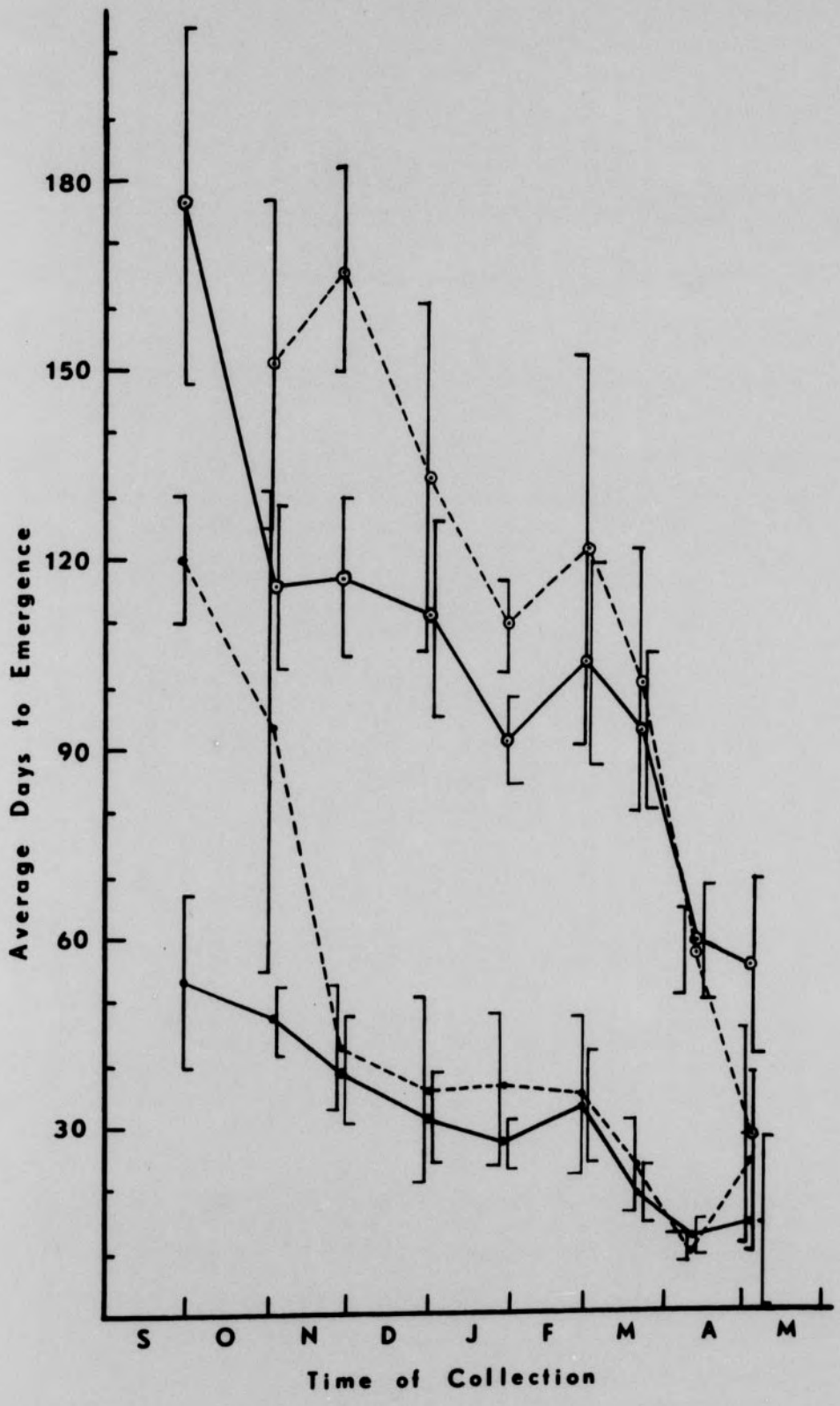

180
150
120
90
60
30
Average Days to Emergence
S
O
N
D
J
F
M
A
M
Time of Collection

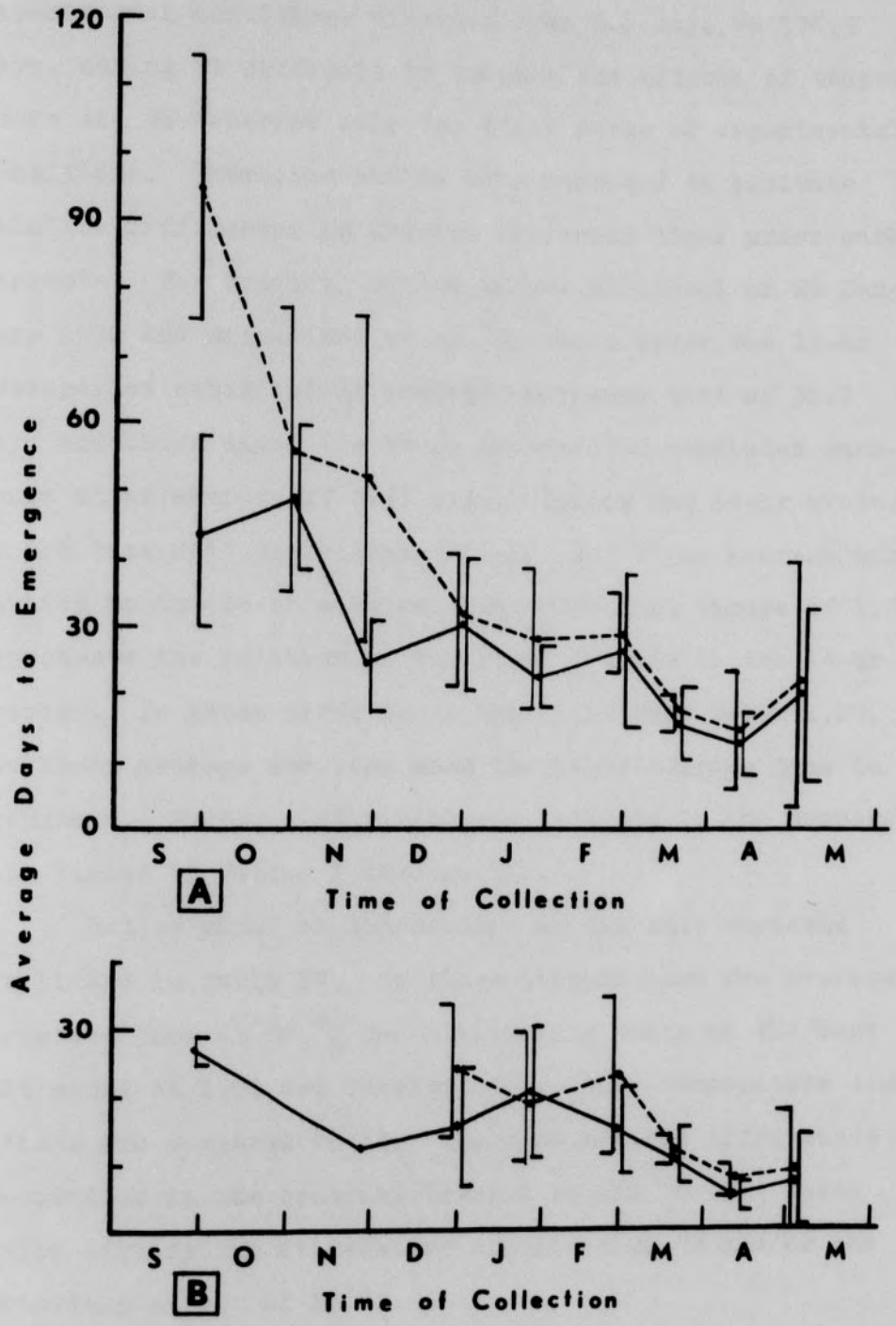
Figure 7. Seasonal variation in responses of larvae to constant temperatures of 25 and 30 °C.

A: Average days to emergence at 25 °C

B: Average days to emergence at 30 °C

Broken line = responses of larvae under 11-hr photoperiod

Solid line = responses of larvae under 14-hr photoperiod


120
90
60
30
0
Average Days to Emergence
S O N D J F M A M
A
Time of Collection
30
S O N D J F M A M
B
Time of Collection

The numerical averages of all collections under all experimental conditions extended from 5.0 days to 176.5 days, making it difficult to compare the effects of temperature and photoperiod over the total range of experimental conditions. Therefore ratios were computed to indicate relative differences in average emergence times under each variable. For example, of the larvae collected on 29 January 1970 and maintained at 20 °C, those under the 11-hr photoperiod exhibited an average emergence time of 36.9 days and those under the 14-hr photoperiod completed emergence at an average of 27.1 days. Taking the 14-hr average as the base unit equivalent to 1.00, the 11-hr average was divided by the 14-hr average. The resultant figure of 1.36 represents the relation of the 11-hr average to the 14-hr average. In those cases where the ratio fell below 1.00, the 11-hr average was less than the 14-hr average time to emergence. Photoperiod ratios are included in the summary data listed in Tables I through III.

Ratios based on temperature as the sole variable are listed in Table IV. In these computations the average emergence time at 20 °C was arbitrarily taken as the base unit equal to 1.00 and results under other temperature conditions are compared to it. The most notable differences are obvious in the contrast between 15 and 20 °C. These ratios signify the stimulatory effect of 20 °C and/or the inhibitory effect of 15 °C.

TABLE I

Summary of results in the three fall collections, listing number of larvae emerging, average days to emergence, standard deviation, and 11:14 photoperiodic ratio.

Date of Collection	°C		No.	Aver. Days	S.D.	Ratio 11:14
28 Sept 69	15	11-hr:	0	-	-	-
		14-hr:	4	176.5	28.2	
	20	11-hr:	5	120.2	10.2	2.26
		14-hr:	8	53.3	13.9	
	25	11-hr:	3	95.0	19.5	2.18
		14-hr:	3	43.7	14.2	
	30	11-hr:	0	-	-	-
		14-hr:	2	27.0	1.4	
2 Nov 69	15	11-hr:	4	151.3	26.0	1.30
		14-hr:	2	116.0	12.7	
	20	11-hr:	10	93.4	38.0	1.98
		14-hr:	5	47.0	5.4	
	25	11-hr:	7	55.9	21.2	1.14
		14-hr:	4	49.0	10.4	
	30	11-hr:	0	-	-	-
		14-hr:	0	-	-	
28 Nov 69	15	11-hr:	5	166.0	16.1	1.41
		14-hr:	7	117.6	12.9	
	20	11-hr:	8	42.5	9.8	1.11
		14-hr:	10	38.7	8.2	
	25	11-hr:	4	52.3	24.1	2.12
		14-hr:	3	24.7	6.4	
	30	11-hr:	0	-	-	-
		14-hr:	1	12.0	-	

TABLE II

Summary of results in the three winter collections, listing number of larvae emerging, average days to emergence, standard deviation, and 11:14 photoperiodic ratio.

Date of Collection	°C		No.	Aver. Days	S.D.	Ratio 11:14
1 Jan 70	15	11-hr:	5	133.6	27.2	1.20
		14-hr:	6	111.2	13.6	
	20	11-hr:	9	35.4	14.5	1.14
		14-hr:	8	31.1	7.3	
	25	11-hr:	13	31.5	9.7	1.02
		14-hr:	13	30.8	9.7	
	30	11-hr:	6	24.0	9.9	1.57
		14-hr:	6	15.3	8.8	
29 Jan 70	15	11-hr:	2	110.0	7.1	1.20
		14-hr:	4	91.5	6.4	
	20	11-hr:	9	36.9	11.1	1.36
		14-hr:	11	27.1	3.6	
	25	11-hr:	16	28.2	10.3	1.24
		14-hr:	12	22.7	4.7	
	30	11-hr:	6	19.8	9.8	0.96
		14-hr:	5	20.6	10.1	
28 Feb 70	15	11-hr:	2	122.0	31.1	1.17
		14-hr:	6	104.2	16.4	
	20	11-hr:	10	34.7	12.3	1.05
		14-hr:	7	33.0	9.1	
	25	11-hr:	9	28.9	6.0	1.08
		14-hr:	9	26.7	11.7	
	30	11-hr:	5	23.0	11.8	1.47
		14-hr:	5	15.6	7.6	

TABLE III

Summary of results in the three spring collections, listing number of larvae emerging, average days to emergence, standard deviation, and 11:14 photoperiodic ratio.

Date of Collection	°C		No.	Aver. Days	S.D.	Ratio 11:14
21 Mar 70	15	11-hr:	7	101.0	20.8	1.08
		14-hr:	5	93.8	13.0	
	20	11-hr:	9	23.6	7.3	1.22
		14-hr:	11	19.3	4.5	
	25	11-hr:	10	17.1	2.5	1.09
		14-hr:	10	15.7	5.2	
	30	11-hr:	9	11.9	1.7	1.05
		14-hr:	4	11.3	4.3	
11 Apr 70	15	11-hr:	4	57.8	6.8	0.97
		14-hr:	6	59.3	9.4	
	20	11-hr:	7	10.4	1.8	0.87
		14-hr:	12	11.9	2.7	
	25	11-hr:	8	14.6	8.8	1.18
		14-hr:	11	12.4	4.6	
	30	11-hr:	11	7.5	2.5	1.48
		14-hr:	11	5.0	2.1	
3 May 70	*15	11-hr:	3	28.3	17.5	0.51
		14-hr:	6	55.7	14.4	
	20	11-hr:	7	24.3	14.6	1.70
		14-hr:	9	14.3	13.7	
	25	11-hr:	10	21.3	18.4	1.05
		14-hr:	12	20.2	12.9	
	30	11-hr:	11	8.7	9.3	1.19
		14-hr:	12	7.3	6.8	

* 15 °C results recorded as of 22 July 1970. Seven larvae still surviving under 11-hr photoperiod; four larvae surviving under 14-hr photoperiod.

TABLE IV

Ratios of results under four temperature conditions, as evidenced by growth rates of larvae of P. lydia. Under each photoperiod the average time to emergence at 20 °C is taken as a base unit equal to 1.00 and averages at other temperatures are compared to it.

		Ratio			
Date of Collection	Photoperiod	15 °C	20 °C	25 °C	30 °C
28 Sept 69	11-hr:	-	1.00	0.79	-
	14-hr:	3.31	1.00	0.82	0.51
2 Nov 69	11-hr:	1.62	1.00	0.60	-
	14-hr:	2.46	1.00	1.04	-
28 Nov 69	11-hr:	3.91	1.00	1.23	-
	14-hr:	3.02	1.00	0.64	0.31
1 Jan 70	11-hr:	3.78	1.00	0.89	0.68
	14-hr:	3.58	1.00	0.99	0.49
29 Jan 70	11-hr:	2.98	1.00	0.76	0.54
	14-hr:	3.38	1.00	0.84	0.76
28 Feb 70	11-hr:	3.52	1.00	0.83	0.66
	14-hr:	3.16	1.00	0.81	0.47
21 Mar 70	11-hr:	4.28	1.00	0.73	0.50
	14-hr:	4.86	1.00	0.81	0.59
11 Apr 70	11-hr:	5.56	1.00	1.40	0.72
	14-hr:	4.98	1.00	1.04	0.42
3 May 70	11-hr:	*1.16	1.00	0.88	0.36
	14-hr:	*3.89	1.00	1.41	0.51

* 15 °C results recorded as of 22 July 1970. Seven larvae still surviving under 11-hr photoperiod; four larvae surviving under 14-hr photoperiod.

In order to understand better the total nature of the entire overwintering population of P. lydia, a large comprehensive larval sample was taken on 29 January 1970. Assignment of larvae to instars other than the final one could not be made reliably; therefore, the data are shown in increments of 1 mm in Table V. The wide range of the measurements denoted that at least six or seven instars were represented in the winter population, with final-instar larvae comprising 18.0% of the total sample. This corroborates the classification of P. lydia as a summer species with little or no synchronization of the overwintering larval stages.

TABLE V

Random sample of *Plathemis lydia* larvae on 29 January 1970, showing structure of the overwintering population.

	Length in mm	Number	% of Total
	5.0 - 5.9	1	0.4
	6.0 - 6.9	6	2.1
	7.0 - 7.9	11	3.9
	8.0 - 8.9	20	7.1
	9.0 - 9.9	54	19.2
	10.0 - 10.9	9	3.2
	11.0 - 11.9	25	8.9
	12.0 - 12.9	32	11.3
	13.0 - 13.9	5	1.8
	14.0 - 14.9	19	6.7
	15.0 - 15.9	41	14.5
	16.0 - 16.9	8	2.8
	17.0 - 17.9	0	0.0
(Final)	18.0 - 18.9	10	3.6
(")	19.0 - 19.9	26	9.2
(")	20.0 - 20.9	15	5.3
		282	100.0

DISCUSSION

Three general inferences may be drawn from the results of these experiments:

(1) Final-instar larvae of Plathemis lydia responded to length of photoperiod, usually showing more rapid rate of maturation under the longer photoperiod than under the shorter photoperiod.

(2) Temperature influenced growth rate. Higher temperatures enhanced the rate of development although not in a straight-line relationship. The five-degree increase between 15 and 20 °C produced a disproportionate increase in growth rates and therefore may represent some form of temperature threshold for metamorphosis in the life cycle.

(3) Seasonal variation influenced the effect of photoperiod and temperature. Thus, endogenous physiological factors may mitigate the effect of abiotic exogenous factors.

All the above points may be applied to what is presently known about the life cycle of P. lydia. The three topics will be discussed separately although they doubtless operate conjointly in nature.

As a summer species with an extended flight season from April to October, P. lydia displays little of the synchronization of emergence which characterizes spring species of odonates. Corbet (1958b, 1964) defines the

latter as those emerging within a relatively compressed time span in the spring, achieving synchronization either by a diapause stage or by the mechanism of lower temperature thresholds. Synchronization is considered by Corbet (1962, 1964) to be a late adaptation of those species which dispersed into regions having only relatively short seasons suitable for reproduction, due to temperature or rainfall patterns. While synchronization is indeed a favorable adaptation in the breeding of such species, it is not without its disadvantages. Overcrowding of suitable breeding sites, food shortage, increased territorial competition of males, unseasonal weather, all may contribute to the rate of attrition. Conversely, the more primitive species with minimum synchronization, although unable to colonize new zones, face less competition for food, mates, and oviposition sites. Mating success is insured in these species by a longer average adult flight season (Moore, 1952).

P. lydia exhibits minimal synchronization to the extent that its flight season is confined to approximately six months of the year. Final-instar larvae are included in the overwintering population but there is no evidence of a diapause stage which might serve as a synchronizing mechanism. The responses of the final-instar larvae to light and temperature, however, could well determine the broad limits of the emergence range in P. lydia.

The ability to respond to a photoperiodic stimulus

is of particular value to a small, aquatic, poikilothermous insect larva. Cold vernal temperatures show an even more pronounced lag in the aquatic environment than on land. This negates the value of temperature increase as a primary stimulus for metamorphosis in the early spring. However, the increase in daylength in late winter could in itself accelerate the process of maturation within the final instar. By the time metamorphosis is completed the air temperature would be suitable for adult flight and breeding. Once the average temperature rises above a certain threshold level, photoperiod and temperature might serve conjointly as stimuli for metamorphosis.

The results of these experiments support this theory in that the longer photoperiod had its most pronounced effect at the lowest experimental temperature. The effect of the longer photoperiod declined at 20 °C and above.

In the spring the most mature final-instar larvae would naturally emerge first, after being exposed first to increasing photoperiod and later to rising temperatures. Younger larvae would continue to molt, metamorphose, emerge, mature sexually, and reproduce during the spring and summer months when both temperature and photoperiod are stimulatory. Meanwhile, the eggs laid early in the flight season would follow the normal pattern of eclosion and molts presumably entering the final instar in mid-September, although

this has not been corroborated by a detailed life-history study. At the collection site no final-instar larvae were found in early September, only a few were found on 13 September 1969, but 91 were collected on 28 September 1969. Water temperatures in September are, on an average basis, as high as during much of the emergence season, yet the new final-instar larvae delay their emergence until the following spring. The pattern of decreasing photoperiod in the fall may well be one inhibitory factor. Stated conversely, the larvae may fail to metamorphose and emerge due to lack of a long photoperiod as a positive stimulus. Physiological factors discussed under seasonal variation could also inhibit emergence at this season.

Temperature exerts its influence on the life cycle of P. lydia by the fundamental process of increasing or decreasing metabolic rates, although not in a straight-line relationship at the experimental temperatures. In general, it may be said that the larvae exhibited most rapid growth rates coupled with highest survival rates at 20 °C and at 25 °C, and therefore these typical summer temperatures may represent the optimal range for maturation in the final instar. At 30 °C growth rates were high but the mortality rates were likewise high in the fall collections. This may be an example of the fact that the metabolic rate is inversely related to the duration of life. Clark and Rockstein (in Rockstein, 1964) state that accumu-

lation of deleterious substances or loss of irreplaceable materials may cause more rapid aging in insects reared at high temperatures.

At the lowest experimental temperature all larvae matured at a markedly slower rate than at the next higher temperature. Natural winter temperatures in this locality are well below the lowest experimental temperature and it may be inferred that growth in nature proceeds at a minimal rate during these months. Whether or not lower temerature thresholds are operative for synchronization of some of the younger larval stages could not be determined without special experimental collections. Lutz (1968a) presented evidence of such thresholds in another summer species, the damselfly Lestes eurinus, but in that species no larvae overwintered in the final instar as does P. lydia. The effect of diurnal temperature changes has not been studied.

Seasonal variation may explain some of the anomalous results in certain of these experiments. The larvae of the last two collections (11 April 1970 and 3 May 1970) deviated in several instances from patterns established in previous collections. This may have been due to varied ages of the larvae. Some may have already spent months in the final instar and may even have commenced metamorphosis when collected. They presumably had already received natural stimuli and may have been ready to emerge regardless of experimental conditions. Other larvae may have entered the

final instar just prior to collection. They would require a certain minimum time to complete metamorphosis under any circumstances, favorable or unfavorable. Altogether, the spring collections did not produce as many significantly different results due to endogenous factors and variability in age of final-instar larvae.

The first collection in the fall (28 September 1969) also produced some results which may have been due to seasonal variation. No larvae emerged under the 11-hr photoperiod at 15 °C. It should be noted that these larvae had molted into the final instar only shortly before collection. They were immediately placed under a photoperiod less than the natural daylength at the time of collection. In sum, they had spent minimum time under natural conditions, which may well include exogenous stimuli as yet unknown. They received no artificial stimuli of rising temperature or increased daylength. Thus metamorphosis was inhibited despite survival to a maximum of 270 days.

Lack of emergence at 30 °C in the three fall collections, at either or both photoperiods, cannot be traced consistently to any one factor. One possible explanation may be that final-instar larvae have different temperature tolerances which vary with the season. Mortality rates were high at 30 °C in the fall collections and were low at the same temperature in the spring collections.

Developmental variation doubtless operates to com-

plicate the response of larvae to photoperiod and temperature at any time of year; however, such endogenous factors appear to exert minimum influence in late fall and winter. That season would therefore be the optimal time for further study of photoperiodic and thermal influences on the larval growth rate of this species.

SUMMARY

Nine collections of final-instar larvae of Plathemis lydia were made at intervals through fall, winter, and spring months and the larvae were then maintained under controlled conditions of photoperiod and temperature. Two photoperiods (11-hr and 14-hr) were used at each of four constant temperatures (15, 20, 25, and 30 °C). The number of days ensuing between the collection of the larvae and their emergence into adult form was taken as the parameter of growth.

Statistical data from the experiments indicated the following: (1) the longer photoperiod enhanced the growth rate and the shorter photoperiod inhibited the rate of growth; (2) higher temperatures increased the growth rate, particularly at the differential between 15 and 20 °C; (3) seasonal variation caused a decrease in specific effects of experimental photoperiods and temperatures as the time of emergence in the natural life cycle approached.

Both temperature and photoperiod are considered as factors in the seasonal regulation of the life cycle. The suggestion is made that increasing photoperiod, rather than temperature, is the primary stimulus for metamorphosis in the early spring. Following the rise in average temperatures above a certain threshold level, long photoperiod

and optimal summer thermal conditions might serve jointly as stimuli for metamorphosis of the final-instar larvae throughout the remainder of the emergence season.

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