PITTMAN, ANNETTE ROGERS. Life-History Study of <u>Libellula</u> <u>incesta</u> with Emphasis on Egg Development as Influenced by Controlled Temperature (1971) Directed by: Dr. Paul E. Lutz.   pp. 75.

A field and laboratory study was carried out with <u>Libellula</u> <u>incesta</u> to investigate the effects of controlled temperature and photoperiod on egg development and to determine various aspects of the life cycle.

Eggs collected from mating females were subjected to two photoperiods (11 and 14 hours) at each of six different temperatures (15, 20, 25, 30, 35, and 40°C). <u>Libellula</u> <u>incesta</u> eggs were not effected by difference in photoperiods, but total development time decreased with increases in temperature.

Information concerning the life-history was obtained by sampling the larval population and field observation throughout the year. It was established that the eggs of <u>Libellula</u> <u>incesta</u> experienced direct development in about one to two weeks. In addition it was found that <u>Libellula</u> <u>incesta</u> is a univoltine summer species which overwinters in one of four instars prior to the final. Emergence began in mid-May and individuals flew until mid-October. Throughout this flight season the reproductive activity was observed. Males established territories in which they interacted with other males and mated with females. The females frequented the water only to mate and to exophytically oviposit large quantities of eggs.

LIFE-HISTORY STUDY OF LIBELLULA INCESTA WITH

EMPHASIS ON EGG DEVELOPMENT AS INFLUENCED
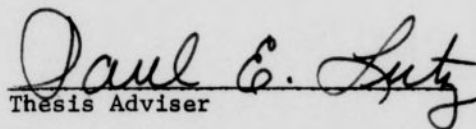
BY CONTROLLED TEMPERATURE

by

Annette Rogers Pittman

A Thesis Submitted to
the Faculty of the Graduate School at
the University of North Carolina at Greensboro
in Partial Fulfillment
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Approved by

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APPROVAL SHEET

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To Dr. Bruce M. Eberhart, Head, and faculty and staff of the Department of Biology, I express sincere appreciation for their efforts related directly to this study as well as their high level of instruction which greatly broadened my view of biology.

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CHAPTER I

INTRODUCTION

In the past, complete life-history studies have been a rather neglected aspect of odonate biology. Some investigations have established life-cycle stages by experimentally rearing larvae in the laboratory. The most notable of these were by Gardner (1950a, 1950b, 1951a, 1951b, 1953). Those life-history studies employing a program of population sampling in nature throughout a complete year are considerably more accurate. This method was used successfully by Corbet 1956, 1957a), Eller (1964), Kormondy (1959), and Lutz and Jenner (1964) to study species of Anisoptera. The present life-history study likewise used sampling from a natural population to establish the larval population structure and growth trends. Corbet (1962) distinguished between the two typical larval population structures by designating one group as spring species and the other as summer species. This designation was made on the basis that a spring species overwinters in the final instar and has a well synchronized emergence period, while a summer species overwinters in one of several instars prior to the final and has a longer, less synchronized emergence period.

The only reports concerning the larval growth within the genus Libellula were by Gardner (1953) and Wilson (1917). Gardner's

investigation of _Libellula depressa_ was a life-history study based on laboratory-reared larvae, and Wilson in his study gave a description of two species of _Libellula_ larvae and eggs and explained how they related to pondfish cultures.

Field observations on particular aspects of adult behavior within the suborder Anisopters have been carried out by Wilson (1917), Montgomery (1945, 1947), Moore (1951, 1952a, 1952b, 1953, 1962), Fraser (1953), Jacobs (1955), Kormondy (1959), Corbet, Longfield, and Moore (1960), Johnson (1962), Pajunen (1962a, 1962b, 1963, 1964a, 1964b, 1966), Lutz and Jenner (1964), Andrew (1966), and Lutz and Pittman (1970), but little has been done in depth on adult behavior within the genus _Libellula_. Corbet, Longfield, and Moore (1960) reported on a typical day in the life of an adult dragonfly and used _Libellula quadrimaculata_ as the example. Moore (1951) discussed length of life in dragonflies and included _Libellula quadrimaculata_, _Libellula depressa_. In a later report Moore (1962) discussed the territory size of the same two species. Wilson (1917) included brief mention of adults of _Libellula pulchella_, _Libellula auripennis_ and _Libellula luctuosa_. Lutz and Pittman (1970) included two species of _Libellula_ in the study we undertook to a community of Odonata. They discussed the interactions and duration of flight periods as it related to time, light intensity, and temperature for _Libellula luctuosa_ and _Libellula incesta_.

The present study was undertaken to determine both the effects of controlled temperature and phptoperiod on the development of _L. incesta_

eggs and to determine the life history of this species in nature. Particular emphasis was placed on the adult reproductive behavior concerning such aspects as timing the extent of flight, mating, and oviposition periods.

CHAPTER II

MATERIALS AND METHODS

Field studies for this investigation were made at a small pond
located in northwest Greensboro, Guilford County, North Carolina. The
pond, about 0.5 ha in surface area, was surrounded by lawns and forested
areas. Members of the species Libellula incesta patrolled all the
shore areas, but only those who frequented the northwest shore were
used for this study. Figure 1 is a diagram of the pond and surrounding
area; the study area for this report is also indicated.

Data regarding adult flight periods and reproductive behavior
were collected during the spring and summer of 1968, 1969, and 1970.
Observations for this study spanned a period from June 20, 1968 to
October 25, 1970. As in the case of Lutz and Pittman (1970) "all
observations were recorded as specific clock times which were then
converted to exact local mean times corrected for latitude and
longitude. Further corrections were made to arrive at sun-dial time
or apparent solar times (AST) where midnight is 0000 hr. and the
exact solar noon is 1200 hr. Corrections made to local mean time
during the study period varied from 1 min. 28 sec. to 6 min. 25 sec.
with a median of 4 min. 59 sec. (Northcutt, 1968). All times in this
study reflect this median correction of 5 min. and are presented as
hours AST" (Lutz and Pittman, 1970, p. 279).

Figure 1

A Diagram of the Pond with the Study Area Indicated
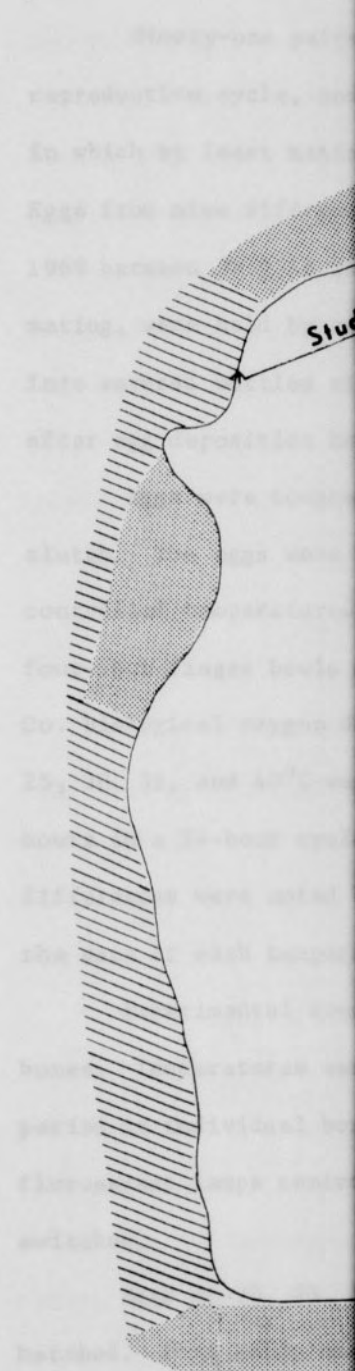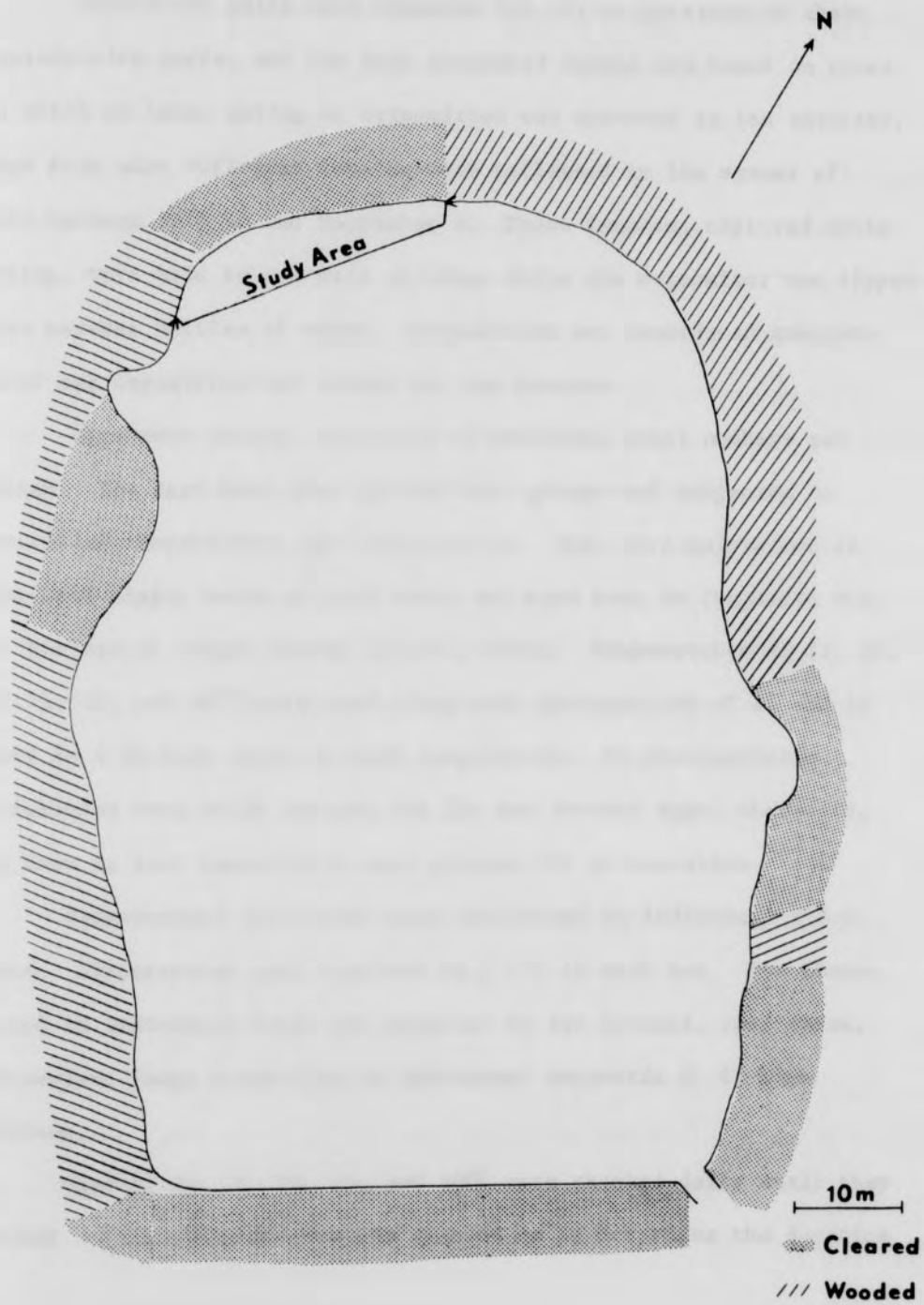
Ninety-one pairs were observed for all or portions of their reproductive cycle, and the data presented herein are based on those in which at least mating or oviposition was observed in its entirety. Eggs from nine different females were collected in the summer of 1969 between July 18 and September 4. These females, captured while mating, were held by one pair of wings while the ovipositor was dipped into several bottles of water. Oviposition was considered complete after egg deposition had ceased for two minutes.

Eggs were counted initially to determine total numbers per clutch. The eggs were then divided into groups and subjected to controlled temperatures and photoperiods. Eggs were maintained in four-inch finger bowls of pond water and were kept in Precision Sci. Co. biological oxygen demand (B.O.D.) boxes. Temperatures of 15, 20, 25, 30, 35, and $40^{\circ}C$ were used along with photoperiods of 11 and 14 hours in a 24-hour cycle at each temperature. No photoperiodic differences were noted between the 11- and 14-hour eggs; therefore, the data at each temperature were grouped for presentation.

Experimental conditions were maintained in individual B.O.D. boxes. Temperatures were constant to $\pm$ $1^{\circ}C$ in each box. The photoperiod in individual boxes was supplied by two 15-watt, cool white, fluroescent lamps controlled by individual automatic G. E. time switches.

Eggs at 20, 25, 30, 35, and $40^{\circ}C$ were checked daily until they hatched. This daily observation enabled me to determine the duration

and development rate of the egg stage and also calculate percentage of hatching. Those at 15°C were checked on alternate days since they developed much more slowly. Some eggs from two collections were maintained in plastic containers (a capacity of approximately 0.75 l) submerged about 20 to 30 cm deep in the pond which had an estimated temperature of between 28 and 32°C at that time. Estimated pond temperatures were based on data collected furing August, 1967, in a similar pond in the area. To determine first evidence of hatching, these eggs were checked every third day after which they were returned to the pond.

Additional egg observations were made on the eggs maintained at 15°C by removing small numbers of eggs after 30, 40, or 50 days and placing them at temperatures of 20, 25, 30, and 35°C. The number of eggs removed from 15°C varied in relation to the total number being maintained at that temperature from each collection. Daily checks were made to determine the relative amounts of development which had already occurred at the lower temperature.

Collections of larvae were made by sampling the bottom detritus with a Cable-Turtox scraper net. Thirteen collections were made from October 28, 1969 to October 10, 1970 at three-or four-week intervals. The number of larvae per collection ranged from 0 to 138.

Larvae were brought into the laboratory and measured using a binocular microscope, an ocular micrometer, and a Bogusch measuring slide. Total length (to 0.1 mm) and head width (to 0.1 mm) were the

measurements used to separate instars. After being measured, most
larvae were returned to the pond; however, a portion was reared to
maturity to verify instar separations.

Instar designations used in this study will follow the procedure
of previous authors and are abbreviated as follows: ultimate = final =
F; penultimate = F-1; antepenultimate = F-2; antepenultimate-minus-
one = F-3; and antepenultimate-minus-two = F-4.

Observations of adult behavior were made visually during 75
days before, during, and after the flying season and spanned a period
from May 15 to October 25 for the three year period. The duration of
each observation period varied in length from 1 to 7 hours, and the
periods included times from 0430 to 2030 hr. AST. Weather conditions
varied considerably during this study and in order to contrast clear
and cloudy conditions some observations were made during periods in
which typical summer thunderstorms occurred.

CHAPTER III

RESULTS

Hatching - The average length of time to hatching for eggs at each of the experimental conditions varied with temperature. No significant differences were noted between eggs reared under the two photoperiods. Therefore, all data about eggs at a given temperature were grouped. These data are based on a total of 1295 eggs that hatched at all conditions. Numbers of eggs that hatched at the various temperatures were as follows: 13 at 15; 502 at 20; 350 at 25; 317 at 30; and 113 at 35°C. The averages for each temperature condition are shown in Figure 2 along with the standard deviation for each of them.

The failure of most eggs at 15°C to hatch and the extreme difference in average hatching time for 15 and 20°C eggs indicate that the lower temperature threshold for eclosion for Libellula incesta eggs was somewhere around 15°C. Those few eggs which did hatch at 15°C took an average of 64.8 days which is more than four times as long as those maintained at only 5°C warmer at 20°C which averaged 14.7 days. The complete failure for eggs to hatch at 40°C suggests an upper temperature threshold between 35 and 40°C. A few of the eggs maintained at 40°C developed to the stage where eye spots were visible even though they failed to hatch.

Figure 2

Average Hatching Time in Days for Eggs of <u>Libellula</u> <u>incesta</u>

70

60

50

40

Days

30

20

10

15 20 25 30 35

Temp.,°C
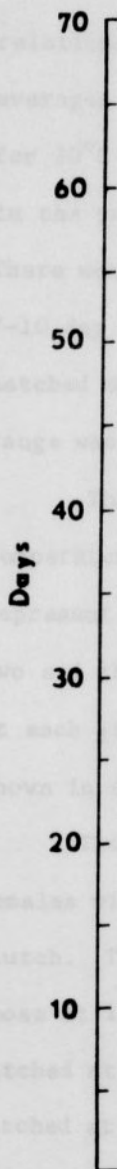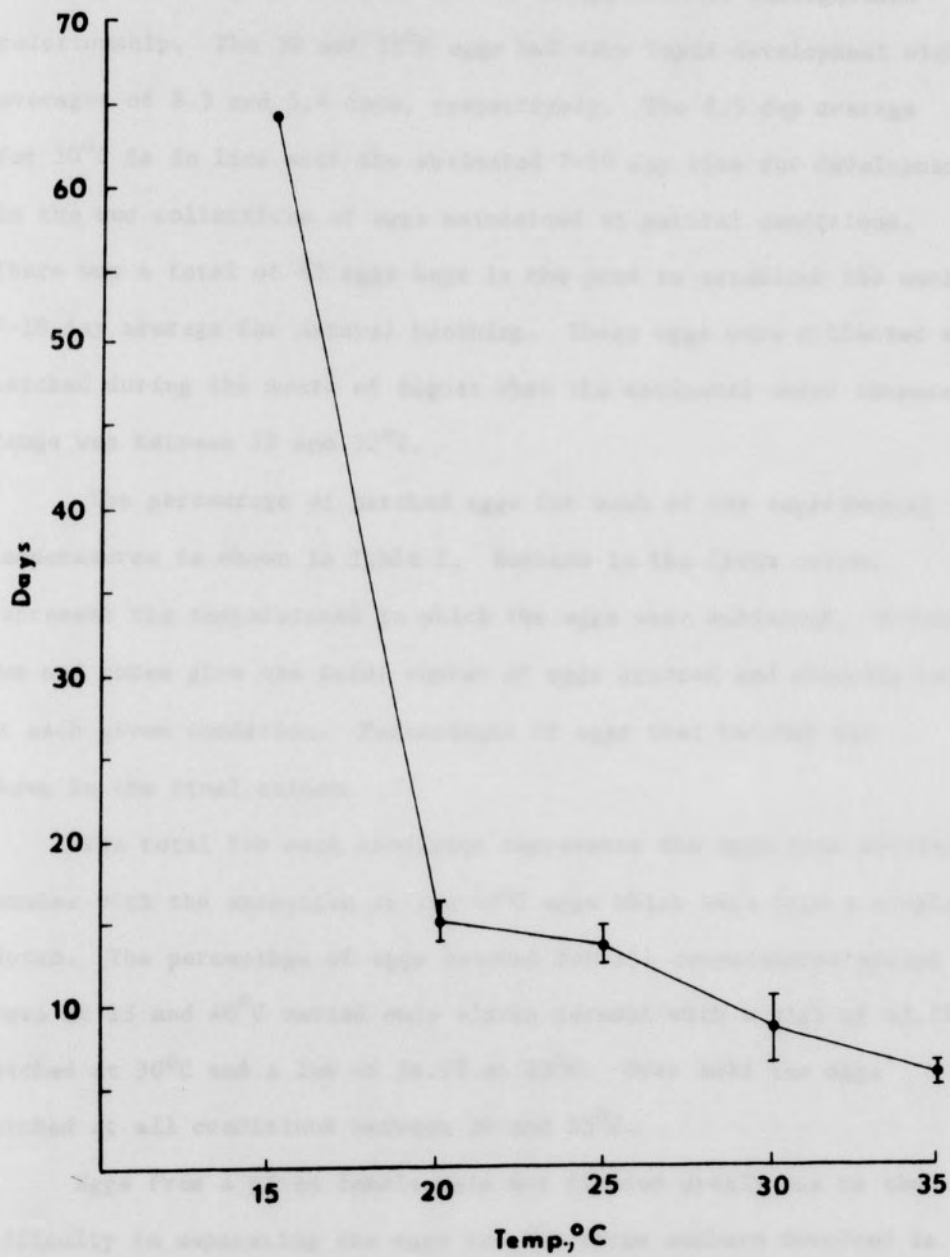
The average number of days for development decreased as temperatures increased for eggs maintained at temperatures between 20 and 35°C. Figure 2 shows this in an approximate straightline relationship. The 30 and 35°C eggs had very rapid development with averages of 8.3 and 5.4 days, respectively. The 8.3 day average for 30°C is in line with the estimated 7-10 day time for development in the two collections of eggs maintained at natural conditions. There was a total of 63 eggs kept in the pond to establish the estimated 7-10 day average for natural hatching. These eggs were collected and hatched during the month of August when the estimated water temperature range was between 28 and 32°C.

The percentage of hatched eggs for each of the experimental temperatures is shown in Table 1. Numbers in the first column represent the temperatures to which the eggs were subjected. Columns two and three give the total number of eggs started and actually hatched at each given condition. Percentages of eggs that hatched are shown in the final column.

The total for each condition represents the eggs from several females with the exception of the 40°C eggs which were from a single clutch. The percentage of eggs hatched for all temperatures except those at 15 and 40°C varied only eleven percent with a high of 65.7% hatched at 30°C and a low of 54.7% at 20°C. Over half the eggs hatched at all conditions between 20 and 35°C.

Eggs from a given female were not divided evenly due to the difficulty in separating the eggs and the large numbers involved in

TABLE 1

Percentage of Eggs Hatched at Controlled Temperatures

Temperature, $^{\circ}$C	Number Started	Number Hatched	Percent Hatched
15	1825	13	0.71
20	917	502	54.75
25	537	350	65.15
30	463	304	65.70
35	206	113	54.80
40	100	0	0.00

some clutches. These two variables may figure in this 11% difference among those reared between 20 and 35°C.

The percentage of eggs that hatched at a particular condition for each of the nine collections varied considerably from a low of 18% hatched at 20°C in one collection to a high of 93% hatched at 20°C in another clutch.

Those at 25 and 30°C had a higher percentage that hatched than those conditions five degrees higher or lower. The 25 and 30°C temperatures most nearly approximated the naturally occurring pond temperatures during the development period.

There was an extremely low percentage hatched at 15°C with only 0.71% hatching. At 40°C the percentage was zero, and even though all the 40°C eggs were from a single female, those eggs of that particular female subjected to other temperatures exhibited normal developemnt.

The total number of eggs that hatched at each experimental condition varied greatly due in part to the different numbers of eggs placed in each situation and the differences in the eclosion percentages. Figure 3 presents the range of eclosion as it relates to the total number of eggs hatched at each experimental condition during the entire hatching period. Since no hatching occurred at 40°C these results are not shown. The four temperatures with large numbers of eggs are shown on the same scale. Data pertaining to the 15°C eggs are shown on a different scale since the total number of eggs was too small and the length of time in days too long to be shown in the same

Figure 3

Number of <u>Libellula incesta</u> Eggs Hatched in Relation to the Number
of Days Since Oviposition
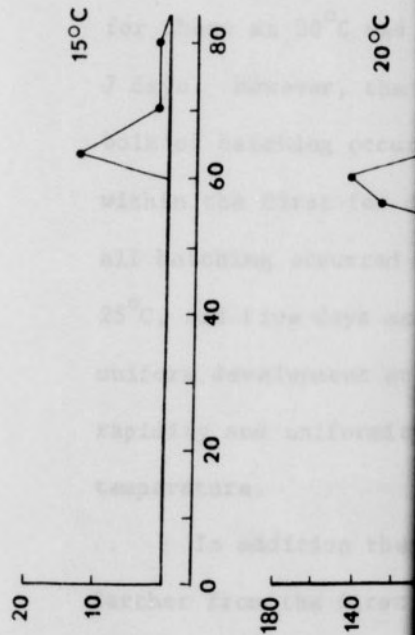
manner as those for the other temperatures.

The eggs of _Libellula incesta_ developed and hatched at a rate relative to the temperatures at which they were maintained. Comparison of the first day of hatching at each condition indicates a decrease in total length of egg development with an increase in temperature but not as gradual a decrease as anticipated. The 20 and 25°C conditions are more similar to each other as are the 30 and 35°C. There is at the same time a considerable difference of five days in development time between those at 25 and 30°C.

The length of the hatching period varied with the conditions and showed no particular trend. For example, the hatching period for those at 30°C was 14 days, while those at 20°C all hatched in 7 days. However, the curves do show a trend in rate with which the bulk of hatching occurred. The vast majority of hatching occurred within the first few days after the onset of eclosion. About 90% of all hatching occurred within two days at 35°C, four days at 30°C and 25°C, and five days at 20°C. There is obviously more rapid and uniform development at 35°C than at the lower temperatures, and the rapidity and uniformity decreases in relation to decreases in temperature.

In addition the peaks in the hatching curves were displaced farther from the first day of hatching as eggs were subjected to lower temperatures. At 35°C the peak occurred on the first day, at 30°C it is the second day, at 25°C the second or third day, and about three or four days at 20°C.

As is shown in Figure 3 the eggs at 15°C developed much more slowly than those at 20°C, had a very low total number hatched and a prolonged hatching duration. A number of those 15°C eggs were subjected to additional experimentation to determine the degree to which the lower temperature affected their rate of development and viability. After maintaining eggs at 15°C for 30, 40, and 50 days, a small number of eggs was transferred to each temperature of 20, 25, 30, and 35°C. The results are shown in Table 2. Each of the temperature columns gives the total number of eggs transferred as N, followed by the number and percentage hatched. The average time to hatching is given as well as the standard error of the hatching time.

As the time spent at 15°C increased there was a tendency toward lower percentage hatched; an exception was those eggs kept forty days before being subjected to 25°C. The percentage in question was based on only twelve eggs while the other 40-day eggs and all the 30-day eggs were represented by larger samples.

The 50-day eggs were also represented by small samples, but the tendency to lower percentage of eggs that hatched is shown in all conditions and probably is a representative trend. As shown in Table 1, average hatching time for eggs at 15°C was 64.8 days with 0.71% hatched which supports the general reduction of the percentage figure as previous treatment time increases.

Comparison of the average hatching time for the 30-day and 40-day eggs indicate less time required for hatching as previous treatment

TABLE 2

Hatching results in eggs exposed to 15°C for specified lengths of time

Duration of Previous treatment 15°C	20°C					25°C					30°C					35°C				
	N[1]	Hat.[2]	%	Ave.	S.E.	N[1]	Hat.[2]	%	Ave.	S.E.	N[1]	Hat.[2]	%	Ave.	S.E.	N[1]	Hat.[2]	%	Ave.	S.E.
30 days	122	39	32.0	8.94	0.32	55	9	16.4	6.14	0.34	85	31	36.5	5.30	0.37	93	22	23.6	3.81	0.19
40 days	98	18	18.4	7.85	1.28	12	3	25.0	4.67	0.67	65	22	33.8	4.60	0.28	68	14	20.6	2.33	0.26
50 days	27	3	11.1	10.8	4.10	14	0	--	--	--	13	2	15.4	2.25	1.25	11	2	18.2	2.25	1.25

[1] Numbers started

[2] Numbers hatched

time was increased. This fact, along with the 62-day hatching at 15$^\text{o}$C, indicates continuing development at 15$^\text{o}$C even though considerably slower than at even slightly warmer conditions.

So few eggs were used in the 50-day samples that it is difficult to make comparisons with these figures. However, if one considers that it was the long duration of only one egg that raised the average time at 20$^\text{o}$C to 10 days then these figures appear more in line. Disregarding the one egg which took 15 days to hatch the average would be 7.25 days at 20$^\text{o}$C.

The average time from oviposition to hatching decreased as the temperature increased within each group. In addition the average time to hatching in all cases except the 50-day treatment before transfer was less than the normal development time at that specific temperature.

Eggs - Eggs from captured Libellula incesta females were extruded in masses which separated when washed from the ovipositor and sank singly to the bottom of the containers. They had sticky transparent gelatinous fibers which anchored them to the containers and to each other, such that agitation was required to release the eggs for counting. Individual eggs were uniform in size and shape. They averaged about 0.5 mm long by 0.3 mm wide and, as shown in Figure 4, were ellipitical in shape with an anterior pole bearing a short, pointed, colorless pedicel. The eggs were a transparent cream color when oviposited but within a few hours, the egg case darkened slightly and within twenty-four hours darkened to a reddish brown.

Figure 4

Shape of the Egg of Libellula incesta

0.5 mm

Complete development time varied with the temperature as noted in the experimental results on development and hatching. After about two-thirds of the average development time had passed, the eye spots were clearly visible, and a day or two later the entire embryo was visible.

From the portions of the hatching process observed in a number of eggs, several facets were obvious. Just prior to eclosion, rhythmic peristalic pulsations occurred in the head region of the prolarva resulting in swelling in the head region. This swelling helped to rupture the embryonic sheath allowing the prolarva to emerge from the egg case.

The prolarva was enclosed in a sheath which restricted the legs. After a short period (up to a few minutes) the prolarva expanded in the region of the thorax and split the cuticle in the dorsal thoracic area. The second instar larva emerged leaving a chitinous cast skin as was typical of subsequent molts. According to Kormondy (1959) several workers have recognized the period between the splitting of the embryonic sheath and the shedding of the pro-larval sheath as the first instar, while others consider this stage merely an embryonic condition. Since the cast skin shed by the pro-larva was comparable to subsequent molts, the prolarva was considered as the first instar and the next molt was into the second instar. The second instar was the first one that the larva had a typical odonate appearance.

Larvae were not reared in the laboratory but returned to the pond after hatching occurred. Larval growth and population structure was determined by systematic sampling of the naturally occurring population throughout the year.

Larval Development - Head width proved to be the best morphological character by which to separate instars. Total body length was also measured, but the variation within this characteristic proved it to be less reliable for instar differentiation. Eleven of the fourteen collections are presented in Figure 5 as histograms showing the relative frequencies of head width as percentages of the total collection. The three collections not shown in this illustration had either no animals or only a very small number of larvae in the collections. Overlap of head width measurements between two instars appeared to occur in the F-3 and F-4 instars. In actual measurements, however, those which were in the F-3 instar were 2.36 mm or larger, and those which were in the F-4 instar were 2.23 mm or smaller. Since both these sizes are included in the 2.35 $\pm$ 0.12 mm bar there is an apparent overlap which is separated by the actual data.

The collection made on October 28, 1969, and shown in Figure 5 indicated that the majority of the animals were in the F-2 and F-3 instars. Since the F-4 animals were very small there may have been a greater percentage of F-4 and younger instars in nature which were overlooked in the sampling and were not represented in the collection. This problem existed in all collections, however, so the number of animals obtained in the later instars can be compared without regard

Figure 5

Relative Head-Width-Frequency Histograms of <u>Libellula incesta</u>
Larvae Collected in 1969-70. Crosshatched Area = F-4 Instar;
Coarsely Stippled Area = F-3 Instar; Diagonally Lined Area =
F-2 Instar; Finely Stippled Area = F-1 Instar; Clear Area =
Final Instar
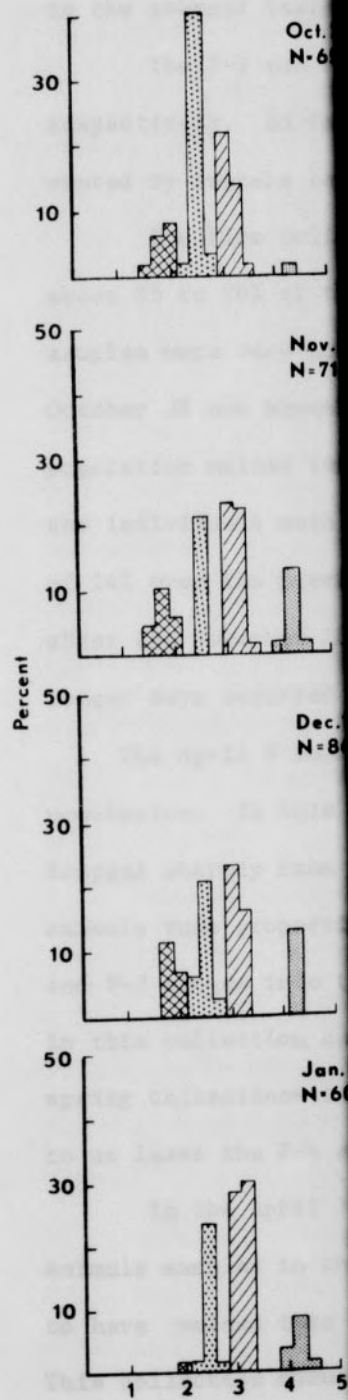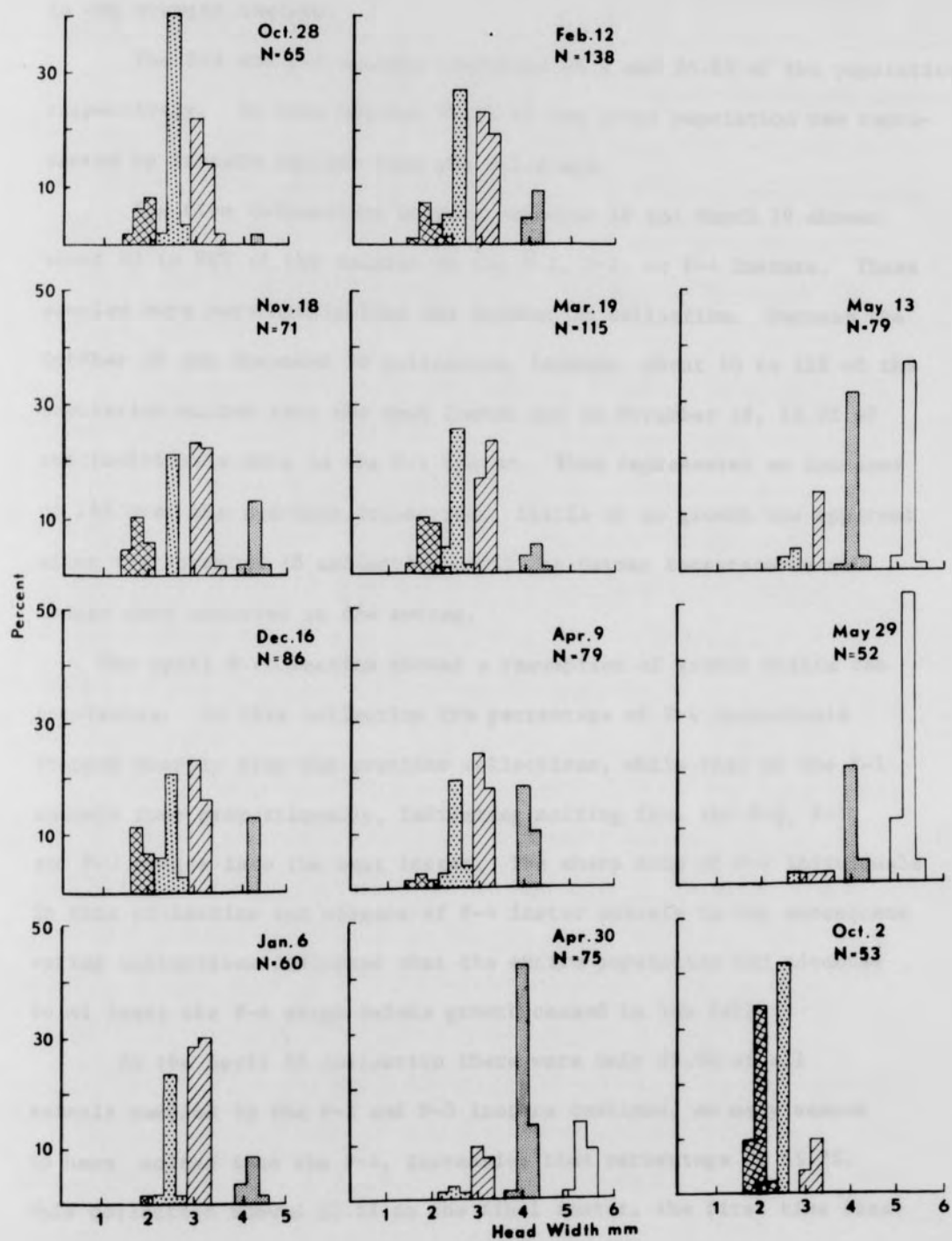
to the younger instars.

The F-3 and F-2 instars comprised 46.1 and 36.8% of the population, respectively. In late October 98.5% of the total population was represented by instars earlier than the F-1 stage.

The five collections between November 18 and March 19 showed about 85 to 90% of the animals in the F-2, F-3, or F-4 instars. These samples were very nearly like the October 28 collection. Between the October 28 and November 18 collection, however, about 10 to 15% of the population molted into the next instar and on November 18, 15.5% of the individuals were in the F-1 instar. This represented an increase of 14% over the previous collection. Little or no growth was apparent after the November 18 collection until the warmer temperatures and longer days occurred in the spring.

The April 9 collection showed a resumption of growth within the population. In this collection the percentage of F-4 individuals dropped sharply from the previous collections, while that of the F-1 animals rose proportionally, indicating molting from the F-4, F-3, and F-2 stages into the next instar. The sharp drop of F-4 individuals in this collection and absence of F-4 instar animals in the subsequent spring collections indicated that the entire population had advanced to at least the F-4 stage before growth ceased in the fall.

In the April 30 collection there were only 21.0% of all animals sampled in the F-2 and F-3 instars combined, as many seemed to have molted into the F-1, increasing that percentage to 55.7%. This collection showed 23.1% in the final instar, the first time final

instar individuals were collected. This was about one month before mature adults were first sighted on May 29.

The remaining two spring collections on May 13 and May 29 showed increasingly more animals molting into the final instar and the percentage of earlier instar representatives decreasing at about the same rate. By the May 29 collection 67.3% were in the final instar and 25.0% in the F-1 stage with only 7.7% of the population in earlier instars.

Based on the length of this species' flight season, it can be assumed that it probably had an extended emergence period but there was little supporting evidence in this study. Emergence must have commenced between April 30 and May 29. Teneral individuals were found as late as July 19 but this probably did not signify the end of emergence since adults were still flying as late as October. It was improbable that the adults lived for three months as these dates would indicate.

The three collections which were not shown on the histograms due to the small number or complete absence of larvae were made on June 23, July 9, and September 4. The June 23 collection yielded only nine animals of which eight were in either the F-1 or final instar. A histogram of this collection would have been almost identical in structure to the May 29 histogram.

The July 9 collection resulted in no specimens and the September 4 collection produced only 14 animals. Histogram presentation of this collection would show a distribution which did not differ significantly

from those of the October 2, 1970, or October 28, 1969, collections.
Obviously the population structure present in early fall of the
previous year had already been established, but the animals were
located in a different area of the pond and therefore not found in
large numbers. By the October 2 collection they were found in fairly
large numbers in the same areas as collections the previous year.

The histogram structure indicates that growth resumed between
the March 19 and April 9 collections for the earlier instars. As
the average ambient temperatures shown in Figure 6 rose to between 8
and 15°C the water temperature realized a similar rise resulting
in earlier instar molting.

Animals in the F-4, F-3, and F-2 stages molted into the next
most advanced instar before the F-1 individuals showed any apparent
molting into the final instar. This resulted in a large increase in
the F-1 group and significant decrease in the size of the F-4 portion
of the population.

Growth resumed between the April 9 and April 30 collections
when the ambient temperature averages rose 5°C and were between 15 and
20°C. The April 30 collection showed all the F-4 instars having
molted into a later instar and most of the F-3 individuals had pro-
gressed to the F-2 and F-1 instars just as the first final instar
larvae appeared. By the May 13 and May 29 collections the emergence
period was in progress and 44.3% of the May 13 collection and 67.2%
of the May 29 collection were in the ultimate instar.

Figure 6

Average Ambient Temperature Between January 1969 and July 1969 in °C, with Collection Dates Indicated by Arrows

_____ Behavior - Mature adult Libellula _____ males were observed in the field as early as May 29, 1969 and their flight season lasted until October 10. These limits may have varied a few days at either end of the period but could not have varied much since observations were made just prior to May 29 and after October 10 and no adults were seen at these times. The May 29 starting date does not include the maturation period which took place prior to this date and for which there is not data available on this species. The first collection of larvae which included the final instar stage occurred on April 30, so emergence probably began within a few days of May 15. This would allow about two weeks for maturation which is a reasonable time for species such as this one.

A summary of the timing for various developmental stages of this species is shown in Figure 7. The limits of each stages could not be determined exactly, and the uncertain areas are represented by dotted lines. However, enough information was available to show the general timing of developmental stages in the life-history of Libellula luctuosa and also to determine that it was a univoltine species.

Quantitative observations of the adults were not begun until June 20, 1969. By that time a substantial population size was available as their territories had been established and reproductive behavior had commenced.

Territoriality studies in adults of Libellula luctuosa were attempted by marking individuals and noting their tendency to return

Temp.,°C

30   20   10   0   -10

Adult Behavior - Mature adult Libellula incesta males were observed in the field as early as May 29, 1969 and their flight season lasted until October 10. These limits may have varied a few days at either end of the period but could not have varied much since observations were made just prior to May 29 and after October 10 and no adults were seen at these times. The May 29 starting date does not include the maturation period which took place prior to this date and for which there is not data available on this species. The first collection of larvae which included the final instar stage occurred on April 30, so emergence probably began within a few days of May 15. This would allow about two weeks for maturation which is a reasonable time for species such as this one.

A summary of the timing for various developmental stages of this species is shown in Figure 7. The limits of most stages could not be determined exactly, and the uncertain areas are represented by dotted lines. However, enough information was available to show the general limits of developmental stages in the life-history of Libellula incesta and also to determine that it was a univoltine species.

Quantitative observations of the adults were not begun until June 20, 1969. By that time a substantial population size was available so that territories had been established and reproductive behavior had commenced.

Territoriality studies in adults of Libellula incesta were attempted by marking individuals and noting their tendency to return

Figure 7

Timing and Duration of Various Events in the Life-History
of *Libellula* *incesta*

Earlier instars

F-2 instar

F-1 instar

F instar

Emergence

Flight

Oviposition

Egg

Eclosion

Nov.   Dec.   Jan.   Feb.   Mar.   Apr.   May   June   July   Aug.   Sept.   Oct.
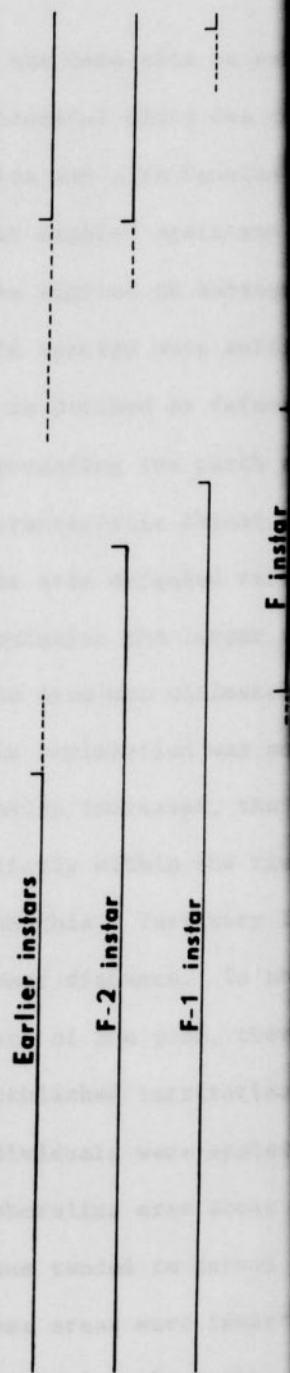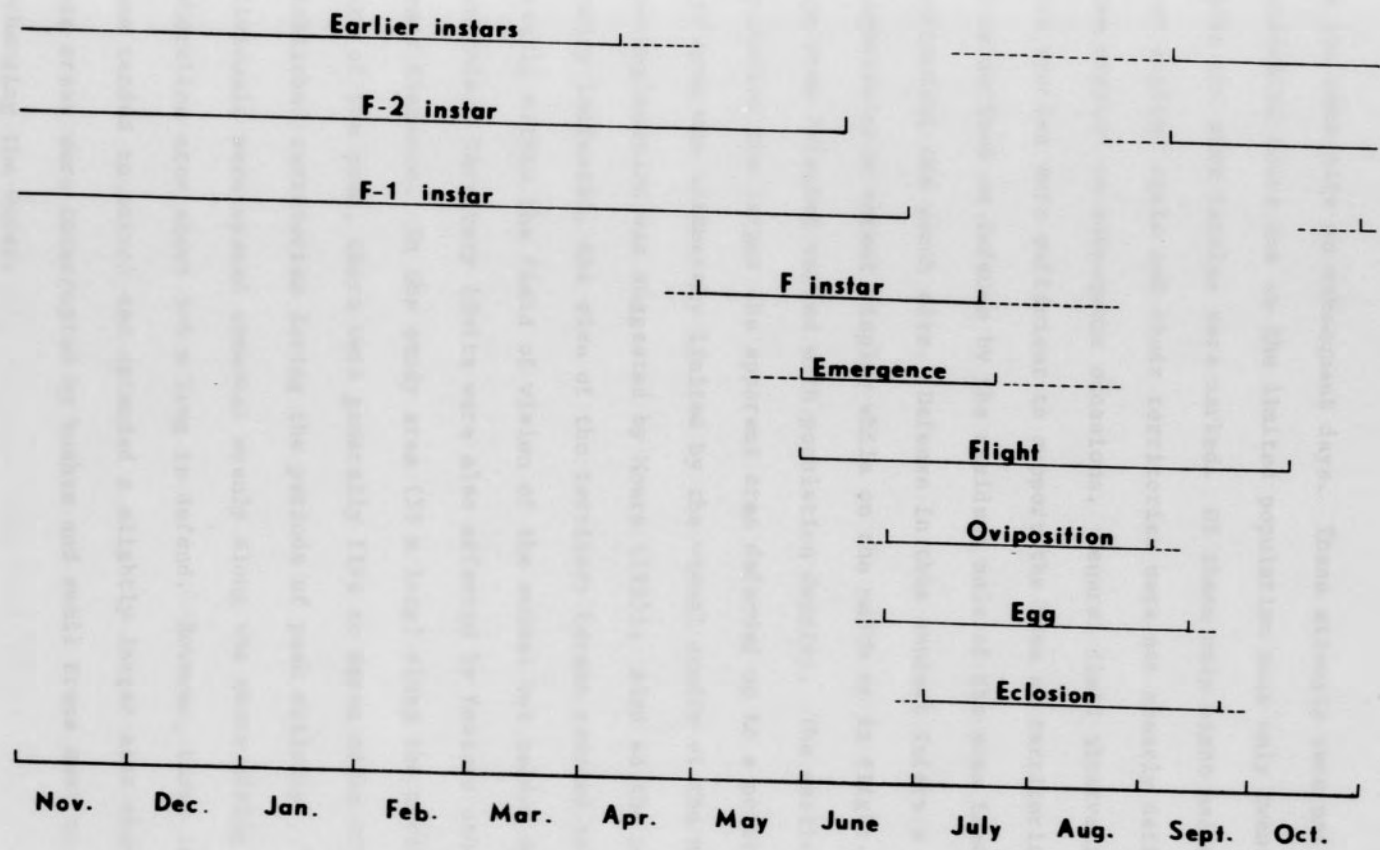
to the same site on subsequent days. These attempts were not
successful since due to the limited population size only twenty-six
males and nine females were marked. Of these only eight males were
ever sighted again and their territories were not clearly defined
when sighted on subsequent occasions. General field observations for
this species were sufficient to support the idea of territoriality when
it is defined as defense by the resident male of the area immediately
surrounding the perch site. Defense in this context infers a
characteristic threat display while on the perch or in flight. The
size area defended varied with population density. The smaller the
population the larger the apparent area defended up to a point. The
size area was ultimately limited by the visual acuity of the species;
this explanation was suggested by Moore (1953). Also as the population
density increased, the size of the territory became reduced to an area
strictly within the field of vision of the animal but seldom smaller
than this. Territory limits were also affected by factors other than
linear distance. In the study area (35 m long) along the northwest
shore of the pond, there were generally five to seven males with
established territories during the periods of peak activity. These
individuals were spaced somewhat evenly along the shore giving each
a shoreline area about 5-6 m long to defend. However, those in open
areas tended to patrol and defended a slightly larger area than those
whose areas were interrupted by bushes and small trees near to or
overhanging the water.

Another example of territory was a small cove located at one end of the study area and measuring about 8-10 m across the end and 12-15 m along the curve of the shore. This cove consistently supported two males without interaction unless a third male entered the area or if one of the resident males exhibited excessive activity. There was nothing to restrict the view of the two resident males in the cove. It may therefore be assumed that the 8-10 m distance between the males perched on either side of the cove was more than a sufficient distance to prevent the presence of one from affecting the other.

Males averaged about eight to nine in the study area at peak periods on clear days. However during the prime flying time, both in relation to time of day and season, there were never enough spaces for all males. Therefore, there were always several males found away from the water in the edge of the woods and these periodically approached the pond and aroused the resident male by crossing some predetermined boundary. When aroused the resident male usually reacted in a threatening manner which seldom resulted in contact but, neverthe-less, generally displaced the intruder whether of the same species or another. From a community study on this same pond Lutz and Pittman (1970) found that Libellula incesta dominated the community in numbers of both intraspecific and interspecific interactions. During the study period, adults of Libellula incesta interacted intraspecifically 691 times and of these, 29 interactions involved contact. Interspecific interactions for Libellula incesta adults with all other species combined totaled 300, and 2 of these involved contact. These numbers were about twice as high as the next most prevalent species.

Libellula incesta males were not patrollers as were Anax or Epicordulia, but generally remained perched when not engaged in defense of territory or when copulating. However, interactions with both males and females were so frequent for this species that they gave a general appearance of flying abundantly.

The observed extremes of the daily flight period of Libellula incesta adults were from 0737 to 2018 hr. AST at temperatures between 25 and 40°C, as shown by Lutz and Pittman (1970). Maximum activity occurred during the midday hours at temperatures in the 30 to 36°C range. This pattern was altered noticably by storms or extreme cloudiness. Figure 8 reproduced from Lutz and Pittman (1970) shows the effect of changes in light intensity, temperature, and Libellula incesta activity in relation to time of day. The upper portion of this figure (8A) shows the average number of adults present on a typical summer day and (8B) the effect that a storm had on this number. The days were hypothetical and represented composite data for both climatological factors and numbers of individuals. This general pattern was actually observed on several occasions.

Males arrived daily at the pond around 0730 hr. or later and established a territory. The time at the water, exclusive of that spent in chasing other males, mating, or making short patrol flights, was spent perched on an overhanging bush, tree limb, or high grass near the water's edge about a meter above the water. Males remained perched as long as possible between intra- or interspecific inter- actions. About one-half hour after the males arrived at the water

Figure 8

A Composite of Flight Activity of Adults of <u>Libellula</u> <u>incesta</u>,
Light Intensity, and Temperature in Relation to Time of Day

    A. Pattern for a hot clear day

    B. Pattern for a hot day interrupted by an early
          afternoon thunderstorm

Dotted line = ambient temperature

Broken line = light intensity

Solid line = number of adults of <u>Libellula</u> <u>incesta</u>

(From Lutz and Pittman, 1970).
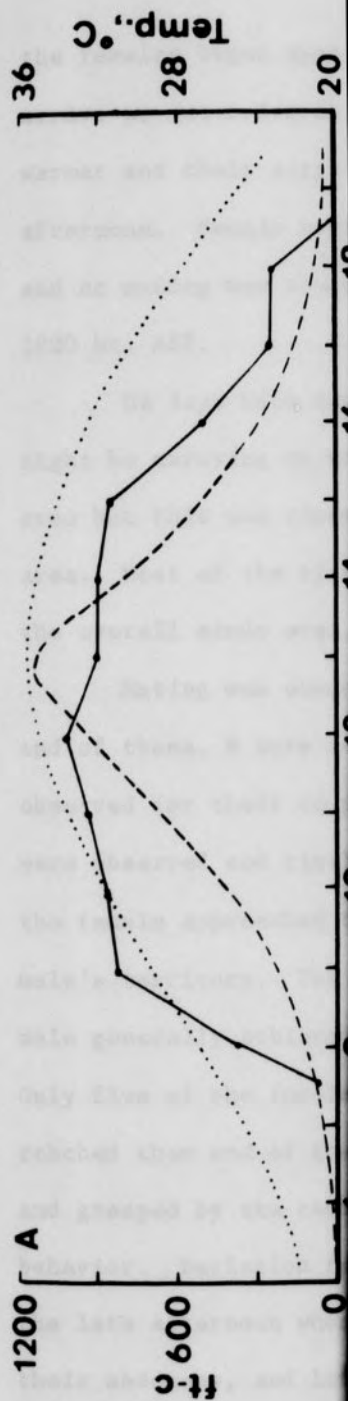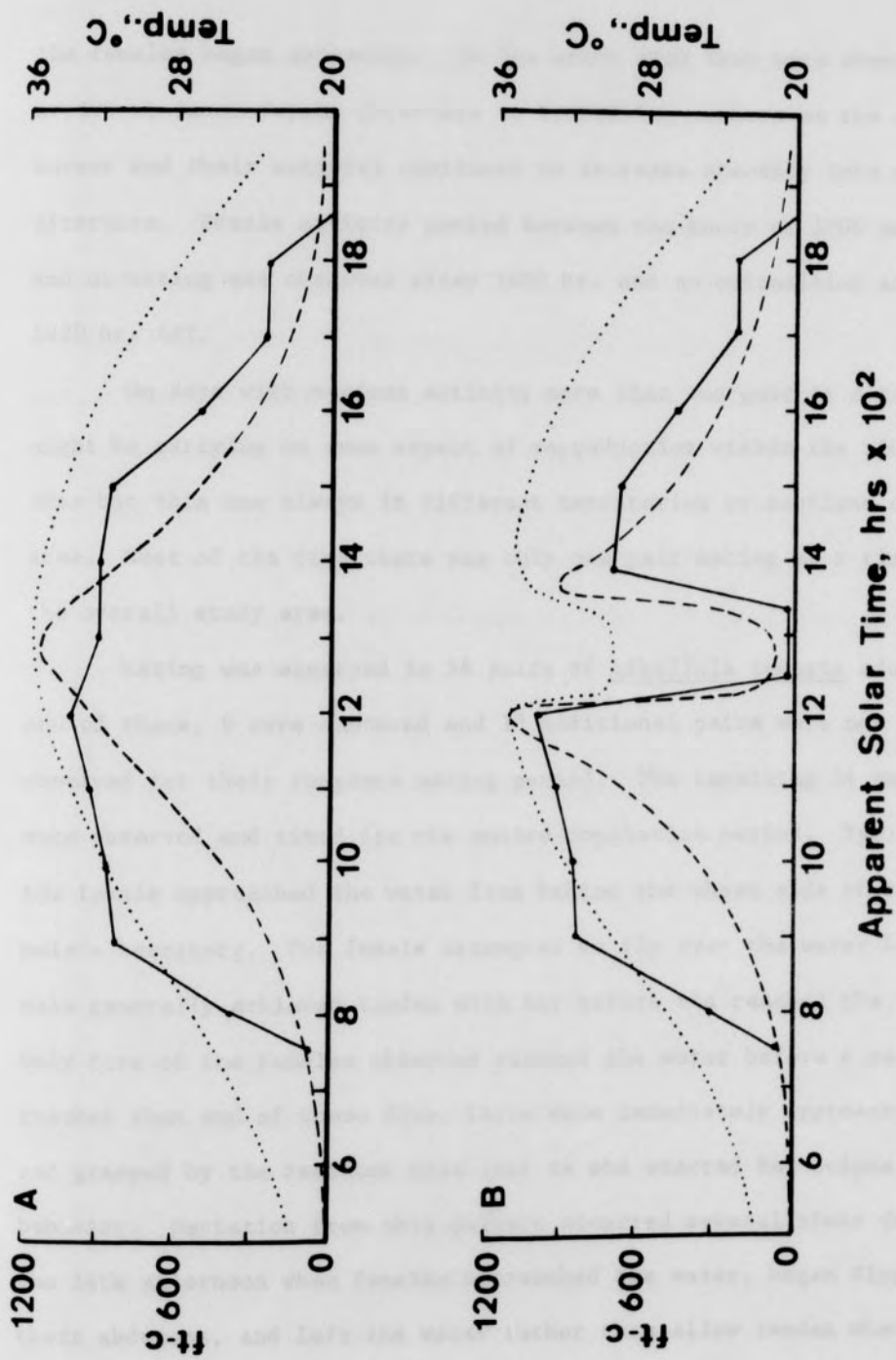
the females began appearing. In the study area they were observed to arrive at inconsistent intervals in increasing numbers as the day grew warmer and their activity continued to increase steadily into mid-afternoon. Female activity peaked between the hours of 1200 and 1500 and no mating was observed after 1600 hr. and no oviposition after 1820 hr. AST.

On days with maximum activity more than one pair at a time might be carrying on some aspect of reproduction within the study area but this was always in different territories or sections of the area. Most of the time there was only one pair mating at a time in the overall study area.

Mating was observed in 56 pairs of *Libellula* *incesta* adults and of these, 9 were captured and 13 additional pairs were not observed for their complete mating period. The remaining 34 pairs were observed and timed for the entire copulation period. Typically, the female approached the water from behind the shore side of the male's territory. The female attempted to fly over the water but the male generally achieved tandem with her before she reached the water. Only five of the females observed reached the water before a male reached them and of these five, three were immediately approached and grasped by the resident male just as she started her oviposition behavior. Deviation from this pattern occurred several times during the late afternoon when females approached the water, began dipping their abdomens, and left the water rather than allow tandem when approached by a male. This behavior was observed only in the late

afternoon after territorial and reproductive activity had slowed considerably.

In all observed mating the male flew immediately to the female as she approached and with extreme accuracy grasped the back of her head with his anal appendages thus achieving tandem. Ten of the 56 mating pairs observed spent a good portion of the mating time in flight and four of these completed mating while in flight. The normal procedure, however, was a quick flight in the copulatory position to a nearby perch which was generally a bush or some grass where most of the actual mating took place. The average mating time for the 34 timed pairs was 46.5 sec. ranged from 25-78 sec., and had a standard deviation of 11.21.

Upon completion the male released the female and she either began ovipositing immediately or rested briefly before commencing. Thirty-three pairs were observed for the entire mating time and into at least a portion of the oviposition period. Of these 17 rested between mating and oviposition while 16 started immediately to oviposit. The complete resting period was timed for only nine individuals and averaged 27 seconds. The range for eight of the nine was between 10 and 40 seconds with the other individual resting 80 seconds before ovipositing.

The approach to the pond after mating had no apparent ritual but consisted of a direct flight from the copulatory site to a suitable site over the water within a few feet of the shoreline.

Oviposition amounted to a series of repeated bending and dipping motions with the tip of the abdomen going just under the water's surface. The procedure consisted of either one or several quick dips making contact with the water's surface, rising above the water from 15-45 cm and bending the abdomen in the same manner as when contacting the water. The number of actual water contact dips were timed for 21 females for at least a portion of their oviposition period and these averaged 0.646 dips per second, and the range of dips per second was from 0.317 - 1.190. These averages do not include the bending motions which did not actually touch the abdomen to the water.

While oviposition was in progress the male generally hovered over the female or circled around her. The male's circle had a radius of about 1-2 meters. Almost all females were attended by the male during at least part of the oviposition period. Only two completely unattended females were recorded, and these were not observed to mate prior to oviposition. The attending male dashed at other males which approached the female as she was laying eggs. Often while the male was engaged in chasing away another male, a third or fourth male would approach the ovipositing female who generally left the pond rather than mate again at that time.

The oviposition period ranged from 51-166 seconds in the seven females for which the entire process was timed. Twenty-two other females were timed for most of their oviposition. These observations were incomplete because the female ceased to oviposit when disturbed by males, and it was difficult to allow for these interruptions in the oviposition period.

Nine mating pairs were captured during copulation and the eggs were collected for the egg development experiments. The entire clutch from each female was counted, but only seven were used in the averages since the completeness was uncertain in two of the clutches, which contained 381 and 884 eggs each. The sizes of the seven complete clutches ranged from 206-2058 with a mean of 1060.4 and a S.E. of 244.56. This range for total number of eggs per female was quite wide, but it was impossible to determine if the females had oviposited earlier in the day or season and, if so, how that would affect the numbers. It was equally impossible to determine the age of the individuals or the effect of age on numbers of eggs laid. Nevertheless, the average of 1060 eggs for the seven females indicated that the reproductive protential for this group of insects was considerable.

Egg deposition in nature was also impossible to determine since the captured females were induced to oviposit until no more eggs were released for two minutes, and this often involved several minutes. Under natural conditions, however, the counts probably approached these figures. The vast majority of those eggs collected experimentally were released in the first minute of dipping, and the average time of oviposition in nature was 110.1 sec. for uninterrupted females.

Upon completion most of the females observed flew directly out over the water, up, and away into the protection of the nearby woods. Often the retreating female was pursued by either her mate or another male. The male would attempt to grasp the female but only on one

occasion was he actually observed to accomplish this. With this one pair the male actually achieved tandem and mated again. The released female repeated the oviposition procedure briefly and left the pond.

Two females were observed to rest among the shore vegetation after oviposition before leaving the general area of the pond, but both of these were in the late afternoon when male activity had slowed somewhat.

Many of the ovipositing females were not allowed to follow this normal pattern due to competition between the males of the area. Thirty-nine females were timed during oviposition but only seven of these completed the process without interruption. Fifty-six other females were observed for at least short portions of their oviposition time and most of these were interrupted before completion. Interrupted females reacted in one of three ways to the intruding male. If the disturbance was slight the female continued to oviposit; however, if the male was aggressive at all with his approach, the female either left the pond without finishing or retreated temporarily to the shore vegetation while her mate chased off the intruder, after which she returned to complete her task. Some females were observed to return after more than one interruption, while others left after only one disturbance and did not return.

The only time females were observed at the pond was during the reproductive activities and their location for the remainder of the day is uncertain. Due to this behavioral pattern the presence of

females was not realized in the accounts of flight activity, population
density, and other daily non-reproductive routines. This behavioral
pattern presented for the population occurred day after day during the
portion of the season when numbers of individuals remained at a high
level.

CHAPTER IV

DISCUSSION

The eggs of Libellula incesta, like those of many other odonates, exhibited direct development both experimentally and in nature. Several authors have reported rates of development without specifying temperature of development, while others have presented data which included temperatures. In Table 3 is presented development times for a number of anisopteran species with temperatures (when available). Under experimental conditions the average rates of development varied with temperature. The results for eggs of Libellula incesta are presented in Table 3 for the 20, 25, and 30°C experimental conditions for comparison with the results by other workers.

The eggs of all species represented in Table 3 exhibited direct development and ranged from 6 to 39 days. Such a range might be expected for species which hatch and develop to a fairly advanced stage before lower winter temperatures occur in nature. The various species of the genus Libellula exhibited an 8 to 39 day range. The observations of the experimental eggs for Libellula incesta ranging from 8.3 to 14.7 days are well within the limits observed for this genus.

According to Corbet (1960) little data exist to support or refute his idea of a positive thermal growth coefficient in Odonata

TABLE 3

Average Days from Oviposition to Hatching in a Number of
Species of Odonata

Species	Authority	Average	Temperature
Epiophlebia superstes	Asahina (1950, in Corbet, 1962)	30	20°C
Epiophlebia superstes	Asahina (.950, in Corbet, 1962)	20	25.5°C
Erythemis simplicicollis	Bick (1941)	11.6	
Hemicordulia tau	Hodgkin & Watson (1958, in Corbet, 1962)	10	21.1°C
Hemicordulia tau	Hodgkin & Watson (1958, in Corbet, 1962)	6	29.4°C
Ischnura damula	Johnson (1965)	15	22°C
Ischnura damula	Johnson (1965)	11.5	23-28°C
Leucorrhinia intacta	Wilson (1917)	12*	
Libellula auripennis	Wilson (1917)	8*	
Libellula depressa	Gardner (1953)	28	
Libellula depressa	Gardner (1953)	14	
Libellula fulva	Gardner (1951, in Corbet, 1960)	11	
Libellula incesta	Pittman (this report)	14.7	20°C
Libellula incesta	Pittman (this report)	13.5	25°C
Libellula incesta	Pittman (this report)	8.3	30°C
Libellula luctuosa	Wilson (1917)	10*	
Libellula pulchella	Wilson (1917)	39*	
Libellula quadrimaculata	Gardner (1951, in Corbet, 1960)	29	
Plathemis lydia	Wilson (1917)	10*	

Table 3 (Continued)

Species	Authority	Average	Temperature
Sympetrum fonscolombei	Gardner (1951a)	23	21°C
Sympetrum rubicundulum	Wilson (1917)	10*	
Sympetrum sanguineum	Gardner (1950b)	22	
Sympetrum striolatum	Corbet (1956)	20	20-25°C
Sympetrum striolatum	Gardner (1950a)	24	20°C ± 1°C
Sympetrum striolatum	Gardner (1950a)	13	20°C ± 1°C
Sympetrum striolatum	Corbet (1956)	22*	24°C
Sympetrum striolatum	Corbet (1956)	9*	25-30°C

*beginning of hatching period and not average

egg development. The experimental results presented by Corbet (1956, 1962) and Johnson (1964) are given in Table 4 along with those from this study. These data seem to support the theory that an increase in development time occurs with a decrease in temperature.

Only in one case, Sympetrum striolatum, is there any apparent discrepancy, and the circumstances of this species are not covered sufficiently well by Corbet (1956) to explain the deviation from the anticipated.

The eggs of most Odonata which experience direct development hatch in 2-5 weeks in nature according to Corbet (1962). He is supported in this idea by the eggs of Anax imperator which had a 22-26 day range (Corbet, 1957) and those of Sympetrum fonscolombei with a 21-day period from oviposition to hatching (Gardner, 1951a). However, the eggs of Libellula incesta hatched in about 7-10 days which was less than the 2-week lower limit. This 7-10 day range received additional support from the laboratory eggs at 30°C which corresponded to temperatures within 5°C of natural conditions and which hatched in an average of 8.3 days.

The range for total hatching time was shown by Corbet (1962) to vary considerably within the eggs of a specific female odonate. He reported that Lamb (1952, in Corbet, 1962) had Pantala flavescens eggs hatch for a period of 35 days and Leiftinik (1933, in Corbet, 1962) reported that eggs of Procordulia artemis hatched for 31 days. This variation was greater for those species which laid egg-strings.

TABLE 4

Average Days to Hatching as a Function of Temperature in
Selected Species of Odonata

Temperature	Species	Authority	Average
22°C	Ischnura damula	Johnson (1964)	15 (13–18)
23–28°C	Ischnura damula	(same)	11.5 (10–14)
20–25°C	Sympetrum striolatum	Corbet (1956)	20[*]
24°C	Sympetrum striolatum	(same)	22[*]
25–30°C	Sympetrum striolatum	(same)	9[*]
20°C	Epiophlebia superstes	Asahina (1950, in Corbet, 1962)	30
25.5°C	Epiophlebia superstes	(same)	20
7.8–24.4°C	Hemicordulia tau	Hodgkin & Watson (1959, in Corbet 1962)	20
21.1°C	Hemicordulia tau	(same)	10
29.4°C	Hemicordulia tau	(same)	6
20°C	Libellula incesta	Pittman (this report)	14.7
25°C	Libellula incesta	(same)	13.5
30°C	Libellula incesta	(same)	8.3
35°C	Libellula incesta	(same)	5.4

[*]beginning of hatching period and not average

Kormondy (1959) found that Tetragoneuria cynosura eggs which are laid in strings hatched over a period of 40 days.

The results of the present study showed the longest hatching period to be 14 days. These data were based on the combined results of eggs kept at 30°C from several females. The longest period for a single female's eggs maintained at 30°C was 13 days which deviates very little from the combined data.

In some species such as Tetragoneuria cynosura (Kormondy, 1959) which lay egg strings, the hatching range was considerable. Yet Aguesse (1959, in Corbet, 1962) found that in species which dispersed their eggs like Libellula incesta, this hatching period range was much less and more nearly approximated the 3-14 day range similar to my results obtained at all temperatures for Libellula incesta. Aguesse also observed that 96% of the eggs of Sympetrum meridionale hatched in 22 days, and all of the eggs of one female of Crocothemis erythraea hatched in 13 days.

The difference in the hatching ranges for dispersed eggs versus clustered eggs in strings is no doubt related to the manner of oviposition. Dispersed eggs were exposed more equally to the environment and able to develop at a more uniform rate, while in clustered eggs those inside were retarded due to lower available O_2 and excess accumulation of waste products around the eggs.

Odonate egg development at various controlled temperatures has not been investigated previously, but based on the results of this study several generalizations seem apparent. The 15°C condition

represented the approximate lower temperature threshold for egg development since only 0.71% hatched at 15°C after 60-70 days following oviposition. Additional supporting evidence comes from the low percentage of eggs that hatched even after the 15°C eggs were transferred to higher temperatures. Obviously, the low temperature greatly retarded development and reduced viability. Yet a temperature of 15°C was not lethal as some of the eggs developed and a few hatched successfully. That development occurred in these eggs at low temperature was evidenced by the embryonic eye spots which were clearly visible 35 days after oviposition. This period in development to the eye-spot stage was recorded at 7-8 days in eggs developing at just 5°C higher.

The idea of retarded growth is supported by comparing the number of days to hatching after removal from 15°C to higher temperatures. Those eggs removed from 15°C and placed at higher temperatures hatched in less time than the total development time of freshly laid eggs at the specified higher temperatures, with the exception of those kept for 50 days at 15°C and then placed at 20°C.

Little can be said about the results obtained on eggs maintained at 40°C since none of these hatched even though some exhibited partial development. A temperature of 40°C obviously was above the upper limit for survival. It is probable that 40 and 15°C closely approximate the upper and lower thermal limits for egg development.

Those eggs developing between 20 and 35°C hatched within the expected period for eggs occurring in nature at this latitude. These results strongly support Corbet's (1962) idea of a positive thermal coefficient for growth. An optimal temperature for development may have been inferred by my results. Approximately 11% more eggs hatched at 25 and 30°C than at the 20 and 35°C conditions. Even larger differences occurred when comparing these figures with the 15 and 40°C hatching percentages. Two factors might possibly account for these differences. The procedure for handling the eggs may have varied from one condition to the next, but this was unlikely since they were all prepared at the same time in the same manner. Secondly, the 25 and 30°C conditions, which closely approximated naturally occurring temperatures, were closer to the optimum for hatching for Libellula incesta. Therefore, these eggs experienced a higher percentage viability.

With no previous comparable studies it is difficult to do more than state a few possibilities based on the data collected in the egg development experiments. It will be interesting to compare my results with those of other species presently being studied by Lutz.

Growth and development patterns for both natural and experimental animals for the various stages in the life cycle of Libellula incesta were investigated in this study. Fertilized eggs of this species darkened within 24 hours just as those of other reported species. Gardner (1950a, 1950b, 1951a, 1951b) found this circumstance in several studies involving the genus Sympetrum, and also in his (1953) study of

Libellula depressa. In addition the eggs of Libellula incesta had other
typical exophytic egg characteristics such as a broad elliptical shape,
a nipple-shaped pedicel at the anterior end, and a sticky gelatinous
covering which caused the eggs to adhere to any object with which they
came in contact. Gardner in his studies found this to be true, and
Corbet (1962) presented this description as typical of exophytic
odonates.

The egg size of other species within the genus Libellula
compared favorably with that of Libellula incesta eggs. Wilson (1917)
reported the eggs of Libellula pulchella measured 0.48 mm long by 0.29
mm wide and the eggs of Libellula luctuosa were 0.57 mm by 0.30 mm.
In addition, Gardner (1953) established that Libellula depressa eggs
were about 0.80 mm by 0.50 mm. In this study, Libellula incesta eggs
averaged about 0.50 mm long by 0.30 mm wide, well within the
anticipated range.

Development was observed to be direct as reported for other
members of this genus by Wilson (1917) and Gardner (1953). The
hatching process for this anisopteran was almost identical to that
reported by Grieve (1937) for the damselfly Ischnura verticalis.
First instar, or prolarvae animals proceeded to molt into the second
instar in a manner consistant with that reported by Corbet (1962) for
the generalized odonate. Individuals of Libellula incesta developed
through several stages during the summer months, became inactive
with the approach of cold weather, and overwintered in one of four
instars. Upon resumption of growth in the spring they molted into the

final instars and emerged throughout the summer over a period of nine or more weeks. This pattern is typical of what Corbet (1962) called a "summer species". Corbet recognized this and "spring species" as the two general catagories of Odonata. He based this designation on their pattern of development and emergence. A "spring species" over-winters in the final instar and has a well synchronized emergence in early spring. *Tetragoneuria cynosura* studied by Lutz and Jenner (1964) is a typical example, and Corbet, Longfield, and More (1960) placed several British species of *Libellula* in this group.

Typical "summer species" such as *Libellula incesta* and *Aeshna cyanea* (Corbet and Corbet, 1958) overwintered in one of several instars prior to the final and exhibited a rather long unsynchronized emergence pattern during the summer. The lack of synchronization resulted from the instar spread within the winter population structure.

Libellula incesta was shown to be a univoltine species, but there were few comparative records in the literature concerning the development of other species within the genus *Libellula*. Corbet, Longfield, and Moore (1960) reported on several species of British odonates, but those species were subject to quite a different climate than that found in this area, which would affect the rate of development. Also they based their conclusions on what they referred to as "slight evidence from various sources including small larvae samples and information from larvae reared in captivity" (Corbet, Longfield, and Moore, 1960, p. 82). Based on these data they concluded that *Libellula depressa*, *Libellula quadrimaculata* and *Libellula fulva* all required

two or more years to complete development. Libellula incesta, on the other hand, developed in a single year in a manner comparable to that found for Pachydiplax longipennis by Eller (1964). This species was studied in the same geographical area as Libellula incesta and differed only in that Eller found 2-3% of his population was semivoltine and that about 1% overwintered in the final instar. Neither of these factors were present in the Libellula incesta population used in the present study.

The larval results obtained by Eller (1964) were typical of a summer species except for those small percentages mentioned. His population overwintered in six instars, the most advanced being the penultimate or F-1 instar. The Libellula incesta population structure contained only four recognizable instars in its overwintering population with the most advanced being the F-1.

Growth resumption occurred in two surges partly supporting Corbet's (1957) theory of lower temperature thresholds for successive developmental stages. Corbet (1962) explained this using a hypothetical situation involving a semivoltine summer species which overwintered in the last four instars. He postulated that if the lower temperature threshold for ecdysis and thermal growth coefficients for each of these four instars were in ascending order, the rise in vernal temperatures would cause an accumulation of larvae in the less advanced instars before passing to the next more advanced one. This would eventually result in an accumulation of larvae in the final instar. He further stated that in order for this system to work fully the larval stages

needed to be spread out in time. Such a temporal range would permit an accumulation of larvae in each successive stage and finally in the ultimate instar, causing a high degree of synchronization in the emergence of the population. He noted that the development time for an univoltine species was insufficient for and the vernal temperatures rose too rapidly to allow the population to take full advantage of a lower temperature threshold phenomenon. This resulted in a widely dispersed unsynchronized emergence pattern.

My results clearly show that growth resumed between March 19 and April 9 for the less advanced instars (F-2, F-3, and F-4) before it resumed in the F-1 instar. Therefore, a marked decrease in the F-4 instar and accumulation in the F-1 instar were realized before any individuals molted into the final instar. This was followed by a second growth wave in which the F-1 instars molted between April 9 and April 30 causing and accumulation of individuals in the final instar in the Arpil 30 and May 13 collections.

Corbet's (1962) idea of a widely dispersed emergence pattern for univoltine summer species was supported by Libellula incesta. This species experienced neither a particularly large accumulation in any one instar nor a synchronized emergence period. It had a rather unsynchronized emergence period lasting from 2-4 months.

Comparing the well-synchronized emergence period lasting 5-6 weeks as reported by Lutz and Jenner (1964) for T. cynosura, the emergence period for Libellula incesta was quite widely dispersed and

in line with other "summer species". Jacobs (1955) reported an emergence period from May through August for Plathemis lydia, and Eller (1964) found that another "summer species", P. longipennis, emerged from May 23 to August 18. His timing for the various life-history events correlated amazingly well with those of Libellula incesta, possibly indicating a general pattern for summer species in this part of the world. Additional studies will be needed to substantiate such a trend.

The 134-day (May to October) flight season for mature adults of Libellula incesta was considerably longer than that of 70 days for Libellula quadrimaculata or the 76 days for Libellula depressa as reported by Moore (1951). Comparison of Libellula incesta with these species is of little consequence since both were British "spring species" which flew from the end of May to the first week in August. In spite of the large obvious overlap in the flight seasons of these species, of which one was a summer species while the other two were spring species, the climate affected both the time of the flight season and its length. It is the flight season in relation to the geographical area and larval population structure which differentiates between spring and summer species.

The climitalogical effect is evidenced by Kormondy's (1959) work in Michigan and Lutz and Jenner's (1964) study in North Carolina involving the same species. Kormondy found that T. cynosura in his study area flew from May through July, while Lutz and Jenner found the flight season in North Carolina occurred during April and May.

No complete records were available on summer species of Libellula, but Westfall (1942) in a North Carolina collection reported that Libellula luctuosa adults were taken from June until mid-September and Libellula incesta adults from June 17 to August 17. Based on observations by Lutz (unpublished data), Libellula luctuosa flew in piedmont North Carolina from June 3, 1970 to September 19, 1970. Even though these limits may not represent the full extent of the flying season, Libellula luctuosa would be classed as a summer species in this area, and its flight season and overwintering larval population structure were almost identical to that of Libellula incesta.

The maturation period for adult odonates represented the first distinct phase of adult life. The estimated two weeks as a maturation period for Libellula incesta compared reasonably well with that of other Odonata species. Corbet (1962) indicated that odonates normally took from one to three weeks to mature in nature. He found an average maturation period of 14.5 days for A. imperator (1957a) and 15 days for Pyrrhosoma nymphula (1952). Pajunen (1962a) indicated 8-12 days for males of Leucorrhinia dubia to mature, and Jacobs (1955) found male Plathemis lydia took 8-14 days while females required 13-24 days.

The second or reproductive phase of the adult life generally lasted longer than the maturation period. Rough estimates for this period have been established previously for two species of Libellula. Moore (1951) subtracted the date of the last teneral individual from the date of the last adult flying to derive a figure for the maximum total life span. This established a value of 48 days for Libellula

quadrimaculata and 35 days for Libellula depressa. Applying this
process to the Libellula incesta data, a value of about 85 days was
obtained which was considerably longer than that shown for other
Libellula species or any other reported odonate species. Corbet (1957a)
reported the longest, carefully observed life span which was 60 days
for A. imperator. Without benefit of sufficient emergence data, it
seems unreasonable to assume that adults of Libellula incesta lived
for 85 days, but it would be within reason to assume a life span figure
within the reported limits of other odonates, that is, between 35 and
60 days.

During the reproductive phase males of Libellula incesta spent
long periods of time protecting their territories at the pond, typical
of the behavior of other odonates. Considerable arguments as to whether
or not odonates actually possess territories have been presented; yet
the matter remains unresolved. Libellula incesta males exhibited what
appeared to be a form of territorality the size and existence of which
was not measured experimentally as was done for several other species.
Their territories were, however, estimated with reasonable accuracy
to be about five to six meters each. Moore (1962) found Libellula
depressa density to be eight per 100 meters of pond edge and Libellula
quadrimaculata concentration was about 28 per 100 meters which was an
average of 12.5 meters and 3.6 meters, respectively. In his investig-
ation of P. lydia and Perithemis tenera, Jacobs (1955) found they
occupied territories of 11 meters and 6 meters, respectively. Kormondy
(1959) found the territories of T. cynosura varied from 3 to 10 meters.

Moore (1953) postulated that territory size varied with the population density. He found that at lower densities the males in his study occurred about one every 50 to 100 yards, whereas at high population densities this was cut to about one every 3 to 10 yards. He seemed to think that territory size decreased in relation to density increase to a point where the size of the territory was proportional to the range of motion perception for the individual species. His idea was supported at least in part by this study but certainly nothing was conclusive.

In defending their territories odonates were forced to interact. Moore (1952a) thought interactions were due to the inability of dragonflies to differentiate between the sexes, but Pajunen (1962b) disagreed. Pajunen filmed accounts of Libellula dubia interactions and was able to distinguish between approaches of a sexual and those of an agressive nature. This problem remains unresolved due to insufficient studies, but interactions certainly accomplished population dispersal as evidenced by this and other studies. Adult males of Libellula incesta were fairly evenly dispersed along the length of the study area and Jacobs (1955) found a similar situation with P. lydia and P. tenera.

It is interesting to note that the greatest number of inter-specific interactions by adult Libellula incesta were with the two species which most nearly approximated the general size and appearance of Libellula incesta. Lutz and Pittman (1970) found the highest

number of interspecific interactions occurring between Libellula incesta
and Libellula luctuosa which were present in about equal numbers.
Adults of Libellula luctuosa closely resembled the females of Libellula
incesta except that Libellula luctuosa males were marked with white on
their wings.

In spite of the fact that there were never more than one or two
Tramea lacerata flying in the study area at any given time, the second
largest number of interspecific interactions involving Libellula
incesta occurred with this species. Tramea lacerata is a species
which is slightly larger than Libellula incesta but otherwise quite
similar. Moore (1962) postulated that more interspecific interactions
occurred between species which superficially closely resembled each
other. The interaction observations between Libellula incesta and
Libellula luctuosa and Libellula incesta and T. lacerata supported
this idea.

During the day only males remained at the pond with females
appearing only to mate and oviposit. Where females spent their time
while not at the pond were not established. Females of Libellula
incesta were never observed anywhere other than the immediate vicinity
of the pond, but since they were not particularly colorful they would
be difficult to spot away from the water. Therefore, it was possible
that they were present in the vicinity and just not recorded.

The 12- to 13-hour flight period of males of Libellula incesta
was somewhat longer than the 10- to 11-hour one found by Lutz and
Pittman (1970) for Libellula luctuosa. Libellula incesta started

flying later than some other species of Odonata but flew as late as any except _Epicordulia_ _princeps_ and _Enallagma_ _signatum_.

Libellula _incesta_ females were observed arriving at the pond about thirty minutes after the males appeared. This difference in arrival time may be significant since according to Corbet (1962) there is a possibility that in some species the two sexes have different temperature thresholds for activity. He cited examples of _Aeshna_ _viridis_ (Rantalinen and Kanervo, 1928, in Corbet, 1962) in which males were active some four hours before the females arrived and _Platycnemis_ _pennipes_ (Buchholz, 1956, in Corbet, 1962) in which the males arrived 10-60 minutes before the females. Jacobs (1955) found a similar lag of 10-60 minutes in _P. tenera_. Lutz and Pittman (1970) reported the arrival time for males and females of five species of Anisoptera other than _Libellula_ _incesta_ and in only one, _Celithemis_ _fasciata_, did both sexes arrive at the same time. The remainder showed a thirty minute lag for _P. tenera_ and _Celithemis_ _eponina_ and an hour difference in arrival time of _Lepthemis_ (_Erythemis_) _simplicicollis_ and _Libellula_ _luctuosa_. It was difficult to determine if this response was temperature-dependent because in thirty minutes there was little change in ambient temperature. This study did not calculate the relationship of temperature to this aspect of behavior. Subsequent studies will be necessary to relate arrival time of the sexes to temperature.

Corbet, Longfield, and Moore (1960) noted that reproduction is the main theme of a dragonfly's life, but only a small fraction of its

time is spent in actual reproductive activities. Whenever females
of Libellula incesta were present at the pond, mating and oviposition
occurred. Pajunen (1964a) and Johnson (1962) stated that all repro-
ductive activity occurred within the territory and these were located
at the pond. One exception to this rule was reported by Pajunen
(1963). After six years of observing adults of L. dubia, he saw one
case of mating deep in the woods away from the pond.

The major mating differences in the various species were the
length of time spent copulating and whether this was accomplished in
flight or perched. Table 5, reproduced from Corbet (1962) with the
exception of data obtained from other sources indicated by an
asterisk (*), shows the undisturbed copulation time for both
zygopteran and anisopteran species. Libellula incesta was rated in
this study as a medium duration species and placed there in the table.

Whether a species copulated in flight or while perched normally
was related to the length of mating time. Those experiencing short
duration completed the process in flight (Corbet, 1962). Perithemis
tenera was the exception because even though it began copulation in
flight, it finally perched to complete the process. Others in the
medium and long range groups, including Libellula incesta, often
started in flight but almost always completed this process while
perched. There were four instances in this study when adults of
Libellula incesta completed mating while flying, but this represented
only a small percentage.

TABLE 5

Recorded Durations of Undisturbed Copulation in a Variety of
Odonata Species

Species	Authority	Duration
Plathemis lydia	Jacobs (1955)	3 seconds
Tetrathemis camerunensis	Neville (1960a)	5 "
Libellula quadrimaculata	Robert (1958)	5-20 "
Crocothemis erythraea	Aguesse (1959b)	7-15 "
Tholymis tillarga		15 "
Perithemis tenera	Jacobs (1955)	17.5 "
Calopteryx splendens	Buchholtz (1951)	1½ minutes
C. virgo	Robert (1958)	2-5 "
Libellula incesta	Pittman (this study)	½-1½ "
Gomphus flavipes	Robert (1958)	5-10 minutes
Lestes barbarus	Neilsen (1954)	6 "
Sympecma fusca	Loibl (1958)	9½-21 "
Enallagma civile	Bick (1963)[*]	11-44 "
Anax imperator		10 "
Anax parthenope	Munchberg (1932)	10-15 "
Sympetrum striolatum	Aguesse (1956b)	10-15 "
Pyrrhosoma nymphula	Robert (1958)	15 "
Leucorrhinia caudalis	Pajunen (1964)[*]	20-30 "
L. dubia	Pajunen (1964)[*]	25-40 "
Lestes unguiculatus	Bick (1965)[*]	25 "
Macromia picta		31 "
Lestes dryas	Loibl (1958)	51-145 "
Aeshna juncea	Robert (1958)	60-75 "
Ischnura elegans	Krieger and Krieger-Loibl (1958)	180-340"

[*](From Corbet, 1962)

Pajunen (1963) reported that L. dubia and Leucorrhinia rubicunda females rested for up to a minute after mating and before oviposition. These were the only species of Anisoptera about which there was mention of a rest period. In this investigation about one half the observed female Libellula incesta rested before ovipositing.

The average oviposition time was not reported for other Libellulidae, but the manner of Libellula incesta oviposition was similar to that reported by Gardner (1953) for Libellula depressa. The eggs were extruded onto the flattened, spoon-shaped ovipositor were washed off the abdomen when the tip submerged below the surface of the water. It had been thought that odonates squirted eggs out above the surface with above-water pumping action observed in many species. Fraser (1953) found this to be untrue, and the eggs actually had to be washed off by dipping the tip of the abdomen into the water.

Corbet, Longfield, and Moore (1960) noted that Libellula quadrimaculata did not attempt to remate immediately with the ovipositing female but protected her while oviposition occurred. Males pursued females upon completion of oviposition but only rarely succeeded in remating. This was in accord with observations on Libellula incesta. Adults of Libellula dubia and L. rubicunda (Pajunen, 1963), Leucorrhinia caudalis (Pajunen, 1964), and P. tenera (Jacobs, 1955) also exhibited the same behavioral patterns.

No reports concerning the rapidity with which exophytic dragonflies lay their eggs have been published, but in Libellula incesta it was a very rapid process. The slowest oviposition average

was 0.317 abdominal submersions per second and the fastest was 1.19 submersions per second. Total length of oviposition time seemed to have little to do with rapidity of oviposition. Those with the longest total time had either average or above-average oviposition rates. The average for all observed females was 0.646 submersions per second. The two females with the longest total oviposition times of 141 seconds and 166 seconds had averages of 0.816 and 0.632, respectively.

Corbet, Longfield and Moore (1960) expected exophytic dragonflies to lay more eggs than endophytic species due to the broad spacious abdomen of the former group. Whether or not this is true has not been established. Females of Libellula incesta, with an average of 1060.4 eggs per female, certainly had a large reproductive potential. Table 6, taken from Corbet (1962), with the exception of those species shown by an asterisk (*), shows the number of eggs laid by various species of dragonflies. The egg output by females of Libellula incesta was equaled or surpassed only by T. cynosura and Gomphus externus, both of which lay eggs in strings rather than individually. The number laid by females of Libellula incesta was almost twice that laid by the other species of Libellula.

Jacobs (1955) found that competition between male and ovipositing females of P. tenera often made oviposition impossible, and the females left without completing the process. The same reaction was observed during this study for Libellula incesta and also for a similar species, Libellula luctuosa.

TABLE 6

Number of Eggs Laid in Single Clutches by Different Species of Dragonflies

Species	Authority	Number of eggs laid
Gomphus externus	Needham and Heywood (1929)	5,200[a]
Epitheca bimaculata	Robert (1958)	1,924[b]
Tetragoneuria cynosura	Kormondy (1959)	1,060[b]
Somatochlora metallica	Robert (1958)	900
Pantala flavescens	Warren (1915)	816
Epiophlebia superstes	Asahina and Eda (1958a)	600–1,000
Libellula fulva	Robert (1958)	500–600[c]
Tetragoneuria spinigera	Kormondy (1959)	524[b]
Leucorrhinia dubia	Robert (1958)	200–300
Aeshna isosceles	Robert (1958)	243
Sympetrum danae		233
S. sanguineum	Gardner (1950)	200[a]
S. striolatum		200[a]
Procordulia artemis	Lieftinck (1933)	150[a]
Perithemis tenera	Jacobs (1955)	150[d]
Erythemis simplicicollis	Curri (1961)*	323.16
Libellula incesta	This study*	1,060.4

[a] known to be less than whole complement

[b] egg-string

[c] female caught mating

[d] average of 13 unusually productive females

* (From Corbet, 1962)

Late in the afternoon females of <u>Libellula</u> <u>incesta</u> often came to

the pond and oviposited without being observed to accept a mate.

Pajunen (1962a) found this reaction in lone females of <u>Libellula</u> <u>dubia</u>.

Whether these females were ones that mated earlier in the day and had

been chased off before completing oviposition was uncertain, but judging

from the large number of disturbed ovipositions this was entirely

possible. Corbet (1962) noted that disturbance during oviposition was

quite common but failed to note that often the protecting males were

unable to stand off the attacks and the females were forced to leave

as in the case of <u>Libellula</u> <u>incesta</u> females.

CHAPTER V

SUMMARY

1. Eggs of <u>Libellula</u> <u>incesta</u> exhibited direct development and the hatching rate was related to the temperature at which they were maintained without regard to photoperiod. Eggs maintained at natural temperatures required 7-10 days to hatch, while the experimental eggs receiving temperatures between 20 and 35°C had a hatching range from 5.4 - 14.7 days. There was a decrease in development time with each 5°C increase in temperature. Development at any given condition was fairly uniform, and eggs hatched over a period of 3-14 days. Those eggs at 15 and 40°C experienced the upper and lower temperature thresholds for development, resulting in reduced viability and retarded development.

2. The exophytically-oviposited eggs were cream colored when laid but turned reddish brown within 24 hours. They averaged 0.50 mm x 0.30 mm, were elliptical with a short colorless pedicel at the anterior end, and were covered with a sticky, transparent, gelatinous film. Hatching was in a typical odonate manner into a short term prolarvae or first instar and quickly into the second instar.

3. Larvae of this univoltine species overwintered in one of four instars, the most advanced being the F-1. Larval growth and emergence lent support to the positive thermal growth coefficient theory; however, this support was not conclusive. The unsynchronized emergence period began in mid-May and ended somewhere between mid-July

and September.

4. The 134-day adult flight period begain in late May and ended in October. During this period males of the population established and protected restricted territories for 12-13 hours daily. The individual territory measured 5-6 meters in length and in order to protect it there were numerous intraspecific interactions and frequent interspecific interactions particularly with Libellula luctuosa and Tramea lacerata. Males arrived at the pond about one-half hour prior to females who came to the water only long enough to mate and oviposit.

5. Mating between 34 timed pairs of Libellula incesta averaged 46.5 sec. per copulation, after which the females either rested briefly or went directly to the water to oviposit. Oviposition time averaged 110.1 sec. per female for those allowed to complete the process undisturbed. The process consisted of rapidly dipping the tip of the abdomen into the water at an average speed of 0.646 sec. per dip. Meanwhile the male protected her by circling, hovering nearby, or chasing other males away. Upon completion the females retreated to the protection of the surrounding woods.

6. The seven females contributing an entire clutch of eggs had an average of 1060.4 eggs per female.

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