ENVIRONMENTAL CONTROLS IN THE SEASONAL SUCCESSION AND SYNCHRONIZATION OF DEVELOPMENT IN SOME POND SPECIES OF DAMSELFLIES (ODONATA: ZYGOPTERA)

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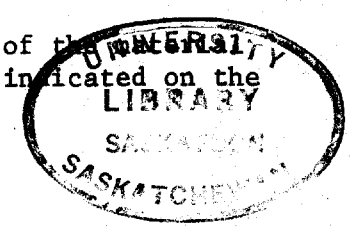

ABSTRACT

Environmental factors which control seasonal succession and synchronization of development in seven species of pond-dwelling Zygoptera were investigated. A three phase succession was observed. Three species of Coenagrionidae, Coenagrion angulatum, C. resolutum and Enallagma boreale which overwintered as nymphs, emerged simultaneously and synchronously commencing during the last week of May. They were followed by Lestes disjunctus disjunctus, L. unguiculatus and L. dryas in late June and early July, then by L. congener near mid July.

Synchronization of development in the coenagrionid species is produced by a temperature and photoperiod influenced diapause in the penultimate and final instars, by differences in thermal growth coefficients in different instars during spring development, and by a threshold temperature for emergence higher than that for nymphal development.

Members of the second group overwinter as eggs in late stages of embryonic development, and are prevented from hatching in the fall by a diapause whose development is accelerated first by low temperature then by long photoperiod. Absence of suitable temperatures in the winter prevent post-diapause development. Further synchronization is obtained through simultaneous wetting which initiates development in the spring.

Finally, L. congener overwinters in the pre-blastokinesis stage of embryonic development. Diapause development is accelerated by low temperature; however, no effect of photoperiod was observed. Winter conditions prevent hatching when diapause is terminated. Hatching is synchronized by diapause, by simultaneous wetting of the eggs and by differential embryonic development and hatching temperatures in the spring. Rapid nymphal

development in the latter two groups sustains the developmental synchrony which was established at the time of hatching.

Seasonal succession was discussed in relation to the factors which control hatching and nymphal development in the three species types.

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1. INTRODUCTION

The multitude of organisms that occupy a typical prairie pothole provides ideal material for research into factors which govern the seasonal succession and synchronization of development in aquatic invertebrates. Among them the Odonata of the suborder Zygoptera (damselflies) have qualities which make them especially suitable for such studies. Several species inhabit these potholes, some sharing a common niche. The dominant species occur in large numbers and are readily available in the egg, nymphal, and adult stages. The eggs and nymphs are easily maintained in the laboratory and the later nymphal instars are easily identified.

This investigation stems from the provocative ideas of Corbet (Corbet et al., 1960; Corbet, 1963) which emanate from his earlier work on the life histories and ecology of British dragonflies (Corbet, 1954, 1956a, b,c; 1957a, b, c). Corbet (1954) found among British dragonflies some species which emerge synchronously and early in the season and others which emerge later in the summer without a distinct early emergence peak. The first group, which he called 'spring species' (Corbet, 1954), is typified by Anax imperator Leach in which 50% of the annual population emerges within three days (Corbet and Corbet 1958) and more than 90% within ten days (Corbet, 1954). The second group, called 'summer species' (Corbet, 1954), is represented by Aeshna cyanea Müll. and Lestes sponsa Hansemann. They only reach 50% emergence after the twenty-fifth day (Corbet and Corbet, 1958). In his search for mechanisms which regulate development and produce this type of synchronized emergence, Corbet (1954, 1956c) found in Anax imperator evidence of a distinct diapause in the final instar. This he applied as a condition defining 'spring species'. 'Summer species', on the other hand, over-

wintered either in the egg stage or some stage of nymphal development other than the final instar (Corbet, 1954).

In 1958 Corbet described two groups of 'summer species', those which developed within one year and exhibited asynchronous emergence and those which required two years to develop and had a relatively synchronous emergence. In his review Corbet (1963, p. 96), noted that:

> A fact which nevertheless requires explanation is that, even in summer species, emergence is sometimes better synchronized than would have been expected from the size-variation shown by the larval population. It is evident therefore that some factors must operate to reduce temporal variation shortly before emergence.

This statement was succeeded by speculation on the factors which might be operating in order to synchronize emergence in the 'summer species'. In 1964, Corbet concluded that while the 'spring species' concept still had some value "... they can no longer be defined as possessing a diapause only in the final instar, since development may be subject to environmentally-induced delay in earlier instars as well". He then suggested that perhaps the term 'spring species' should not be used generally to describe those species possessing synchronized emergence but restricted to those species in which this synchronization is accomplished in the simplest way. Other species achieving this degree of synchrony by more complex methods and those which emerge asynchronously should perhaps be reclassified (Corbet, 1964). The latter conclusion came after the publications of Jenner (1958) and Montgomery and Macklin (1962) which showed a possible involvement of photoperiod in synchronization of nymphal development in some North American odonates.

Several North American odonatologists tried to fit their species to Corbet's hypothesis. Lutz (1963) classified Tetragoneuria cynosura Say as

a 'spring species' with a definite diapause in the final instar. Eller (1964), who worked on seasonal regulation in a libellulid Pachydiplax longipennis Burmeister, described it as a 'summer species' in his introductory remarks. His concluding statements (Eller, 1964, P. 179) indicate, however, that he was frustrated with the classification scheme. He noted that:

> Pachydiplax longipennis is notably different from any 'summer species' studied by Corbet (1956b). His univoltine 'summer species' passed the winter either in the egg stage (Lestes sponsa) or as very small nymphs (Sympetrum striolatum). The prominent role of diapause in seasonal regulation in P. longipennis demonstrated in this study, indicates that Corbet's denial of a synchronizing role for diapause in 'summer species', will be subject to major revision as our knowledge of Odonata biology increases.

Kormondy and Gower (1965) also found shortcomings in Corbet's theory when applied to species which they studied. They observed that Enallagma ebrium Hagen exhibited a highly synchronized emergence pattern typical of spring species or semivoltine summer species while itself possessing only a one-year life cycle.

Benke (1969) concluded that Corbet's classification of Odonata on the basis of life cycles and emergence patterns was inoperative, at least for nearctic species. He proposed that species should be identified simply as "synchronous" or "asynchronous", depending solely on their emergence pattern. As an example of an "asynchronous" species he suggested Plathemis lydia Drury. This species had a fairly stable age distribution during the warm season; during the winter growth continued without emergence, causing an accumulation of individuals in the larger instars. However, this accumulation was apparently not a significant synchronizing mechanism. Other species tentatively placed in this group were Pachydiplax longipennis and Erythemis simplicicollis Say. Ladona deplanata Rbr. characterized the typical

"synchronous" species by showing a constantly increasing mean instar until most individuals were in the final instar.

The controversial classification schemes reviewed here set the stage for the present research into the life-histories and emergence patterns of several species of Western Canadian odonates. The study is of particular interest since to date no investigations have been undertaken on species subject to the rigorous environmental limitations presented by Canadian prairie winters.

Recognizing the role of diapause as a synchronizing mechanism in Anax imperator, Corbet (1954, 1956c) initiated a search for environmental factors governing diapause induction and termination. He found that nymphs entering the final instar are able to detect the amount by which successive days are changing in length irrespective of absolute day length involved. If day length increases by more than two minutes per day the larvae bypass diapause and emerge (Corbet, 1955, 1956c, 1957a). Schaller (1960) found that a response to changing day length played an important role in breaking diapause in Aeshna cyanea. These results were criticized, first by Danilevsky (1961) and later by Eller (1964). Both felt that the experimental data presented were inconclusive and that explanations based on absolute values of photoperiod were possible.

Jenner (1958), in a preliminary investigation, showed a differential response in one species of dragonfly, Tetragoneuria cynosura, and five species of damselflies to short and long photoperiods. Long photoperiods of 13 to 14 hours promoted nymphal development while an 11 hour photoperiod induced diapause. Diapause was induced in the final nymphal instar in T. cynosura and Ishnura posita Hagen, in the final and penultimate instars in Enallagma signatum Hagen and E. basidens Calvert, in the penultimate in E. divagans Selys and in several instars in E. traviatum Selys.

In 1960 Lutz and Jenner reported that while development rate at the short photoperiod was only about one third of that at the long photoperiod in T. cynosura in the fall, the difference in response to 11 and 14 hour photoperiods was all but erased in nymphs collected in March and April. This investigation also included the first monitoring of physiological responses to different photoperiods through observations on changes in oxygen consumption. Subsequent experiments on this species (Lutz, 1963; Lutz and Jenner, 1964) demonstrated that while long photoperiod induced diapause prior to the fall equinox, a reversal in response to day-length occurred after this date with the longer photoperiod inducing more rapid development in the specimens collected in the fall and winter.

Although Corbet recognized the presence of diapause in instars other than the final, he remained unconvinced of its role in synchronization of emergence (Corbet, 1963). In its stead he proposed, for species which diapause in instars other than the final, a scheme for synchronization based on temperature thresholds and/or thermal growth coefficients in a progressively ascending series (Corbet, 1957b, 1963); that is, the younger the instar the lower will be its development and moulting temperature threshold. At that time he could find no example in odonate literature to support his theory. However, he later noted that Lloyd (1941) found such a mechanism operative in the chironomid, Spaniotoma minima Mg, which breeds in sewage beds. Lutz (1968) has since discovered such a mechanism synchronizing nymphal development in Lestes eurinus Say where generally development in the younger instars was completed at a faster rate at 15°C than at higher temperatures. Conversely, final instar development took place more rapidly at temperatures of 25°C and 30°C.

The work of Jenner (1958) and the apparently unpublished information exchanged between Jenner and Corbet (Corbet, 1963) suggest yet another way in

which synchronization might be achieved in a population overwintering in the nymphal stage. Jenner found that nymphs of T. cynosura, Ishnura posita and several Enallagma species reared under photoperiods of 11, 12, 13 and 14 hours underwent pre-emergence development at a rate inversely proportional to the photoperiod. The photoperiodic influence affected different stages of development to different degrees depending on the species. This work has apparently never been followed up. Brief attention was given to this aspect in the present study.

Eller (1964) studied a so-called 'summer species' Pachydiplax longipennis in which he tested the effect of 11 and 14 hour photoperiods on various instars of an overwintering population. He expanded on the approach of Lutz and Jenner in determining seasonal changes in response to these photoperiods. In addition to corroborating the findings of Lutz and Jenner (1964), he noted that nymphs of this species were capable of going into diapause in any of five instars and that diapause was induced by short photoperiod at a critical intermoult stage, its intensity depending on the time of the year. He also found that in the course of post-diapause development younger nymphs developed more rapidly than later instars, on occasion completing development more quickly than the specimens overwintering in the late instars. The differential development rate permitted establishment of synchrony in the emerging population. Eller's work was incomplete in two respects. First, he used only two photoperiods, 11 and 14 hours, in his laboratory experiments. From his field studies he showed that a 15½ hour photoperiod might have been more appropriate than 14 hours. Better still, he might have used a range of photoperiods. Secondly, all of his laboratory experiments were conducted at 22°C; no temperature-photoperiod interactions were examined. The present investigation examines the effect of a broader spectrum of temperatures and

photoperiods in an attempt to broaden the scope of the interpretations presented by Eller.

The presence of a diapause stage in the eggs of certain odonates has been recognized for many years. It has generally been associated with species occupying temporary habitats and is believed to be a means by which the species can survive extended periods of adverse conditions during which the aquatic habitat may completely disappear (Needham, 1903). In this instance and others where the aquatic habitat remains, diapause occurs in the overwintering egg stage. Corbet (1956b, 1958) suggested that diapause in Lestes sponsa may be a mechanism which insures that all the individuals pass the winter in the egg stage. He discounted the possibility that diapause in the egg stage might play a role in synchronizing subsequent development "... since the whole larval life is interposed between it and emergence" (Corbet, 1958).

In his review of the odonate literature, Corbet (1963) noted that many dragonflies, especially the Lestidae,lay their eggs above water. This caused him to speculate that perhaps diapause development was completed by October. Hatching could subsequently be induced by wetting and an appropriate temperature,both of which occur in the spring. These triggering mechanisms would insure a suitable environment for the newly hatched nymphs.

Corbet (1956b) investigated temperature effects on diapause development in eggs of Lestes sponsa. He found that the termination of the obligate diapause was most rapidly completed at 10°C and that hatching was subsequently achieved in about two weeks at 20°C. Corbet's results were essentially replicated by Schaller (1968) in his study of diapause development in eggs of Aeshna mixta Latr. He determined an optimal temperature for diapause development as 5 to 10°C. Both Corbet and Schaller noted that a prolonged exposure

of eggs to cold resulted in a synchronization of hatching after a return to 20°C.

A large portion of this thesis will be devoted to the investigation of environmental controls in pre-diapause, diapause, and post-diapause development in eggs of several species of Lestes in relation to synchronization and temporal spacing of nymphal development and emergence.

Odonate research has, up to now, centred around the study of life-histories of individual species, generally under laboratory conditions. Corbet (1963, P. 109) indicated an awareness of the shortcomings of such an approach in his statement: "... The season at which emergence occurs, as well as its degree of synchronization within the emergence period, are presumably associated ecologically with longevity and interspecific competition..." Few people have, however, attempted to investigate species associations in Odonata in order to verify this. While the work of Corbet, Lutz and Jenner, and Eller involved regular sampling of nymphs in the field, species interactions and the position of the species they were studying in relation to other species present were seldom mentioned.

Moore (1953) was among the first to study populations of dragonflies under natural conditions to gain an insight into seasonal occurrences and species interactions. His efforts were concentrated on the activities of sexually mature adults with no attempt to account for their appearance at a particular time of year.

Kormondy and Gower (1965) studied seasonal development and emergence patterns in an association of seventeen species under natural conditions. They observed differences in timing of appearance of the different species and speculated that this might be associated with peak feeding and reproductive demands of the adults. This would enable efficient utilization of the

available resources throughout the season. They also observed inconsistencies in emergence patterns within and among species in the two year study period, and drew the conclusion that different regulatory mechanisms involving light and temperature operated in different species and/or there was a different relative influence of these factors at different stages of the life cycle. These were not investigated. Perhaps a more rigorous sampling program might have eliminated some of the apparent variability.

Benke (1969) investigated associations of nymphal Odonata in an attempt to determine the role of temporal distributions of species in relation to interspecific interactions. He attempted quantitative evaluations of nymphal populations in a search for factors explaining coexistence of several closely related species. While his study was intensive, it centred around the nymphal stage. No attention was given to other parts of the life cycles of these species, nor was any attention paid to environmental influences on various phases of the life cycles.

In summary, the literature cited demonstrates the need for further research in order to clarify a variety of aspects of Odonate biology. Some of the objectives of the present study are:

(a) To make observations on the life-histories of damselflies inhabiting a prairie pond through a system of regular sampling and to relate the findings to the classification schemes of Corbet and Benke.

(b) To look for evidence of species interactions and seasonal succession of damselfly species under field conditions.

(c) To search for environmental and behavioral factors which control life cycles and seasonal succession in damselflies.

(d) To search for evidence of synchronized nymphal development and emergence in the species of damselflies in the study area.

(e) To investigate the role of temperature and photoperiod as controlling factors in egg and nymphal development in damselflies under laboratory conditions and to correlate these findings with field observations.

(f) To search for evidence of diapause in the damselflies.

(g) To evaluate diapause as a mechanism which controls seasonal succession and synchronization of development in damselflies.

2. MATERIALS AND METHODS

2.1 Study Area

Material for the present study was obtained from a single slough, a typical example of the numerous waterfilled depressions occupying the glacial drift of the parklands and prairies of central Saskatchewan (Figs. 1, 2, 3). It is located on the S.W. ¼ Section 21, Township 36, Range 4, West of the 3rd Meridian approximately 6.4 Km (4 mi) east and 1.6 Km (1 mi) north of the southeast boundary of the city limits of the city of Saskatoon, Saskatchewan (Lat. 52° 15' N, Long. 106° 30' W).

The slough lies in a large, flat depression occupying approximately 26.3 hectares (66 acres). It is divided by an earth dam which retains water in a dugout excavated immediately behind it. This arrangement provides a relatively deep water area adjacent to a large shallow marshy expanse wherein habitat preferences of the species could be conveniently studied.

2.1.1 Physical Features of the Study Site

(a) Depth of water

Seasonal fluctuations in water level are considerable. The slough depends entirely on runoff from melting snow and local summer rains for its water supply. Nevertheless, it is considered a permanent body of water. The average depth during the study remained about one metre.

(b) Water temperature

Temperature measurements were not taken in the study pond. However, water temperature data were obtained from the Saskatchewan Research Council which maintains continuous recording instruments in a rather similar pond 4.8 Km (3 mi) away. Temperatures of water within 15 cm of the surface were provided for the period between the first week in May and mid-October. Air

Figure 1. Study area, shallow portion, summer, 1970.

Figure 2. Study area, shallow portion, winter, 1971.

Figure 3. Study area, deep portion, summer, 1970, showing stands of Scirpus which served as oviposition sites for Lestes species.

temperature data for the area were obtained from the meteorological section of the Saskatchewan Research Council.

The slough water tends to have a higher temperature than daily mean air temperatures during the ice-free periods before mid-May and after mid-October. During the intervening period daily fluctuations in shallow water temperature are a reflection of changes in air temperature. A maximum water temperature of 31.5°C was recorded on August 7, 1970.

Though the slough became filled with runoff water on April 6 in 1970 and April 10 in 1971, it was not free of ice until the final week in April in both years. The ice-free period extended to the first week in November in 1969 and 1970. Ice formation was rapid in 1970, reaching a thickness of 38 cm by December 2 under 5 cm of snow. The rate of ice formation is a function of the prevailing air temperatures and the depth of the protective snow cover. Ice thickness reached a maximum of 1 metre by March 4, 1971 in sections of the pond where snow had blown off. In areas of the pond protected by deep snow, ice thickness was considerably less. Neither the deep nor the marshy sections of the pond were ever frozen to the bottom.

Snow, a factor of vital importance in the survival of overwintering stages of all odonates studied, was distributed unevenly over the pond surface. It accumulated early in the season in clumps of reeds and other emergent vegetation to depths of 60 cm or more. There it became extremely hard-packed and stable as a result of wind action. Snow cover over exposed sections of the pond ranged from nil to drifts of 30 cm or more. These drifts tended to shift with each storm.

Ice temperatures were not measured. Data from measurements made near Saskatoon in 1969 by Mr. L. Fertuck of the Saskatchewan Research Council

are presented in Figure 4.

(c) Water analyses

Chemical analysis of the water from the pond was not essential to the study but since such data might provide useful information about the habitat, a single analysis was carried out on water collected July 5, 1971 by the Sanitary Engineering laboratory, University of Saskatchewan, Saskatoon. The data are presented in Table 1. It should be remembered that ion concentrations do not remain stable throughout the ice-free season but tend to change, at times several-fold, corresponding to the extent of evaporation of the water (Sawchyn, 1966).

Oxygen measurements were not taken. However, the appearance of the distinct odour of hydrogen sulfide soon after the slough became covered with ice indicated that oxygen reserves in the water were very rapidly used up, probably by decomposition of the thick layer of ooze and organic material which covers the bottom. The hydrogen sulfide concentration was so high by March that water collected from under the ice was greenish-black in colour. Sulfides were quickly deposited on the walls of the container when the water was brought to room temperature. Even amphipods and Chaoborus larvae became encrusted by sulfide deposits.

2.1.2 Biotic Features of the Study Site

(a) Flora

The study area is very much exposed, surrounded on all sides by cultivated fields. Remains of dry and dying willows border the immediate shoreline of the deep portion of the slough. The large shallow area is devoid of arboreal vegetation (Fig. 1). There is little sign of living aquatic vegetation until near the end of May. Emerging clumps of Beckmania syzigachne (Steud.) Fern. begin to appear in the very shallow water, followed

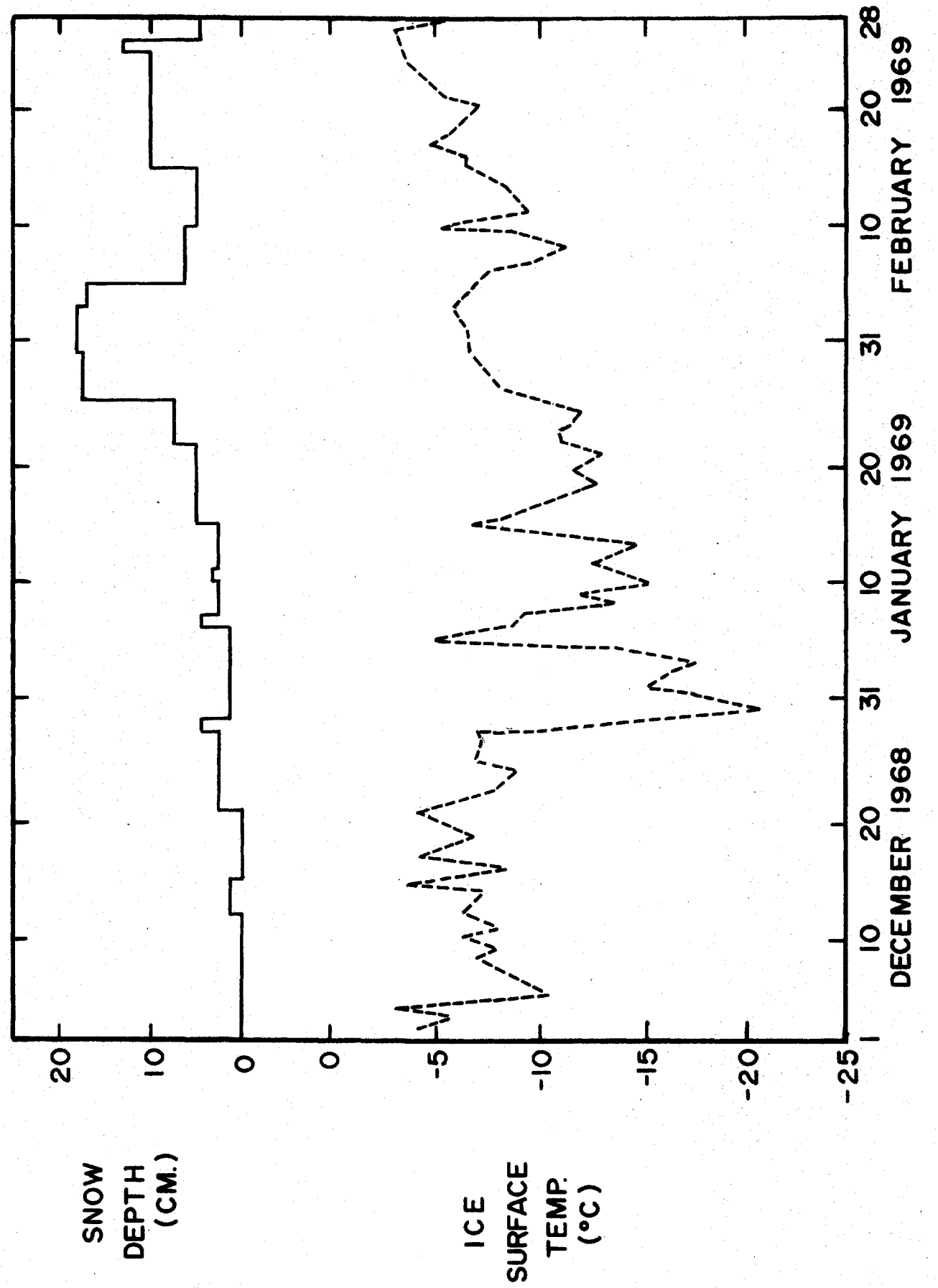

Figure 4. Ice surface temperatures in relation to snow depth in a pond near Saskatoon. (Modified from Fertuck et al. 1971).

Table 1. Report on water quality based on a single water sample collected from the study pond July 5, 1971. Figures are presented in parts per million by weight.

Dissolved solids by evaporation at 103°C	376
Bicarbonate as CO_3 produced by evaporation	184
Carbonate as CO_3	Nil
Chloride as Cl	Nil
Sulphate as SO_4	14
Nitrate as NO_3	Nil
Iron	0.13
Manganese	Nil
Sodium	9
Potassium	18
Fluoride	0.17
Total Hardness	274
Calcium Hardness	142
Magnesium Hardness	132
Phenolphthalein alkalinity	Nil
Total alkalinity	306
pH	8.2
Appearance	Yellow
Odour	Nil

by clumps of Typha latifolia L. and Scolochloa festucaceae (Willd.) Link. The latter becomes the dominant emergent plant in the marshy area, growing over about 50% of the water surface by mid-summer. Flowering stalks of the smartweed, Polygonum amphibium L., appear throughout the shallow water. Later in June the arrowhead, Sagittaria cuneata Sheldon, becomes quite common. The deep water portion is relatively devoid of emergent vegetation with the exception of conspicuous beds of Scirpus (Fig. 3). Floating vegetation which becomes prominent in both sections of the pond after mid-June includes Utricularia vulgaris L., Ranunculus aquatilis L., Potamogeton richardsonii (Ar. Benn) Rydb. and later in the season Myriophyllum exalbescens Fern. Flowering stalks of these plants provide oviposition sites for various odonates as will be discussed later.

Floating mats of the filamentous green algae, Spirogyra and Mougeotia, are common in shallow water throughout much of the summer along with the duckweed Lemna minor L. Other macroscopic algae are rare.

(b) Fauna

Only superficial attention was given to fauna other than the Zygoptera pertinent to the study. Larvae of the phantom midge Chaoborus americanus Johannsen predominated numerically in the overwintering insect population. They were present in large numbers, particularly in the deep water, at all times of the year, with the exception of late May and early June, and provided a valuable source of food for odonate nymphs. Chironomid larvae were abundant in the bottom ooze and in the stems of decaying vegetation. Mass emergence of these two insect groups occurred during the last week in May and the first two weeks in June, providing an important food supply for adult odonates.

Larvae of several species of Dytiscidae became numerically conspicuous by mid-June. Several species of aquatic Hemiptera were found. Odonates other

than those to be discussed were essentially non-existent. Specimens of Aeshna juncea L. and Libellula quadrimaculata L. were occasionally observed. Sympetrum rubicundulum Say was the only anisopteran occurring in large numbers.

Non-insect arthropods worthy of mention were several species of Daphnia and Diaptomus which appeared in large numbers during June. The amphipod, Gammarus, was abundant in the deep water throughout the year.

Vertebrates of possible significance to the study because of their predatory habits were small numbers of redwinged blackbirds, Agelaius phoeniceus L., and yellowheaded blackbirds, Xanthocephalus xanthocephalus Bon., and a colony of black terns, Chlidonias niger L.

2.2 Sampling

2.2.1 Summer

The study area was visited at approximately one-week intervals during the ice-free period throughout the course of the three year study. Approximately one hour was devoted to sampling on each visit, yielding, generally, no fewer than one hundred nymphs. The same portion of the pond, extending along about 100 m of shore, was sampled on each occasion. Nymphs were collected at all depths up to 60 cm. A long handled bottom net was used to sweep the nymphs from their perches in the dense stands of emergent and floating vegetation. The relatively coarse mesh of the net (8 meshes/cm.) biased the sampling against the first four or five instars of all species, however, this bias was not detrimental to the study as a whole. Specimens were sorted from the accumulated debris and preserved in 70% alcohol for identification and analysis in the laboratory. Specimens required for experimental purposes were returned to the laboratory and placed under experimental conditions an the date of collection. Observations on emergence, mating and oviposition were made during these weekly visits. Additional visits were made during critical periods of emergence and oviposition.

Adult damselflies were collected with their exuviae immediately after emergence for identification and confirmation of species. Sexually mature specimens were collected in tandem in hedges away from the slough area. These were preserved in a formalin-acetic acid-chloral hydrate fixative (Weaver and Thomas, 1956) for counting and measurement of mature oocytes.

Newly laid eggs were obtained by collecting plant stems in which damselflies were seen to oviposit. Newly laid eggs of some Lestes species were collected from stems of Scirpus in which fresh incisions could be easily distinguished from those made several hours prior to the collecting time. Eggs of various species of Lestes could be identified by the patterns of incisions on the stems (Fig. 5) and by the morphological characteristics of the eggs.

2.2.2 Winter

Nymphal collections were made during the winter only when experimental material was required. Until the ice thickness exceeded 15 cm nymphs could easily be obtained by cutting a hole in the ice with an axe and sweeping through the vegetation with a bottom net in a manner similar to summer collecting.

By December the overwintering nymphs of most species had become embedded in the ice. Subsequent to this time, specimens were obtained in large numbers by cutting out large pieces of ice in areas heavily occupied by nymphs in the fall. These occurred in water 15 to 20 cm deep heavily overgrown by Scolochloa. The pieces of ice were returned to the laboratory and thawed in open vessels at room temperature. Nymphs were subjected to experimental conditions as soon as they were free and displayed normal

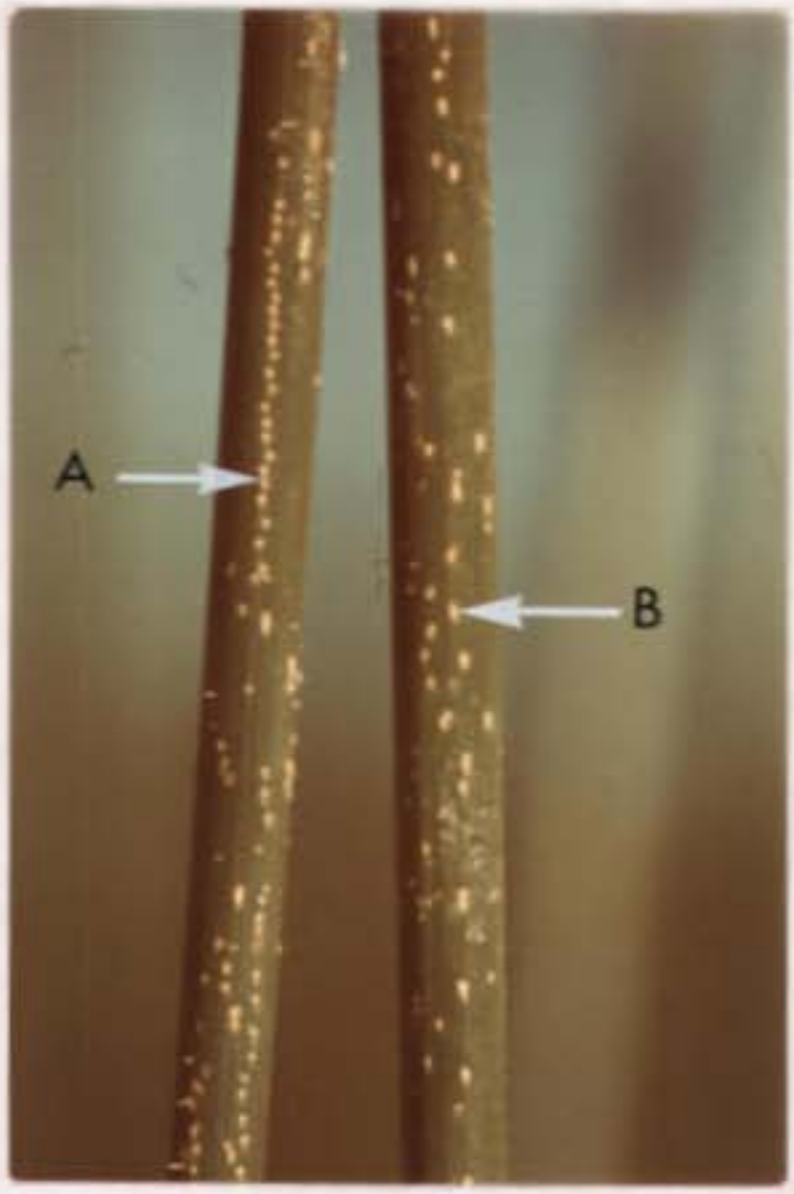

Figure 5. Oviposition incisions in stems of Scirpus made by two species of Lestes. (a) L. unguiculatus, (b) L. disjunctus.

behavior. Complete thawing often took more than one day. During this time care was taken to maintain the photocycle experienced by the insects in the field by covering the containers with black polyethylene for the appropriate length of time.

Nymphs were occasionally obtained from the deep portion of the pond during the early winter by collecting and sorting through Myriophyllum, Potamogeton and Lemna which sank to the bottom after freeze-up.

Eggs were collected during the winter simply by locating under the snow stems known to be oviposition sites from the previous summer's observations.

Food for nymphal damselflies, in the form of Chaoborus larvae, was readily obtainable throughout the winter. The Chaoborus larvae appeared to be attracted to the light of a hole in the ice where they were easily collected and concentrated in large numbers. The larvae were stored in a refrigerator, serving as a source of live food material for several weeks.

2.3 Laboratory experiments

The experimental portion of the project would have been impossible without the use of six controlled-environment cabinets, each having independent temperature and photoperiod controls. The cabinets, model RT24B, are distributed by Sherer-Gillett of Marshall, Michigan. Temperatures were maintained within approximately ± 1 degree of the desired level. The fluctuation was dampened by the aquatic medium of most of the experiments so that the experimental organisms were maintained at relatively constant temperatures.

Light was provided by pairs of fluorescent tubes suspended approximately nine inches above the experimental material. Light provided by

this arrangement yielded from 135 to 200 foot candles. Photoperiod was controlled by timers built into the cabinets. Bubbling air into the experimental trays in initial experiments proved to be unnecessary and was discontinued. Low temperatures were provided by a household refrigerator equipped with a sensitive thermostat and a household chest-type deepfreeze.

2.3.1 Rearing and Handling of Nymphs

Damselfly nymphs were reared in specially constructed containers (Fig. 6). The outside vessel, measuring approximately 28 x 18 x 10 cm, was made from plexiglass 0.6 cm thick. These dimensions easily accommodated 15 cylindrical containers. The latter were made of plexiglass tubing 5 cm in diameter cut into 8.25 cm lengths. The bottom ends were covered by plastic screen, 8 meshes per cm. The cylinders were later modified to consist of a plexiglass framework wrapped with plastic screening. This permitted freer circulation of water through the containers and provided a surface on which nymphs could perch and emerge without the need of providing extra strips of screening for this purpose. The cylindrical containers were suspended by stiff wires approximately 1.5 cm above the bottom of the tray. The apparatus permitted rearing of up to 30 specimens, one male and one female per container, under identical conditions, allowing individual development to be observed with relative ease.

Nymphs were reared in pond water. A supply of live Chaoborus larvae was maintained in each cylindrical container as food throughout the course of the experiments.

The experimental insects were observed approximately every second day. Moults into successive instars were identified by the presence of exuviae in the containers. Food and water were replenished when necessary.

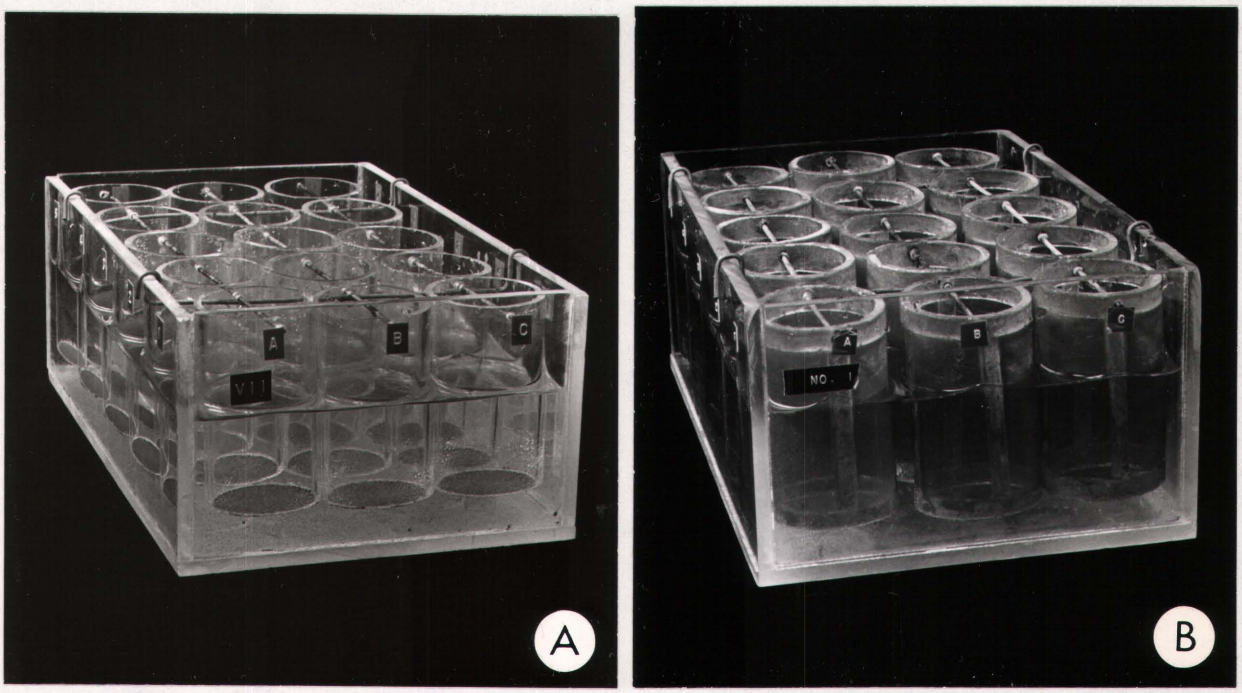

Figure 6. Rearing trays for damselfly nymphs.

(a) Plexiglass type.

(b) Modified, walls constructed of screen.

Water was changed only when algal growth on the sides of the containers made observation difficult.

The number of nymphs subjected to any experimental condition generally varied between five and fifteen in a particular instar, depending on the availability of space and insects. Experimental effort was concentrated on the last three instars in which most overwintering occurs.

Experiments to determine the lethal temperature of nymphs were conducted in the freezing compartment of a refrigerator equipped with a sensitive control. Variable numbers of insects were placed in open plastic bags containing pond water. The water was allowed to freeze. Thermocouples of a Speedomax continuous temperature recorder were placed in and around the bags to determine temperature fluctuation and the time when the water was completely frozen. The insects were retained under these conditions from twelve to twenty-four hours after complete freezing. The bags were then removed, thawed and the number of insects surviving determined.

2.3.2 Incubation and Handling of Eggs

Eggs of damselflies were collected in the field, returned to the laboratory, and removed from the stems in which they were laid. They were placed in Petri dishes lined with wet filter paper and subjected to the appropriate experimental temperature and light conditions. Water was added when necessary in order to prevent desiccation.

The rate of embryonic development in non-diapause eggs was established by subjecting eggs to 16, 21 and 26.5°C at a 16½ hour photoperiod.

Eggs destined for diapause development studies were collected when newly laid. They were subjected to various conditions to study the pre-diapause development rate. Pre-diapause development was judged to be complete when no further embryonic development was apparent following the appearance

of dark eye spots and distinct tracheal tubes (Fig. 7). At least one further week was allowed to insure complete pre-diapause development in all eggs. Pre-diapause development in eggs of L. congener could not be traced because of the presence of a thick, darkly pigmented chorion. Therefore, eight days were allowed at 21°C beyond the last collecting date to insure that this phase was complete. Eggs also were collected in the field at regular intervals for comparison with those in which embryonic development took place in the laboratory. Details of experiments concerning diapause development will be presented in the appropriate section of the results.

Eggs of the four species of Lestes were collected in March and incubated at 21°C and a 16½ hour photoperiod, conditions considered optimal for hatching of all damselfly eggs. The results were used to establish a criterion against which hatch date could be compared in order to determine whether or not Phase I of diapause development was complete. Eggs of L. disjunctus, L. unguiculatus, and L. dryas required 4 to 8 days to complete hatching. Eggs of L. congener required 13 to 23 days. If hatching took longer than 8 or 23 days respectively, Phase I of diapause development was not completed prior to the test. Hatching in less time than the lower limit indicated that some degree of post-diapause development had occurred. Those pronymphs which died in the process of hatching were counted as hatched.

Lethal temperatures for eggs, as for nymphs, were determined by placing eggs in Petri dishes of water and subjecting them to the appropriate sub zero temperature until freezing of the water was complete. Controls were run at temperatures near the freezing point. The eggs were then subjected to 21°C and a 16½ hour photoperiod to determine survival rate.

Eggs of L. congener were subjected to the following relative humidities to determine the effect of desiccation on egg survival: 100%, 75%,

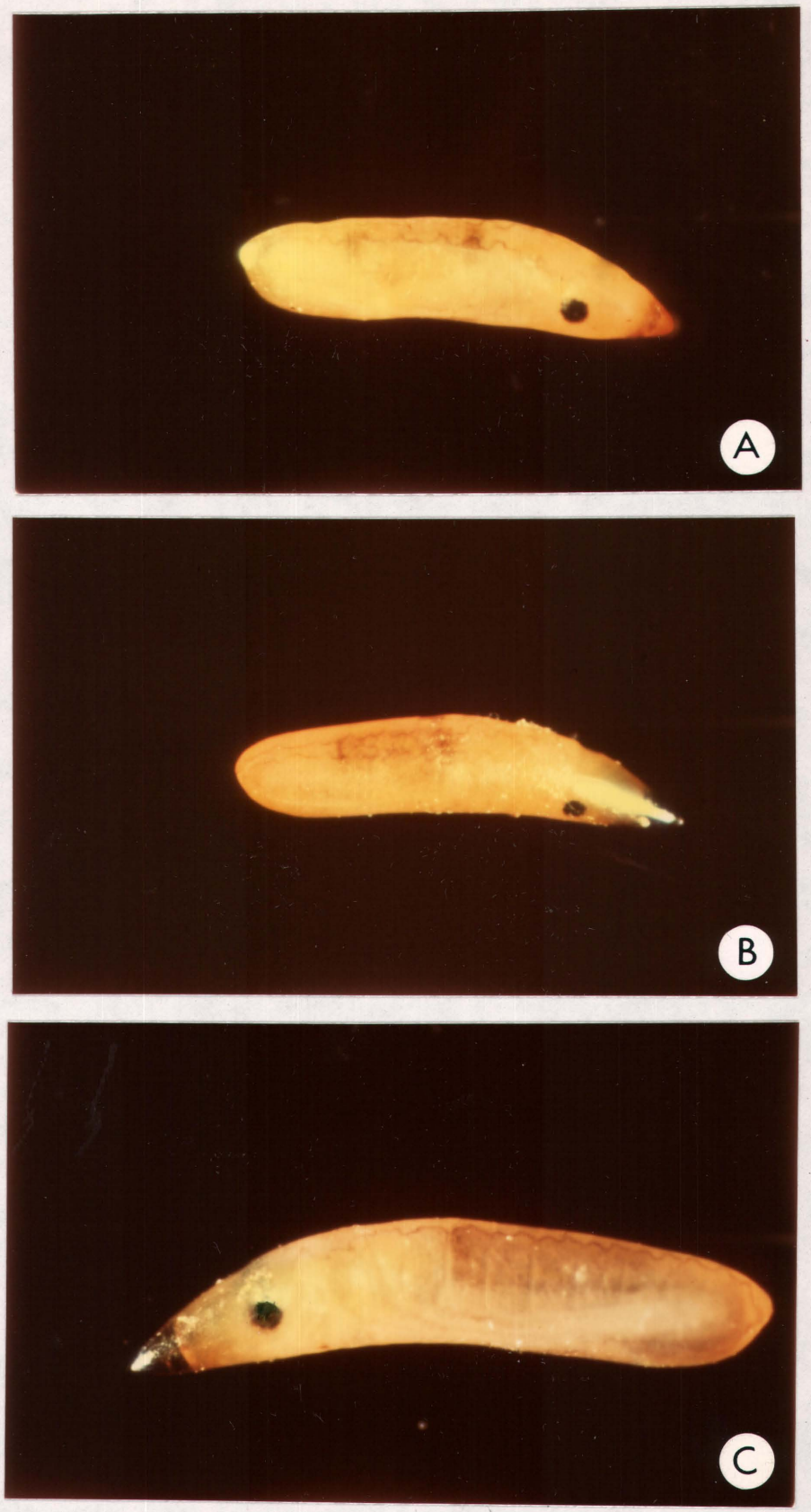

Figure 7. Eggs of Type B Lestes, showing eye spots and tracheae.(30X)

(a) L. disjunctus
(b) L. unguiculatus
(c) L. dryas

50% and 25%. Small Petri dishes containing the eggs were floated in larger dishes containing KOH solution prepared in concentrations necessary to produce the desired relative humidity according to Hales (1965). The outer dish was then sealed. Observations were made through the glass. A control was run using wet eggs. After 36 days the lids were removed and water was added to all eggs to test survival. All experiments were conducted at 21°C and a 16½ hour photoperiod.

A similar experiment was conducted with eggs of L. disjunctus and L. unguiculatus to test whether wetting was necessary for hatching to occur.

2.4 Identification of nymphs

Nymphs in the final instar were identified with the aid of the keys prepared by Walker (1953). Identification was confirmed by collecting newly emerged adults with their cast skins in the field and by rearing experiments in the laboratory. Once the population structure was established, nymphs in the earlier instars were identified by certain specific characteristics of the final instars. Nymphs of Coenagrion angulatum develop characteristic nodal constrictions at approximately the fourth nymphal instar. Coenagrion resolutum nymphs have dark pigmented spots associated with the bases of the setae found over the general tergal surface. Their caudal lamellae lack pigmentation. The tracheae in the lamellae are numerous and almost parallel to the long axis. The young nymphs of Enallagma boreale appear to lack characteristics which easily distinguish them from other Enallagma species. The nymphs identified as E. boreale may have included a very small percentage of E. cyathigerum. The labial characteristics used in Walker's (1953) description of final instar nymphs of various Lestes species applied equally well to the identification of the earlier instars of the Lestidae in this study.

3. RESULTS

Field collections of damselfly nymphs revealed a distinct succession of species in the study pond (Fig. 8). All members of the Coenagrionidae overwinter in the nymphal stage and will henceforth be designated as Type A species. Zygoptera overwintering in the egg stage consisted of four species of Lestidae. Though there was considerable overlap of nymphal and adult stages between these species, they could be further subdivided according to the stage of embryonic development in which the eggs overwinter. Those overwintering as fully formed embryos as in Figure 7 were designated as belonging to Type B. Type C species are those in which the embryo overwinters in a stage immediately preceding blastokinesis. The biology of each species type will be described separately.

3.1 Type A Species

Three species fitting the above description were collected in numbers significant enough to warrant individual attention, namely Coenagrion angulatum Walker, Coenagrion resolutum Hagen and Enallagma boreale Selys. Others present in extremely small numbers were Coenagrion interrogatum Hagen, Enallagma cyathigerum Charpentier and Enallagma clausum Morse.

3.1.1 Coenagrion angulatum - Field observations

(a) Pre-emergence spring development

This species was easily the numerically dominant one of the Type A group in the study area (Fig. 8). The last three nymphal instars constitute most of the overwintering nymphal population with only a small fraction of the population appearing in the antepenultimate-1 (A-1) instar. A weighted mean instar established for the overwintering population (Fig. 9)

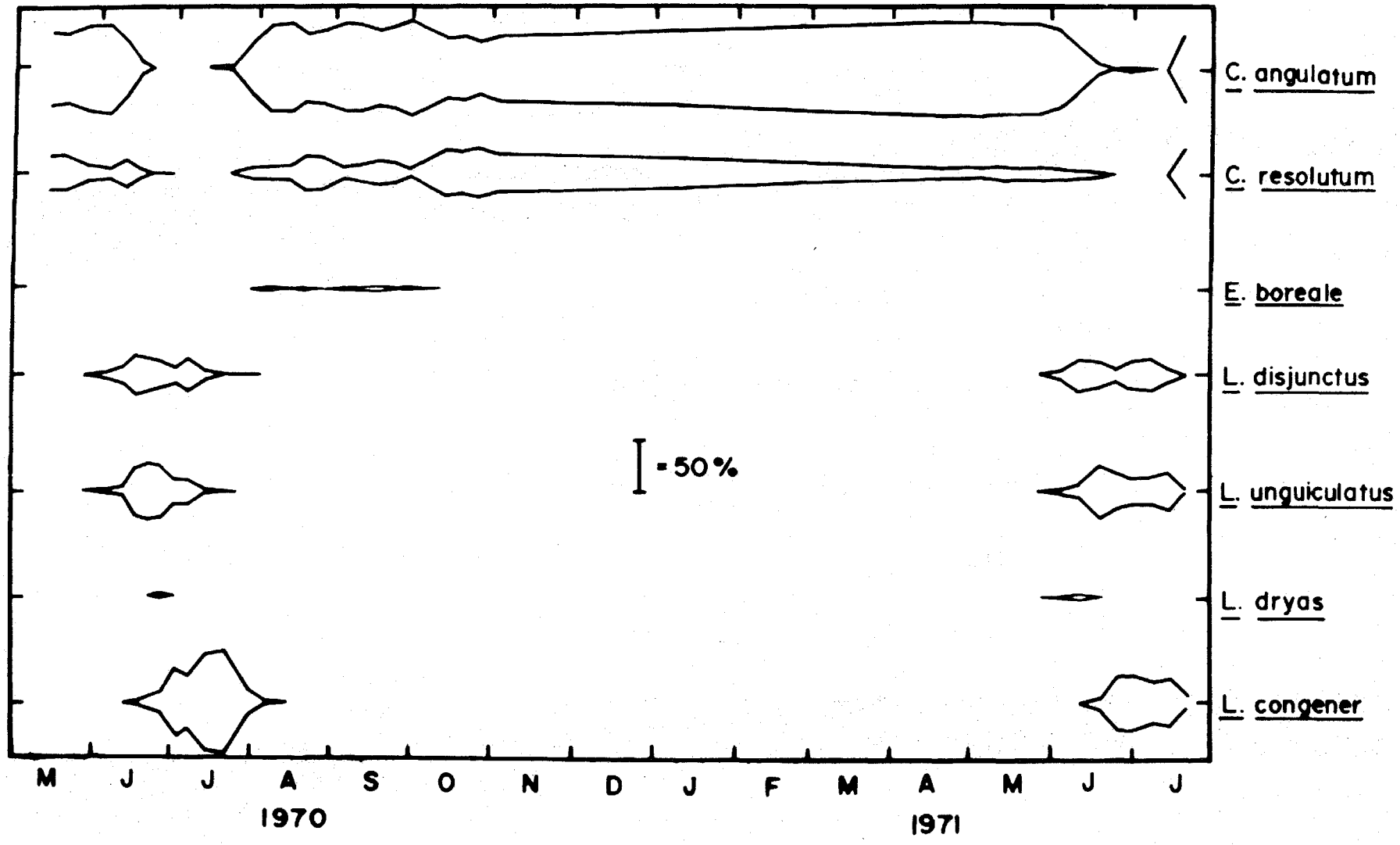

Figure 8. Seasonal distribution and relative abundance of damselfly nymphs in the shallow part of the study pond, shown as percentages of total nymph collection. (See also Appendix A.)

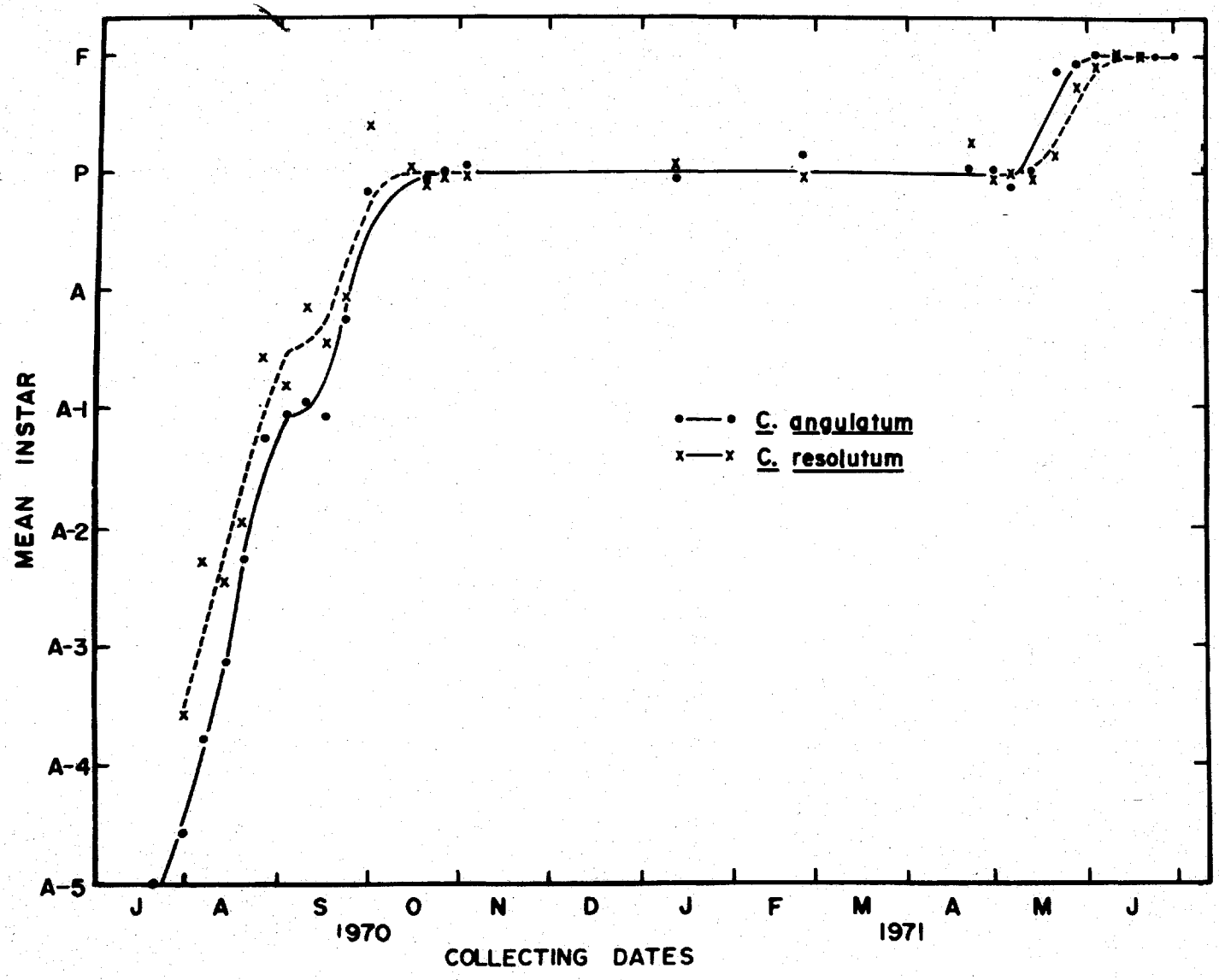

Figure 9. Development rate of Coenagrion nymphs under field conditions. Curves were fitted by eye using a three-point moving average. (See also Appendices B and C.)

(Benke 1970) showed the average specimen to be in the penultimate instar. Twenty-eight per cent of the individuals were in the final instar. Although the study pond filled with water on approximately April 10 in each year of the study period, no changes in the population structure of the nymphs occurred until after May 5. By May 20 the proportion of individuals in the final instar had increased to 59%, 97% and 86% in the years 1969, 1970 and 1971 respectively.

The date of commencement of emergence varied somewhat from year to year. Newly emerged adults were first observed on May 27 in 1969, May 31 in 1970 and May 24 in 1971. The emergence date is governed by prevailing water and air temperatures at the completion of nymphal development. Emergence, which occurred only if mean daily water temperatures exceeded 12°C and the mean maximum air temperature reached 20 to 21°C, could be delayed for a week or more until the appropriate temperature was attained. Emergence may not be indefinitely postponed because nymphs at this stage of development are no longer capable of feeding or obtaining their normal oxygen supply (Corbet, 1963).

The rapid decrease in the proportions of C. angulatum within nymphal collections indicated that peak emergence occurred within the first 10 days following appearance of the first adults and was complete by June 20 in all three years of the study.

(b) The adult stage

Newly emerged adults retreat from the slough area as soon as their wings have hardened sufficiently for flight. Large numbers of tenerals were observed in a lilac hedge approximately 100 metres from the water. These were actively feeding on numerous chironomid and Chaoborus adults.

Male to female sex ratio was 1.15 to 1 in the nymphal stages, based on 3052 specimens. The disproportionately large number of males was maintained

throughout nymphal development. It is therefore probable that the same ratio was retained in the newly emerged adult population. There was no indication from the sex ratio of final instar nymphs of differences in emergence dates in the two sexes.

Sexual maturation required approximately one week. Pairs were found in tandem and copulating in the hedge away from the water after this time. There was no oviposition following these initial copulatory acts as described for most other species.

A single observation was made of sperm transfer from the genital opening in the ninth abdominal segment to the copulatory apparatus in the second and third abdominal segments of the male. It took place immediately after the tandem position was achieved and lasted approximately 10 seconds. It was immediately followed by copulation which often required 20 minutes or more. One pair, already copulating when observation began, remained in copula for a further 37 minutes. Copulation always began while the insects were perched and only when disturbed would they continue to copulate in flight. Pairs in tandem or copulating were never disturbed by the presence of single male individuals of the same species.

Oviposition in this species is endophytic and begins within two weeks of the first emergence. With rare exceptions, females oviposited while in tandem with the male. Preferred oviposition sites are the flowering stalks of the floating plants, Utricularia, Ranunculus and Potamogeton which temporarily emerge 3 to 5 cm above the water surface. In the absence of these, C. angulatum will oviposit in fleshy leaves, petioles and stems of emergent vegetation.

Eggs are always deposited below the water surface. When oviposition occurs in stalks of floating vegetation, the female backs down the stem until her abdomen is submerged, and deposits her eggs within 3 cm of the surface.

Female C. angulatum appear able to distinguish between floating and emergent vegetation. If the latter is used for oviposition the female backs down well below the surface dragging the male with her. One pair submerged to a depth of 45 cm and remained below the surface for 30 minutes before reappearing. The only response of the submerged pair to an approaching object was to move around to the opposite side of the stem in which the female was ovipositing without interrupting oviposition. When oviposition was complete the pair simply released their grasp on the stem and floated to the surface. They then crawled out of the water, fluttered their wings to free them of water droplets and flew away.

Egg clutch size in this species was determined by counting mature oocytes in females caught in tandem away from the water prior to oviposition. The mean clutch size in five females was 172. The number of egg clutches actually produced by females was not determined. However, up to 8 oocytes were observed per ovariole. It is doubtful whether many females live long enough to utilize their maximum egg laying potential.

Oviposition in this species takes place at the rate of approximately five eggs per minute, each laid in a separate incision. The incisions are made in two rows 0.4 mm apart. The eggs in each row are spaced 1.2 to 1.9 mm apart.

The flying season for C. angulatum extended from May 24 to July 29 in 1971. Specimens were extremely rare on the latter date and disappeared during the following week. The last field observation of the adults of this species was made on July 30 in 1970.

(c) The egg stage

Newly laid eggs of C. angulatum are soft and creamy white in colour.

Within 48 hours the eggs become considerably more firm. The pointed anterior ends become orange-brown. The eggs are 1.15 mm long and 0.19 mm wide when first laid and increase in size only slightly during the course of embryonic development. The pointed anterior ends of the eggs are capped with a funnel-shaped loosely connected structure similar to that shown in Figure 10. These structures extend to the surface of the stem in which the eggs are laid and appear to prevent scar tissue from developing over the incision, thereby insuring a passageway for the newly hatched nymph. From observations of hatching under experimental conditions it was noted that the funnels themselves do not serve as an exit for the pronymphs.

Embryonic development commences as soon as the eggs are deposited. No observations were made on embryonic development under field conditions. However, hatching occurred after 16 days in the laboratory at 21°C. Similar development probably took place in the field since nymphs which had undergone two or three moults were collected on July 15, about five weeks from the time that oviposition first occurred.

(d) Summer and autumn nymphal development

Nymphal development proceeds rapidly during the summer under field conditions. Development rate was established from weekly sampling and is presented in Figure 9. Nymphs in the final instar were first collected on September 10 in 1970. These failed to develop any further and remained in the final instar throughout the winter. Development, expressed as change in mean instar, came to an end during the first two weeks of October. During this time water temperature had dropped from means of 10° to 15°C down to 2°C, probably a factor contributing to cessation of development.

(e) Overwintering of nymphs

Nymphal development in C. angulatum takes place in shallow water, generally less than 60 cm deep.Nymphs were most numerous in water less than

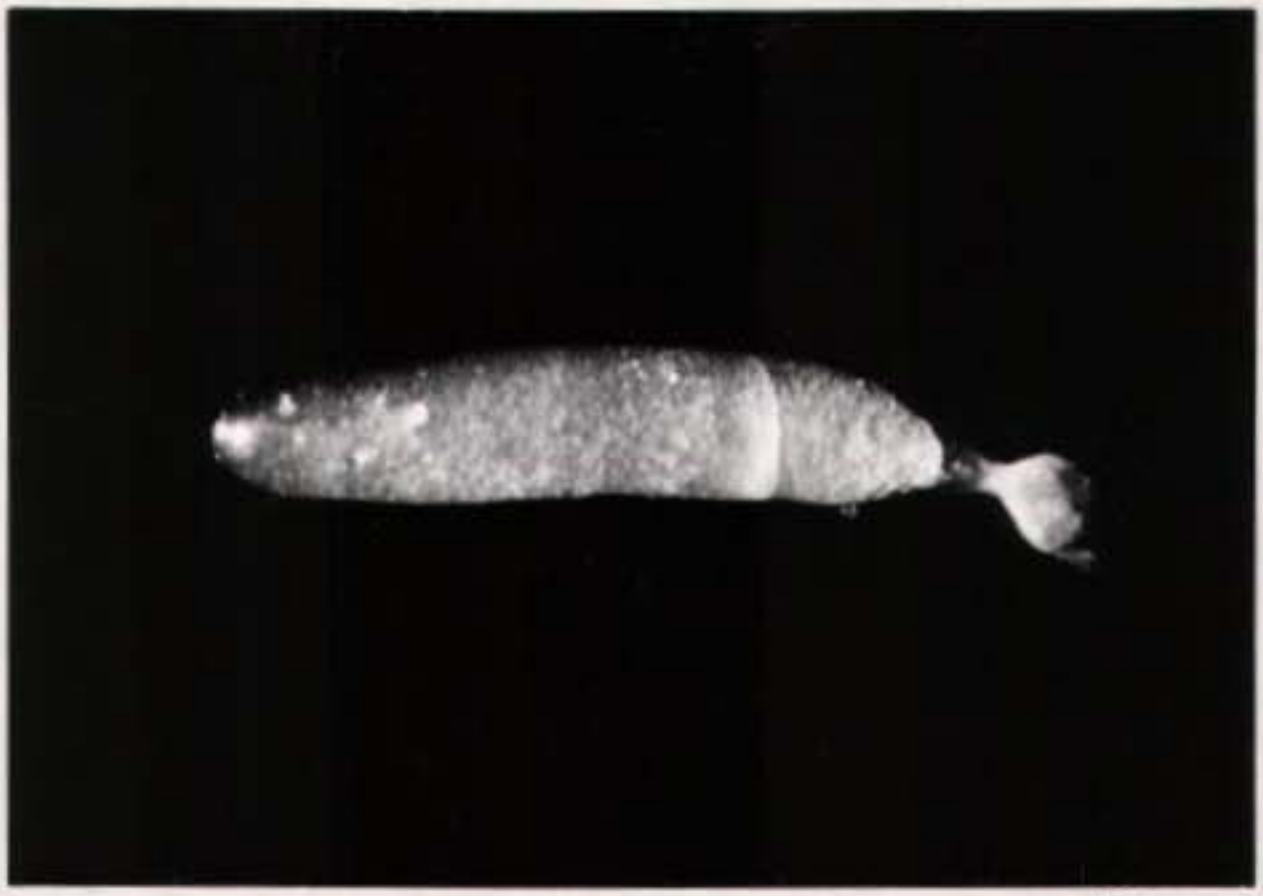

Figure 10. Newly laid egg of C. resolutum showing funnel-shaped structure at anterior end.

30 cm deep overgrown by dense stands of Scolochloa. When the first ice appeared on the slough, they made no obvious attempt to retreat to deeper water as the ice thickened. It was, therefore, not surprising to find the nymphs embedded in the ice as the slough froze to the bottom in the shore areas (Fig. 11). The nymphs were found in highly clumped distribution (Fig. 12), embedded in ice 15 to 20 cm below the surface. Sometimes they clung to plant stems but more frequently they were found frozen upside down presumably trapped while walking on the lower surface of the ice. Their absence in the upper 10 cm of ice suggests that stagnating conditions created by the ice cover slowed their activity sufficiently to allow them to become embedded in the ice.

Although trapped in ice, the bodies of the insects were not themselves frozen. Specimens cut from the ice in the field in January were soft and pliable. When they were pulled apart, hemolymph oozed from the broken ends.

3.1.2 Coenagrion angulatum - Laboratory experiments

The relatively high degree of synchrony in emergence of a species which overwinters in four instars, the ability of nymphs to cease development in the fall while environmental conditions were apparently conducive to emergence, and the unusual mode of overwintering prompted experiments to determine the effects of photoperiod and temperature on the development of the later nymphal stages.

(a) Photoperiod effect on nymphal development

Final instar specimens were collected in the field on October 14, 1970 and reared under conditions of 21°C and a 16½ hour photoperiod and 21°C and an 8 hour photoperiod according to the method described earlier. Fifteen specimens were subjected to each condition. All fifteen emerged at the long

Figure 11. Overwintering habitat of nymphs of C. angulatum and C. resolutum.

Figure 12. Distribution of nymphs of C. angulatum and C. resolutum in ice.

photoperiod with a mean emergence time of 29.5 days. Only two specimens emerged at the short photoperiod with a mean emergence time of 86 days (Fig. 13). Three died as a result of parasitism by nematodes. With one exception, the remaining 9 specimens died after spending an average of 97 days in the final instar with no visible signs of preparation for emergence.

Specimens collected in the penultimate instar were reared under similar conditions. Normal moulting into the final instar occurred after an average of 23.7 days at the long photoperiod. Those reared at 21°C and an 8 hour photoperiod spent the unusually long time of 65.6 days in the penultimate instar (Fig. 13). Then, rather than moulting to the final instar, these specimens underwent two or occasionally three additional moults during which there was a gradual morphological change towards the characteristics of the final instar (Table 2). Original data are presented in Table 3. Specimens collected in January or later and subjected to the above conditions developed more rapidly under both photoperiods (Fig. 13).

Penultimate instar nymphs subjected to 16°C and a 16½ hour photoperiod moulted into the final instar in 41.6 days (Table 2). Those reared at an 8 hour photoperiod at this temperature remained in the penultimate instar for an average of 140.9 days and then moulted directly into the final instar.

Replicate experiments conducted on final instar nymphs in January and February showed that the photoperiod effect on emergence is maintained throughout the winter. However, although an 8 hour photoperiod led to several intermediate moults in nymphs collected in the penultimate instar in October and reared at 21°C, this form of photoperiodic inhibition was essentially erased by January. Only two specimens out of fifteen displayed this type of behavior at an 8 hour light period, one out of fifteen at 10 hours

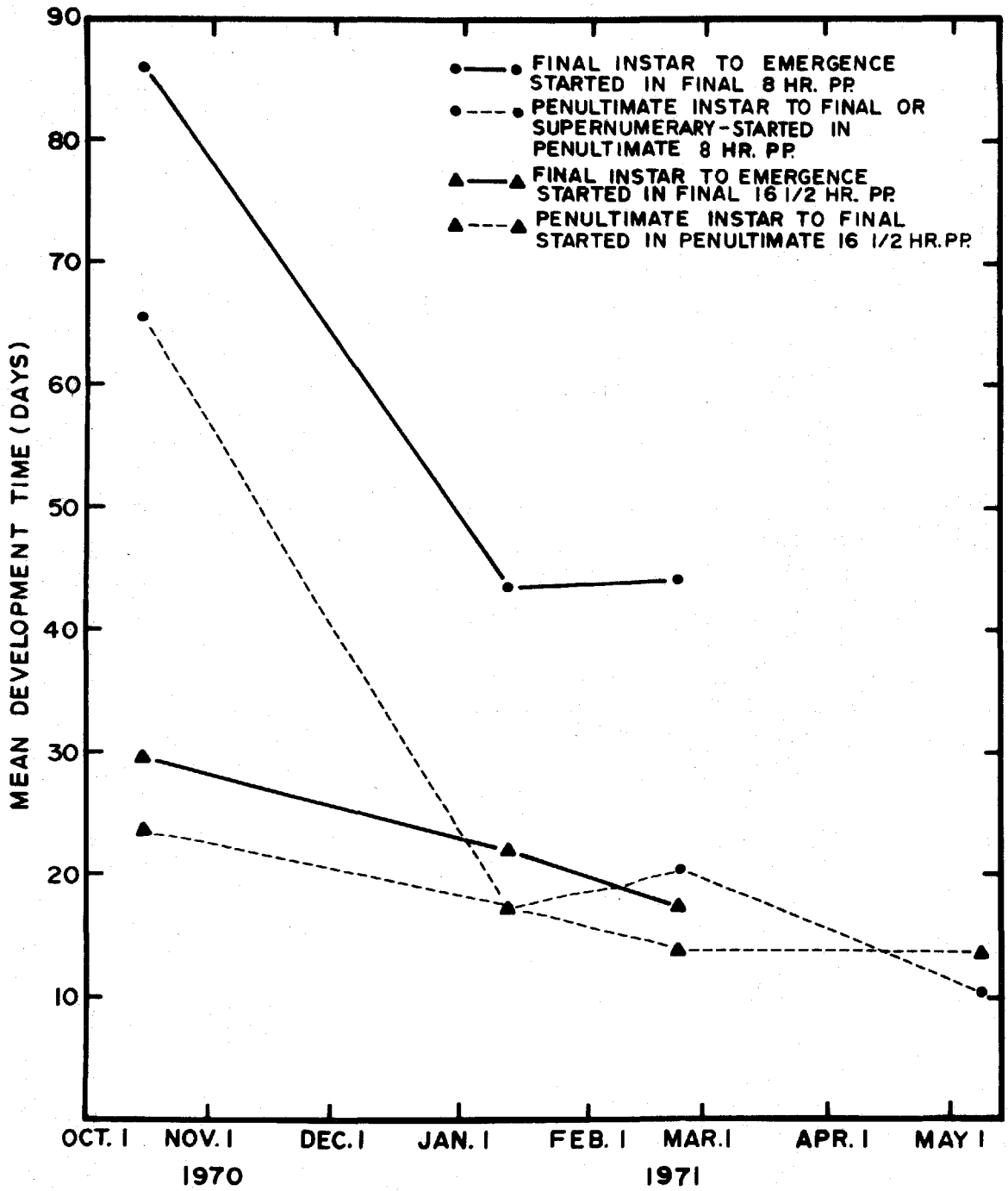

Figure 13. Seasonal changes in development rate of C. angulatum nymphs reared at 21°C. (See also Appendix H.)

Table 2. Effect of photoperiod on stadium duration (Days) in nymphs of C. angulatum collected in the penultimate instar October 14, 1970, and reared at 16 and 21°C.

	16°C 16½ hr			16°C 8 hr		
	Stadium		Mean	Stadium		Mean
	Penultimate	Final	Total	Penultimate	Final	Total
N	15	15	15	13	10	10
$\bar{X}$	41.6	29.8	71.4	140.9	37.4	178.4
95% C.L.	± 1.95	± 1.28	± 2.26	± 15.50	± 3.99	± 20.00
	21°C 16½ hr			21°C 8 hr		
N	15	11	11	8	6	6
$\bar{X}$	23.7	21.0	45.4	131.2*	25.8	160.0
95% C.L.	± 1.95	± 4.23	± 5.05	± 27.12	± 12.72	± 30.73

*Includes time spent in supernumerary stadia.

Table 3. Effect of photoperiod on stadium duration (Days) in nymphs of C. angulatum collected in the penultimate (P) instar October 14, 1970, and reared at 21°C and an 8 hour photoperiod.

Specimen No.	To P + 1	To P + 2	To P + 3	To Final	To Emergence
1	37	34	——	26	4 Died
2	67	——	——	44	18
3	97 Died				
4	76	29	36	32	22
5	82	19	30	30	24
6	1 Died				
7	31	17	27	1 Died	
8	78	34	——	1 Died	
9	89	22	——	42	24
10	——	——	——	78	51
11	57	32	——	40	16
12	59 Died				
13	15 Died				
14	17 Died				
15	61	49	——	38	1 Died

and two out of fifteen at 12 hours. Other specimens showed no significant differences in response to photoperiod in the penultimate instar (Table 4). Specimens collected in the antepenultimate instar responded in a similar manner (Table 4). Although significance among the means was demonstrated at the 5% level among the latter insects the means do not show the dramatic differences described earlier.

Nymphs of C. angulatum which overwinter in the final instar appear not to become completely free of photoperiodic inhibition before spring emergence. Nymphs collected from ice in January and subjected to a series of photoperiods at 21°C exhibited a distinct development threshold between a 12 and 14 hour photoperiod, below which the development time was doubled. By the end of February the threshold appeared to have shifted towards a lower photoperiod (Fig. 14). It was maintained at 12 hours at a temperature of 16°C (See Fig. 17).

Nymphs collected in January in the penultimate and antepenultimate instars were unaffected by photoperiod in their moult to the succeeding instar (Table 4). However, the effect of short photoperiod in these instances is expressed in the succeeding instar. A decrease in photoperiod produces lengthening of the time required for emergence in those nymphs which were subjected to these experimental conditions in the penultimate instar at 21°C (Fig. 15).

Antepenultimate instar nymphs collected in January and subjected to 21°C and an 8 hour photoperiod yielded penultimate nymphs which subsequently underwent successive stationary moults similar to those of penultimate instar nymphs collected and subjected to these same conditions in October (Table 5).

(b) Temperature effect on nymphal development

The effect of low overwintering temperatures on development is

Table 4. Effect of photoperiod on stadium duration (Days) in nymphs of C. angulatum collected in (a) the penultimate instar and (b) the antepenultimate instar on January 11, 1971, and reared at 21°C.

	8 hr	10 hr*	12 hr	14 hr	16½ hr
(a) Penultimate stadium (excluding specimens with stationary moults).					
N	12	13	13	15	15
$\bar{X}$	17.2	14.5	20.0	16.5	17.7
95% C.L.	± 2.44	± 1.66	± 5.77	± 1.59	± 1.61
(b) Antepenultimate stadium					
N	10	7	10	9	10
$\bar{X}$	10.8	9.7	11.8	11.3	8.4
95% C.L.	± 1.93	± 2.91	± 1.57	± 1.27	± 0.60

*Temperature at 23.5°C for two days.

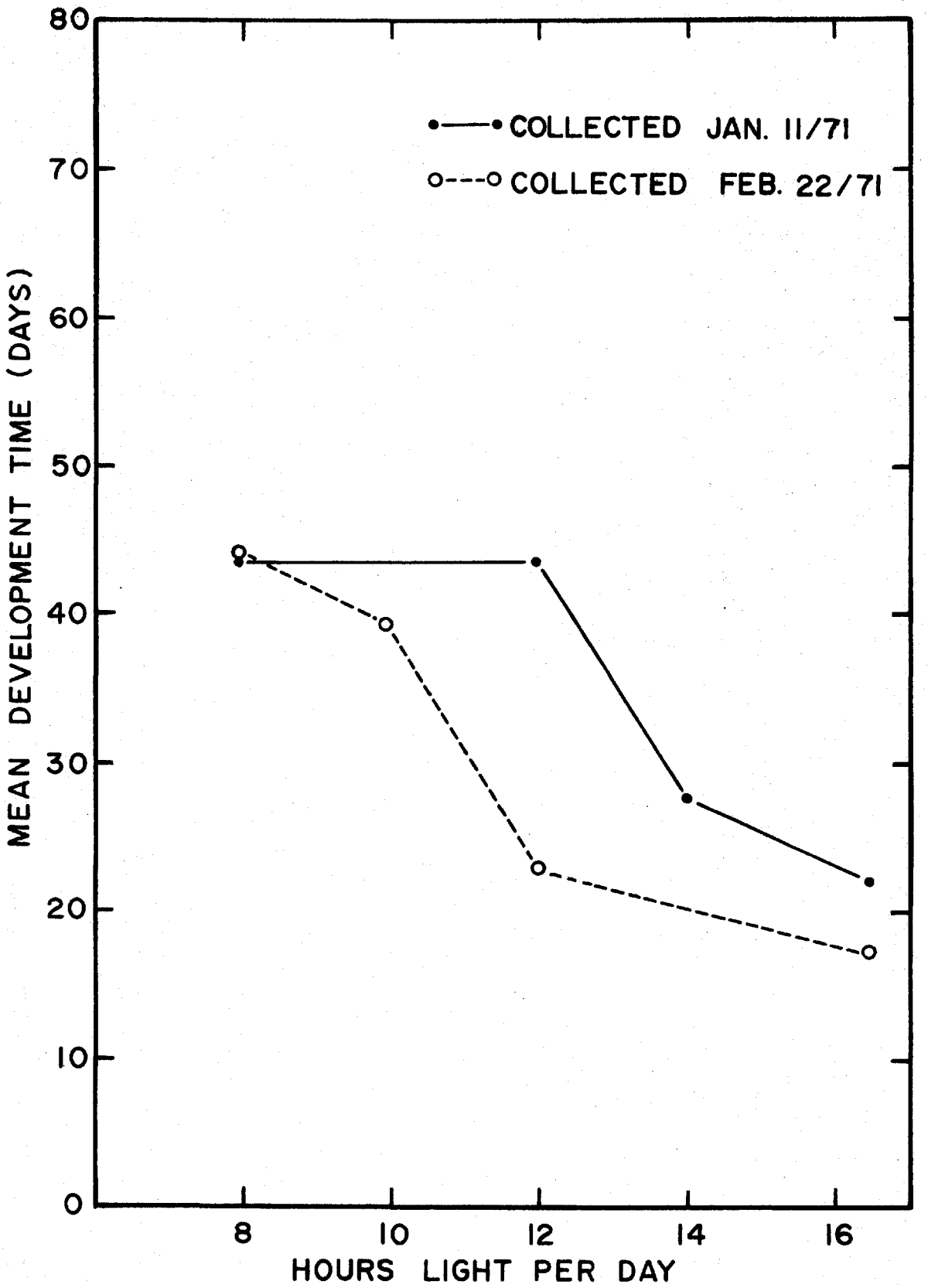

Figure 14. Effect of photoperiod on duration of final stadium in nymphs of C. angulatum collected in the final instar and reared at 21°C. (See also Appendix I.)

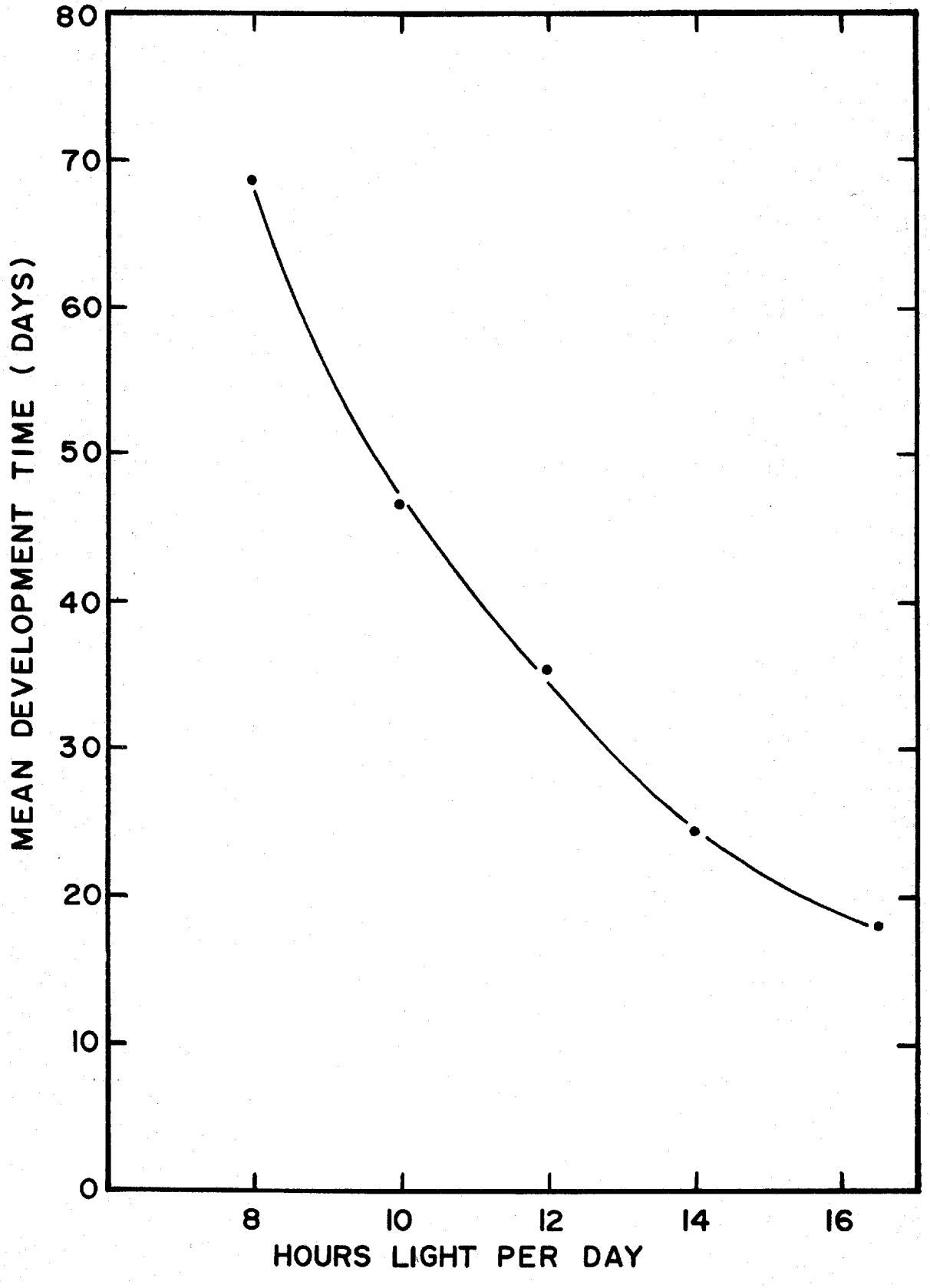

Figure 15. Effect of photoperiod on duration of final stadium in nymphs of C. angulatum collected in the penultimate instar January 11, 1971, and reared at 21°C. (See also Appendix J.)

Table 5. Effect of photoperiod on stadium duration (Days) in nymphs of C. angulatum collected in the antepenultimate (A) instar January 11, 1971, and reared at 21°C and an 8 hour photoperiod.

Specimen No.	To P	To P + 1	To P + 2	To Final	To Emergence
1	10	———		30	20
2	12	30	66	12 Died	
3	14	56	———	44	33
4	10	———		32	20
5	10	26	———	38 Died	
6	8	44	22	24	2 Died
7	16	36	28	55	32
8	8	42	———	36	24
9	12	54	30 Died		
10	8	———		24	1 Died

expressed in a seasonal decrease in development time in successive replicates of nymphs reared under conditions of constant temperature and photoperiod (Fig. 13). While the temperature controlled inhibition of development in the final and penultimate instars appears to have been completed in January at the short photoperiod, experiments at the 16½ hour photoperiod demonstrate continued effects of low temperature on development throughout the winter by a progressive lowering of development time in both instars.

The effect of low overwintering temperature is further demonstrated in an apparent seasonal change in the photoperiod threshold necessary for rapid emergence of adults from nymphs collected in the final instar (Fig. 14).

Data presented thus far have been derived from photoperiod experiments conducted at a constant temperature of 21°C. Further experiments were carried out at temperatures held constant at 16°C to correspond with the 21°C experiments already described. A comparison of nymphal development between 16 and 21°C at 16½ and 8 hour photoperiods is shown in Table 2. There was a difference of 26 days in the mean total development time from the penultimate instar through to emergence between the two temperatures at the long photoperiod. The most significant effect appeared to be on the penultimate instar where a mean decrease in development time of 18 days was recorded for the 5 degrees increase in temperature.

A more striking effect of temperature was shown at the 8 hour photoperiod. As demonstrated earlier, nymphs of C. angulatum collected in the penultimate instar in October and subjected to an 8 hour light period underwent up to three additional moults at 21°C before emerging (Table 3). Nymphs exposed to this photoperiod at 16°C remained in the penultimate instar for considerably longer and failed to exhibit stationary moulting (Table 2). The final outcome was,however,the same. Mean development time to emergence at

21°C was 160.0 days while at 16°C emergence occurred in 178.4 days. The difference between the two means was not significant.

An experiment was begun on March 10, 1971 to determine whether certain temperatures might overcome the delay in development induced by short photoperiods. A constant 12 hour photoperiod was used at 16, 21 and 26.5°C. Results in Figure 16 indicate a considerable retardation of development in insects collected in the penultimate and final instars and reared at 16°C. Insects collected in the antepenultimate instar and subjected to these conditions responded only slightly to decreasing temperatures, maintaining a consistently high development rate even at the lower temperature.

Subsequent development in the insects which were collected in the penultimate instar appeared to be governed more by the short photoperiod than by the temperature. At all temperatures, time spent in the final instar was about twice the time required in the absence of photoperiodic inhibition (Fig. 17).

Approximately 30% of the specimens collected in the antepenultimate instar in March and reared at a 12 hour photoperiod underwent stationary moults similar to those specimens collected in the penultimate instar and subjected to a short photoperiod at 21°C in October. The remaining specimens spent an average of 50.6 days in the penultimate instar at 26.5°C, 28.6 days at 21°C and 26.4 days at 16°C (Fig. 18). Development between the final instar and emergence took a course similar to that of insects started in the final instar with a lesser degree of inhibition at the 16°C temperature (Fig. 17).

A summary of total development has been prepared for all instars reared at the three temperatures (Fig. 18). Of particular interest are total development rates at 16°C and 21°C temperatures. These temperatures are the

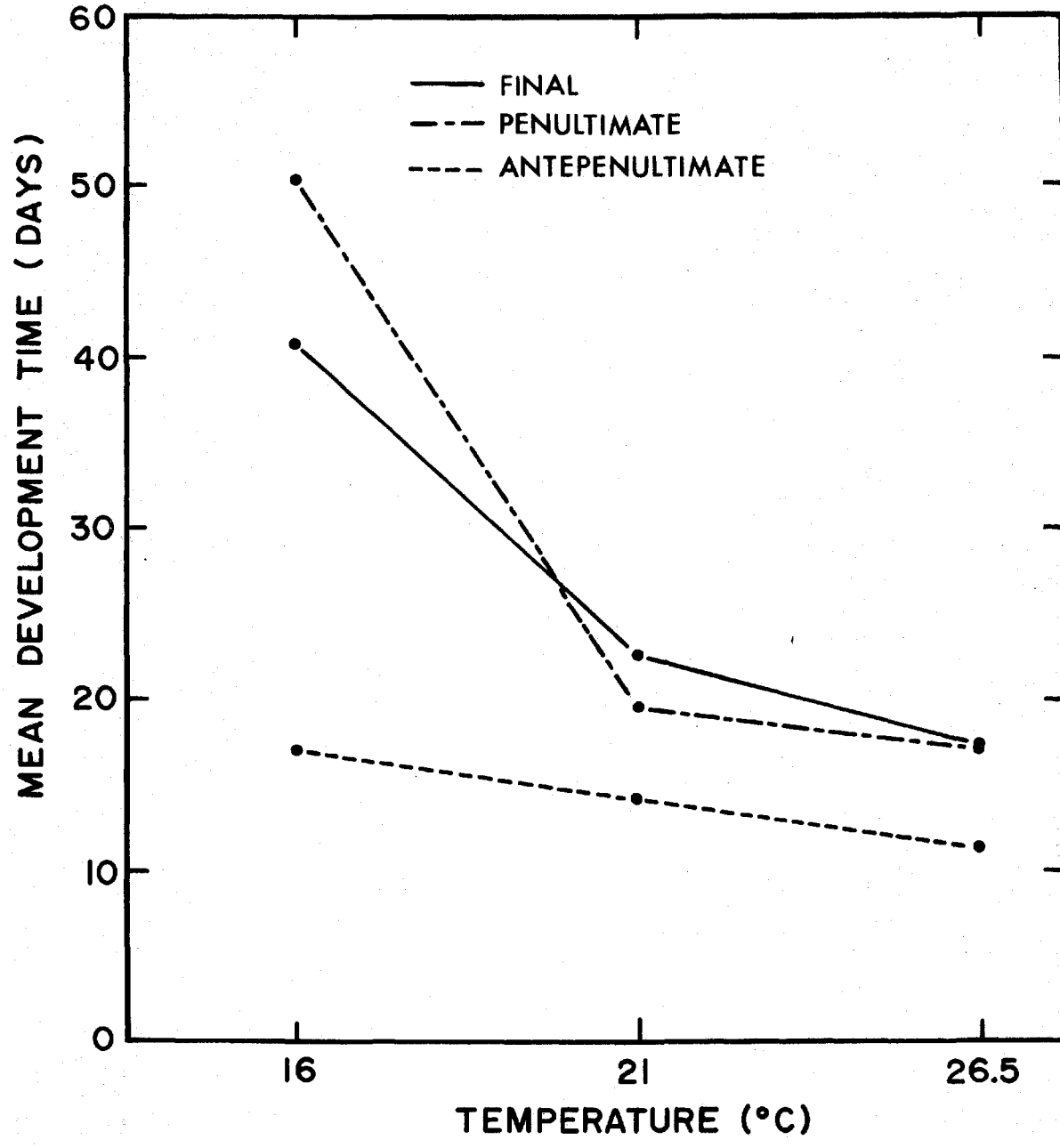

Figure 16. Effect of temperature on stadium duration in nymphs of C. angulatum collected March 10, 1971, and reared at a 12 hour photoperiod. (See also Appendix K.)

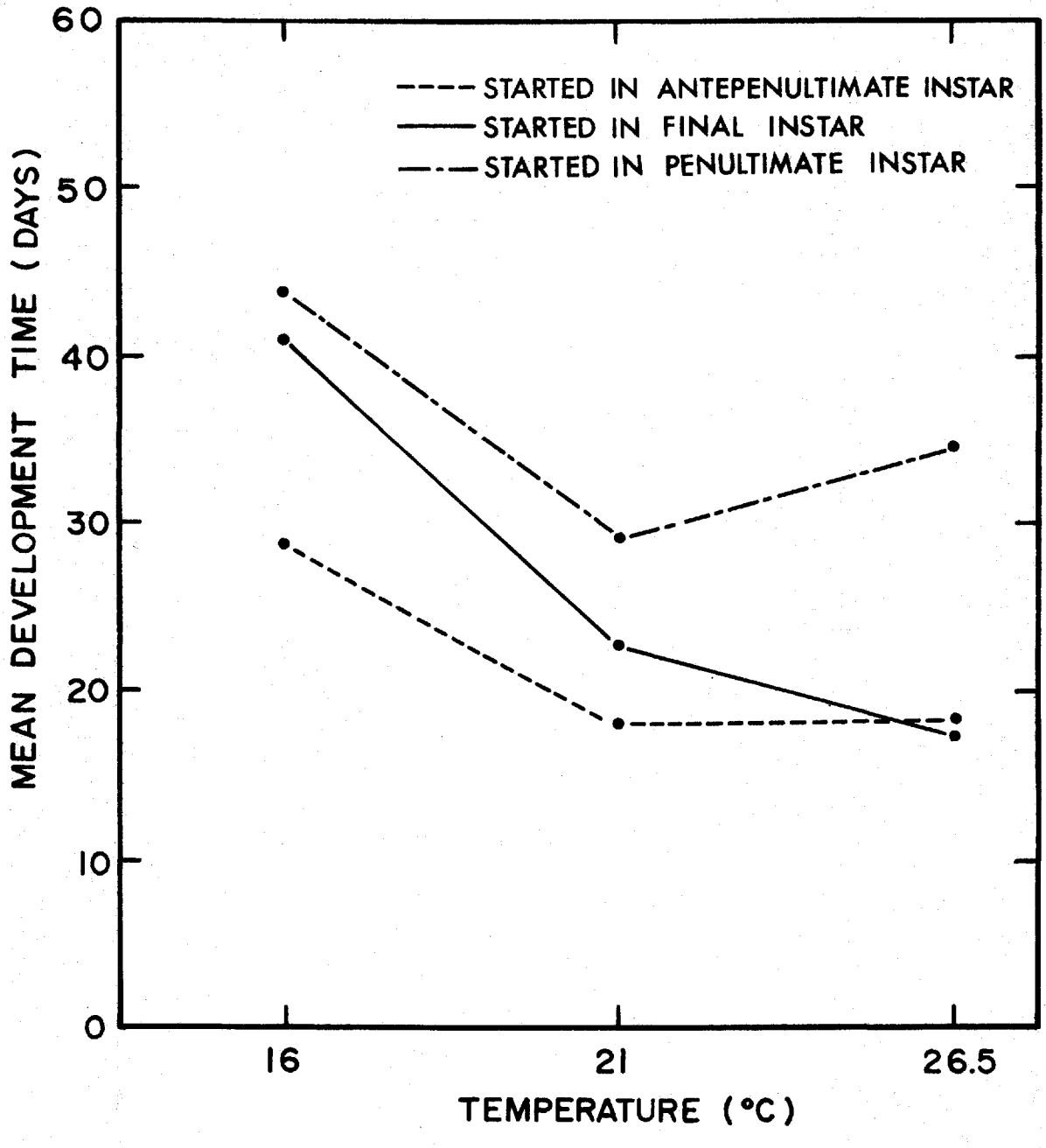

Figure 17. Effect of temperature on duration of final stadium in nymphs of C. angulatum collected in various instars March 10, 1971, and reared under a 12 hour photoperiod. (See also Appendix K.)

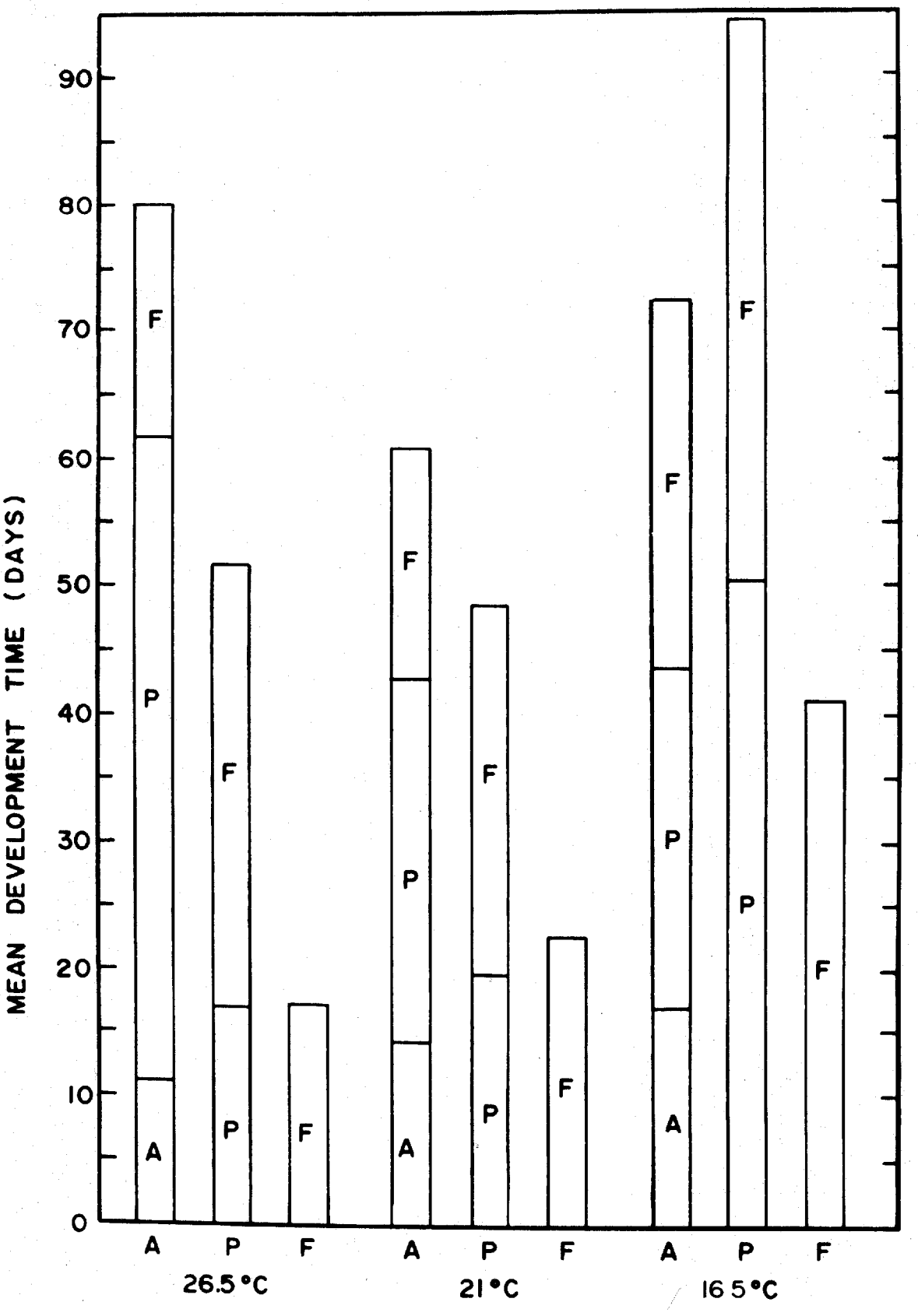

Figure 18. Effect of temperature on stadium duration in nymphs of C. angulatum collected in the antepenultimate (A), penultimate (P) and final (F) instars March 10, 1971, and reared under a 12 hour photoperiod. (See also Appendix K.)

most likely to be experienced under field conditions in the spring. It is apparent that while a 16°C temperature has a mild retarding effect on overall development in nymphs reared from the antepenultimate instar, the development time of nymphs collected in the penultimate instar is double that experienced at 21°C. The antepenultimate instar nymphs at 16°C were thus able to complete development sooner than the penultimate in spite of having to pass through an additional instar. From the available data it appears that, at a constant development temperature somewhere between 18 and 21°C, nymphs collected in the penultimate and antepenultimate instars should emerge synchronously. Nymphs reared from both antepenultimate and penultimate instars require considerably more time to emerge at all three temperatures than the nymphs which overwinter in the final instar. Final instar nymphs reared to emergence at 21° and 26.5°C and a 12 hour light period are no longer inhibited in their development by the photoperiod (See Fig. 14). Insects in this instar reared at 16°C require 41 days to emerge. This figure falls in the category of insects still inhibited by photoperiod, suggesting that a lower temperature increases the threshold photoperiod to which final instar nymphs respond normally.

Final instar nymphs reared from the penultimate and antepenultimate instars do not have a threshold photoperiod, as do the insects overwintering in the final instar. Their development rate increases progressively with increasing photoperiod (Fig. 15) at 21°C. Similarly, nymphs reared from the penultimate instar May 2, 1971 at 16°C and a 16½ hour photoperiod spent an average of 27.6 days in the final instar, compared to 43.8 at 12 hours, (Fig. 18).

(c) Effect of supernumerary moults on adult size

Nymphs undergoing extra moults were recognized in C. angulatum

under several experimental conditions. Particular attention was paid to nymphs collected in the penultimate instar in October and subjected to 21°C and an 8 hour light period. With one exception all specimens which emerged underwent at least three extra moults. Observations on the length of the wing pads showed that these insects advanced morphologically to stages intermediate between the penultimate and the final instar. Several of the nymphs in this experiment overshot the normal morphological characteristics of a final instar in their last moult resulting in nymphs somewhat larger than normal with considerably longer wing pads (cf. Figs. 19 and 20). These specimens then went on to complete pre-emergence development. Three of the nymphs attempted emergence and all had difficulty in withdrawing their wings from the large wing pads. They died as a result, completely emerged except for the wings which became dried to the exuviae. Successful emergence might have been possible under more humid conditions than those present in the growth chamber.

The mean maximum width of the head capsule in these insects measured 4.84 mm as compared to 4.07 mm in those insects reared at 16°C and an 8 hour photoperiod which remained in the penultimate instar for a considerable time rather than undergoing stationary moults.

(d) Lethal ice temperatures

Nymphs collected in the field March 10, 1971 were allowed to become embedded in ice in shallow dishes of water frozen under controlled conditions. After being frozen overnight, the ice was thawed and nymphal survival was determined. A temperature of -5 to -6°C was established as lethal. At this temperature 50% of the nymphs failed to recover. All three instars of nymphs tested were equally susceptible.

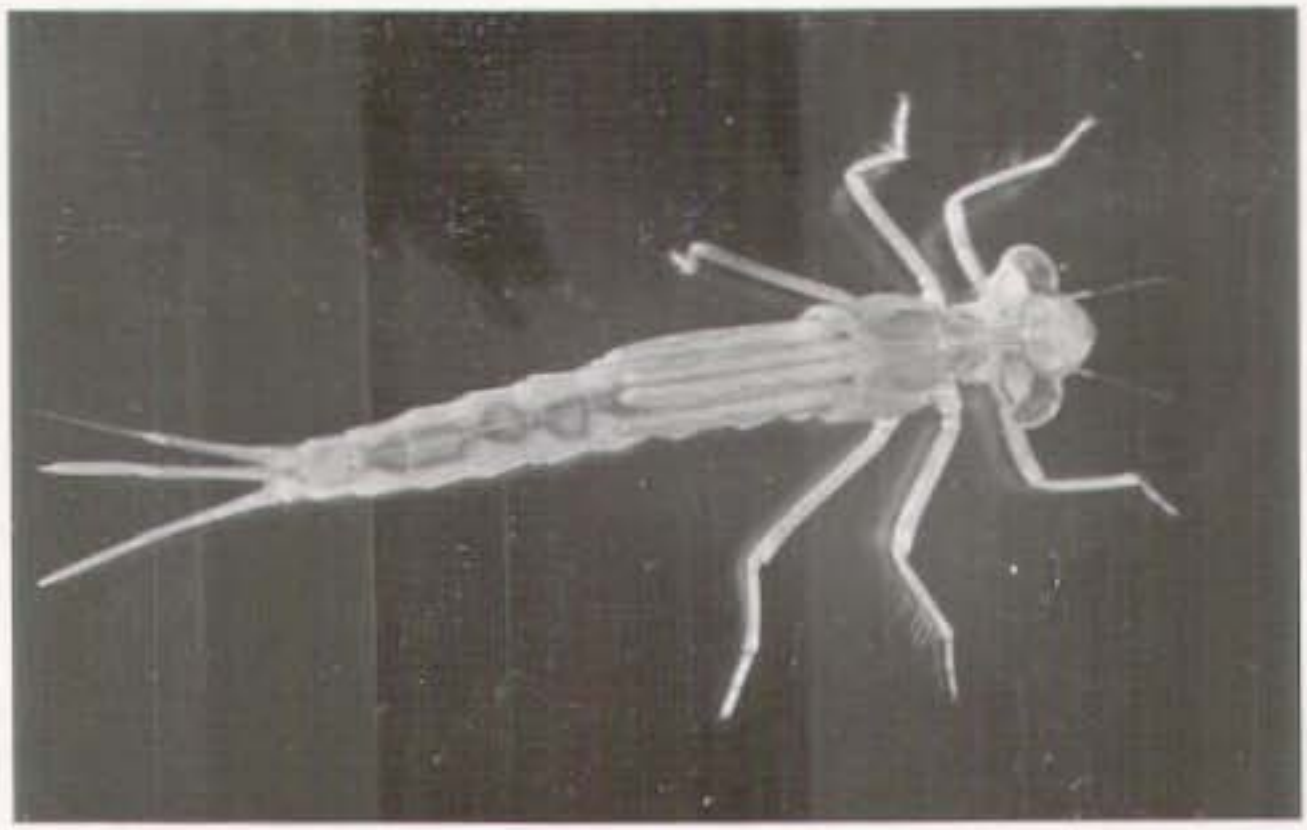

Figure 19. Final instar nymph of C. angulatum just prior to emergence.

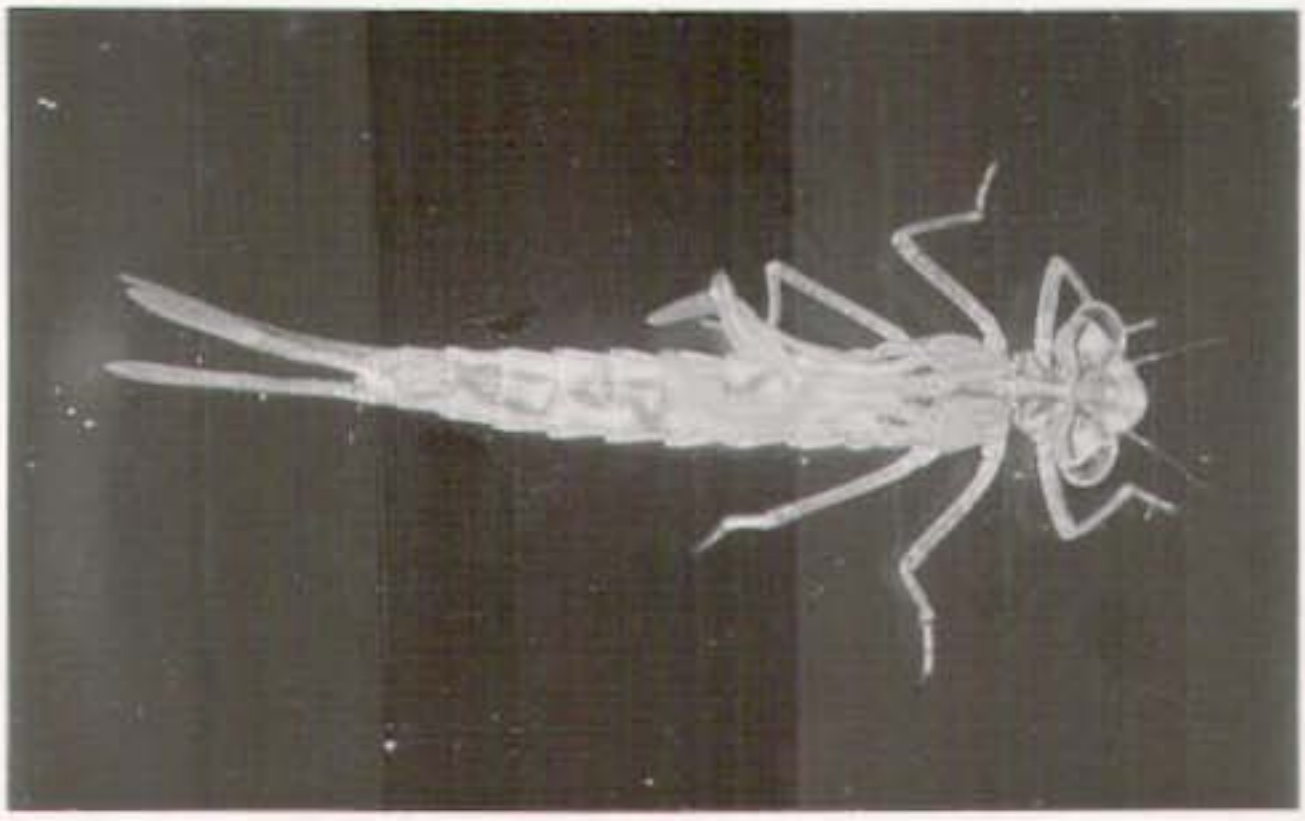

Figure 20. C. angulatum nymph displaying supernumerary instar characteristics.

Further experiments were conducted in May to determine whether the nymphs retain their tolerance to these low temperatures. Eighteen percent of the nymphs collected on May 6 survived -5.5°C. Those collected on May 13 and tested at -3.3°C all died while 57% recovery occurred at -2.2°C. Nymphs collected on May 20 could no longer survive any temperature appreciably below the freezing point of pond water.

3.1.3 Coenagrion resolutum - Field observations

(a) Pre-emergence spring development

Coenagrion resolutum, though almost entirely absent from the deep-water portion of the slough, was collected consistently in small numbers in the shallow area along with C. angulatum (Fig. 8). Like C. angulatum, this species overwinters in the late stages of nymphal development. Winter collections yielded 10.3% in the final instar, 76.4% in the penultimate instar and 13.2% in the antepenultimate. Changes in these proportions were not evident until May 20 in 1971, somewhat later than in C. angulatum. These are expressed as changes in mean instar of the nymphal population (Fig. 9).

Newly emerged C. resolutum adults were observed in the field for the first time on June 1, 1970 and May 27 in 1971. As in C. angulatum, emergence reached a peak during the first week in June, then dwindled during the next two weeks. Emergence was completed in this species shortly after June 18 in 1970 and 1971.

(b) The adult stage

Adults of C. resolutum were numerous in the hedges away from the water, apparently living on the abundant supply of adult chironomids and Chaoborus. Male to female sex ratio calculated on the basis of 944 nymphs collected was 1 : 1. This ratio was characteristic of nymphs in the final instar and undoubtedly expressed itself in the newly emerged adult population.

Sexual maturation was quickly completed in 1971. Adults in tandem were seen flying over the water on June 1. Oviposition was observed on June 3.

Eggs were deposited below the water surface in flowering stems of Ranunculus and Utricularia. The insects were never observed submerging completely, although this possibility is not ruled out since only a limited number of ovipositing females were observed. Oviposition always occurs in tandem. Oviposition sites are selected by the females who often settle on stems which barely protrude above the surface, leaving the male balancing in mid air with legs folded throughout the oviposition procedure. The ovipositing pairs move frequently from stem to stem, depositing approximately 8 to 10 eggs in each. Clutch size in this species was not determined.

The flying season of C. resolutum lasts approximately two months. Specimens were extremely difficult to find on July 29, 1971 and disappeared completely during the following week.

(c) The egg stage

Newly laid eggs, as in C. angulatum, are elongate, pointed at one end. They are soft and creamy white in colour. They later harden, the pointed end becoming dark brown. The anterior ends are capped by funnel shaped structures (Fig. 10) which probably function in the manner described for C. angulatum. Egg dimensions are 1.00 by 0.19 mm, excluding the funnel.

Embryonic development begins immediately. Newly laid eggs were collected and allowed to hatch under three temperature conditions. Temperature effect on embryonic development is shown in Figure 21. On the basis of these results embryonic development under field conditions would be expected to take approximately 17 days.

(d) Summer and autumn nymphal development

Nymphs of the new generation began to appear in field samples on

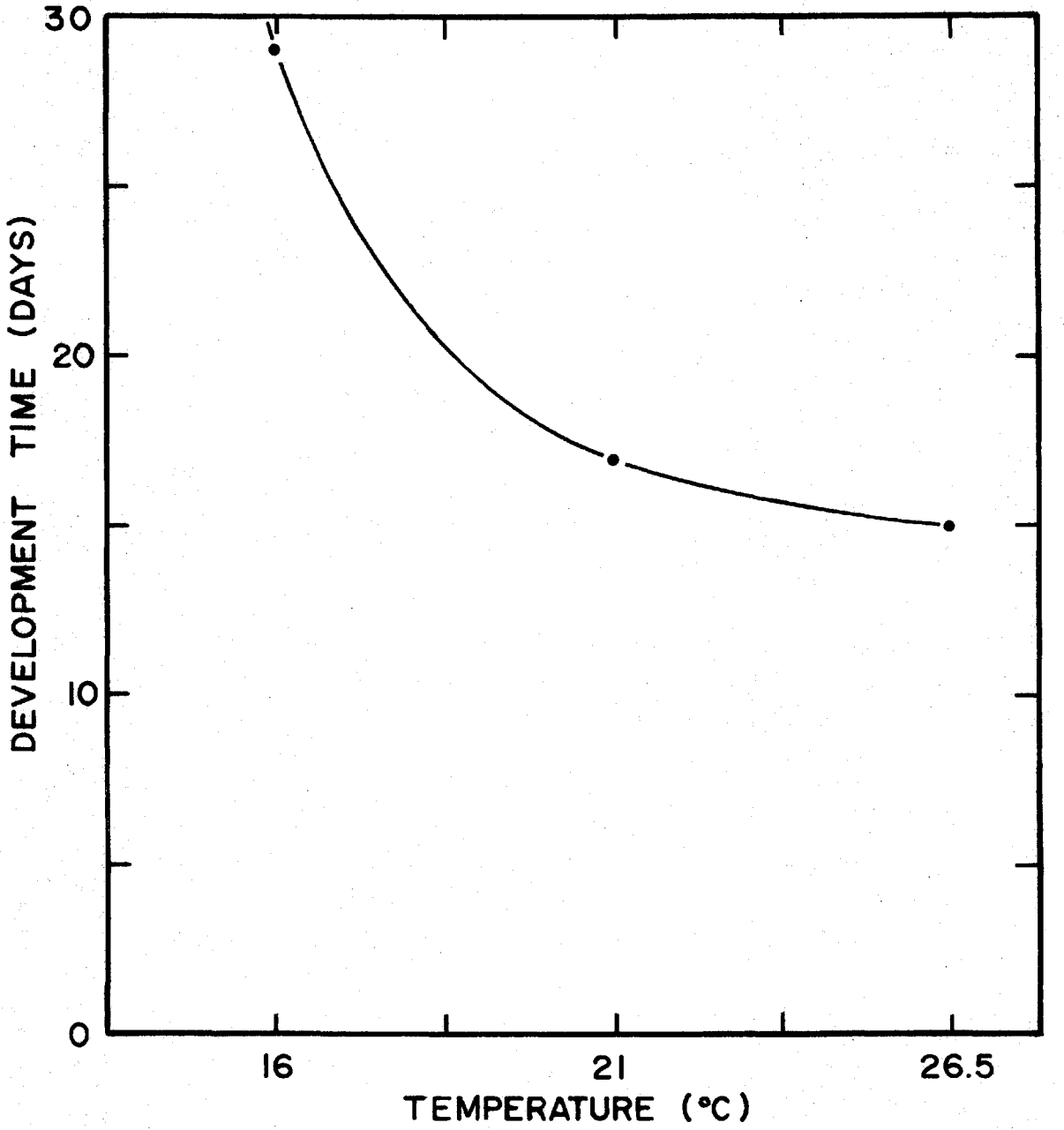

Figure 21. Effect of temperature on embryonic development in C. resolutum, shown as time required to complete hatching.

July 30, 1970 and July 22, 1971. Mean instar was established from the specimens collected at regular intervals. These were plotted (Fig. 9) in order to establish a growth curve for the species. Nymphal development appears to stop for the winter at approximately the beginning of October, slightly earlier than in C. angulatum. The mean overwintering instar was the penultimate in 1970-71.

(e) Overwintering of nymphs

The factors influencing the overwintering of C. angulatum also apply to C. resolutum. Both species were found together throughout the collecting period, both summer and winter.

3.1.4 Coenagrion resolutum - Laboratory experiments

The similarity in the life cycles of C. angulatum and C. resolutum prompted experiments with nymphs of the latter species to compare with those of C. angulatum whenever specimens were available.

(a) Photoperiod effect on nymphal development

Autumn development in nymphs of C. resolutum also appears to be under the influence of photoperiod. Experiments conducted on the last two instars in October showed that insects reared at 21°C and an 8 hour photoperiod required approximately 80 days to undergo a moult (Fig. 22). Under these conditions, approximately 40% died from no apparent cause without moulting. This mortality is, however, considerably lower than that experienced by nymphs of C. angulatum. While almost all nymphs of C. angulatum collected in the penultimate instar at this time underwent several successive stationary moults, only one out of ten specimens demonstrated this type of behavior in C. resolutum. Development time was shortened by 35 days at the long photoperiod in both final and penultimate instars (Fig. 22).

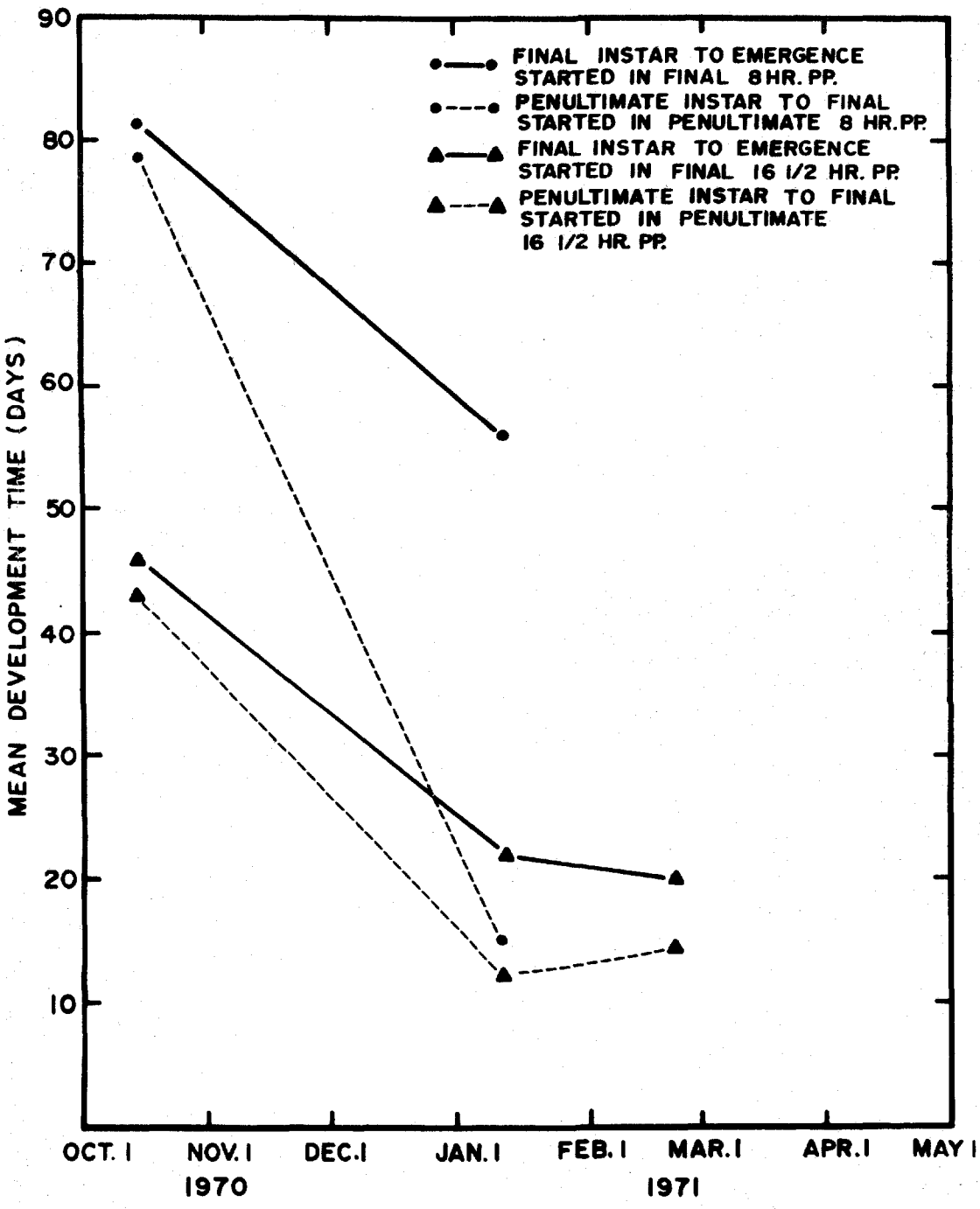

Figure 22. Seasonal changes in development rate of C. resolutum nymphs reared at 21°C. (See also Appendix L.)

The photoperiod controlled aspect of nymphal development persisted, in the insects collected in the final instar, throughout the winter. Experiments conducted at a constant temperature of 21°C at several photoperiods, using final instar nymphs collected on January 11, 1971, demonstrated a definite threshold between 12 and 14 hours light above which development was considerably accelerated (Fig. 23). Results of this experiment were similar to those obtained for C. angulatum. Two specimens collected March 10 and reared at 21°C and a 12 hour photoperiod emerged in 22 days, suggesting that there was a shift in threshold towards the shorter photoperiod, as was demonstrated in C. angulatum.

Experiments similar to the above were conducted on C. resolutum nymphs collected on January 11 in the penultimate instar. As is shown in Figure 24, little or no photoperiod effect was indicated in development rates preceding moulting into the final instar. A photoperiod effect was, however, expressed in the final instar of these specimens by an increase in development time inversely proportional to the photoperiod at which the nymphs were reared (Fig. 24). The relationship is better illustrated in Figure 25. The unexpectedly low development time obtained at the 10 hour photoperiod may have been caused by the inadvertent exposure of the nymphs to a long photoperiod for two days early in the penultimate instar. The curve presented is similar to that obtained for C. angulatum.

(b) Temperature effect on nymphal development

Comparison of October photoperiod experiments with replicates conducted in January and February shows that there has been a drop in development time in all phases of the experiment between nymphs collected in October and in January (Fig. 22). This drop may be attributed to certain physiological changes brought about in the nymphs by cold temperature under field conditions

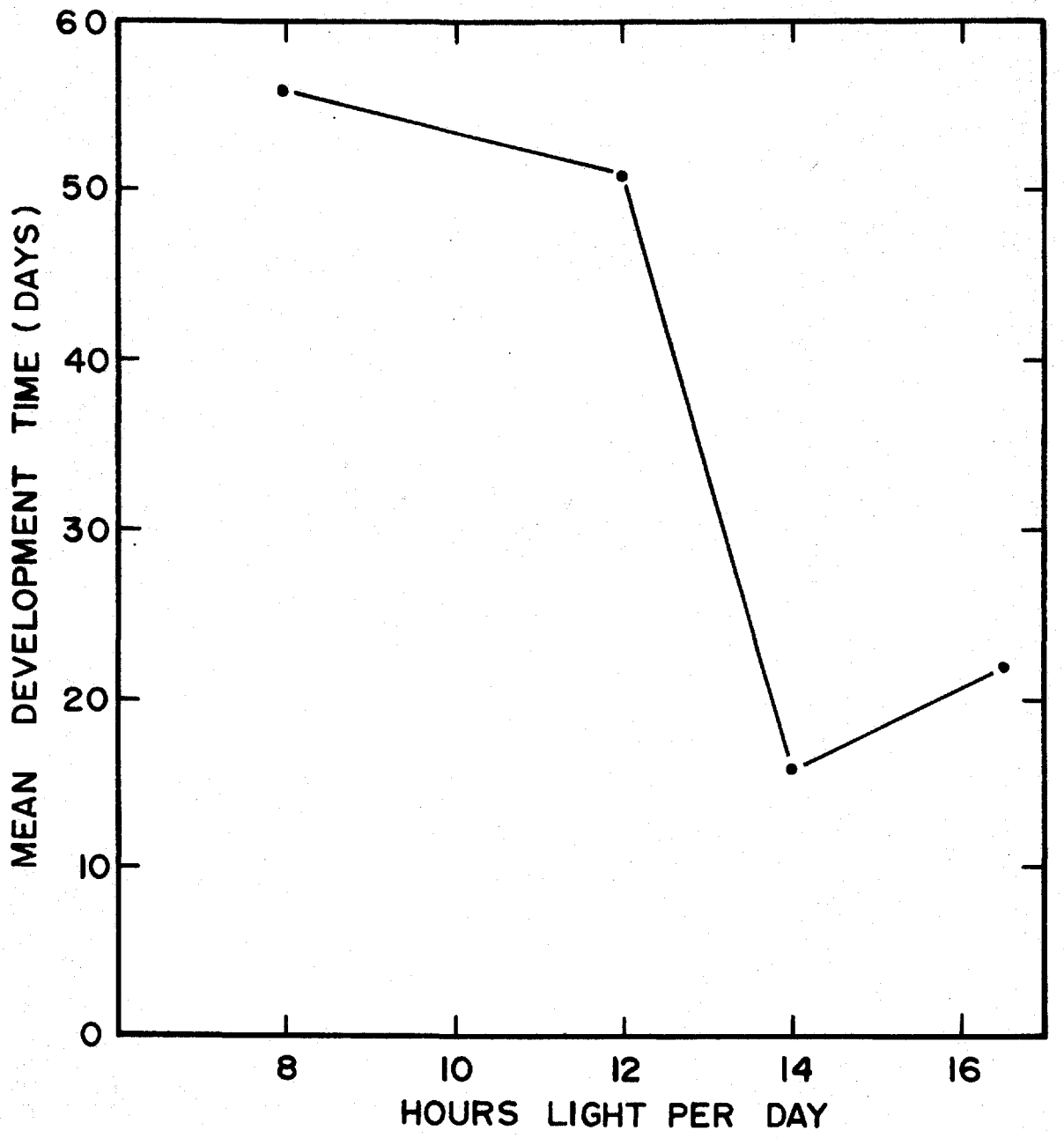

Figure 23. Effect of photoperiod on duration of final stadium in nymphs of C. resolutum collected in the final instar January 11, 1971, and reared at 21°C. (See also Appendix M.)

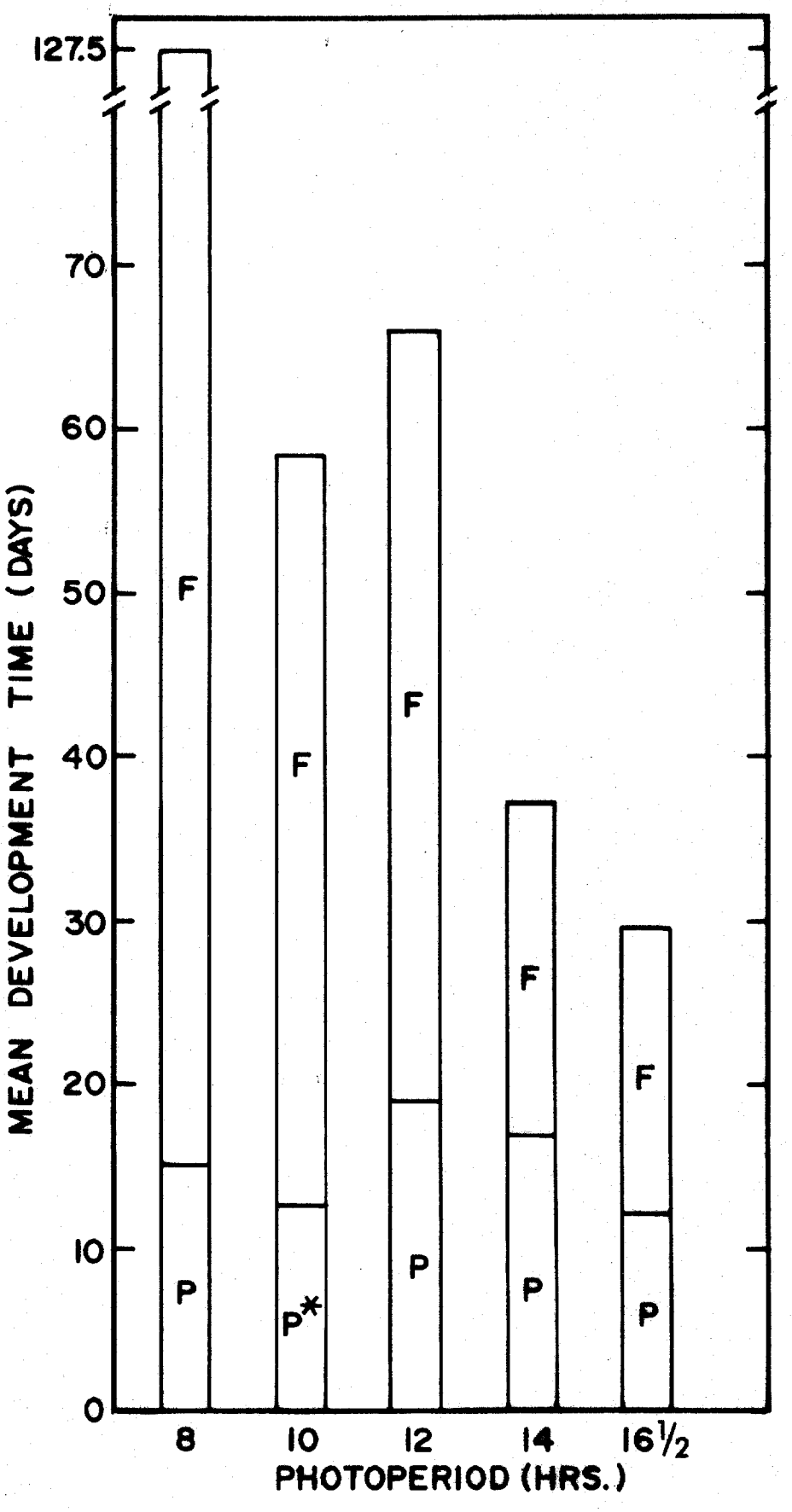

Figure 24. Effect of photoperiod on stadium duration in nymphs of C. resolutum collected in the penultimate (P) instars January 11, 1971, and reared at 21°C. (See also Appendix N.)
*Inadvertently exposed to a long photoperiod for two days.

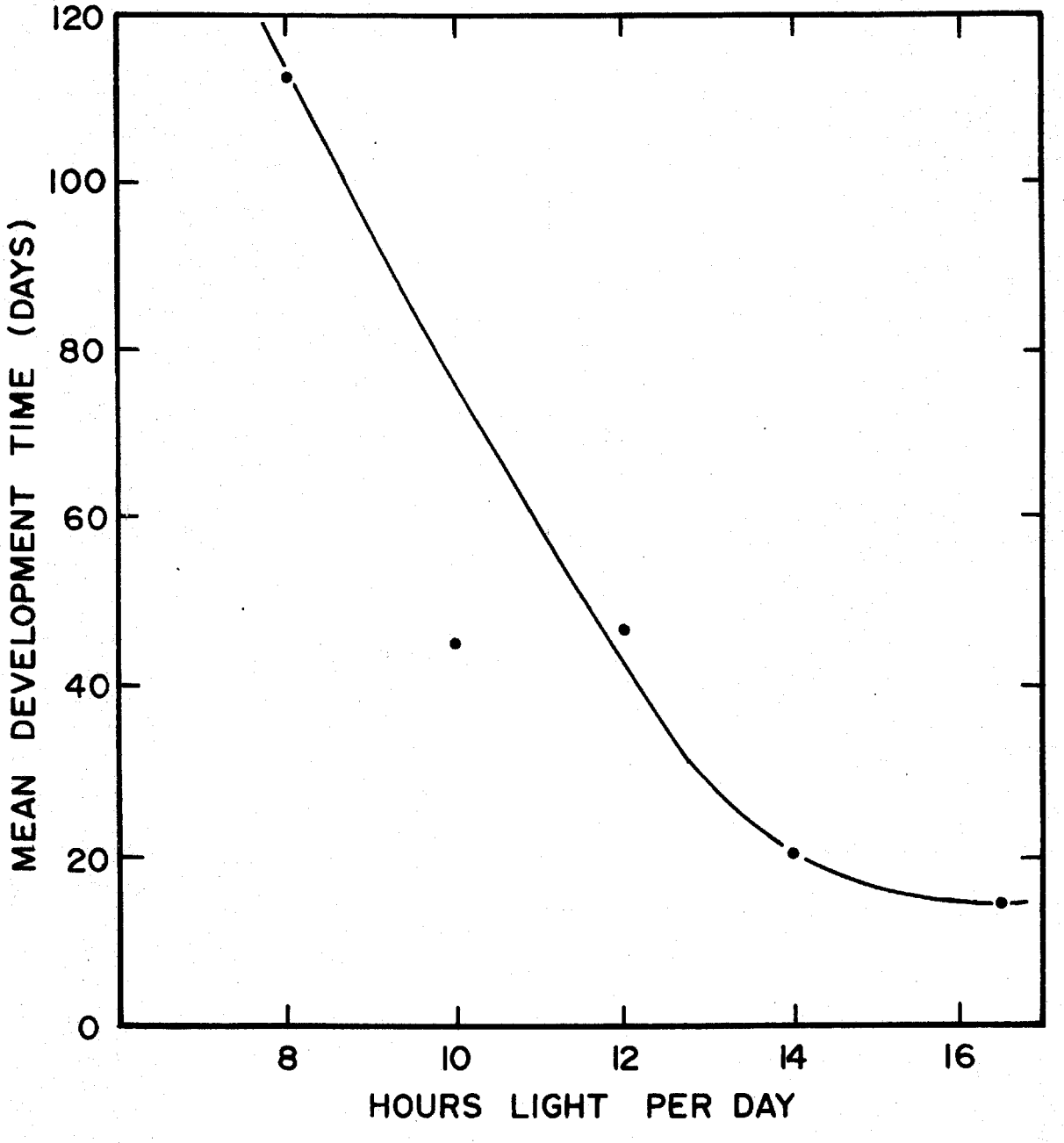

Figure 25. Effect of photoperiod on duration of final stadium in nymphs of C. resolutum collected in the penultimate instar January 11, 1971, and reared at 21°C. (See also Appendix N.)

during the early winter months. Comparison of January and February data at the 16½ hour photoperiod show no further decrease in development time, suggesting that the phase of nymphal development controlled by cold temperature had been completed by January 11. Temperature effect on nymphal development was studied by subjecting penultimate and final instar nymphs collected March 10, 1971 to 16, 21 and 26.5°C at a 12 hour photoperiod. Results of the experiments are shown in Figure 26. The figure indicates that 21°C is an optimal development temperature for the two instars examined. Original data indicate a high mortality in insects reared at 26.5°C. Forty percent of those insects placed at 26.5°C in the penultimate instar died without moulting. Another 30% died before or during emergence. At 16°C, mortality was low in the penultimate instar. However, several specimens had difficulty emerging, suggesting that 16°C is only marginal as an emergence temperature. At 21°C emergence was normal. Only two out of ten specimens at this temperature were lost throughout the entire experiment. Increase in the length of time spent in the final instar at 16°C and the relatively normal development in the penultimate instar suggest a certain amount of synchronization of development might be possible under field conditions in the spring. The cool spring temperatures might permit more rapid development in the earlier instars while retarding development in the final. Unfortunately, insects were not collected in the antepenultimate instar. No comparisons of development could be made to further test this hypothesis. However, the overall responses of C. resolutum nymphs to the experimental conditions appear similar enough to those of C. angulatum to suggest that the two species respond similarly to temperature in all aspects of nymphal development.

(c) Lethal ice temperatures

Specimens of C. resolutum collected from ice March 10, 1971 were "refrozen" according to the method described earlier for C. angulatum.

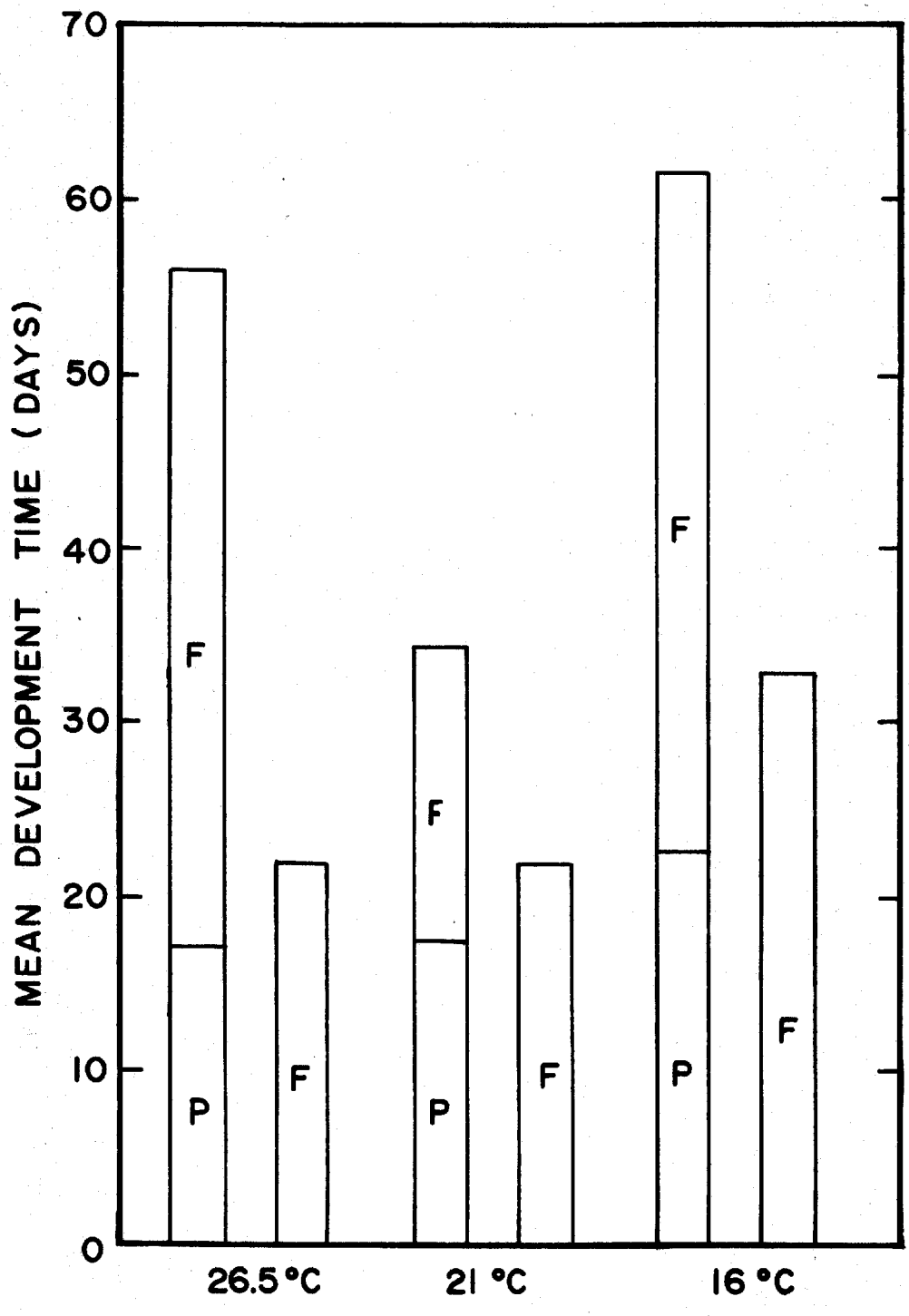

Figure 26. Effect of temperature on stadium duration in nymphs of C. resolutum collected in the penultimate (P) and final (F) instars March 10, 1971, and reared under a 12 hour photoperiod. (See also Appendix O.)

Approximately 50% mortality was obtained at a temperature of -5 to -6°C. All overwintering stages of this species were equally susceptible.

Tolerance of specimens collected in May was not tested.

3.1.5 Enallagma boreale - Field observations

(a) Pre-emergence spring development

Enallagma boreale overwintered successfully only in the deep portion of the study area. Since sampling during two of the three years was concentrated in the shallow marshy part of the slough, only limited data on this species are available. The nymphs overwinter in 60 cm or more of water but migrate in the spring to the shallow grassy areas near the shore which are more suitable for rapid development. The shallow water warms up more rapidly and attracts a variety of organisms which can be used as food. The species overwintered in four instars in 1968-1969, most specimens occurring in the penultimate and final instars. Spring nymphal development appeared similar to that of C. angulatum and C. resolutum. Newly emerged adults were observed on May 27, 1971, along with newly emerged individuals of the other two species. Most of the specimens had emerged by mid June in 1969. However, a single final instar nymph was collected July 8. In 1970, newly emerged adults were seen on June 30.

(b) The adult stage

Unlike the two species of Coenagrion, E. boreale adults appear more frequently near the water during their maturation period. Maturation in this species appears to take longer. In spite of frequent visits to the study area during 1970, I did not observe copulating pairs until June 22, about three weeks after the beginning of emergence.

Sexually mature E. boreale showed a definite preference for the deep water of the slough, seldom occurring over the heavily vegetated shallow

water frequented by C. angulatum and C. resolutum. Adults were common, flying over water a metre or more in depth, or resting on stems of Scirpus far from shore.

E. boreale females were observed ovipositing in emerging flowering stalks of Potamogeton, Ranunculus and, later in the season, Myriophyllum (Fig. 27). The insects always oviposited in tandem. The female dips her abdomen below the surface to insert the eggs. Occasionally eggs were deposited in portions of stems just above the water surface. In this species also, the female selected the oviposition site, probing the vegetation on which the pair settled until a suitable stem was encountered by the ovipositor. This activity often left the male fluttering helplessly without a perch to rest on. As in C. resolutum, E. boreale females shift frequently from stem to stem, depositing a few eggs in each.

The flying season in E. boreale extended to August 6 in 1970 and August 12 in 1971.

(c) The egg stage

Egg clutch size was determined from three sexually mature females caught in tandem prior to oviposition. These females contained an average of 592 mature eggs each.

The newly laid eggs measured 0.89 to 0.96 mm in length. As in the other two species, they were creamy white and soft but soon darkened and became harder. A funnel-shaped hood was present over the pointed end of each egg in this species (Fig. 28). However, it was considerably smaller than those in Coenagrion.

Embryonic development begins soon after oviposition. Newly laid eggs were hatched at two temperatures under laboratory conditions. Hatching required 18 days at 21°C and 25 days at 16°C. No field hatch data are available.

Figure 27. Oviposition scars left by E. boreale in flowering stalk of Myriophyllum.

Figure 28. Newly laid egg of E. boreale displaying the funnel-shaped structure at the anterior end.

(d) Summer and autumn nymphal development

Young-of-the-year nymphs were first collected on July 31 in 1969. By this time many specimens had already reached the antepenultimate-2 (A-2) instar. Subsequent development proceeded rapidly during the remainder of the summer. Specimens in the final instar were first recorded September 12, 1969. However, on this date the population was made up of individuals in seven different instars. Sampling was terminated early in 1969 but specimens collected early the next spring indicated that this species overwintered in the last three instars.

(e) Overwintering of nymphs

Nymphs of E. boreale were found overwintering only in the deep portion of the study pond. They were collected through the ice in December and in the water soon after the ice lifted in the spring. The nymphs were never found embedded in ice as were nymphs of C. angulatum and C. resolutum. In December they were collected from vegetation dredged from about 1.2 metres of water. When collected, they appeared lifeless. The specimens were returned to the laboratory and placed in clean water. At first no sign of life was observed even when they were viewed through a microscope. However within ten to fifteen minutes, faint pulsation of the heart was detected. Subsequent recovery was rapid and within one hour the specimens looked completely normal.

3.1.6 Enallagma boreale - Laboratory experiments

(a) Photoperiod effect on nymphal development

The effect of photoperiod on nymphal development and emergence in Enallagma boreale was studied in eight specimens collected October 14, 1970. A final instar nymph required 77 days to emerge at 21°C and an 8 hour photoperiod. A second nymph died ready to emerge after 149 days under these conditions. One nymph reared at 21°C and a 16½ hour photoperiod emerged in

only 23 days. A specimen collected in the penultimate instar and reared at 21°C and an 8 hour photoperiod moulted to the final instar after 129 days, requiring an additional 40 days to emerge. Four insects collected in the antepenultimate and antepenultimate-1 instars were unaffected by photoperiod in their moult to the succeeding instar.

In spite of the small sample size, the data presented indicate a response to photoperiod similar to that in nymphs of C. angulatum and C. resolutum.

(b) Temperature effect on nymphal development

Nymphs of E. boreale were collected in August, 1969 in five instars ranging from penultimate to antepenultimate-3 (A-3) and reared under a 16½ hour photoperiod at constant temperatures of 4.5, 10, 21, and 26.5°C. Data are presented in Table 6.

Nymphs reared at 26.5°C appeared to undergo a weak developmental arrest in the penultimate instar. Emergence proceeded without delay once the insects passed through this stadium. Further developmental inhibition was demonstrated at this temperature by the appearance of stationary moults in three of the specimens. At 21°C nymphal development proceeded without arrest up to the final instar. A mean development time of 84.3 days in the final instar demonstrates that at this temperature developmental arrest has been bypassed at the penultimate stage and occurs in the final instar only. In addition to the expected inhibitory effect of low temperature on early instars subjected to a 10°C temperature, development of the nymphs was considerably retarded in the penultimate instar. An average of 114.8 days elapsed before the nymphs moulted to the final instar. Emergence took place after the nymphs had spent an average of 55 days in the final instar. Ten degrees appears to be near the minimum threshold for emergence and may account for the rather long emergence time.

Table 6. Effect of temperature on stadium duration (Days) in nymphs of E. boreale collected August 13, 1969, and reared at a constant 16½ hour photoperiod.

	To A - 2	To A - 1	To A	To A + 1	To P	To P + 1	To Final	To Emergence
26.5°C								
	-	-	-	-	-	-	26	32
	-	-	-	-	22	-	10	10
	-	-	-	-	19	32	29	15
	-	12	7	-	21	-	40	13
	-	-	9	9	16	-	42	12
	-	-	-	12	19	-	60	8
$\bar{X}$		12	8	10.5	19.4	32	34.5	15.0
21°C								
	-	-	-	-	19	-	21	100
	-	-	-	-	20	-	17	51
	-	-	16	-	12	-	29	56
	-	-	-	-	27	-	20	232
	-	15	14	-	15	-	11	-
	-	-	-	-	28	-	26	107
	-	27	12	-	40	-	21	57
	-	30	41	-	30	-	40	-
	-	14	15	-	14	-	35	95
	10	9	15	-	40	-	26	18
	-	24	10	-	15	-	33	43
$\bar{X}$	10	19.8	17.6	-	23.6	-	25.4	84.3
10°C								
	-	-	-	-	26	-	125	55
	-	-	27	-	42	-	105	32
	-	-	26	-	22	-	-	-
	-	29	24	-	35	-	112	55
	-	-	27	-	22	-	235	49
	-	34	66	-	41	-	35	63
	45	39	24	-	28	-	105	63
	-	26	26	-	30	-	205	53
	21	62	35	-	41	-	81	56
	-	36	77	-	30	-	31	62
	-	-	35	-	25	-	114	62
$\bar{X}$	33.0	37.7	36.7	-	31.1	-	114.8	55.0

Essentially no development occurred at 4.5°C. Four out of twelve specimens moulted once after several months at this temperature but died soon after. The others died without moulting generally after 100 to 250 days. One specimen lived for 16 months without moulting a single time. Data from this experiment were excluded from Table 6.

It appears from these results that developmental arrest is, at least in part, under the influence of temperature. Under optimal conditions nymphs are able to develop to the final instar before entering developmental arrest while apparently suboptimal conditions experienced at 10 and 26.5°C resulted in the appearance of the diapause phase in the penultimate instar.

(c) The overwintering phase

An attempt was made to reintroduce the inactive overwintering state in nymphs of E. boreale collected early in May. Chilling alone was insufficient. However, when placed in sealed bottles of pond water high in dissolved hydrogen sulfide and then chilled, the specimens quickly became lethargic, then inactive. One specimen survived four months in this condition without food or oxygen, despite having spent the preceding winter in this state. Independent effects of oxygen deficiency and high hydrogen sulfide concentrations were not tested since both conditions existed simultaneously in the ice-covered pond.

Nymphs of E. boreale were subjected to sub zero temperatures according to the procedure described earlier for C. angulatum and C. resolutum. Numerous attempts to revive specimens embedded in ice at various temperatures were unsuccessful, leading to the conclusion that E. boreale nymphs are incapable of tolerating temperatures below the freezing point of pond water.

3.2 Type B Species

Characteristics distinguishing Type B damselflies have been attributed

to three species collected in the study area. All belong to the family Lestidae, and include Lestes disjunctus disjunctus Walk., Lestes unguiculatus Hagen and Lestes dryas Kirby. Lestes disjunctus and L. unguiculatus constitute a significant portion of the pond's zygopteran population while L. dryas was collected only occasionally. Preliminary collections in the vicinity of the study area showed a preference by the latter species for shallow, temporary habitats, such as road-side ditches. Sufficient information was, however, gathered on the species to warrant its inclusion in this study.

3.2.1 Lestes disjunctus - Field observations

(a) Hatching and nymphal development

Hatching in L. disjunctus began approximately May 5 in 1971, slightly less than one month after the slough had become filled with runoff water from melting snow. Hatching is highly synchronous and appears to be complete within one week.

Nymphal development is very rapid in spite of the relatively cool temperatures at this time of the year. When nymphs were first netted on June 3, 1971 all specimens were in the antepenultimate-2 (A-2) instar. Final instar nymphs were present in the June 22, 1970 and June 24, 1971 collections. At no time were there more than three instars in the nymphal population sampled. The mean instar was determined for each sampling date, allowing the establishment of a growth curve for field development (Fig. 29).

The final stages of nymphal development in L. disjunctus were completed soon after the emergence of the Type A species (Fig. 8). During this time the nymphs became conspicuous in the shallow water previously occupied by the Type A species.

Emergence in L. disjunctus began on July 4 in 1970 and between July 3 and July 9 in 1971. It was virtually complete within ten days.

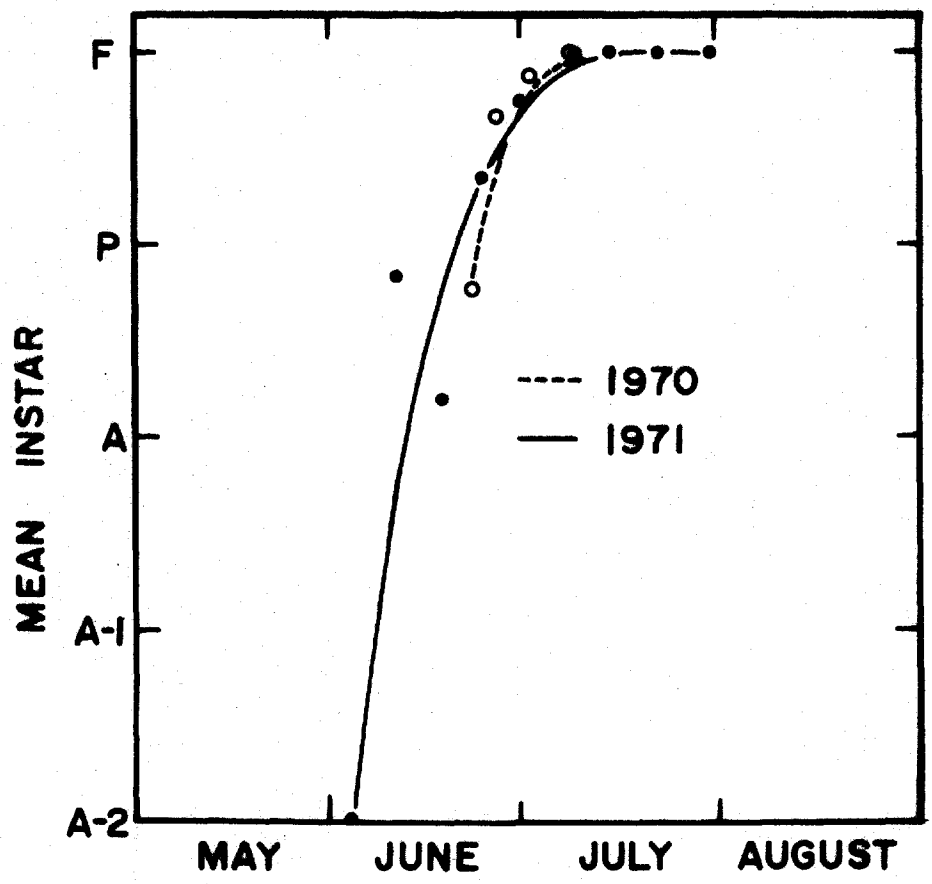

Figure 29. Development rate of L. disjunctus nymphs under field conditions. Curves were fitted by eye using a three-point moving average. (See also Appendix E.)

F = Final, P = Penultimate, A = Antepenultimate, A-1 = Antepenultimate-1, A-2 = Antepenultimate-2.

Only one nymph was collected after July 15 in both 1970 and 1971.

Nymphs of L. disjunctus about to emerge migrated into very shallow water and became especially abundant in depths of 8 cm or less. Emerging nymphs crawled out onto any vegetation that was available. Cast skins were found as high as 20 cm above the water surface although 8 to 10 cm was a more common height for emergence.

(b) The adult stage

Adults of L. disjunctus appear to follow the same pattern of post-emergence behavior as Type A species, moving away from the water as soon as they are able to fly. The maturation period is considerably longer than previously observed in other species. Pairs in tandem were rarely observed away from water. Males appear to complete sexual maturation and return to the water before the females. They were seen in large numbers in the grass surrounding the pond on July 20 and 21, 1970. Females were conspicuous by their absence. Mating and oviposition were taking place on July 23. The presence of scarred stems of Scirpus indicated that oviposition had begun a day or two earlier.

On returning to the water females were soon caught by a male, either in flight or while perched. Often a struggle occurred between two males to achieve tandem, but as soon as one succeeded the other disappeared. The pair were then no longer disturbed by single males, who often flew past at close range. Sperm transfer took place immediately after tandem was achieved and lasted approximately 30 seconds. Copulation occurred at once and lasted approximately 15 minutes, seldom uninterrupted. Mating generally occurred in the grass along the shore. At the end of copulation, the pair immediately flew to the clumps of Scirpus growing in 30 to 60 cm of water which served as oviposition sites (Fig. 3).

Oviposition in this species always occurs in tandem. Eggs were observed to be deposited only in the green stems of Scirpus in the deep portion of the pond, although in the absence of these plants in the shallow portion of the pond other plants probably are used. The stems selected for oviposition were either those standing in small numbers apart from the large clumps, or the stems bordering the large clumps, never in the centre. The eggs were deposited above the water surface, at a height ranging from 5 cm to 60 cm or more. Some eggs were collected from stems of Scirpus growing in the soft mud away from the edge of the water.

Eggs of this species were deposited in the pith of the Scirpus stem, just beneath the epidermal and vascular layers. Up to six eggs were deposited per incision, a distinguishing characteristic of the species. The eggs were inserted in two rows approximately at right angles to one another, the pointed anterior ends directed towards the incision in the stem. Following completion of egg deposition in an incision, the female then carefully replaced the flap of plant tissue torn back during oviposition by pulling her abdomen over it. Whether or not some form of sealant was deposited is unknown. The flap eventually dried to form a complete seal over the wound (Fig. 5). The incisions were spaced well apart in the stem, approximately 5 to 8 mm, compared to those made by other members of the same genus studied. This factor was also used as an identifying characteristic when collecting eggs.

Only two females were dissected for oocyte counts. They contained 112 and 45 mature eggs respectively. It is believed that the larger figure is more indicative of the average clutch size in this species. The average number of recognizable oocytes per ovariole was nine. Apart from these observations the reproductive potential of the species was not investigated.

(c) The egg stage

Eggs of L. disjunctus, as in all endophytic odonates, are elongate, and pointed at the anterior end. The eggs are 1.3 mm long. They do not possess a funnel-shaped structure at the anterior end as was observed in the Type A species studied. The eggs, though quite soft when first laid, become firm soon after, much more so than eggs of Coenagrionidae.

Egg development begins soon after oviposition. The embryos develop at approximately the same rate as those of Type A species almost to the point of hatching. The eggs then enter a clearly defined diapause phase (Fig. 7a) demonstrated by a cessation of development during otherwise favorable development conditions. Pre-diapause morphological changes in the embryos were essentially completed in all eggs under field conditions by August 25.

It was noted that the thin egg shell would provide little protection from desiccation since this covering splits as embryonic development progresses. The green plant tissue in which the eggs are laid performs a vitally useful function in this regard providing a moist environment for the developing eggs. A severe frost early in the fall of 1970 caused the Scirpus stands to dry out long before the first snowfall. The result was an extremely high egg mortality through desiccation. Normally Scirpus stays green until late in October, later than any other plants in the study area. Heavy dew and frost associated with late fall conditions are subsequently able to maintain moisture conditions previously provided by the green stems. During the winter moisture is maintained by snow cover.

The eggs, although somewhat resistant to low temperatures, are unable to withstand the rigors of prairie winters, during which temperatures occasionally drop to -35 or -45°C, without additional protection. The protection

comes in the form of snow cover. Eggs collected from exposed stems during the winters of 1969-70 and 1970-71 were not viable. The stems selected for oviposition by this species are the ones which become broken early in the fall and are covered by snow which accumulates in the stands of Scirpus. Stems in the centre of a stand remain standing throughout the winter and for this reason are likely to be unsuitable oviposition sites.

Wind and snow during the winter, ice action during the breakup in the spring, and rise in water level cause the dry Scirpus stems to become submerged. Wetting is essential for hatching in this species (see page 93). Wind may be a hazard to the survival of the eggs for it may blow the floating stems of the rushes up on shore before hatching takes place.

3.2.2 Lestes disjunctus - Laboratory experiments

Several aspects of the life cycle of L. disjunctus and other members of the Type B species lent themselves to laboratory investigation. The diapause in the egg evidently plays a role in synchronization of subsequent development. Events leading up to the onset of diapause were investigated under controlled conditions, as were factors affecting diapause and post-diapause development. Effects of freezing temperatures on survival and moisture conditions on hatching also were explored.

(a) Effects of temperature and photoperiod on pre-diapause development

Newly laid eggs of L. disjunctus were collected, removed from the stems and placed in dishes on wet filter paper. They were subjected to experimental temperatures of 16, 21 and 26.5°C under photoperiods of 8 and 16½ hours. The effect of temperature on the time required to complete pre-diapause development is shown in Figure 30. Pre-diapause development was considered complete when the embryonic eye spots had turned black, when the tracheal

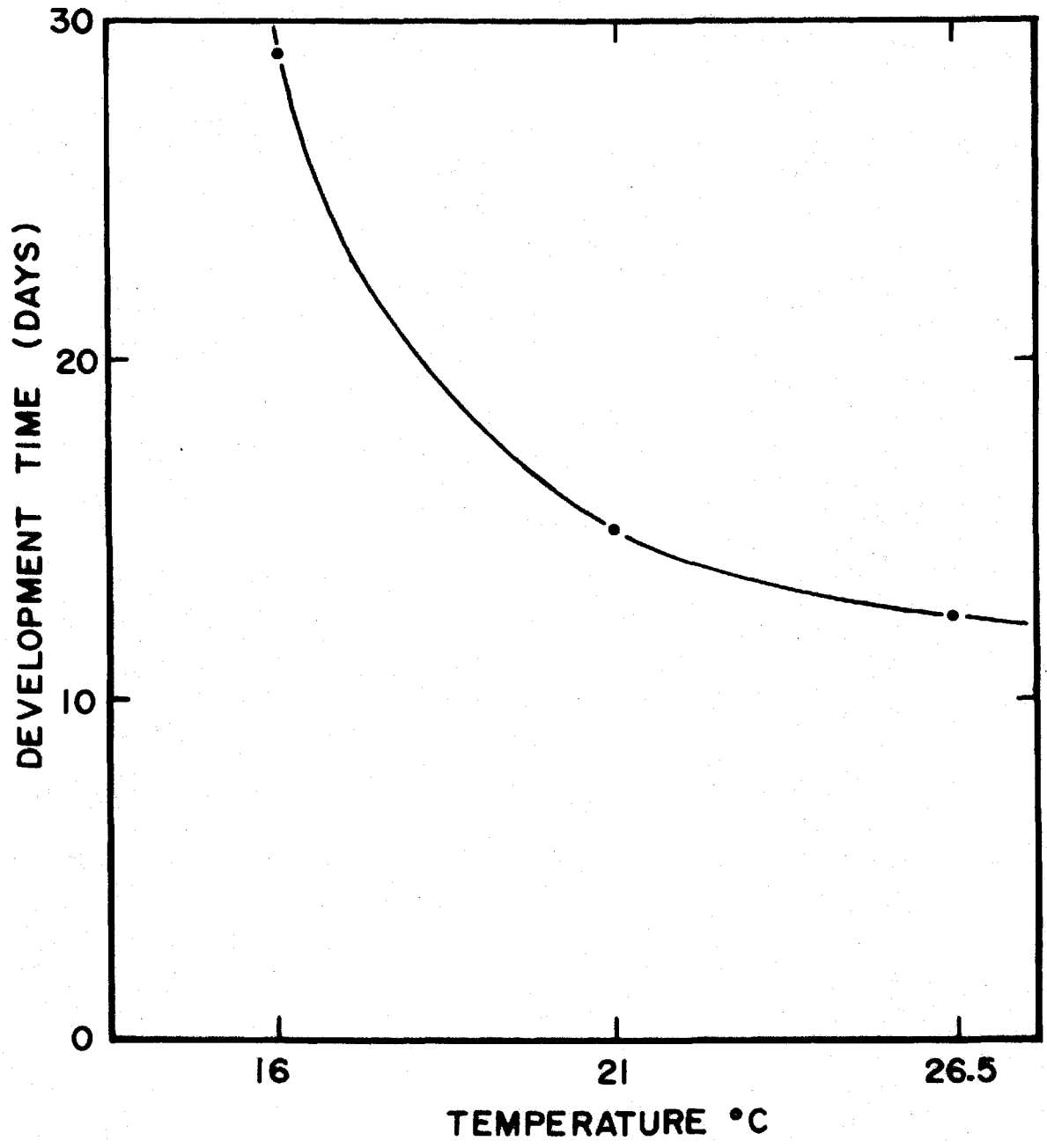

Figure 30. Effect of temperature on pre-diapause development in eggs of L. disjunctus, shown as time required for all eggs in the sample to complete pre-diapause development.

system had become clearly visible, and when no further morphological change in the embryo was observed (Fig. 7a).

The reciprocals of the number of days required to complete pre-diapause development were plotted against temperature. Figure 31 predicts the minimum temperature for pre-diapause embryonic development as approximately 6°C.

No photoperiod effect was observed on pre-diapause development.

(b) Effect of temperature and photoperiod on diapause development

Diapause development in L. disjunctus eggs appears to be governed by two independently operating factors. The stage of diapause development which is under the influence of temperature will be referred to as Phase I. Phase II is that portion of diapause development under the control of photoperiod.

Eggs of L. disjunctus collected during the winter hatch within four to eight days at 21°C and a 16½ hour photoperiod. This range was taken as a standard for post-diapause development and was used to establish whether or not Phase I of diapause development had been completed. Eggs which required longer than eight days to hatch under these conditions were considered as having still been in Phase I of diapause when the test was initiated. Those which hatched in fewer than four days were believed to have undergone some post-diapause development prior to being subjected to the test conditions. Pronymphs which died in the process of hatching were counted as hatched.

Newly laid eggs were collected on July 21 and August 3 and incubated at 16, 21 and 26.5°C and photoperiods of 8 and 16½ hours until hatching was complete. Results are presented in Table 7. Even under the best hatching conditions eggs failed to hatch in less than two months after the beginning of diapause development. The high mortality is probably associated with the

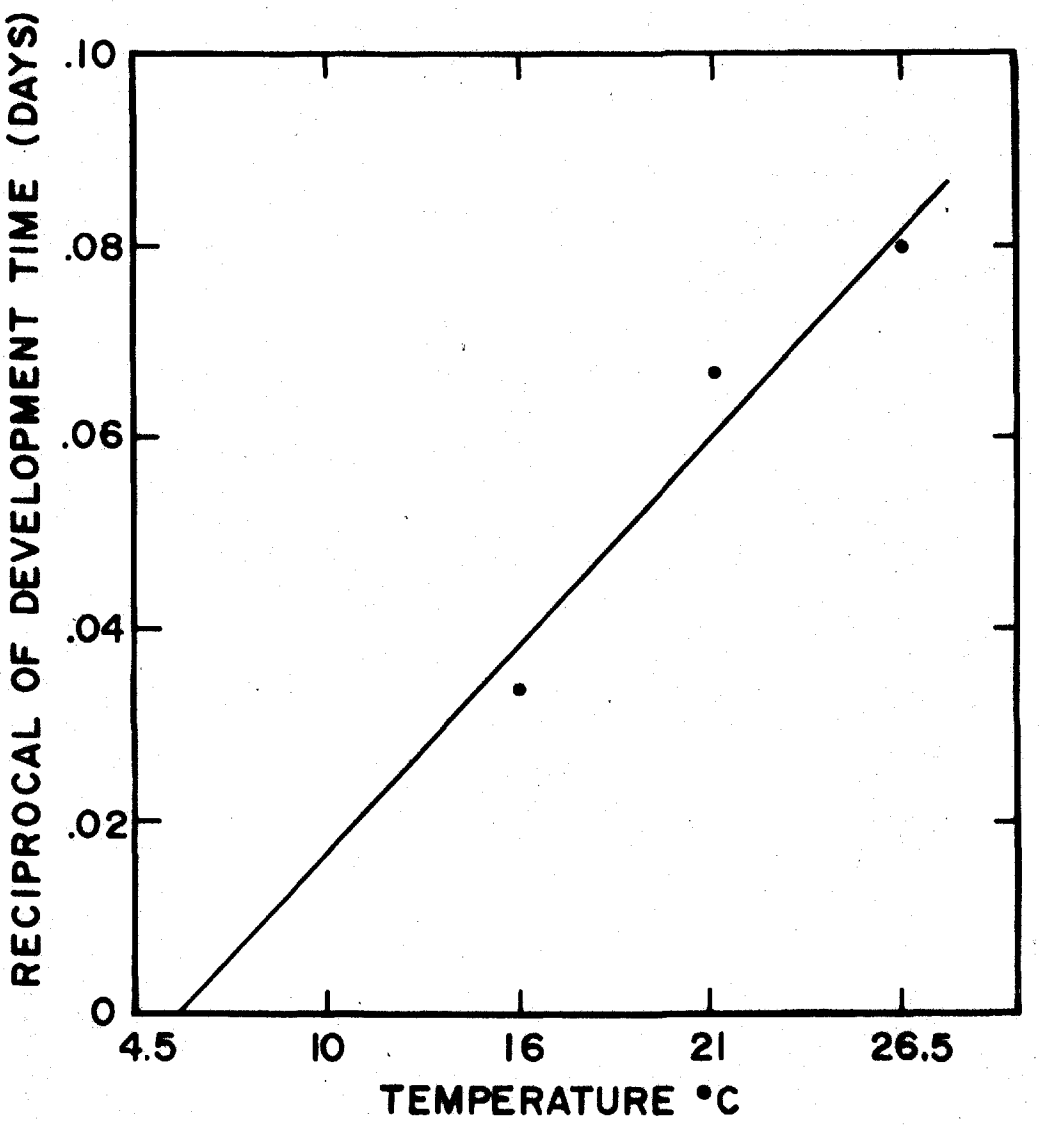

Figure 31. Effect of temperature on pre-diapause development in eggs of L. disjunctus.

Table 7. Development of newly laid eggs of L. disjunctus incubated at constant temperature and photoperiod.

Incubation conditions		Collecting Date	Pre-diapause Development (Days)	% mortality in Pre-diapause Development	Hatching Range (Days after beginning of diapause)	Days to 50% hatch	% Diapause mortality
26.5°C	16½ hr	3.8.70	12	21	53-113	74	37.5
26.5°	8	3.8.70	12	20	67-126	77	58.3
21°	16½	21.7.70	14	44.3	77-105	85	35.3
21°	8	21.7.70	14	29.6	85-120	89	26.3
16°	16½	21.7.70	29	40.6	69-149	97	10.5
16°	8	21.7.70	29	34.6	110-170	145	30.2

excessively moist environment in which the eggs were maintained. The results demonstrate that diapause development is able to proceed at relatively high temperatures.

Experiments were conducted on eggs of L. disjunctus to determine the effect of lower temperatures on Phase I of diapause development. Eggs were collected in the field between July 21 and August 7, 1970, and incubated at 21°C until August 28 to insure that pre-diapause development was complete. Subsequent experiments showed no relationship between collecting date and diapause development, nor was there a difference in diapause development between the eggs which were incubated in the laboratory prior to diapause and those in which pre-diapause development was completed in the field.

On August 28, 260 eggs were put into each of four Petri dishes lined with wet filter paper and incubated at 10, 4.5, -1 and -6.5°C in the dark. Samples of 20 eggs were removed biweekly and subjected to 21°C and a 16½ hour photoperiod for hatching. Hatch data are presented in Figure 32. The maximum rate of diapause development was attained at 10°C. Those eggs at 4.5° required up to 3 to 4 weeks longer to attain the same level of development. Response to -1 and -6.5° temperatures was very much slower.

The time in which 50% of the specimens completed Phase I of diapause development was determined for each temperature. Five days were deducted from the figures shown in Table 7 to account for post-diapause development time to hatching at 26.5, 21 and 16°C.

26.5°C	-	69 days
21	-	80
16	-	92
10	-	35
4.5	-	56

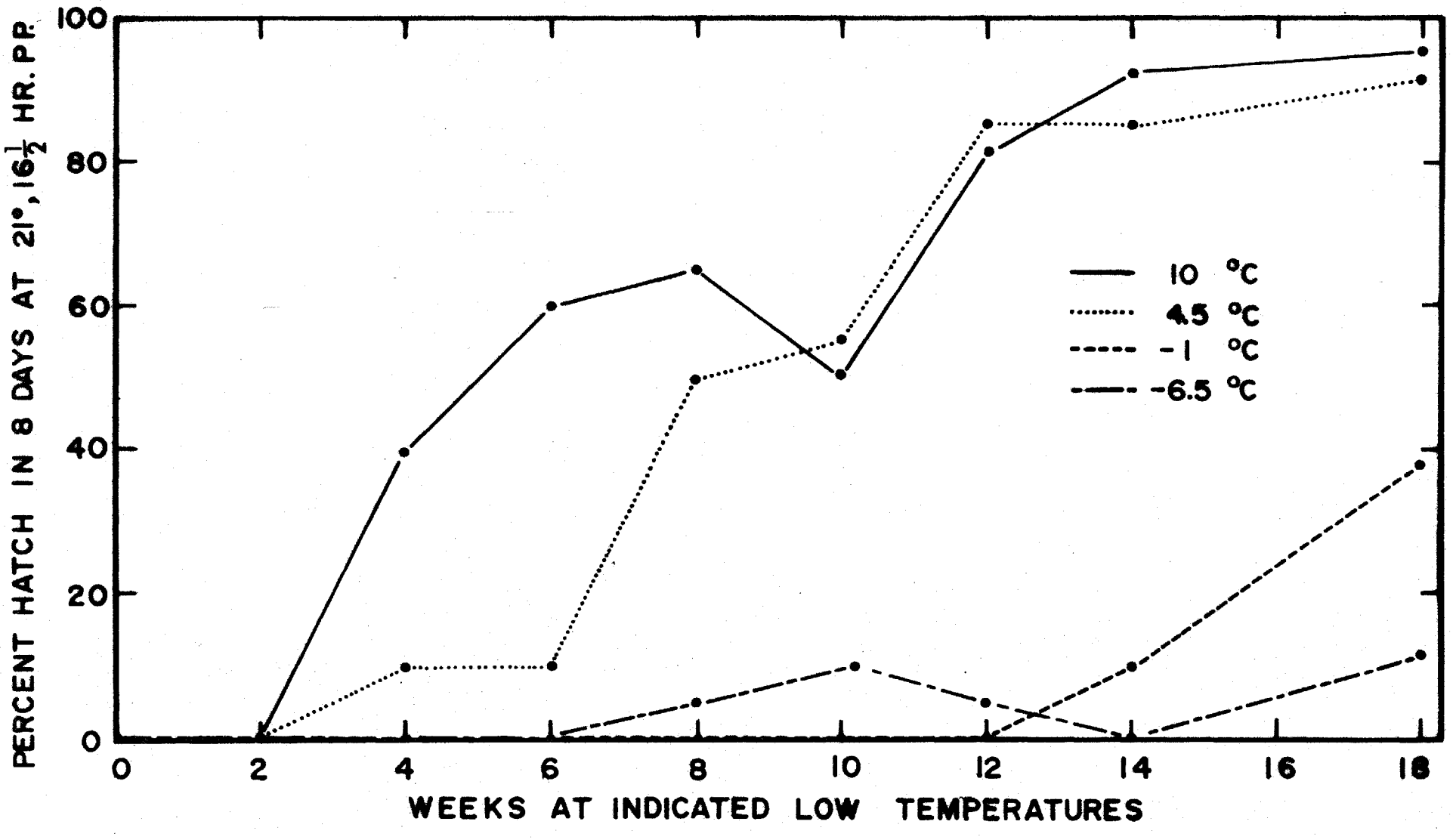

Figure 32. Effect of temperature on Phase I of diapause development in eggs of L. disjunctus incubated in the dark at various temperatures then subjected to test conditions of 21°C and a 16½ hour photoperiod.

-1 °C - 126+ days

-6.5 - 126+

Diapause development occurred most rapidly at 10°C. The results at temperatures above 10°C are inconsistent with the trend established by the low temperatures and remain unexplained.

Experiments were conducted to determine the effect of photoperiod on Phase I of diapause development. Eggs were collected between July 21 and August 27, 1970, and treated in the manner described earlier to insure that pre-diapause development was completed. Two hundred and sixty eggs were subjected to each of three photoperiods, 16½ hour, 8 hour and 0 hours at 4.5°C. Samples were removed and incubated at two-week intervals as described earlier. Results presented in Figure 33 indicate a definite, but not especially large, enhancement of diapause development by the long photoperiod. The slowest diapause development took place under conditions of total darkness.

Twenty-five to thirty eggs were collected in the field at two-week intervals from September 10 to November 19 and subjected to 21°C and a 16½ hour photoperiod to determine the rate of Phase I diapause development under field conditions. Some hatching within the criteria for completed Phase I of diapause development took place in eggs collected on October 8. By October 23 nearly 80% of the eggs had completed this stage of diapause development in the field (Fig. 34).

Following completion of the first phase of diapause development in the fall, hatching remains under the control of photoperiod (Table 8). This photoperiod controlled phase of development has been designated as Phase II of diapause development. Not only does shorter photoperiod retard hatching, it also broadens considerably the range over which hatching occurs. The combined effect of these responses is reflected in a considerable loss in viability of

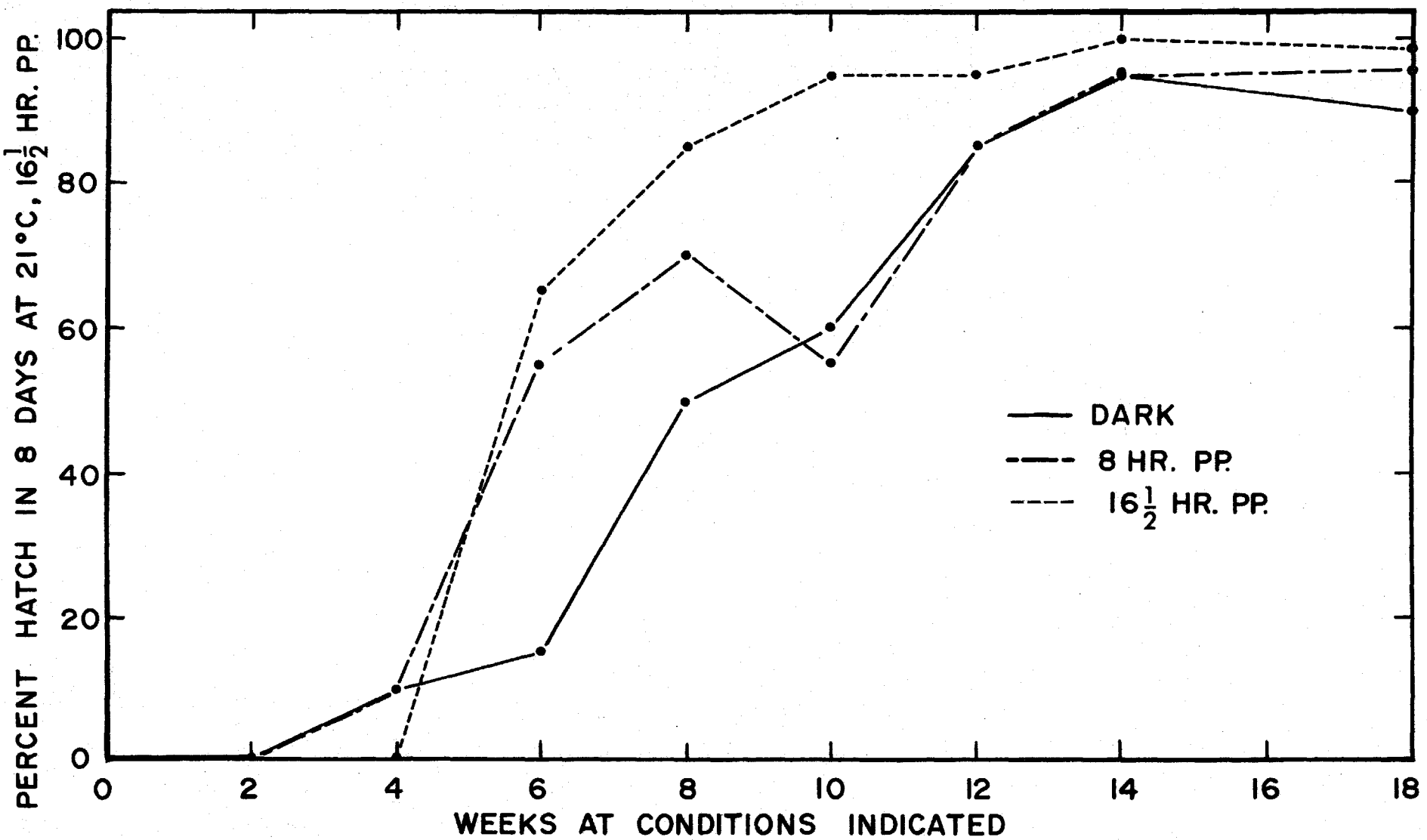

Figure 33. Effect of photoperiod on Phase I of diapause development in eggs of L. disjunctus incubated at 4.5°C then subjected to test conditions of 21°C and a 16½ hour photoperiod.

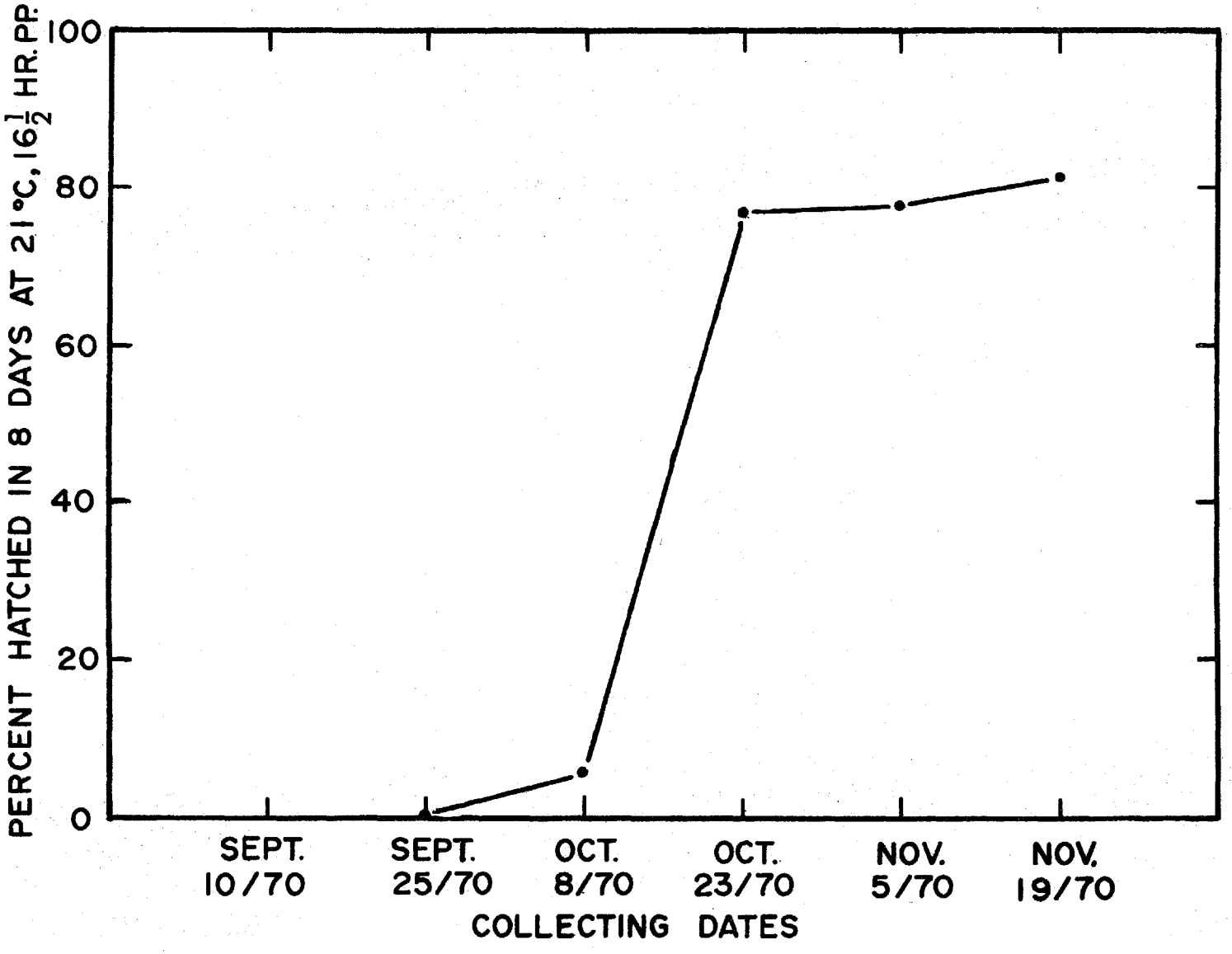

Figure 34. Percent of field collected eggs of L. disjunctus which have completed Phase I of diapause development.

Table 8. Effect of photoperiod on hatching in L. disjunctus, showing percentage of total hatch occurring within the criteria for completed diapause.

Collecting Date	Incubation conditions				
	21°C 16½ hr	21°C 14 hr	21°C 12 hr	21°C 10 hr	21°C 8 hr
5.11.70	84	-	-	-	0
19.11.70	81	-	-	-	0
21.1.71	100	11	22	36	77
9.4.71	100	-	56	74	54
30.4.71	100	100	100	-	-

Table 9. Effect of photoperiod on hatching in eggs of L. disjunctus collected in the field November 19, 1970.

Incubation conditions	Hatching Range (Days)	Days to 50% Hatch	% mortality
21°C 16½ hr	6-16	8	7
21° 8	49-80	66	49
16° 16½	8-19	11	4
16° 8	67-118	95	28

eggs incubated at short photoperiods (Table 9). The critical photoperiod for hatching at this time appears to be somewhere between 14 and 16½ hours. The inhibitory effect of shorter photoperiods is gradually lost during the winter, disappearing completely sometime between April 9 and April 30 when photoperiod under field conditions changes from 14.4 to 15.8 hours.

(c) Effect of temperature on post-diapause development

Eggs of L. disjunctus and L. unguiculatus were collected on April 6, 1970, and incubated at 4.5, 10, 16, 21 and 26.5°C at a 16½ hour photoperiod in order to determine the effect of temperature on post-diapause development and hatching. Results shown in Figure 35 illustrate the incubation period required to achieve 50% hatch. Temperatures between 16°C and 26.5°C seem about equally suitable for hatching. The development rate decreased drastically in eggs incubated at 4.5°C. The reciprocals of incubation periods necessary to obtain 50% hatching were plotted in Figure 36. These results predict the critical threshold temperature for post-diapause development at approximately 0°C. Although hatching can occur at temperatures near 4.5°C it does not occur normally. The lower the incubation temperature the greater is the range of hatch from a given egg sample. For example, hatching range of eggs incubated at 21°C is approximately 5 days. At 10°C it is 13 days and at 4.5°C it is 21 days.

Under field conditions hatching in L. disjunctus began approximately May 5 in 1971, soon after mean air temperature exceeded 10°C (Fig. 37). Figures for water temperature for this period are unavailable. However, mean air temperature differs only slightly from water temperature at this time of year.

(d) Role of moisture in post-diapause development

Pre-diapause and part of the diapause phase of embryonic development occur in the green tissues of Scirpus stems. The stems freeze and become

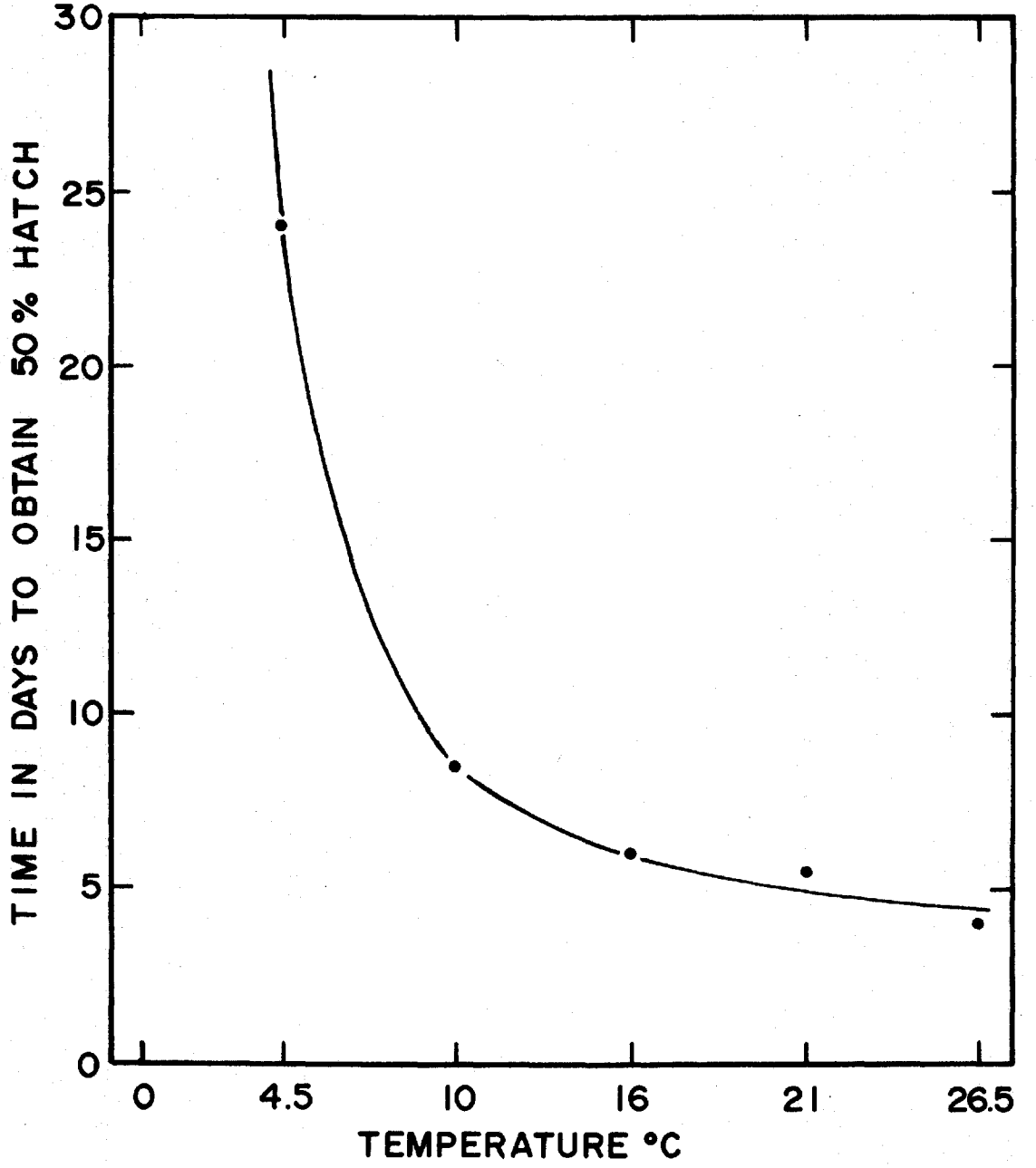

Figure 35. Effect of temperature on hatching in L. disjunctus and L. unguiculatus at a 16½ hour photoperiod following completion of diapause development.

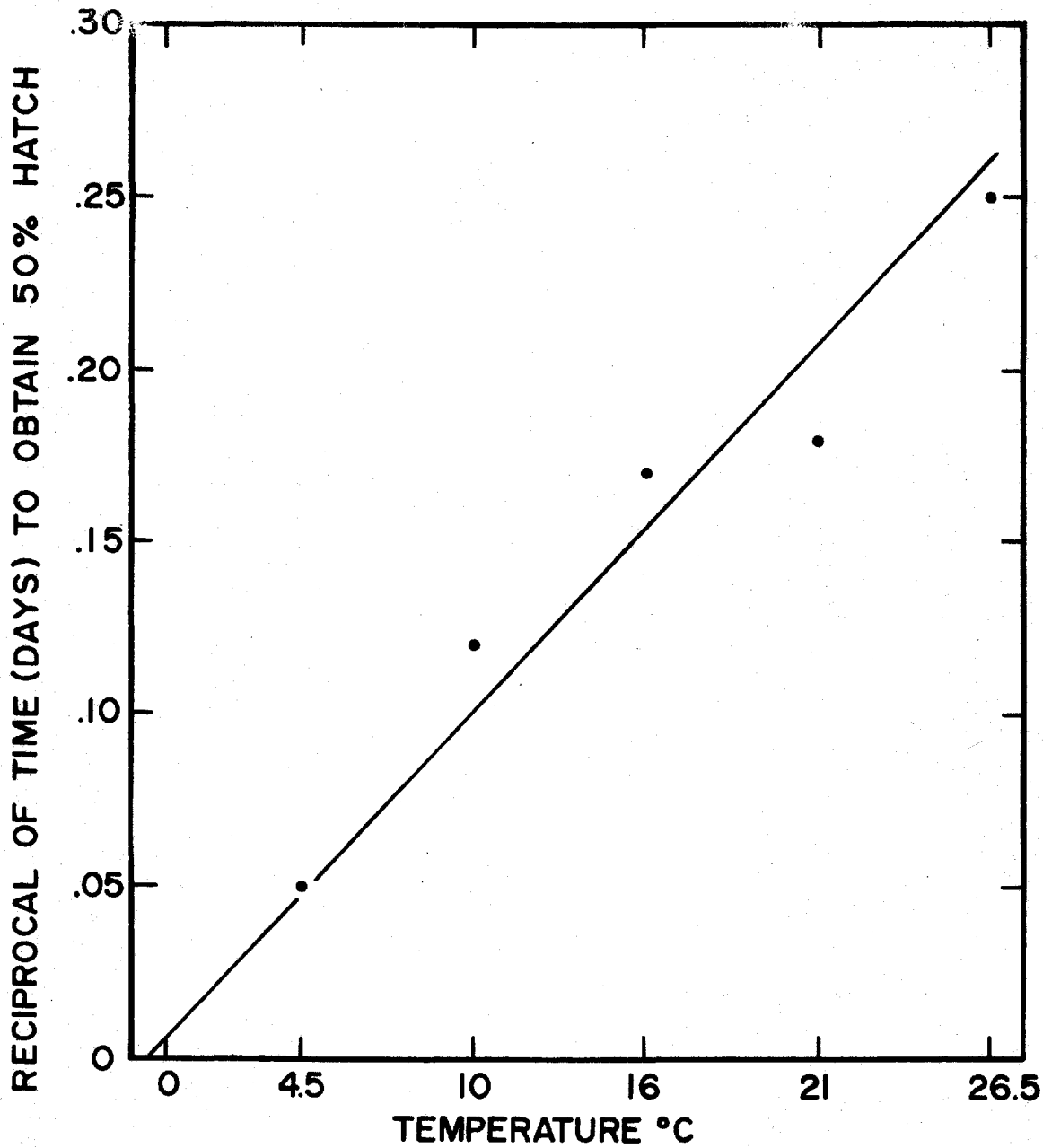

Figure 36. Effect of temperature on hatching in L. disjunctus and L. unguiculatus at a 16½ hour photoperiod following completion of diapause development.

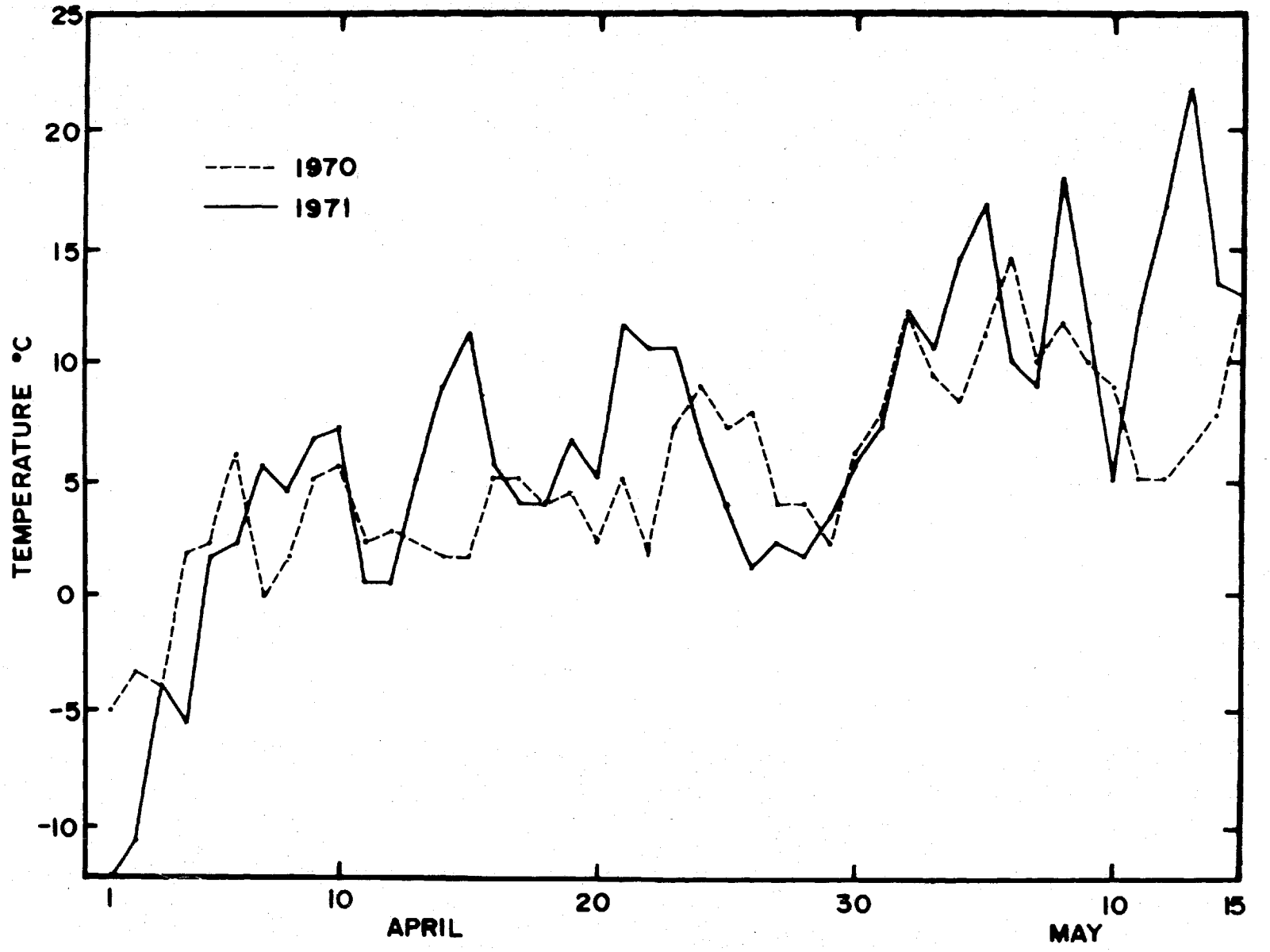

Figure 37. Mean daily air temperatures at Saskatoon, provided by the Saskatchewan Research Council.

partially desiccated during the winter months. Post-diapause development will not take place under these conditions. A necessary requirement for the completion of embryonic development is for the eggs to be wet. Experiments conducted in the laboratory showed that an atmosphere of 100% relative humidity was inadequate, that eggs required direct contact with water in order that post-diapause development be initiated. This requisite may be of considerable importance in the synchronization of hatching in the field since all eggs become wet at approximately the same time when the slough fills with water in the spring.

(e) Determination of freezing temperatures lethal to eggs of L. disjunctus

Field observations described earlier indicated that overwintering temperatures are lethal to eggs of L. disjunctus in the absence of adequate protective snow cover. Laboratory experiments were conducted according to procedures described earlier to determine the temperatures lethal to eggs of this species. Eggs were collected during mid March in 1971 and subjected to several temperatures between -3 and -25°C for periods of no less than 24 hours. Controls were run at near 0°C. The eggs were then incubated at 21°C and a 16½ hour photoperiod. Normal hatching was obtained at all temperatures above -20 to -22°C. At the latter temperatures 50 to 75% hatch was obtained as compared to the controls in three replicates of the experiment. The specimens which did hatch failed to moult out of the prolarval form and died soon after. Many of the eggs which failed to hatch had ruptured.

3.2.3 Lestes unguiculatus - Field observations

The life cycle of Lestes unguiculatus is essentially the same as that described for L. disjunctus. Only the main features expressing the similarities and differences between the two species will therefore be presented.

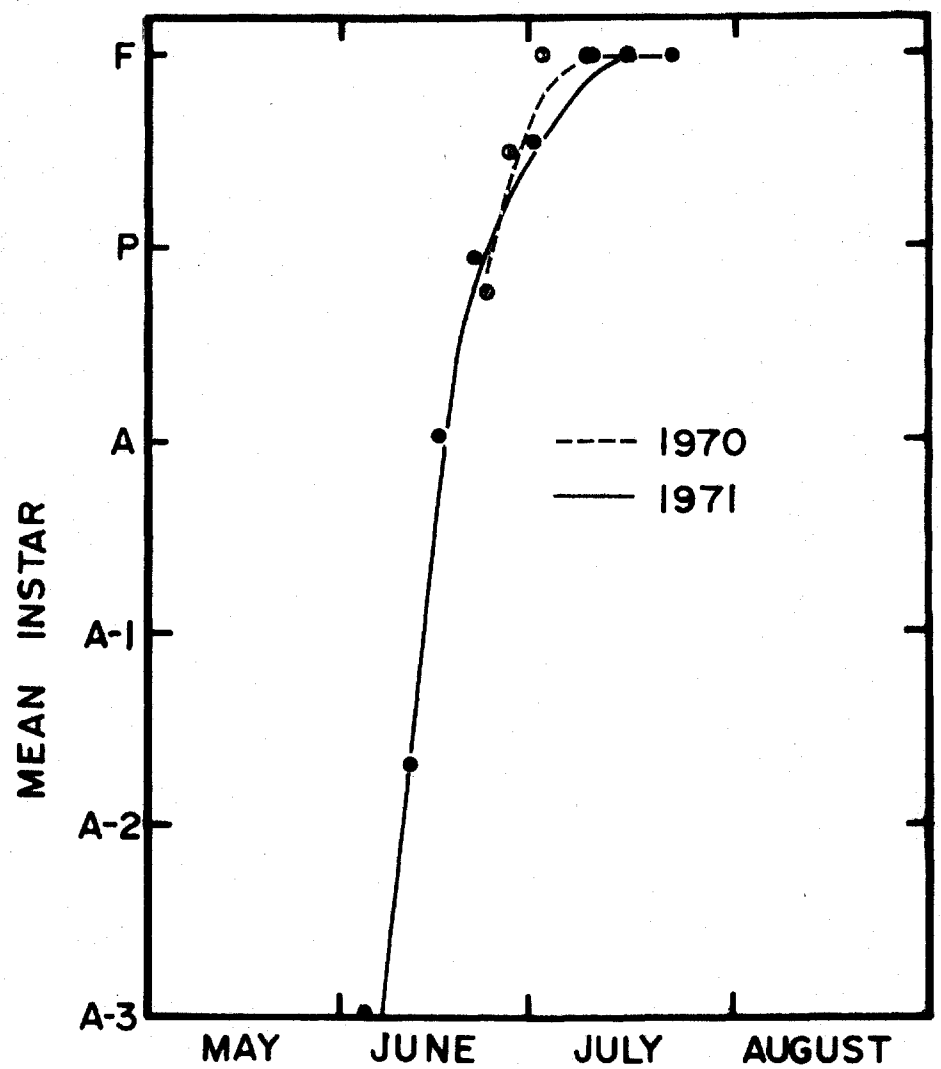

Figure 38. Development rate of L. unguiculatus nymphs under field conditions. Curves were fitted by eye using a three-point moving average. (See also Appendix F.)
F = Final, P = Penultimate, A = Antepenultimate, A-1 = Antepenultimate-1, A-2 = Antepenultimate-2.

The appearance of the various nymphal and adult stages of L. unguiculatus corresponds very closely with that of L. disjunctus (Fig. 8). Both species are present in about the same numbers.

(a) Hatching and nymphal development

Hatching of eggs in the field occurred approximately May 5 in 1971. Estimates established from collections of stems made on May 6 showed hatching to be approximately two-thirds completed. Of the eggs collected on this date, almost all hatched overnight at room temperature. Eggs of L. unguiculatus could no longer be found in the field one week later.

Nymphs were obtained in the field for the first time on June 3, 1971. At this time they had completed approximately one-half of their nymphal moults. Development was well synchronized; no more than three instars were collected at any one time. Subsequent development was very rapid. A growth curve established from the mean instars of nymphs obtained during weekly random sampling is presented in Figure 38. Specimens were obtained in the final instar on June 22, 1970 and June 24 in 1971. Emergence began in the latter part of the week preceding July 9 in 1971. A single adult was collected in Saskatoon on July 3. The peak of emergence was passed shortly after July 15. Nymphs were collected for the last time on July 22 in 1970 and 1971.

(b) The adult stage

Adults of L. unguiculatus are difficult to distinguish from L. disjunctus. The males can be separated on the basis of the shape and size of the abdominal claspers (Walker, 1953). In the field females were identified by their association with males in tandem. Ovipositing adults could be identified by the oviposition scars that the females left in the plant stems (see Fig. 5).

Nothing can be added to information already presented on emergence, maturation and mating of L. disjunctus which would distinguish the two species.

Oviposition occurs in tandem in green stems of Scirpus up to 60 cm above the water level. As in L. disjunctus, the stems selected as oviposition sites were never in the centre of large clumps of bullrushes but rather, around the outside margin, or in stems occurring in small groups.

Eggs deposited by L. unguiculatus could be distinguished from those of L. disjunctus by the pattern of incisions in the stems. Females of L. unguiculatus generally deposit only a single egg per incision, occasionally two. The incisions are small, spaced close together in a vertical column in contrast to those of L. disjunctus (Fig. 5).

(c) The egg stage

The eggs of L. unguiculatus are elongate and pointed at the anterior end. The eggs measure 1.46 mm in length. They can be tentatively distinguished from those of L. disjunctus by their more tapered anterior end and by the darker brown colouration which the pointed tip acquires (Fig. 7b).

The eggs begin embryonic development soon after they are laid. As in L. disjunctus, the absence of further morphological change suggests that they enter developmental arrest after the appearance of dark eye spots and tracheal tubules just prior to hatching (Fig. 7b). Pre-diapause embryonic development is essentially completed in all eggs during the latter part of August.

The eggs are susceptible to desiccation and are therefore highly dependent on the green plant tissue in which they are embedded for their survival. Frost is also damaging to eggs contained in unprotected stems.

3.2.4 Lestes unguiculatus - Laboratory experiments

Experiments under controlled temperatures and photoperiods were conducted on eggs of L. unguiculatus to correspond to those of L. disjunctus on various aspects of embryonic development.

(a) Effect of temperature and photoperiod on pre-diapause development

Newly laid eggs were incubated at 16, 21 and 26.5°C to determine the effect of temperature on early embryonic development. The results presented in Figure 39 are not much different from those obtained for L. disjunctus, though L. unguiculatus developed a little more slowly at the lower temperatures. The reciprocals of the values (Fig. 40) permit an estimate of the temperature threshold below which pre-diapause embryonic development will not occur. The value obtained in this manner was 9°C, somewhat higher than in L. disjunctus.

The preceding experiments were conducted simultaneously at two photoperiods, 8 hours and 16½ hours. Development rate was identical at both, indicating that early embryonic development in this species proceeds independently of photoperiod.

(b) Effect of temperature and photoperiod on diapause development

Diapause development was shown to follow the same pattern as in L. disjunctus. The criteria used to detect completion of Phase I of diapause in eggs of L. disjunctus also apply for this species. If hatching occurred in 8 days or less at 21°C and a 16½ hour photoperiod, the first phase of diapause was considered to have been terminated.

Newly laid eggs were collected in the field on August 3, 1970. They were subjected to 21 and 26.5°C at 16½ and 8 hour photoperiods until hatching was completed. Data are presented in Table 10. No eggs hatched in less than 20 days after the beginning of diapause development even under optimal hatching

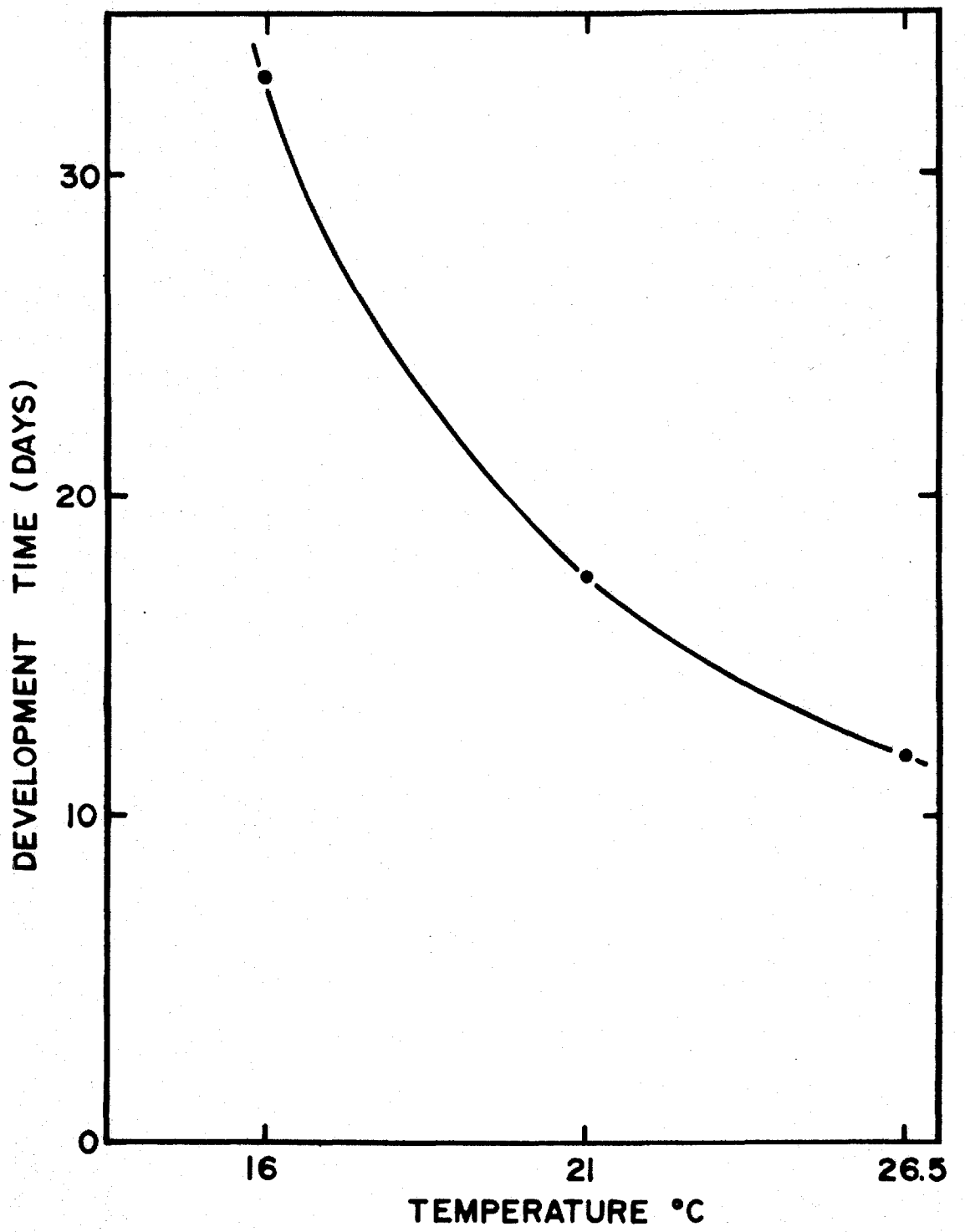

Figure 39. Effect of temperature on pre-diapause development in eggs of L. unguiculatus, shown as time required for all eggs in the sample to complete pre-diapause development.

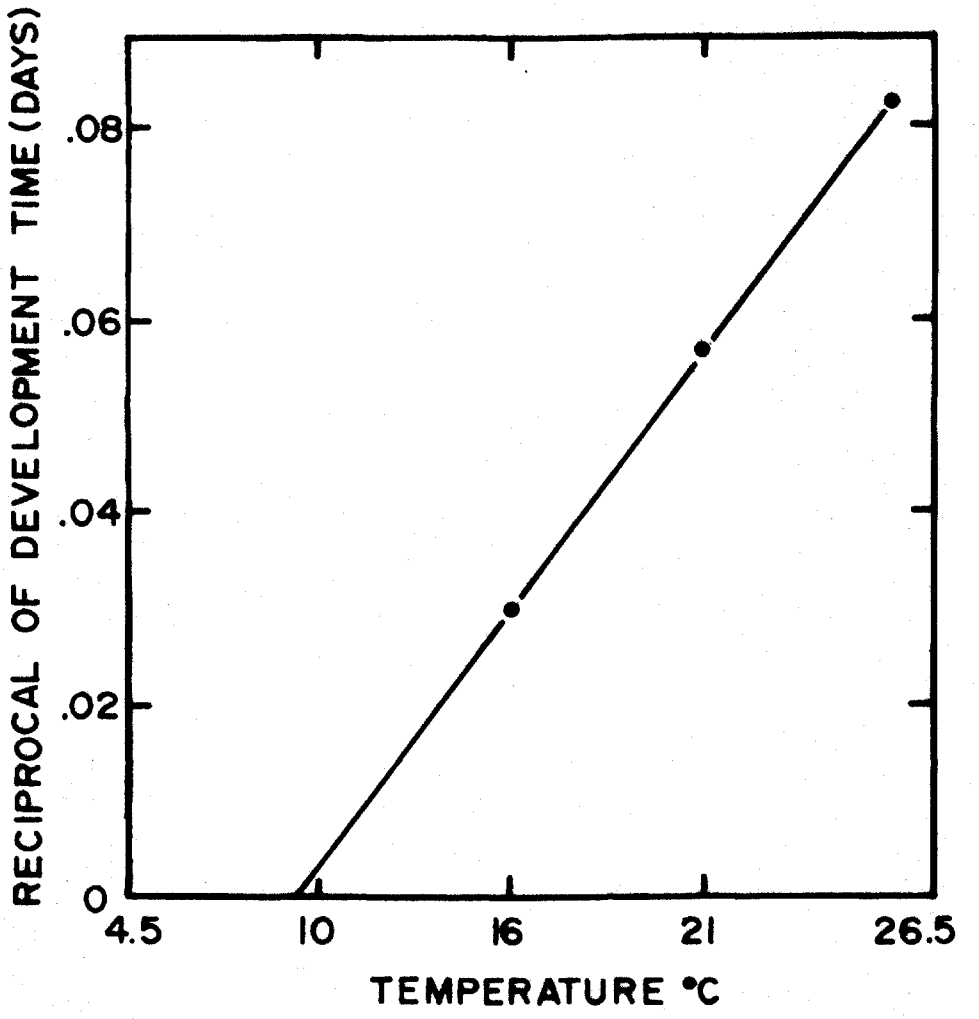

Figure 40. Effect of temperature on pre-diapause development in eggs of L. unguiculatus.

Table 10. Development of eggs of L. unguiculatus collected when newly laid on August 3, 1970 and incubated at constant temperature and photoperiod.

Incubation conditions	Pre-diapause Development (Days)	% mortality in Pre-diapause stage	Hatching Range (Days after beginning of diapause)	Days to 50% hatch	% mortality in Diapause phase
26.5°C 16½ hr	12	5.7	27-152	73	25.8
26.5° 8	12	22.0	67-175	75	63.4
21° 16½	17	8.8	20-111	64	9.7
21° 8	17	7.8	99-164	131	55.4

conditions. The hatching range extended over two to four months.

The results demonstrate that diapause development takes place at both 21 and 26.5°C. The lower hatch and the wide hatching range at 26.5°C suggest that this temperature is approaching the upper limit at which normal diapause development can take place.

Experiments were conducted to determine the effect of lower temperature on diapause development rate. Eggs were collected between July 21 and August 7, 1970, and incubated at 16 and 21°C until August 28 to insure that pre-diapause development was complete. On August 28, 260 eggs were put into each of four Petri dishes lined with wet filter paper and incubated at 10, 4.5, -1, and -6.5°C in the dark. Samples of 20 eggs were removed bi-weekly and tested to determine whether or not Phase I of diapause development was completed according to the criteria described earlier. Results shown in Figure 41 indicate rapid diapause development at 10°C. Sixty to 80% development is complete in 6 weeks. Diapause development at 4.5°C is somewhat slower. Unlike L. disjunctus, substantial diapause development takes place in eggs at -1 and -6.5°C. This suggests that eggs laid late in the season would likely complete the first phase of diapause development earlier than those of L. disjunctus laid at the same time.

The time required for 50% of the eggs to complete Phase I of diapause development was calculated from the data presented. Five days were subtracted from the time required to obtain 50% hatch at 21° and 26.5°C to account for post-diapause development time. No data are available for 16°C.

26.5°C	-	68 days
21.0	-	59
10.0	-	30
4.5	-	70
-1.0	-	80
-6.5	-	126+

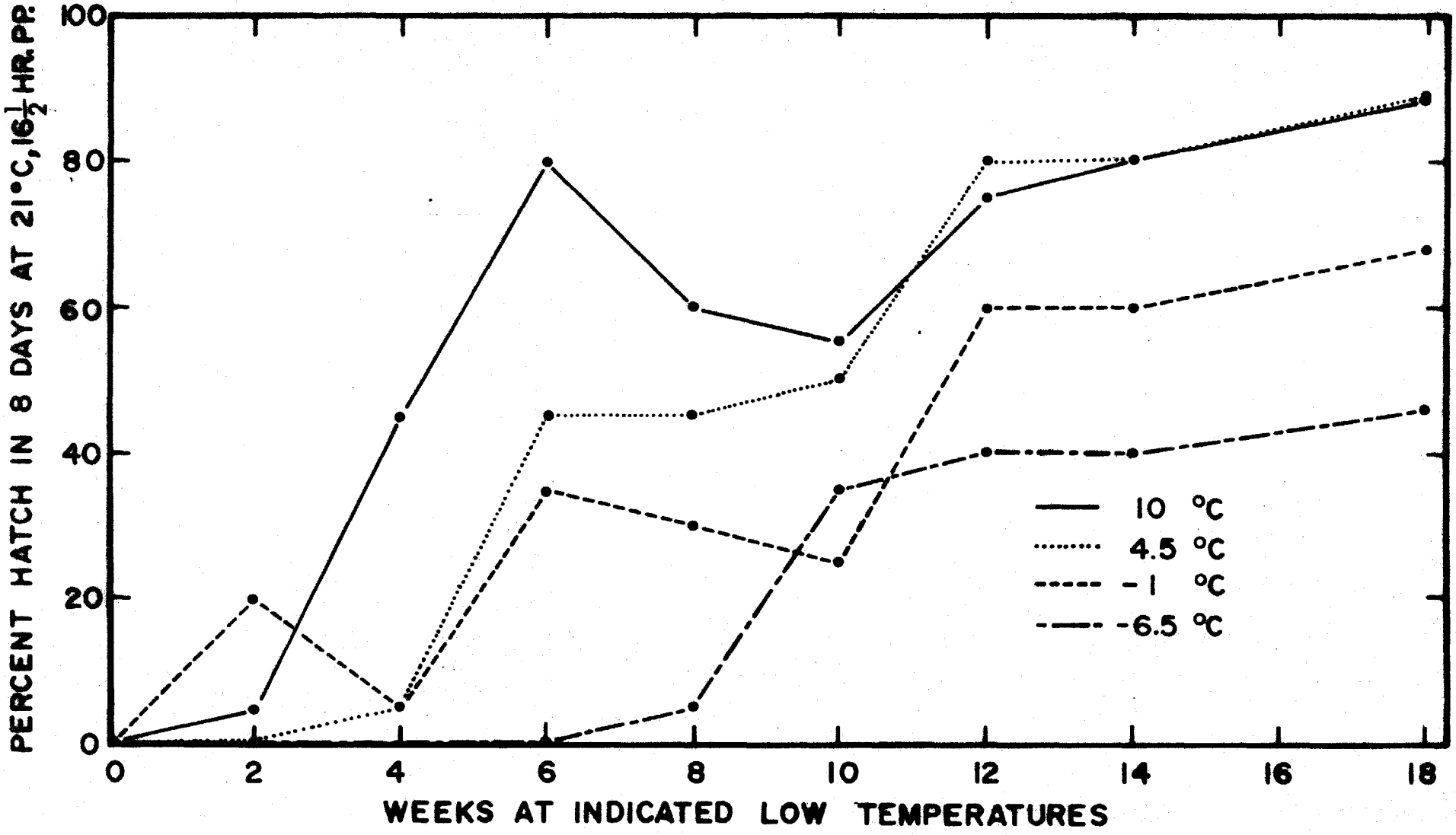

Figure 41. Effect of temperature on Phase I of diapause development in eggs of L. unguiculatus incubated in the dark at various temperatures then subjected to test conditions of 21°C and a 16½ hour photoperiod.

Optimum diapause development temperature is 10°C with increasing time required at warmer and colder temperatures.

Experiments were carried out to determine the effect of photoperiod on Phase I of diapause development in L. unguiculatus. Eggs were collected on four occasions between July 28 and August 27, 1970, and treated in the manner described earlier to insure that pre-diapause development was completed. On August 28, 260 eggs were placed under each of the following photoperiods at 4.5°C: 16½ hours, 8 hours and 0 hours. Samples of 20 eggs were removed at two-week intervals and incubated at 21°C and a 16½ hour photoperiod. Results presented in Figure 42 indicate a positive response of diapause development to increasing photoperiod. The response is, however, not as distinct as in L. disjunctus.

Twenty-five to thirty eggs were collected in the field every two weeks between September 10 and November 19, 1970, and incubated at 21°C and a 16½ hour photoperiod. Figure 43 shows that, while completion of the first phase of diapause development had already occurred in a few eggs on September 25, the majority of eggs terminated this phase of development between October 8 and October 23. Only a few eggs collected in November failed to hatch.

Evidence from the data presented in Table 10 points to a definite involvement of photoperiod in controlling egg hatching in L. unguiculatus. Hatching began much later at the short photoperiod at both temperatures. A considerably higher egg mortality was also experienced at the short photoperiod in both cases. Egg samples were collected during the late fall and winter, after Phase I of diapause development was essentially complete, in order to test the effect of photoperiod on hatching. Results obtained from these experiments indicate the presence of a distinct photoperiodically controlled Phase II of diapause (Tables 11 and 12) with a development threshold

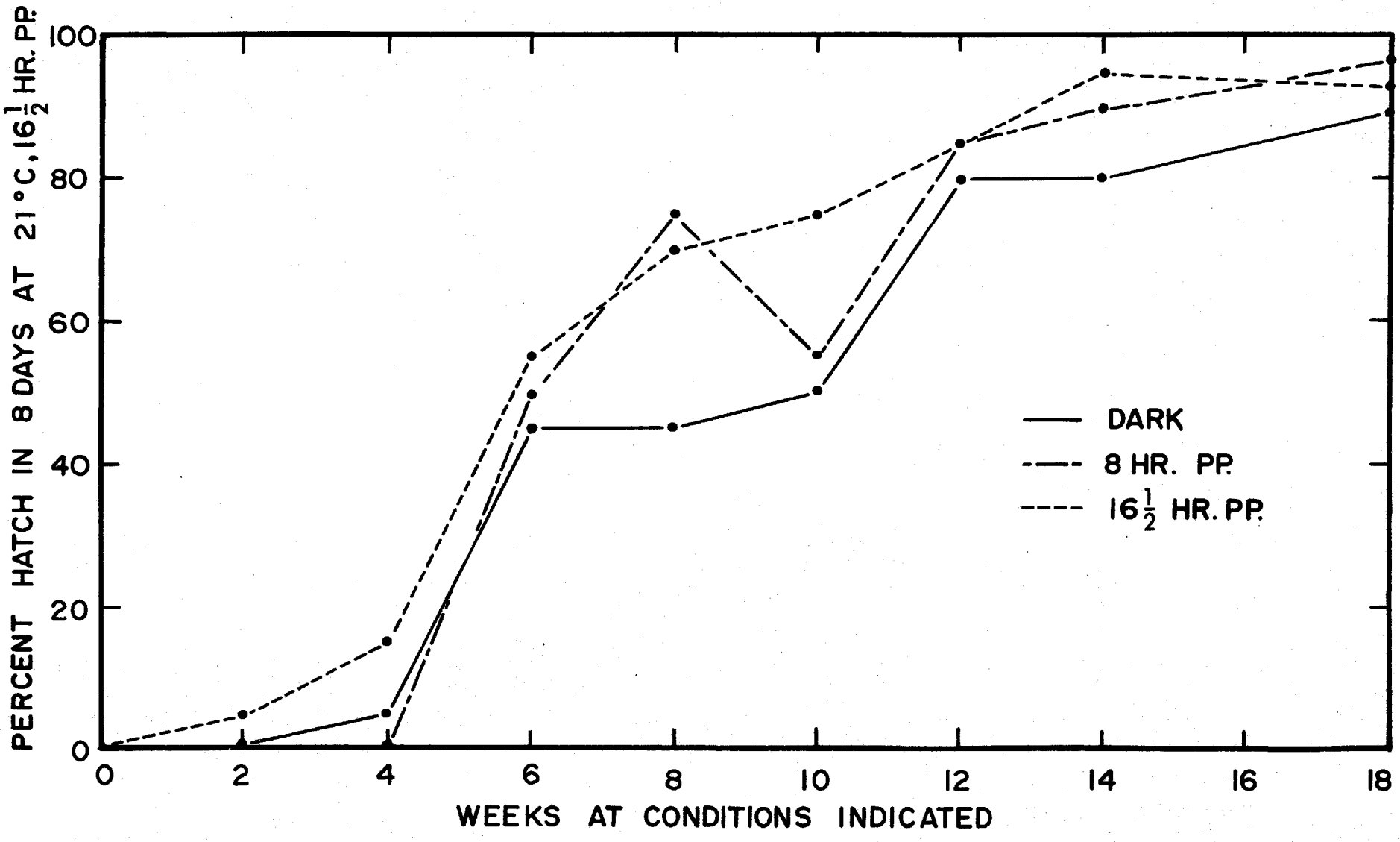

Figure 42. Effect of photoperiod on Phase I of diapause development in eggs of L. unguiculatus incubated at 4.5°C then subjected to test conditions of 21°C and a 16½ hour photoperiod.

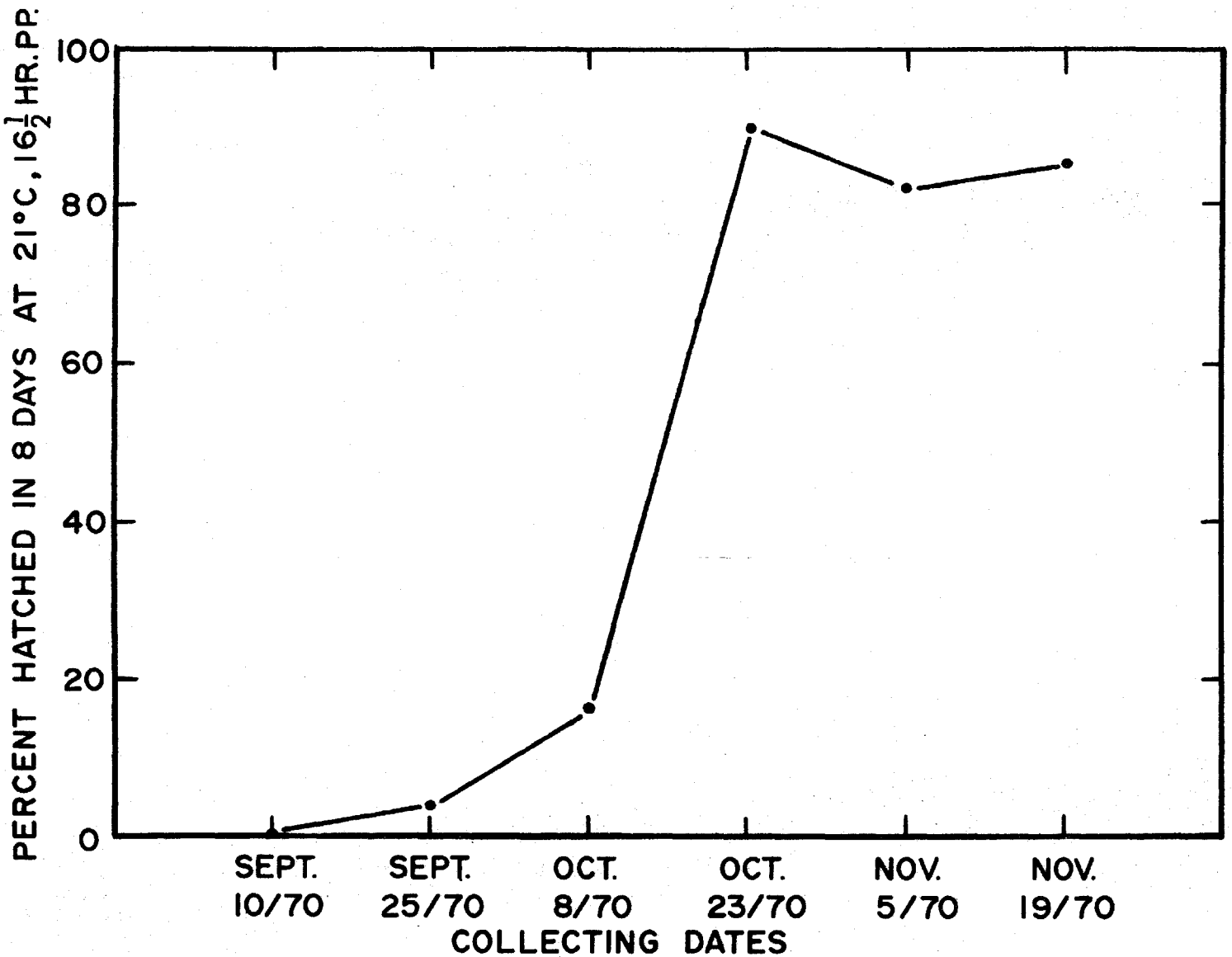

Figure 43. Percent of field collected eggs of L. unguiculatus which have completed Phase I of diapause development.

Table 11. Effect of photoperiod on hatching in L. unguiculatus, showing percentage of total hatch occurring within the criteria for completed diapause.

Collecting Date	Incubation conditions				
	21°C 16½ hr	21°C 14 hr	21° 12 hr	21°C 10 hr	21°C 8 hr
19.1.70	85	-	-	-	0
2.12.70	92	-	-	-	0
21.1.71	100	100	85	50	61
1.4.71	100	-	43	63	43
1.5.71	100	100	100	100	100

Table 12. Effect of photoperiod on hatching in eggs of L. unguiculatus collected in the field November 19, 1970.

Incubation conditions	Hatching Range (Days)	Days to 50% Hatch	% mortality
21°C 16½ hr	6-43	8	4
21° 8	26-88	49	41
16° 16½	8-14	11	11
16° 8	46-115	75	19

at approximately a 14 hour photoperiod. Photoperiodic inhibition at this temperature gradually disappears during the winter months (Table 11).

(c) Effect of temperature on post-diapause development

The effect of temperature on post-diapause development in eggs of L. unguiculatus is shown in the combined results in Figures 35 and 36. The interpretation of the results for L. disjunctus eggs applies equally well for eggs of this species. In essence, temperatures over 10°C are interpreted as suitable for hatching. Although hatching occurred at temperatures as low as 4.5°C under laboratory conditions, the range of the hatching period increased tremendously. Figure 36 indicates that hatching in L. unguiculatus should be possible at any temperature higher than 0°C. However, hatching in the field did not take place until about May 5 in 1971 when mean daily air temperature had exceeded 10°C (Fig. 37).

(d) Role of moisture in post-diapause development

Eggs of L. unguiculatus will not hatch unless they are immersed in or, at least, in contact with water. Eggs placed in an atmosphere of 100% relative humidity failed to hatch.

(e) Determination of freezing temperatures lethal to eggs of L. unguiculatus

As in L. disjunctus, eggs of L. unguiculatus are intolerant of the severe prairie winter frosts in the absence of protective snow cover. Laboratory experiments were carried out on frost tolerance in eggs of this species. Eggs were collected in March, 1971, and subjected to several temperatures between -3 and -25°C according to the procedures described for L. disjunctus. The results obtained were almost identical to those for the latter species. Only about 50% of the eggs held at -20 to -22°C for 24 hours remained viable. Eggs frozen at temperatures higher than this hatched normally. Lower temperatures were lethal to all eggs.

3.2.5 *Lestes dryas* - Field observations

Data on this species are scanty. Certain critical observations, however, justify comparison of its life cycle with those of *L. disjunctus* and *L. unguiculatus*.

Only four nymphs of *L. dryas* were collected in the weekly samples during the study. No inferences could be drawn from these specimens other than that their development at low temperatures may be more rapid than that of nymphs of *L. disjunctus* and *L. unguiculatus*. Alternatively, perhaps eggs of *L. dryas* hatch earlier in the spring.

Newly emerged adults were first seen at the study pond on July 2, 1970 and on June 24 in 1971. Sighting was approximately one week earlier than of the other two species in both years.

Mating and oviposition also began earlier than in the other two species. Eggs were deposited singly in well separated incisions. Oviposition sites were identical to those described for *L. disjunctus* and *L. unguiculatus*.

The eggs of *L. dryas* are large, approximately 1.77 mm in length (Fig. 7c). They are easily distinguished from those of other species by their size. Embryonic development begins soon after oviposition and proceeds to the stage shown in Figure 7c before developmental arrest sets in. All eggs of this species found on September 4 were in diapause. On the basis of laboratory experiments it is believed that pre-diapause development was complete as early as mid-August. Eggs were rarely encountered in the field. Therefore, the progress of diapause development under natural conditions could not be followed.

3.2.6 *Lestes dryas* - Laboratory experiments

Freshly laid eggs of *L. dryas* were collected on July 21 and July 23,

1970. These were subjected to 16 and 21°C at 16½ and 8 hour photoperiods. Pre-diapause development was completed in 16 days at 21°C and in 29 days at 16°C. There was no photoperiod effect on this stage of development. Development rate compares with L. disjunctus and L. unguiculatus.

The first newly hatched nymphs at 21°C and an 8 hour photoperiod appeared on October 1. No hatch occurred under either of the 16°C incubation conditions until December 14, indicating that a strong diapause governs development in this species. Hatching began on this date at both photoperiods, making it impossible to form any positive conclusions about a photoperiod sensitive diapause phase as in the two Lestes species previously described.

One hundred and fifty eggs of L. dryas were collected in the field between July 21 and July 30, 1970. These were incubated at 16 and 21°C until September 3. They were then subjected to -1°C and continuous darkness in order to determine the effect of low temperature on diapause development. Results are shown in Table 13.

Table 13

Percentage of eggs of L. dryas hatching in 8 days at 21°C and a 16½ hour photoperiod in successive samples from stock maintained in the dark at -1°C.

	Incubation Period (Weeks)							
	2	4	6	8	10	12	14	18
% Hatch	0.0	0.0	13.4	6.7	13.4	33.3	40.0	28.3

Results obtained indicate a diapause development rate at this temperature approximately intermediate between those obtained for eggs of L. disjunctus and L. unguiculatus.

3.3 Type C Species

Only one species of Zygoptera found in the study area has the characteristics ascribed to Type C species. Lestes congener Hagen is the last species to hatch in the spring, the last to complete its nymphal development and emerge, and the last to disappear in the fall. It portrays several aspects in its life cycle which are unusual and of considerable interest.

3.3.1 Lestes congener - Field observations

(a) Hatching and nymphal development

Intensive collecting of stems containing eggs showed that hatching in L. congener began on May 30, 1970. It began on May 24 in 1971 and extended to approximately the end of the month. Nymphal development is very rapid under field conditions. Nymphs of this species first appeared in field collections on June 18, 1971. Final instars were present on July 9. Mean instar was calculated for the nymphs at each collecting date. The results plotted in Figure 44 represent the nymphal development rate under field conditions.

Although results shown in Figure 8 display considerable overlap between the nymphs of this species and Type B species, sampling in shallow water and water 60 cm or more in depth showed that the younger L. congener nymphs were predominant in the deeper water while the Type B nymphs which were ready to emerge occupied the very shallow zone. Lestes congener nymphs moved into the shallow water as the niche became vacated by L. disjunctus and L. unguiculatus.

Emergence in L. congener began on July 21, 13 days after the collection of the first final instar nymphs, and continued for approximately three weeks. At the time emergence began, 95 to 100 percent of the nymphs were in the final instar. On only one occasion was the nymphal population represented by more

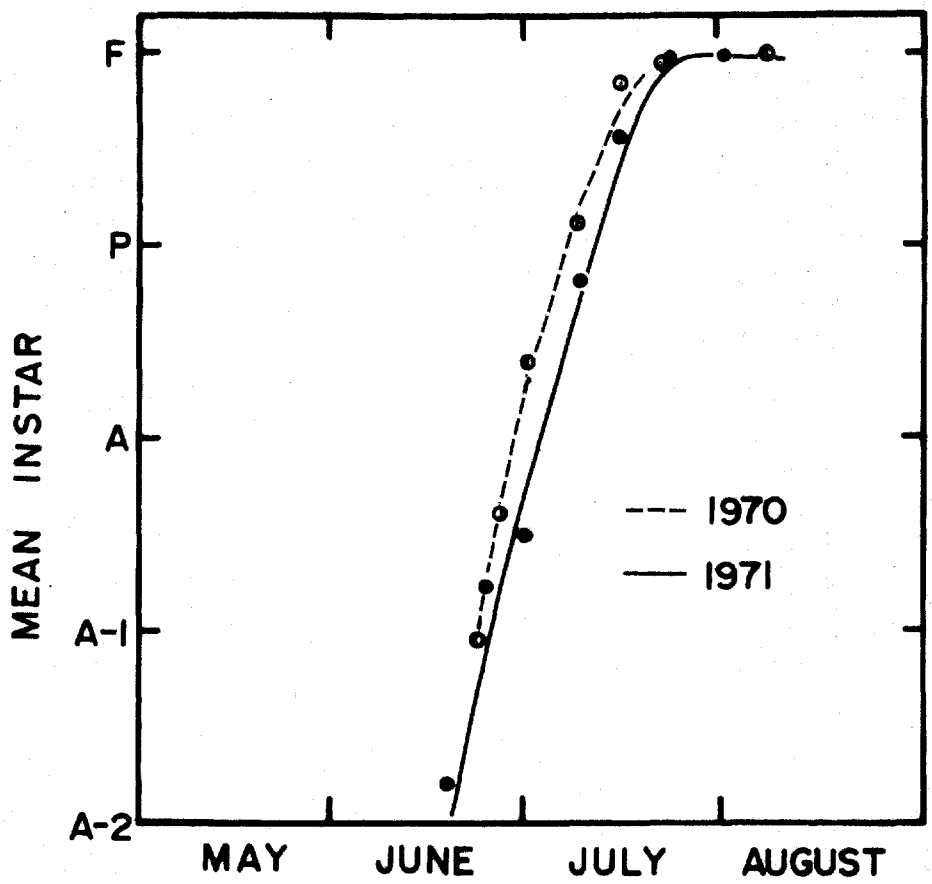

Figure 44. Development rate of L. congener nymphs under field conditions. Curves were fitted by eye using a three-point moving average. (See also Appendix G.)
F = Final, P = Penultimate, A = Antepenultimate, A-1 = Antepenultimate-1, A-2 = Antepenultimate-2.

than four instars. The synchronous nymphal development was reflected in mass emergence of adults during the first ten days of the emergence period. Synchrony may be somewhat disrupted by weather conditions unfavorable for emergence. Nymphs are temporarily capable of postponing emergence to await suitable conditions.

Emergence takes place from very shallow water, 15 cm or less in depth. Nymphs select emergence sites up to a height of 45 cm above the water surface. Any form of emergent vegetation appears suitable. Active emergence was observed generally between 10:00 A.M. and 2:00 P.M.

(b) The adult stage

Maturation in this species takes approximately three weeks. First records of L. congener adults mating were noted on August 11 in 1970, but oviposition was delayed to August 20 by a week of cold weather. Oviposition was observed on August 12 in 1971.

Copulation immediately preceded oviposition. The latter always occurred while the insects were in tandem and was observed to take place only in dry stems of Scirpus. The stems most frequently selected were bent or broken, lying in an angular position over the water. Preference was given to stems located towards the centre of stands of rushes. Eggs were laid from 5 cm to about 30 cm above the water surface. An apparent shortage of stems suitable for oviposition often resulted in deposition of very large numbers of eggs in the available stems (Fig. 45). Up to six pairs of adults were observed ovipositing simultaneously in a 30 cm long piece of stem. The eggs are laid singly in incisions spaced on the average of 1.9 mm apart in vertical rows. They are deposited in the dry pith just beneath the epidermal and vascular layers (Fig. 46). The incisions are left open or partially covered, there being no attempt to seal them as in Type B species.

Figure 45. Incisions in dry stem of Scirpus made by ovipositing L. congener females.

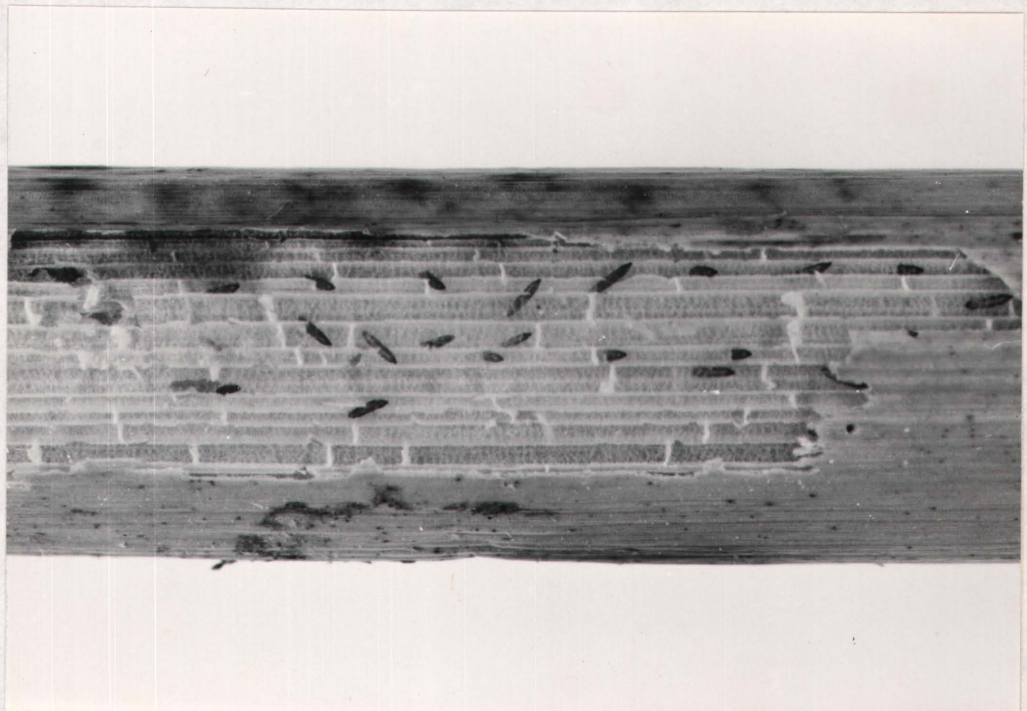

Figure 46. Epidermal and vascular tissue removed from dry stem of Scirpus to show eggs of L. congener deposited in the pith.

Five sexually mature females of L. congener were caught prior to oviposition and the oocytes counted. Numbers of mature oocytes ranged from 87 to 297, with a mean of 205 per female. The number of oocytes is almost three times that found in L. disjunctus. The number of egg clutches produced per female was not determined.

During a warm, dry autumn such as the one experienced in 1970, adults may still be found in October. Adults were observed in tandem October 1, 1970, even after temperatures of -4.5°C had been recorded. None were observed after October 8 and 9 on which days the night air temperature dropped to -9.5° and -13°C respectively.

(c) The egg stage

Eggs of L. congener are elongate and pointed at the anterior end. They measure 1.24 mm in length. When first laid they are a grayish white colour but soon turn a dark gray. Whereas eggs of the Type A and B species were translucent, permitting observation of development, those of L. congener are thick-walled and dark until late in embryonic development. They lose the dark pigmentation at about the time that eye spots appear in the developing embryos.

Embryonic development begins as soon as the eggs are laid. Though undetectable without removing the thick chorion, it proceeds just to the point of embryonic rotation or blastokinesis. Development ceases at this stage and the eggs remain dormant until spring. The filling of the pond with water in the spring and the subsequent wetting of the eggs presumably induces resumption of development. About seven weeks elapse before these eggs finally hatch.

The eggs of L. congener display a high degree of resistance to desiccation. Eggs of this species were never found dried out under field conditions. Similarly, while eggs are usually laid in locations which trap

large quantities of snow, egg mortality due to freezing was not evident even in those stems with relatively little snow cover.

3.3.2 Lestes congener - Laboratory experiments

The failure of eggs of L. congener collected August 28, 1969 to hatch at 21°C and a 16½ hour photoperiod in less than three months led to the suspicion that diapause also occurs in this species.

According to data from eggs collected late in February, 1970, any eggs having completed diapause development hatch between 13 and 23 days when incubated at 21°C and a 16½ hour photoperiod. Eggs which hatch in fewer than 13 days presumably have undergone some post-diapause development. Having established this criterion, I conducted experiments to determine the effects of temperature and photoperiod on diapause development in the laboratory and the rate of diapause development under field conditions.

(a) Effect of temperature and photoperiod on diapause development

High temperatures appear to impede diapause development in eggs of L. congener. Ninety per cent of the eggs collected on September 7, 1970 and kept at 21°C rotted. Hatching began on November 17 and continued sporadically during the next two months. Perhaps the excessively moist conditions at which the eggs were maintained induced the high mortality.

Diapause development at lower temperatures was measured according to the procedures outlined earlier for similar studies on the Type B species. Eggs were collected on August 20 and September 17, 1970. They were incubated at 21°C and an 8 hour photoperiod to September 25 to insure that the pre-diapause phase of development was completed. One hundred and thirty eggs were then put into each of four Petri dishes lined with wet filter paper and incubated at -6.5, -1, 4.5, and 10°C in continuous darkness. Ten eggs were removed from each condition at two-week intervals and tested at 21°C and a 16½

hour photoperiod. Those eggs which hatched within twenty-three days were considered to have completed the temperature controlled phase of diapause development corresponding to Phase I in the Type B species. Results in Figure 47 show a very rapid diapause development rate at 10°C. Complete development was achieved in eight weeks. Diapause development was slower at 4.5° and -1°C. However at -1°C it was much more rapid than in the Type B species. Essentially no diapause development occurred at -6.5°C.

Poor hatch was obtained from eggs incubated at 10°C for more than 10 weeks. It appears that although this temperature is near the optimum for diapause development, it is too low for normal post-diapause development into which the eggs were forced because they were wet. Under field conditions diapause development takes place in dry stems. No post-diapause development at this time is possible, as will be seen later.

Table 14

Relationship between collecting date anddiapause development in eggs of L. congener, indicated as percent hatching in 23 days at 21°C and a 16½ hour photoperiod.

	Time (wks) subjected to 4.5°C, Dark after September 25, 1970						
Collecting Date	2	4	6	8	10	12	14
20.8.70	0	40	20	20	80	100	97
7.9.70	0	40	20	100	80	100	97
17.9.70	0	20	80	80	100	100	94

Eggs of L. congener were collected on August 20 and September 7, 1970, and were pre-treated in the manner described earlier. They were then

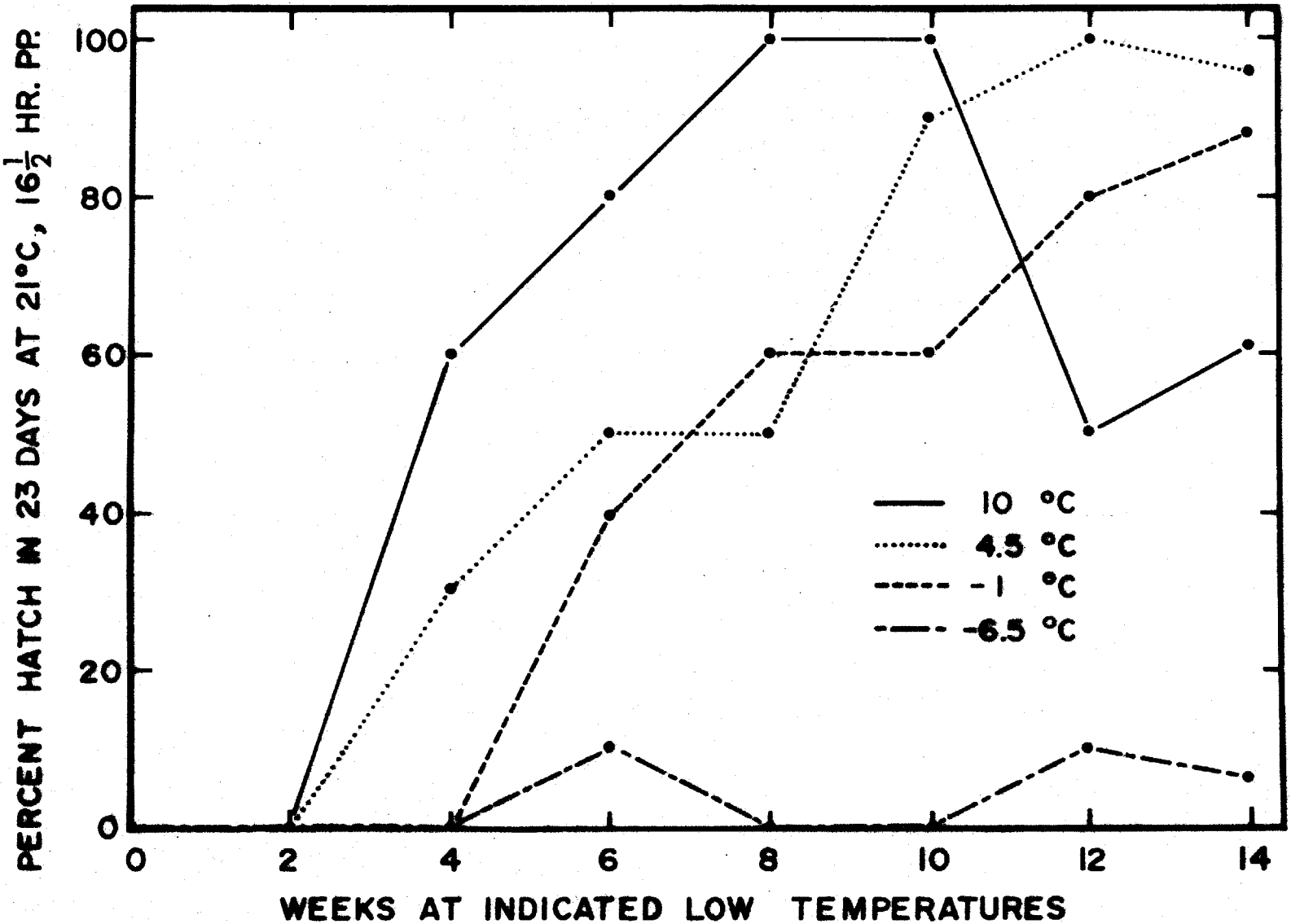

Figure 47. Effect of temperature on diapause development in eggs of L. congener incubated in the dark at various temperatures then subjected to test conditions of 21°C and a 16½ hour photoperiod.

subjected to photoperiods of 16½, 8 and 0 hours at 4.5°C to determine the effect of photoperiod on diapause development. Samples were removed every two weeks and incubated at 21°C and a 16½ hour photoperiod. Results presented in Figure 48 show that photoperiod has no effect on the rate of diapause development.

Field collections of eggs made on August 20, September 7 and September 17, 1970, were kept at 21°C prior to subjecting them to diapause development conditions of 4.5°C in continuous darkness on September 25. The eggs from the three collecting dates were kept separated in the latter condition. Five eggs from each collecting date were removed at two-week intervals and subjected to 21°C and a 16½ hour photoperiod for hatching. Results shown in Table 14 indicate that substantial diapause development took place in the field between August 20 and September 17, being reflected in termination of diapause in the eggs collected September 17 in four weeks less at 4.5°C than in those collected on August 20. Diapause development at the experimental temperature of 21°C was therefore very much slower than it was in the field where the mean air temperature during this period was approximately 14.5°C.

Eggs were collected in the field at two-week intervals, beginning on October 9, and subjected to 21°C and a 16½ hour photoperiod. Approximately 8% of the eggs collected on October 9 hatched in 23 days. By November 19 the figure had risen to 69% (Fig. 49). Another 28% of the latter collection hatched during an additional five days of incubation. Seventy-three percent hatch was obtained in 23 days in eggs collected December 2. The data suggest that diapause development in the field is essentially completed before the arrival of severe winter conditions.

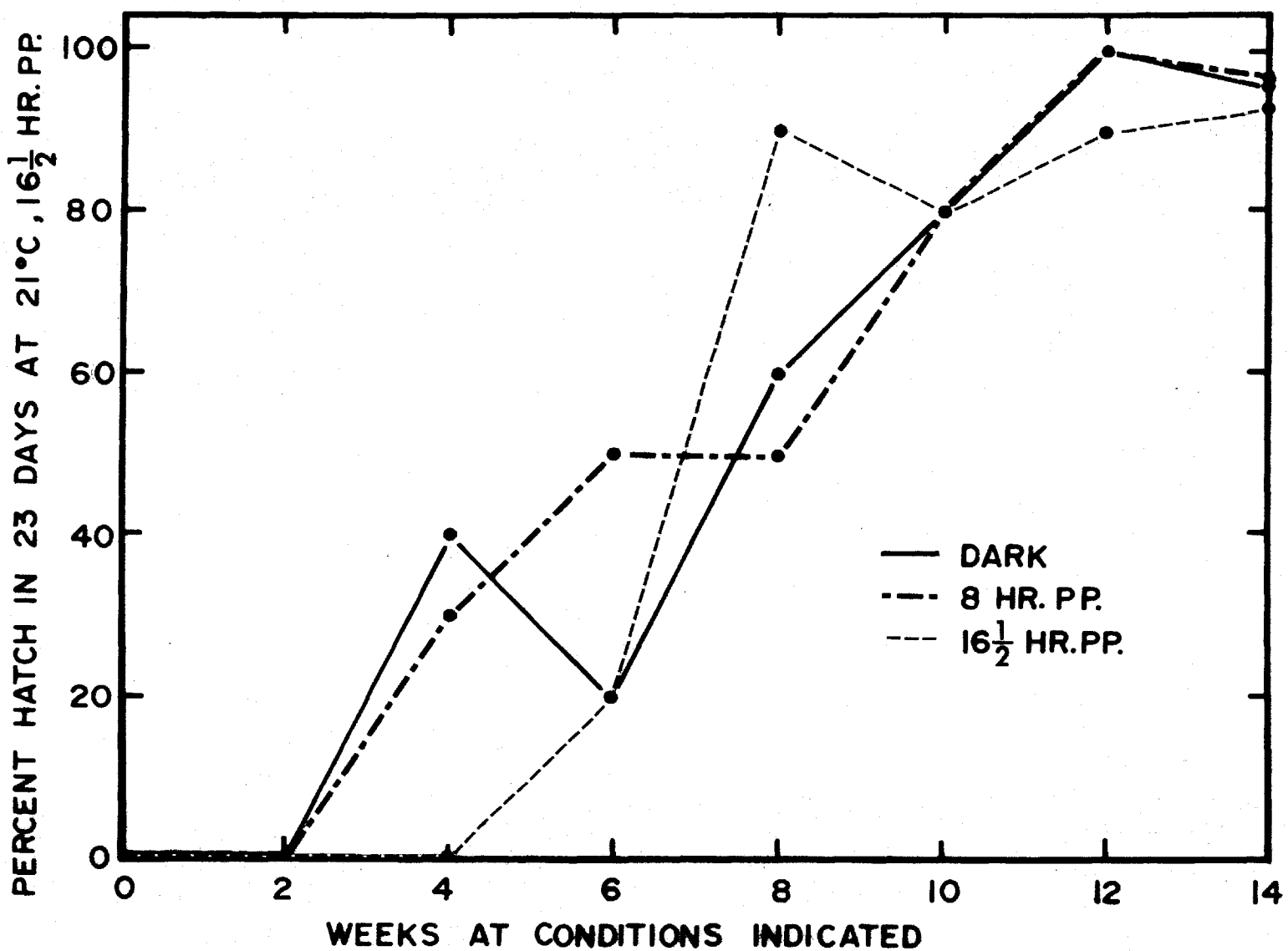

Figure 48. Effect of photoperiod on diapause development in eggs of L. congener incubated at 4.5°C then subjected to test conditions of 21°C and a 16½ hour photoperiod.

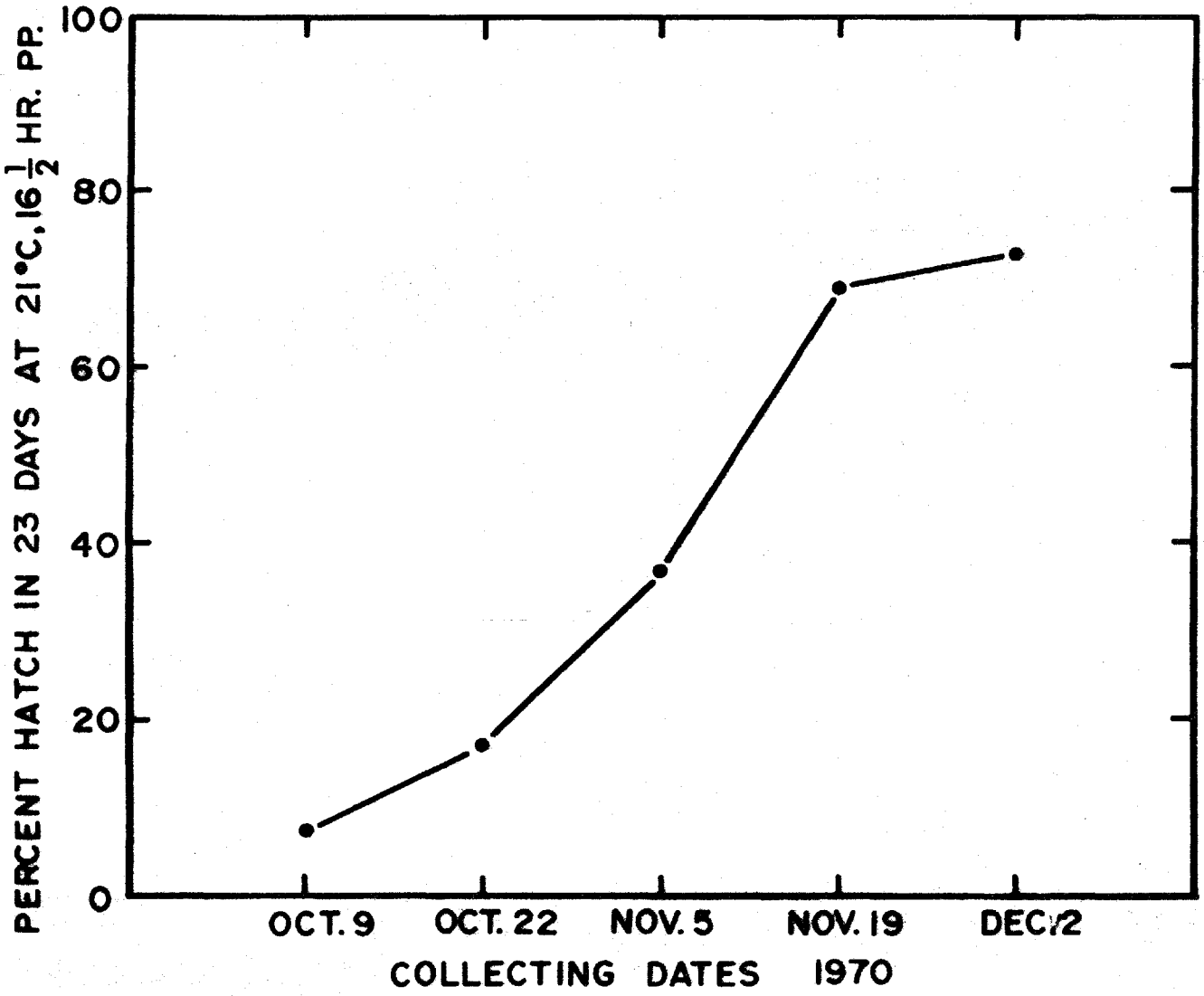

Figure 49. Percent of field collected eggs of L. congener which have completed diapause development.

(b) Effect of temperature and photoperiod on post-diapause development

Eggs of L. congener were collected in February of 1970 and subjected to 26.5, 21, 16, 10 and 4.5°C at a 16½ hour photoperiod. Embryonic development and hatch data are presented in Table 15. Blastokinesis and the subsequent stages of embryonic development in L. congener take place at very low temperatures. Plotting the reciprocals of the time for the appearance of eye spots in 50% of the eggs against temperature (Fig. 50) indicated that embryonic development probably goes on at all temperatures above 0°C.

Comparison of the hatching time with pre-hatch embryonic development between 4.5°C and other temperatures indicates that while 4.5°C is adequate for post-blastokinesis embryonic development, it is very near the threshold for hatching. Ninety-one percent of the eggs at 4.5°C completed embryonic development but only 48% succeeded in hatching. The insects which hatched seldom succeeded in emerging from their prolarval skins. Plotting the reciprocals of hatching time against temperature (Fig. 51), ignoring the value for 4.5°, showed the critical hatching temperature to be about 5°C.

The temperature of 26.5°C appears to approach the upper limit at which normal embryonic development is able to take place. Development at this temperature often was abnormal. Many embryos failed to undergo rotation and died in the early eye spot stage.

Under field conditions of early spring when water temperatures are still below the critical level for hatching one should expect a synchronization of embryonic development to take place. Eggs which have completed pre-hatch development will be prevented from hatching while eggs whose development has been delayed for various reasons are able to continue pre-hatch embryonic development. Field evidence shown in Figure 52 demonstrates that the later the date of egg collection, the narrower the hatching range at 21°C, that is,

Table 15. Effect of temperature on post-diapause embryonic development and hatching in eggs of L. congener collected February 23, 1970, and reared at a 16½ hour photoperiod.

	Temperature °C				
	26.5	21	16	10	4.5
Days to appearance of eye spots	7	9	13	20	38
Days to appearance of eye spots in 50% of the eggs	11	11	16	22	44
Days to 50% hatch	13	16	30	50	-
Hatching range	11-20	9-17	24-54	44-96	107-?
% mortality	32	5.3	12	8.3	52

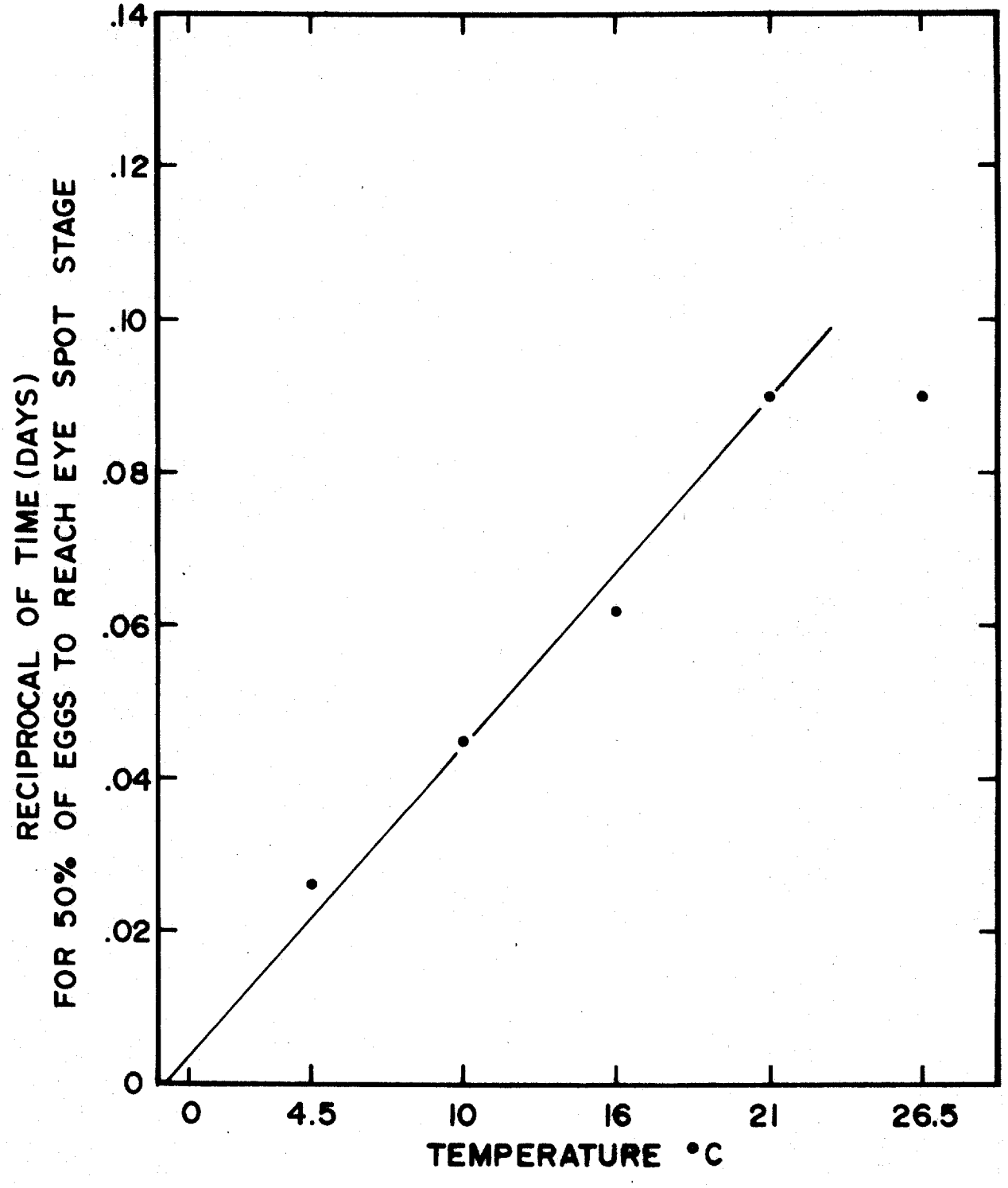

Figure 50. Effect of temperature on post-diapause embryonic development (excluding hatching) in eggs of L. congener collected February 23, 1970, and incubated at a 16½ hour photoperiod.

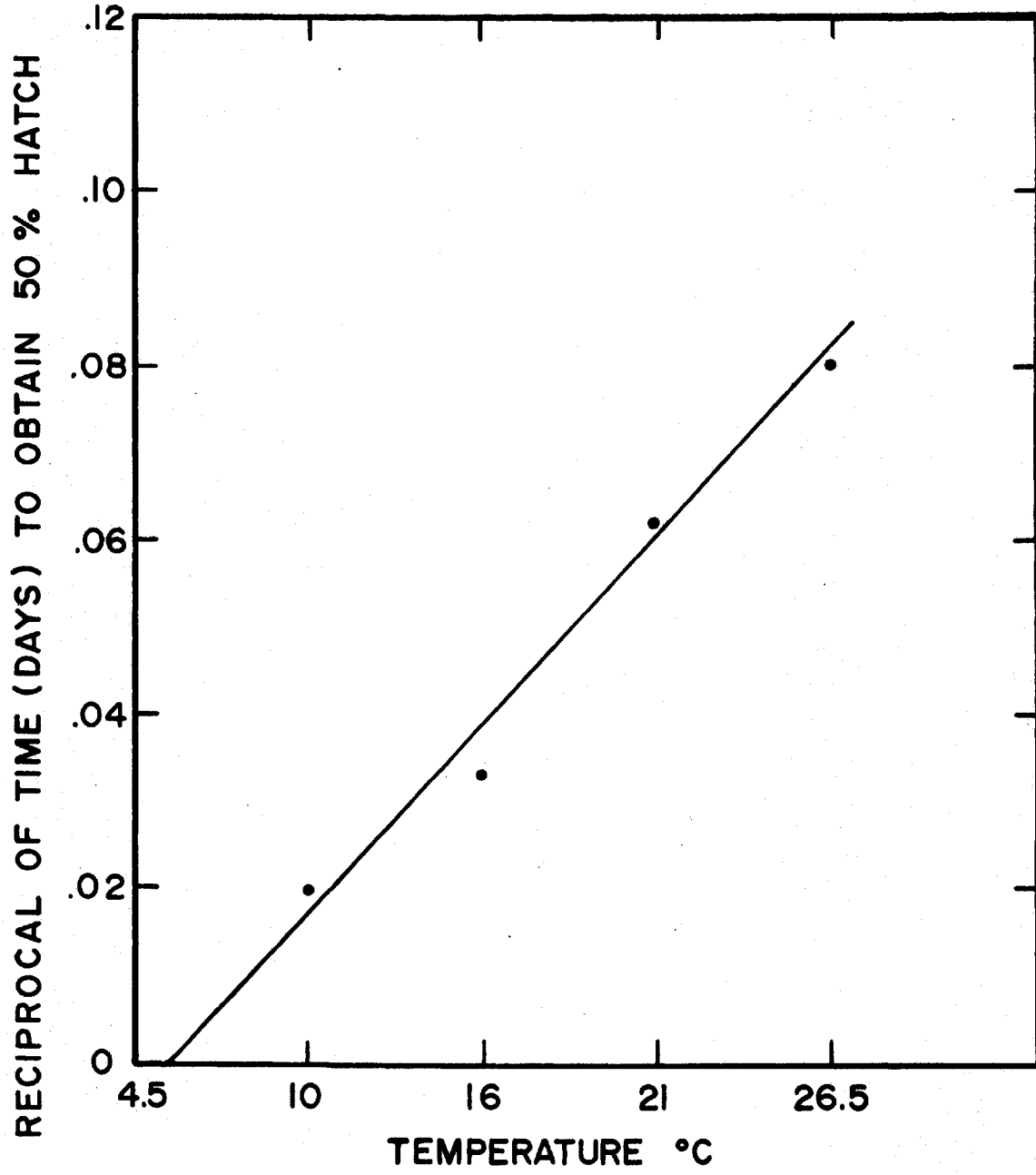

Figure 51. Effect of temperature on post-diapause embryonic development (including hatching) in eggs of L. congener collected February 23, 1970 and incubated at a 16½ hour photoperiod.

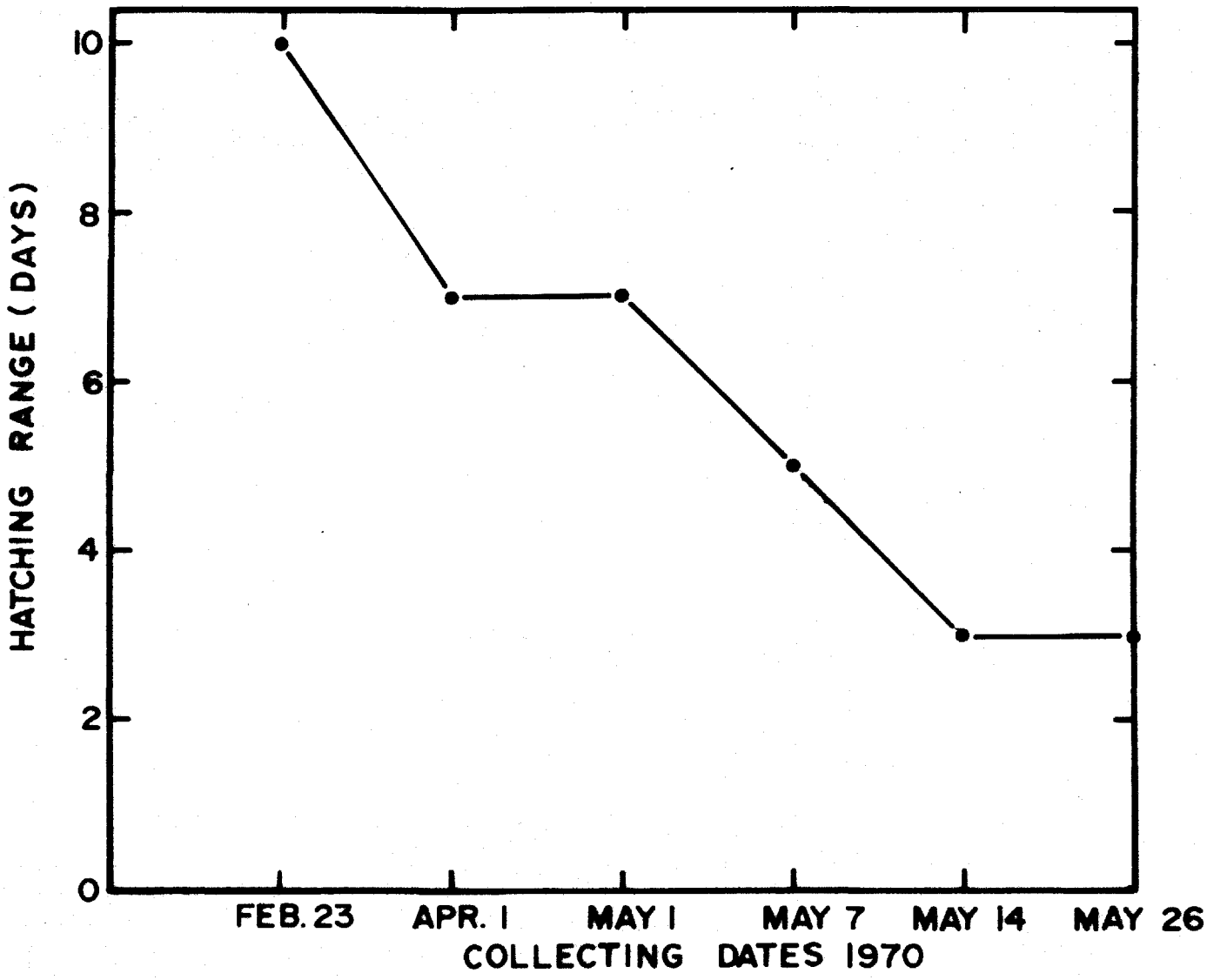

Figure 52. Hatching range of eggs of L. congener collected during late winter and spring, and incubated at 21°C.

the greater the synchronization of hatch.

Under laboratory conditions, eggs incubated at 4.5°C developed to a stage just prior to hatching, then went into a developmental arrest which persisted for up to 6½ months. Transfer of the eggs to higher temperatures allowed hatching to take place. In the absence of higher temperatures the embryos eventually died in this stage. The stage in which this temperature-induced developmental arrest occurred corresponds to the stage of embryonic development in diapausing Type B species.

Eggs of L. congener were collected in the field between November 5 and May 4 and incubated at 21°C at 16½ and 8 hour photoperiods. Results shown in Table 16 demonstrate no photoperiodic effect on hatching. There is, therefore, no photoperiodically controlled diapause phase following the primary phase as there is in the Type B species.

Table 16

Effect of photoperiod on hatching in L. congener showing percentage of total hatch within limits of completed diapause at 21°C and a 16½ hour photoperiod.

Collecting date	21°C 16½ hr	21°C 8 hr
5.11.70	55	42
19.11.70	71	62
2.12.70	100	95
4.5.70	100	100

(c) Role of moisture in post-diapause development

Experiments were conducted to determine the effect of relative humidity and contact water on post-diapause development in L. congener. Eggs

were collected December 15, 1969 and incubated at 21°C at four relative humidities, 100%, 75%, 50% and 25%, with wet eggs as controls according to the procedures outlined on Page 27. These conditions were maintained for 36 days. The control eggs hatched within the normal time for post-diapause eggs. In the experimental conditions there was a progressive increase in egg desiccation with decrease in relative humidity. At the 25% relative humidity all eggs collapsed from loss of moisture. Some hatching occurred at the 100% relative humidity between 32 and 36 days. However, this was probably induced by condensation dripping onto the eggs from the lid of the container.

Water was added to all of the eggs on day 36. The viable eggs in each treatment hatched within the normal 13 to 23 days following the addition of water. These results demonstrate that wetting of the eggs is necessary before post-diapause development will occur even at appropriate incubation temperatures. The uniformity of egg hatch in the random egg sample demonstrates the vital role played by the wetting stimulus in synchronizing egg hatching.

Heavy mortality due to desiccation was observed at the 25% relative humidity, where there was only a 28% hatch. The viable eggs,however, quickly absorbed water and within two days had regained their normal shape. The natural environment of L. congener eggs in this study suggests that it is highly unlikely that mortality due to desiccation will occur.

(d) Determination of temperatures lethal to eggs of L. congener

Post-diapause eggs of L. congener collected on March 16 and April 2, 1971, were subjected to temperatures as low as -28°C for 40 hours with no loss in viability. Under field conditions, eggs in exposed stems survived air temperature down to -33°C. In each case, temperatures survived by eggs of this species were much lower than those tolerated by eggs of the Type B species.

Resistance to cold drops progressively as embryonic development advances. At -16°C eggs "frozen" in the pink eye spot stage survived while those "frozen" just prior to hatching developed a band of scar tissue across the base of each appendage and generally died in the process of hatching. The significance of the scar tissue at the moment remains unexplained. Lestes congener cannot tolerate cold temperatures in the late stages of embryonic development in which L. disjunctus, L. unguiculatus and L. dryas overwinter.

4. DISCUSSION

Kormondy (1959), in his attempt to separate closely related species of Tetragoneuria on the basis of ecological, life history and morphological criteria, was among the first to express concern over the problem of the ability of several closely related species of Odonata to occupy a single habitat simultaneously. His observations appear contrary to the theories developed by Hutchinson (1959), Hardin (1960) and others, which in essence state that no two closely related species can occupy the same niche at the same time. While his efforts were concentrated on the adult stage, he did speculate that vertical and horizontal separation as well as behavioral characteristics of nymphs might permit such coexistence in a single habitat. In an effort to explain the coexistence of seventeen species of odonate nymphs in a single pond in Pennsylvania, Kormondy and Gower (1965) were forced to conclude, because of inconsistent results in their two year study, that there were no recognized differences in responses of various species of nymphal odonata to physical and biological regulating mechanisms. Similarly, although finding differences in spatial and temporal distribution at the generic level, Benke (1969) was unable to detect congeneric differences among species.

The present investigation has emphasized seasonal succession and congeneric associations among seven species of damselflies in Saskatchewan. The interpretation of the results in this respect can be more fully appreciated after an evaluation of the life-histories of the species involved. The egg stage is an appropriate starting point for such a discussion.

4.1 The life-histories of the Type A species

Eggs of the Type A species of damselflies approach 1 mm in length. They showed a slight increase in size as embryonic development progressed,

not unlike the examples given by Ando (1962).

A structure characteristic of all Type A species and apparently found in eggs of all Coenagrionidae and Agrionidae (Ando, 1962) is a membranous funnel-shaped appendage extending from the anterior end of the eggs. While these structures were observed as early as 1869 (Brandt, cited by Ando, 1962) in Calopteryx virgo and later in Coenagrion hastulatum and C. puella (Gardner, 1954), their function still remains obscure. Ando (1962) noted that, while the eggs of these species are inserted into the stem, the membranous funnel extends to the outside. Eggs of Lestes, which are generally deposited above the water surface, lack these structures. Corbet (1963) associated these structures with the blade-like projections in eggs of Hemianax ephippiger, Anax imperator (Corbet, 1955b) and Aeshna isosceles (Gardner, 1955). In these species the structure functions as an escape hatch for the hatching pronymph.

In the Coenagrionidae investigated in this study, the membranous structure extends to the surface of the stem and appears to prevent the formation of scar tissue over the incision in which the egg was deposited. Experiments clearly demonstrated that the funnel does not provide a passage-way for the newly hatched pronymph. The point of rupture of hatching eggs is well behind the membranous extension of the funnel. The pronymph pushes this structure and the operculum in front of it as it emerges from the shell.

Corbet (1963) remarked on the paucity of information on hatching of odonate eggs under controlled conditions. Evidence is presented in this study to confirm his suspicion that those eggs which hatch the same season they were laid have a positive thermal coefficient. An average of 17 days was required to achieve hatching at 21°C in the three Type A species studied. In each instance optimal hatching was obtained at 21°C, with a considerable

decline in development rate demonstrated at 16°C. Hatching under field conditions is estimated to require approximately three weeks in these species.

Early nymphal development is rapid in all species. Although insufficient data prevented the establishment of a growth curve for E. boreale, the appearance of final and penultimate instar nymphs in the fall demonstrates that its development rate is similar to those of the other two Type A species. The structure of the overwintering nymphal population of all these species is similar to that described by Eller (1964) for Pachydiplax longipennis. While his species was dominated by the antepenultimate instar with slightly more than one percent in the final (these belonging to the previous year's population), the three species studied here were generally in the penultimate instar with approximately five per cent in the final instar. All instars entered overwintering conditions in a state of developmental arrest.

It is now possible to evaluate the developmental arrest of the Type A species in terms of Mansingh's (1971) recent classification scheme for dormancies in insects. Nymphs which overwinter in instars below the penultimate appear to be in a state of quiescence or a condition of developmental arrest which is induced directly by environmental factors (Shelford, 1929). The temperature- and photoperiod-influenced developmental arrest occurs in either the penultimate or the final instar in these species. This suggests that it might be described by the term oligopause as defined by Mansingh (1971). Other evidence favoring this term is the requirement of food by these insects when maintained at temperatures over 4.5°C. Mansingh states that oligopausing insects do not feed under cold conditions. It is very probable that none of the Type A nymphs feed during October and November. Certainly no feeding is possible when the nymphs are embedded in ice or

lying in an inactive state at the bottom of the slough during the winter.

Mansingh states that in oligopausing insects: "The 'refractory phase' is probably absent because oligopause insects resume development within a reasonable time of acclimation after the return of favourable conditions". The term 'refractory phase' is used in this instance as defined by Watson and Smallman (1971) to represent that phase of diapause development which is accelerated typically by cold temperature. A definite 'refractory phase' has been demonstrated in the Type A species suggesting that they might more accurately be classified as having a diapause as defined by Mansingh. The latter term will be used to describe developmental arrest in these species with full recognition that all conditions ascribed to it by Mansingh have not been met. Dragonflies such as Anax imperator (Corbet, 1957a) and Tetragoneuria cynosura (Lutz and Jenner, 1964) meet these conditions more closely.

The 'induction phase' of diapause (Mansingh, 1971) was not studied in nymphs of C. angulatum and C. resolutum. However, nymphs of E. boreale went into developmental arrest either in the penultimate or the final instar depending on temperature. At suboptimal temperatures of 26.5°C and 16°C or lower, nymphs reared at a long photoperiod (16½ hours) went into diapause in the penultimate instar. At 21°C the nymphs moulted into the final instar before undergoing developmental arrest. Eller (1964) found that diapause induction at 22°C is photoperiodically controlled. An 11-hour photoperiod induced diapause in the last three instars. He noted that photoperiodic inhibition of development was particularly strong in the penultimate instar. The combined results from Eller's study and the present investigation show that suboptimal temperature and short photoperiod are both effective in

inducing diapause. A combination of the two factors produces a stronger effect than either one acting alone. Evidence for such an effect was presented from rearing experiments on C. angulatum nymphs which showed continued moulting at 21°C and an 8-hour photoperiod but a complete arrest of development at 16°C and an 8-hour photoperiod. Nymphs of C. angulatum and C. resolutum in the penultimate and final instar were already in diapause when fall experiments on these species were begun. Nymphs in the antepenultimate instar ceased development when water temperature dropped to 4.5°C. A similar response was observed in Pachydiplax by Eller (1964).

A differential response to short and long photoperiods was obtained in all three species of diapausing nymphs. This agrees with the results of Jenner (1958), Lutz and Jenner (1960, 1964), Lutz (1963) and Eller (1964). While this response disappeared completely by January in nymphs overwintering in the penultimate instar, as was indicated also by the observations of Lutz and Jenner (1964) and Eller (1964), it was retained in the final instar until spring. Although the differential response was retained in this instar, there was a progressive seasonal decrease in development time at both short and long photoperiods. These seasonal changes in response demonstrate a weakening of diapause which likely can be attributed to the effect of low overwintering temperature. The diapause phase thereby corresponds to Mansingh's (1971) 'refractory phase'.

Nymphs overwintering in the final instar in C. resolutum and C. angulatum had a distinct photoperiod threshold of 12 to 14 hours. In C. angulatum the threshold decreased as the season progressed, a result somewhat different from that in the penultimate instar and those obtained by Eller (1964). This corresponds to the 'activated phase' of diapause according to Mansingh (1971).

Corbet (1963) referred to a personal communication with Jenner in which it was stated that development time to emergence in Tetragoneuria cynosura, Ischnura posita and several species of Enallagma was inversely proportional to the photoperiod they experienced. This effect was observed in several instars depending on species. Lack of information on the conditions under which the earlier instars of these species were reared makes comparisons with the results of this study difficult. Nymphs of C. angulatum and C. resolutum, when subjected to a series of photoperiods in the penultimate instar after mid January, were unaffected by photoperiod in their first moult but emergence followed the pattern observed by Jenner. Corbet suggests this as a possible mechanism which might reduce temporal variation within a species. This mechanism certainly does not function among the Type A species under study here since field conditions are not appropriate for any form of nymphal development in the spring until photoperiod has exceeded 14 hours. It might be a functional mechanism in more southern latitudes.

The combined roles of temperature and photoperiod in nymphal diapause development have not been previously investigated. It has been noted that temperatures of 16°C or lower appear to considerably strengthen diapause under an 8 hour photoperiod in the fall. Nymphs collected in the penultimate instar remain in this instar for more than one hundred days. At 21°C the short photoperiod is not sufficient to arrest the moulting cycle. Moulting takes place at irregular intervals. However, the moults are essentially stationary, resulting in slow advancement toward maturity. The ability of short photoperiods to induce stationary moults under conditions otherwise suitable for nymphal development and emergence is viewed as a vital mechanism for the retention of nymphal stages and the prevention of emergence in the fall. This temperature-photoperiod effect is lost by January.

Stationary moults can be induced in nymphs of C. angulatum by subjecting nymphs collected in the antepenultimate instar to a temperature of 21°C and a photoperiod not exceeding 12 hours. The first moult in such cases occurs rather rapidly. However, subsequent moults occur irregularly, producing individuals with morphological characteristics intermediate between those normally observed in penultimate and final instars.

Eller (1964) found the eye-index, a relationship between head width and the distance separating the compound eyes of Pachydiplax longipennis, a useful morphological criterion for monitoring intermoult development. He found that whether or not a nymph went into diapause depended on the photoperiod during a critical phase of nymphal development early in the moulting cycle. Such an index was not determined in the nymphs in the present investigation. However, comparison of the emergence of nymphs which had overwintered in the final instar with the moulting and emergence of those overwintering in some other instar yielded some interesting results. The synchrony of emergence from overwintering final instars and the synchrony of the first moult in those overwintering in the penultimate and antepenultimate instars clearly demonstrates that diapause begins at the same stage of morphological development in a particular instar, as observed by Eller. The length of time required for post-diapause development to take place in final instar nymphs also suggests that this stage of morphological development is very early in the moulting cycle.

The difficulties of working under conditions presented by Temperate Zone winters have resulted in a paucity of information on the overwintering nymphal stages of the odonate life cycle. Rostand (1935) noted that most of the Odonata spend the winter in the larval form and undoubtedly protect themselves against the cold by burrowing in the bottom mud. He did, however, note an exception:

> D'après Hodge, celles de Cordulegaster annulatus résistent à une basse température; il en a trouvé de bien vivantes dans des blocs de glace.

He doubted whether they were actually frozen since all the odonate nymphs which he subjected to a temperature of -4°C for an hour or two invariably perished. Corbet et al. (1960) came to a similar conclusion:

> We do know that medium and large-sized larvae of several species (including Aeshna and Anax) can survive being frozen into blocks of ice, apparently without suffering any ill effects, for they swim away actively as soon as they have been thawed out. But it is unlikely that they have occasion to use this ability often in nature, because most overwintering larvae seem to retire to the deeper regions of a pond when the cold weather begins in autumn.

Although Daborn (1969, 1971) apparently overlooked the comments of Rostand and Corbet et al. on the subject, he was the first to present evidence of survival of damselfly nymphs in ice under field conditions. His record of specimens surviving such conditions included all Type A species in the present study. While his data for C. angulatum and C. resolutum have been confirmed in the present investigation, his results for E. boreale remain questionable. He noted from the previous year's emergence studies that E. boreale constituted approximately 5% of the population. Without identifying the nymphs collected in ice he concluded that the same proportions were also found there. Nymphs of E. boreale were never obtained in ice in this study. All efforts to revive nymphs of this species subjected to sub-zero temperatures under laboratory conditions met with failure. Specimens of Enallagma were collected during the winter from vegetation on the mud surface in 60 to 90 cm of water. The inability of this species to withstand ice temperatures appears to restrict it to areas which will not freeze to the bottom.

Daborn found in his investigation that nymphal mortality of insects embedded in ice increased as the season progressed and suggested that overwintering in this manner is abnormal to the species investigated. Results contradictory to these were obtained in the present investigation. A collection of nymphs in January yielded a survival rate of 85%. The observed mortality could easily be attributed to mechanical injury inflicted while removing the blocks of ice. No direct counts were taken in subsequent collections. However, there was no obvious indication of increased mortality as the winter progressed. The increasing mortality in Daborn's results was perhaps brought about by an exceptional lowering in temperature caused by the absence of snow cover. Perhaps, his methods of handling the material after it was removed from the pond may have caused the mortality. He noted that the ice was thawed and the water brought to room temperature in closed plastic bags. The contents were then emptied and the active nymphs counted. The anoxic conditions of a sealed plastic bag could result in nymphal mortality at room temperature. Furthermore from experiments in this study it was noted that nymphs under aerated conditions may require several hours to recover. The nymphs which failed to display activity may not necessarily have been dead. For example, nymphs of E. boreale, though not frozen, display no visible sign of life throughout the winter. Rather, they lie in an apparently moribund condition at the bottom of the pond.

The minimum lethal temperature for nymphs of C. angulatum and C. resolutum was approximately -7°C. The results presented by Daborn (1971) showed that ice temperature a few centimetres below the surface dropped below this level only once during the winter of 1967-68. Ice surface temperatures, in the absence of snow cover, measured by Fertuck et al. (in press) dropped below this level more frequently. However, Fertuck

(personal communication) demonstrated a direct relationship between depth of snow cover and ice temperature. Ice thickness had a similar insulating effect. For example, if the surface ice temperature in exposed areas is approximately -7°C, the temperature at a level 15 cm below the surface of ice which is covered by 60 cm of hard-packed snow is between -4 and -1°C, well above the lethal level.

It was concluded that nymphs which are able to withstand being embedded in ice use this as a means of surviving in temporary ponds in temperate and subarctic climates. Their ability to withstand this condition enables them to survive for periods of four or five months without food during a time when food is normally scarce, and protects them from predators. It enables the species to occupy temporary habitats, which may freeze to the bottom, in the absence of an egg diapause which performs a similar function in the Lestidae.

The cold-resistant overwintering nymphal stages appear associated with diapause. Freezing experiments performed with nymphs which were not in diapause showed no resistance to temperatures below the freezing point of pond water.

Since diapause that can occur in several nymphal instars in a species in itself fails to function as a synchronizing mechanism for subsequent development, Corbet (1957b, 1963) suggested a means by which synchronization might be accomplished during spring development through a series of temperature thresholds. He speculated that young nymphs might begin development earlier in the spring at a lower threshold temperature or have a low thermal coefficient for development. The younger nymphs would then be placed at a developmental advantage over those in the more advanced instars and might eventually catch up to them. Such a system would be

operational only during a period of rising spring temperatures. Its efficiency would depend on the differences between successive temperature thresholds, the duration of the successive nymphal stages and the instar variation within the population. While Corbet did not obtain experimental support for his theory, he noted that Lloyd (1941) found such a system operational in the chironomid Spaniotoma.

Lutz (1968), in his studies on development of nymphs of Lestes eurinus, found evidence of increasing temperature threshold with instar. Moulting into the antepenultimate instar occurred at 12 to 14°C, into the penultimate at 13 to 15°C and into the final at 18 to 21°C. Emergence occurred at 20 to 24°C. Lutz also found that younger nymphs developed more rapidly at a lower temperature; the opposite was true for nymphs in the final instar. The lack of complete synchrony was explained by the fact that water temperature rose at a faster rate than maximal development of the nymphs.

The results presented by Lutz (1968) are a good example of the differential temperature response which Corbet speculated as being useful in synchronizing emergence. The response of several instars to successively higher temperature thresholds has not been shown in other Odonata. Since L. eurinus appears to be atypical of the Lestidae in its ability to overwinter in the nymphal stage, and, since Lutz found no evidence of diapause in the nymphs, it may be that this method of synchronization of development is quite restricted.

Eller (1964) did not find different critical temperature thresholds in the earlier instars of Pachydiplax. He noted that development began almost simultaneously in the antepenultimate-2, antepenultimate-1 and antepenultimate instars. However, the penultimate instar began development about two weeks later. Development in the final instar began later still. Eller explained the delay on the basis of persistent diapause in the penultimate and final

instars. Similarly, laboratory experiments in the present study indicated that diapause persisted later in the spring in overwintered final instars than in the penultimates.

Eller noted that, while several instars commence development at the same time, the development rate of the younger nymphs is faster, so that they are able to catch up, or nearly catch up, to those which overwintered in the penultimate instar. Subsequent development occurs synchronously. He concluded that differences in thermal-growth coefficients between the younger and more advanced instars possibly accounts in part for the more rapid development in the earlier instars in spring. Synchronization of development within an instar occurred as a result of diapause. Overall synchronization was accomplished by the differential development rate coupled with higher critical threshold temperatures in the penultimate and final instars.

The results obtained in the present study were similar to those of Eller. Nymphs of C. angulatum that overwintered in the antepenultimate instar went through the penultimate more rapidly at 21°C than those which overwintered in the penultimate instar. As a result, emergence of nymphs overwintering in these two instars was nearly in synchrony. Both antepenultimate and penultimate instars responded similarly to 16, 21 and 26.5°C temperatures while those overwintering in the final instar were distinctly delayed at 16°C. This suggests that, as in Pachydiplax, the final instar has a higher thermal growth coefficient.

Studies on the effect of temperature on emergence demonstrated that a relatively high threshold temperature for emergence existed. While a temperature of 4.5°C completely inhibited both nymphal moulting and emergence, a temperature of 10°C was adequate for nymphal development but barely adequate

for emergence. It was noted that under natural conditions emergence took place only after the air temperature approached 21°C. Water temperature at this time will have exceeded 13°C. If these conditions were not present when the nymphs were morphologically ready to emerge, emergence could be delayed for up to a week. Such delays at the beginning of the emergence period also serve to synchronize emergence.

Emergence in the Type A species thus appeared to be synchronized by several factors. Within an instar synchronization was accomplished by diapause. Differences in thermal growth coefficient allowed synchronization of development of antepenultimate and penultimate nymphs in the spring. The development of nymphs overwintering in the final instar is delayed sufficiently by low spring temperatures to allow the other nymphs to catch up. Finally, emergence temperature may act as a synchronizing mechanism should the group encounter low temperature conditions just prior to emergence.

The important role of temperature in spring nymphal development in these species was reflected in the behavior of the nymphs in the spring. Soon after they were freed from their winter confines, the nymphs migrated to the shallow water where they could take advantage of the direct effects of warm day time temperatures, and more accurately monitor temperatures suitable for emergence.

Emergence begins in this group during the last week of May and extends over a period of approximately three weeks. Emergence in all three species shows no distinct diurnal pattern, usually beginning near 10:00 A.M. when air temperatures become suitable and extending into the mid afternoon. Corbet (1963) associated daytime emergence with higher latitudes and altitudes where night-time conditions are too cool for this process.

All Type A species emerge on any available object, usually in water less than 30 cm deep. Emergence is not so closely synchronized that mortality due to overcrowding on emergence sites occurs. Corbet (1957a, 1963) showed that up to 16% mortality experienced in Anax imperator is attributed to this factor. The adults are capable of weak flight within an hour of emergence. They vacate the emergence sites as soon as they are able to fly.

Maturation in Zygoptera takes place away from the emergence site, as has been well documented. In the Type A odonates of this study maturation took place in hedges perhaps little more than one hundred metres away from the slough. Adults were observed there in very large numbers along with abundant food. From their reluctance to fly more than a few metres it appears highly unlikely that these individuals dispersed to other areas during this phase of their life cycle.

Maturation was completed within a week, approximately one half the time required in Lestes sponsa (Corbet, 1956a) and in the species of Lestidae in this study. Unlike most odonates which return to the oviposition site prior to mating, the males preceding the females, Type A adults begin mating at the site of sexual maturation. There appears to be no courtship or aggressive territorial behavior preceding mating as is common in many Anisoptera (Corbet, 1963; Connor, 1968). Copulation in all species is prolonged, lasting for 10 minutes or more without interruption, falling into the category of 'long copulation' described by Corbet (1963). While the species are able to remain in copula in flight, the latter occurs only when a pair has been disturbed. Mating pairs remain in tandem after copulation and during oviposition. Only on rare occasions were females observed ovipositing alone. Walker (1953) noted that most Coenagrionidae oviposit while

resting on floating vegetation. The females deposit the eggs into stems below the water surface. He noted that several species of Coenagrionidae may descend below the water surface for oviposition. Only C. angulatum was observed to descend below the surface in the present study. This was done while in tandem and only on occasions when suitable floating vegetation was scarce. The species appeared to distinguish between floating and emergent plants. Oviposition far below the surface was necessary in emergent vegetation to insure that the eggs remain below the surface at the time of hatching. High evaporation rate may lower the water level by 15 cm or more between the time of oviposition and hatching.

The selective oviposition of E. boreale in the deep water area where floating vegetation was surrounded by large areas of open water may be associated with the inability of the nymphs to tolerate sub-zero temperatures during the winter.

Corbet (1963) reviewed the findings of several workers on the variability in numbers of eggs laid in a single batch by various dragonflies. They ranged from 150 in Perithemis tenera (Jacobs, 1955) to more than 5200 in Gomphus externus (Needham and Heywood, 1929). Ischnura verticalis, a coenagrionid species, is capable of producing 400 eggs at a time (Grieve, 1937).

Egg clutch size was determined in two of the three Type A species. In C. angulatum the mean egg number of 172 contrasted sharply with clutch size in E. boreale which averaged over 500. The latter is also considerably higher than the egg numbers found in Lestes species in this study.

The flying season of the species extends beyond the range established by Walker (1953). C. angulatum have been recorded by Walker from May 26 to July 4, in Saskatchewan and Manitoba. In this study earliest records of

adults were on May 24, 1971. The last record for the season was obtained on July 29, 1971. The flying season for C. resolutum is only slightly different from that of C. angulatum. Newly emerged adults were first seen on May 27, 1971. Adults were last recorded in the field on July 29. Walker (1953) gave May 26 to July 19 as the flying season of this species near Regina.

Enallagma boreale, being a transcontinental species, has a widely ranging flying period from as early as April 29 (Whitehouse, 1941) to the middle of October in British Columbia (Walker, 1953). No dates were cited for Saskatchewan. In the present study E. boreale adults were seen on May 27 through to August 12 in 1971.

4.2 The life-histories of the Type B species

Needham (1903) described the salient features of life cycles of the Lestidae. His keys include all the Lestes species described in this study. His own studies emphasized L. uncata (L. dryas) and L. unguiculatus. He noted that eggs of these species gathered in the middle of July and in the middle of October were apparently at the same stage of development. However, the eggs collected in October hatched within a week of being placed in a bowl of water under laboratory conditions. These two observations provided evidence of a very important controlling mechanism in the life cycles of these species which many investigators since have recognized but few have investigated -- an egg diapause. Diapause in the Type B species has all the characteristics ascribed to this term by Mansingh (1971).

With rare exceptions such as Lestes eurinus (Lutz, 1968), the Lestidae occurring in temperate climates overwinter in the egg stage. The diapausing stage of embryonic development in these eggs is characterized by dark eye spots, distinct body segmentation, appendages, and the presence

of tracheal tubules. This stage of embryonic development in overwintering eggs is almost exclusively associated with the Lestidae among the Odonata (Ando, 1962). Only Sympetrum of the Libellulidae demonstrates embryonic development characteristics of the Lestes type. Gower and Kormondy (1963) obtained two forms of overwintering eggs in L. rectangularis, one in the normal late eye spot stage and one which showed no visible sign of embryonic development which they called the blastula stage. Unfortunately, they failed to demonstrate the viability of the latter eggs. Eggs of L. disjunctus and L. unguiculatus, in which there was no apparent embryonic development, proved to be infertile. In this study the first certain record is presented of a Lestes species which overwinters in an embryonic stage other than the eye spot stage. The species, L. congener, belongs to the Type C group and will be discussed later.

The eggs of L. dryas, L. disjunctus and L. unguiculatus, representing Type B species, all undergo a diapause in late embryonic development in the fall. Pre-diapause development at 21°C was completed in 15 to 18 days. Corbet (1956b) estimated the time required to complete this phase of development in L. sponsa, Aeshna grandis and Sympetrum danae at approximately two weeks. Pre-diapause development was considerably delayed at 16°C in all Type B species.

Corbet (1956b) observed that in L. sponsa diapause development occurs at all temperatures between 5 and 20°C but is completed most rapidly at a temperature estimated at 10°C which is below the lower threshold for hatching. He concluded that diapause development is completed before temperatures fall to 5°C in the field. Synchronized post-diapause development occurs in the spring when temperatures rise. He did not evaluate diapause development under field conditions to confirm his conclusion.

The observations of Needham (1903) suggest that diapause development is completed early enough in the field in L. dryas and L. unguiculatus to permit fall hatching. He stated that in these species egg development stops till the pools are refilled in late autumn.

The present studies showed that diapause development could take place at all temperatures between freezing and 26.5°C in L. disjunctus and even at -6.5°C in L. unguiculatus. The optimal diapause development temperature was approximately 10°C, which, in contrast to the situation in L. sponsa, was well above the threshold hatching temperature of approximately 0°C for these species.

An aspect of diapause development previously uninvestigated in odonate eggs is the effect of photoperiod on this stage. Both L. disjunctus and L. unguiculatus showed indications of accelerated diapause development at long photoperiods. The effect was more marked in L. disjunctus. This may have been compensated for by the ability of L. unguiculatus to undergo diapause development at a lower fall temperature.

What may be described as the first phase of diapause development was essentially completed in the field by October 23 in all species. Some eggs collected as early as October 8 promptly underwent post-diapause development when incubated at 21°C and a 16½ hour photoperiod. These figures correspond to the observations of Needham (1903). Both observations indicate that the temperature controlled Phase I of diapause is completed early enough that hatching should be able to take place if the eggs became wet in the fall. This phase of diapause in itself is,therefore, insufficient to prevent hatching from taking place under field conditions in the fall.

Evidence from this investigation shows for the first time the presence of a second phase of diapause immediately succeeding Phase I. Eggs

which have completed Phase I of diapause development will develop promptly only at a photoperiod longer than 14 hours. Hatching is delayed for several weeks at short photoperiods. The short photoperiod experienced in the field in October is sufficient to retain these species in the egg stage until winter conditions set in. While Phase I of diapause development prevents hatching early in the fall, it is predominantly Phase II which completes synchronization of embryonic development in preparation for hatching in the spring. Synchrony in hatching is not determined solely by the temperature at which diapause development occurs as suggested by Corbet (1956b) for L. sponsa.

The experiments conducted on eggs of Sympetrum sanguineum by several investigators suggest that a photoperiodically controlled diapause phase may be operative also in this species. Gardner (1950a) was able to hatch the eggs of this species, collected in August, in 22 days, although he did not define the conditions under which development took place. Corbet (1963) noted that Longfield was unable to hatch eggs of S. sanguineum in 129 to 149 days. Gardner (1951), finding that eggs of that species laid late in the season failed to hatch until spring, speculated that eggs laid early hatch immediately while those laid later are prevented from hatching until spring. Since there was no indication that these experiments were conducted under controlled photoperiods it is dangerous to accept the speculation of Gardner (1951) of differential development rate based on the time of oviposition.

The majority of Temperate Zone Lestidae oviposit in stems above the water surface. A notable exception is L. sponsa (Corbet, 1963). Hatching has been associated with wetting of the eggs when the ponds become filled with water during the wet season (Needham, 1903; Gardner, 1952; Corbet, 1963).

Only one exception to this pattern has been recognized. Abbé Pierre (1904a, b) noted that eggs of L. viridis laid in the cambium of Salix well above the water hatch without wetting. The pronymphs then drop into the water.

Experiments in this study confirmed the need for wetting as a prerequisite for hatching. Attempts to hatch eggs of the Type B species in an atmosphere containing a 100% relative humidity were unsuccessful. The need for wetting performs several functions: (1) It assures the newly hatched nymphs of a suitable habitat, (2) it plays an important role in synchronizing emergence, and (3) wetting initiates decay in the surface layers of the plant stems, allowing the newly hatched nymphs to escape.

All Lestes species which achieve a late stage of embryonic development before entering diapause oviposit in green plant stems. Such stems were believed to assist in preventing desiccation ,thereby enabling the species to occupy temporary habitats (Kennedy, 1942; Fischer, 1964). In the present study the plants used as oviposition sites are Scirpus which remain green longest in the fall. Since the eggs of all Type B species were deposited in stems growing in permanent water throughout the year, the problem of desiccation occurred only when the Scirpus was killed by an unusually early frost.

The green stems possibly provide moisture for the developing embryos. The latter was not confirmed in this study. However, Salt (1949) noted that embryonic development in the grasshopper Melanoplus bivittatus will not proceed beyond blastokinesis in the absence of adequate moisture. Dempster (1963) noted only one exception to this type of response among all British acridids. Results from this study show that embryonic development in the Type C species, L. congener, whose eggs are laid in dry stems, will not advance beyond blastokinesis even under ideal conditions of temperature

and photoperiod in the absence of water. It therefore seems probable that embryonic development in the Type B Lestes is dependent on the moisture provided by the tissues of the green Scirpus stems.

The egg has evolved as a cold resistant stage to enable the species to survive temperate and subarctic winters. Fischer (1964) noted that occasionally eggs of Lestes nympha which failed to become wet in the spring hatched in the fall, However, the nymphs perished. She associated their inability to survive with cold fall temperatures, and speculated that the eggs, which have an ion concentration thirty times that of the surrounding medium, are probably a better stage in which overwintering in odonates might occur.

Fischer (1958, 1964) appears to be the only investigator who has attempted to determine survival ability of Lestes eggs at freezing temperatures. She noted that viability of eggs of L. sponsa decreases as temperature is lowered below 0°C. Egg survival in the ice at the bottom of a frozen pond was better than in eggs frozen into the surface layers and better still than in the eggs exposed to the air. She attributed the mortality of eggs exposed to the air in part to desiccation associated with freezing. In laboratory experiments, Fischer (1958) obtained 55% survival in eggs of L. sponsa subjected to -20°C.

A very small percentage of the eggs of the Type B species overwinter in the ice. Most are found in broken stems above the ice, covered by 60 or more cm of snow. Approximately 50% survival was obtained in the laboratory at -20 to -22°C. Viability of eggs collected from under the snow throughout the winter remained near 100%. Those eggs which remained exposed to the air did not survive the winter conditions. The insulating properties of the snow cover appear essential to the survival of the species in Western Canada.

Post-diapause development in Type B species requires only a short time, generally 4 to 8 days, at 21°C. Although hatching can be obtained at any temperature above the freezing point, the time required extends to more than 20 days at 4.5°C. These figures were confirmed by field data. Hatching took place after the first week in May, even though the eggs were wet for about a month prior to this time. Fischer (1958) determined the critical hatching temperature in L. sponsa as 14°C. Data for other species are not available.

Needham (1903) implied that hatching in L. dryas and L. unguiculatus in New York state occurred in late autumn, noting that "... they (the eggs) estivate through the remainder of the summer and early autumn. Development stops apparently entirely, and remains stopped till the ponds are refilled in late autumn, and the stem and leaves, now dead, fall into the water." Gardner (1952) came to a similar conclusion in his studies of Lestes dryas in Britain. He collected eggs in the field on June 20, 1949. These hatched in the laboratory on November 17. He managed to rear a single specimen through the winter to emergence on May 31 the following year. He collected nymphs in the penultimate instar on May 20, 1951. Finding that the stages corresponded to his single lab-reared specimen, he concluded that they, too, hatched in the fall. He estimated the duration of the nymphal stage in L. dryas at 195 days.

In the present study L. dryas hatched in the spring, underwent rapid nymphal development and emerged in less than two months. Lestes disjunctus and L. unguiculatus demonstrated a similar nymphal development rate.

Corbet (1956a) estimated the spread of emergence in L. sponsa on the basis of the return of sexually mature males to the study site. He

concluded that the peak of emergence occurred after ten days. Emergence in L. rectangularis extended from 16 to 23 days (Gower and Kormondy, 1963). In this study emergence in the Type B species based on the disappearance of mature nymphs appeared similar to that described for the Type A damselflies. Emergence was spread over ten days, indicating a higher degree of synchrony in nymphal development than shown by Corbet or Gower and Kormondy. As in other Zygoptera (Corbet, 1963), the Lestes in this study showed a diffuse diurnal emergence pattern. They began to emerge at about 10:00 A.M. and continued throughout the day provided that suitable temperatures prevailed.

Gower and Kormondy (1963) recorded a 21 to 24 day lapse between commencement of emergence and the onset of oviposition in L. rectangularis. Approximately 16 days elapsed between emergence and oviposition in L. disjunctus and L. unguiculatus. This time is comparable to that in other Zygoptera noted by Corbet (1963), but considerably longer than in the Type A species. Males returned to the water earlier than the females. Corbet (1956a, 1957a) noted the same thing in L. sponsa and Anax imperator and suggested that this was caused by more rapid maturation of the males rather than their earlier emergence. Such was the case in this study.

Oviposition occurred in tandem in all Type B species. The choice of oviposition sites is of particular importance to the survival of the species. Most Lestes choose oviposition sites well above the water level, or even along the shore or in depressions containing no water (Needham, 1903; Wesenberg-Lund, 1913; Gower and Kormondy, 1963; Bick and Hornuff, 1965). While a variety of vegetation is usually available, preference is commonly shown for a particular species (Corbet, 1963). Lestes rectangularis oviposited almost exclusively in leaves of Typha (Gower and Kormondy, 1963);

L. eurinus chose Sparganium americanum (Lutz and Pittman, 1968); L. unguiculatus oviposited in flowering stems of Sparganium in Indiana (Bick and Hornuff, 1965); L. disjunctus australis oviposited in stems of Eleocharis (Bick and Bick, 1961). In this study all Type B species oviposited exclusively in green stems of Scirpus. The stems chosen were always those which bordered a stand or those growing singly or in small clumps. It is these stems which are the first to be broken during late autumn storms and become covered with snow early in the season. The stems in the centre of a Scirpus stand often remain standing above the snow throughout the winter. As was noted earlier, a critical factor in the survival of eggs of these species is that they be protected by snow cover. Gardner (1950b) noted similarly that stems in which eggs of Aeshna mixta are laid are broken down and submerged by autumn gales.

The flying seasons of the Type B species in the study area generally fall well within the range set out by Walker (1953) for Canadian species. Emergence was observed as early as June 24 in L. disjunctus in British Columbia (Whitehouse, 1941). The latest seasonal record is from Saskatchewan where the species was taken up to October 16 (Walker, 1953). In this study, L. disjunctus began emergence during the first week of July and were last seen on August 20. The flying period of L. unguiculatus in Manitoba and Saskatchewan as reported by Walker (1953) is from June 19 to October 7. The dates describing the flying season of L. unguiculatus in this study are similar to those recorded for L. disjunctus.

Walker noted that emergence of L. dryas begins usually more than a week before that of L. unguiculatus. A similar observation was made in the present study. While Walker reported June 14 as the earliest date of emergence in the prairie provinces, newly emerged adults were not seen until

the last week in June in this study. Termination of the flying season for this species was not determined. Walker records August 30 as the last date of observation on the prairies.

4.3 The Life-histories of the Type C species

In spite of its widespread distribution throughout temperate North America (Walker, 1953), Lestes congener has passed almost unnoticed by odonatologists. It has a univoltine life cycle which is comparable to that of Type B species. Therefore, it will be discussed only with respect to its differences.

The egg stage of L. congener is unique among the Lestidae studied to date. Rather than entering diapause in the eye spot stage as is typical of other Lestes (Ando, 1962), L. congener diapauses just prior to blastokinesis, similar to Japanese Aeshnidae (Ando, 1962). The diapause was shown to have all the characteristics ascribed to this term by Mansingh (1971). Most of the eggs in the field did not complete the 'refractory phase' of development until November. Diapause development progressed at an optimal rate at 10°C, as in the Type B species discussed earlier. While the Type B species displayed a photosensitive second phase of diapause after the temperature controlled 'refractory phase' was complete, no such photosensitive diapause phase was observed in L. congener. Late oviposition and lengthy diapause development were sufficient protection against fall hatching.

The early stage at which diapause occurs in L. congener necessitates a substantial amount of development in the spring before hatching. This development begins when temperatures exceed 0°C. Eggs are capable of developing to the eye spot stage just prior to hatching, but then stop development if the appropriate hatching temperature is not obtained. Hatching takes place if water temperature exceeds 4.5°C. The stage at which this temperature

induced arrest occurs is coincidental with the diapause stage of Type B species. It is possible that the earlier diapause stage of L. congener has evolved from that in the Type B species. The ability of L. congener to cease development again in the later stage of embryonic development may reflect its evolutionary history.

Moisture plays a critical role in the development of L. congener eggs. As in the Type B species, the eggs will not undergo any post-diapause development unless they become wet.

Photoperiod has no effect on diapause or post-diapause development in L. congener. Synchronization of development is controlled by other mechanisms in this species. Diapause followed by low temperature at its termination play a dominant role in synchronization. The wetting requirement is another obvious synchronizer. The delay of hatching by low temperatures enables those eggs in which embryonic development has lagged to catch up, thus acting as a third synchronizer.

Hatching in L. congener occurs approximately two weeks later than in the Type B species. However, the difference in development is reduced to a week at emergence. This species undergoes the most rapid nymphal development of all Lestes studied, completing this phase in fifty days. Emergence extended over a three week period, the majority of adults emerging in the first ten days.

Sexual maturation requires three weeks, perhaps a week longer than in the Type B species or the species reported by other workers. As a result, oviposition is extended into the late summer and early fall. In 1971 oviposition was first observed on August 12. Bad weather delayed oviposition to August 20 in 1970.

Montgomery (1925) reported on the behavior of an ovipositing L. congener female. The observations made in this study agree with his

description of the egg size and positioning of eggs in the stems. He reported that L. congener used Scirpus stems as oviposition sites but did not state whether green or dry stems were used. Kennedy (1915) recorded this species ovipositing in a small willow stem. Walker (1953) observed oviposition in cat-tail leaves and other vegetation including willow: "They were apparently indifferent as to the condition of the foliage, whether dead or alive, and many were seen ovipositing on dead, dried cat-tail leaves as well as green ones." In this study L. congener almost exclusively selected dry stems of Scirpus as oviposition sites. Those which were bent and broken, lying within a foot of the water surface, were preferred. Oviposition took place in such stems throughout a stand of Scirpus rather than being selective as in Type B species. Since pre-diapause development does not proceed beyond blastokinesis, the eggs apparently have no moisture requirement until the following spring. The eggs of this species are thick-walled and much more resistant to desiccation than eggs of Type B species. Both of these factors permit L. congener to oviposit in the dry stems which are much more readily available in the fall. The choice of bent and broken stems throughout a Scirpus stand insures the accumulation of protective snow cover over the eggs during the winter.

The eggs are considerably more resistant to low winter temperatures than L. sponsa (Fischer, 1958) or the Type B species in this study. Eggs of L. congener hatched after being subjected to -30°C in the laboratory and -35°C in the field, whereas those of the Type B species were no longer viable below -22°C. This may be an evolutionary advance over the other Lestes towards occupation of more northerly habitats.

Lestes congener is the latest Canadian species of Lestes to reach the adult stage (Walker, 1953). Walker recorded the flying season of this

species in Ontario as being from August 9 to October 13. Whitehouse (1941) gave July 10 to November 10 as the flying dates in British Columbia. In this study adults of L. congener were observed from July 21, 1970 to October 1, on which date they were still in tandem.

4.4 Seasonal succession and species associations

Kormondy and Gower (1965) investigated the life history variations of seventeen species of Odonata with regard to their ability to occupy one habitat. Inconsistencies in their data from the two-year study resulted in little progress toward explanation of this association.

Benke (1969) showed distinct differences in temporal and spatial distributions and in food preferences in the nymphs of seven genera of dragonflies which he studied. Although he found niche separation to be quite distinct between genera, the life histories of species within any one genus were extremely similar. The differences between species were too subtle to be separated by his technique.

In the present study temporal segregation was demonstrated among the three Types of damselflies. The Type A species were completing their nymphal development in the spring at the time of hatching of the Type B species. The Type C species hatched when the Type A species began emerging. The Type B species were at this time nearing the mid-point of their nymphal development. These results are similar to those of Benke (1969). He found that nymphs of two genera of mud-dwelling dragonflies, Ladona and Libellula, were temporally separated by 3 to 5 months and were 3 to 4 instars apart at a given time. Tetragoneuria and Celithemis nymphs were separated by 2 to 3 months with at least a 2 instar separation at a given time.

Food preferences at different instars were not investigated. However, Chutter (1961) found that different instars of Pseudagrion salisburyense

eat prey whose size is proportional to their own. Although an overlap exists in the nymphal stages of the three groups during a portion of their life cycles, the newly hatched nymphs of the Type B Lestes, which hatch early in May, are believed to be too small to be captured for food by the large Type A nymphs. By the time the Type B nymphs have obtained prey dimensions, the Type A nymphs have either emerged or ceased feeding in preparation for emergence. Similarly newly hatched Type C species are too small to be preyed upon by the Type B nymphs.

No temporal segregation was present among members of a particular species Type. The Type A species hatched at approximately the same time and subsequently completed their nymphal and adult development at the same time. A large number of nymphal instars was found in late summer but this was reduced to four in the overwintering population. Similarly, synchronous hatching in the Type B species insures that the nymphs develop uniformly. So long as this fairly narrow size differential is maintained in the population, the species in each Type, though very voracious, appear to avoid attacking each other and members of their own kind, thereby allowing species coexistence. In the light of Fischer's (1961) observations on L. nympha, closely synchronized hatching and nymphal development appear more important in the Lestidae. Lestine cannibalism, especially among young nymphs, is higher than in coenagrionids, and occurs whether food is abundant or not. If inter- or intraspecific predation does occur, it serves only to eliminate stragglers, a feature beneficial to the association as a whole.

Evidence of spatial separation was found among the Type A and the Type B species. In the Type A species, E. boreale was restricted to the deep, more exposed portion of the study pond; C. resolutum showed a preference for the shallow marshy area overgrown with vegetation; C. angulatum was found

in both habitats. In the Type B association, L. dryas, because of its larger size and presence in smaller numbers compared to that of L. disjunctus and L. unguiculatus, appears not to fit the requirements of the association. It showed a preference for temporary habitats such as roadside ditches, suggesting that there is only a partial spatial overlap between it and the other two species.

Partial temporal overlap between the late instars of the Type B nymphs and the Type C species was neutralized by spatial separation of the species. Lestes congener remained in deep water during the period of late nymphal development and emergence in the Type B species. L. congener nymphs overlapped with the newly hatched Type A nymphs after July 15, but because of their size differential, it is not expected that nymphs of the Type A species were preyed upon by L. congener even though they occupied the same habitat.

The successive use of the same emergence sites by all members of the Type A, B and C species suggests that perhaps the limiting factor for the survival of the species is the absence of adequate weedy shore area in which completion of nymphal development and emergence might occur. Such habitats are preferred by damselfly nymphs because they warm up rapidly in the spring bringing about temperature conditions suitable not only for nymphal development but also for the proliferation of prey organisms such as Daphnia, Diaptomus and Diptera larvae. The rapidly changing temperature of the shallow water enables the nymphs to determine whether air temperature is suitable for emergence.

Although a considerable degree of synchrony demonstrated in hatching and nymphal development appeared to separate the life cycles of the three species Types, a greater overlap occurred among the adults. While no

species interaction was observed at the adult level at the study pond, a distinct drop in numbers of the Type A species, especially C. angulatum and C. resolutum, was noted soon after the beginning of emergence of the Type B group. The disappearance of the Type A group at this time probably is the result of aging and natural mortality. However, the possibility of predator-prey interaction between the groups should not be discounted and remains open to investigation. A single observation away from the study area was made of the capture of an adult Enallagma male by a Lestes adult. The Enallagma male would undoubtedly have become a meal for the lestid had the two not been separated. It is noteworthy that the largest adults are those belonging to the Type B association. It is, therefore, possible that the smaller Type A species have evolved life cycles which allow minimum interaction with the Type B species for this reason.

The coexistence of L. congener adults with significant numbers of Type B species suggests minimal competitive interaction between these two groups. Emergence and early oviposition in adults of L. congener overlapped with the oviposition period of L. disjunctus and L. unguiculatus. However, since L. congener oviposits in dry plant stems while the Type B species prefer green plants, competitive interaction in this phase was avoided. With the possible exception of Enallagma boreale, L. congener has the longest flying period of any of the damselflies studied, extending from mid July into October. Perhaps this extended flying period compensates for the cool, often inclement weather during which this species must reproduce. Lestes congener fits Moore's (1953) suggestion that the later in the season a species emerges, the longer its flying season.

With the exception of E. boreale which preferred to oviposit in areas having extensive open water, adults of the Type A species displayed

no behavioral differences in mating and oviposition. Similarly, L. disjunctus and L. unguiculatus used the same oviposition sites at the same time. The two species were frequently seen ovipositing in the same stem within one inch of one another.

The striking similarities between the adult females of C. angulatum and C. resolutum of the Type A group, and between L. disjunctus and L. unguiculatus of the Type B group coupled with the similarity in life cycles and behavior of these damselflies, suggest that morphological differences in their copulatory structures allow the species to remain reproductively isolated. Walker (1953) noted such differences in the mesostigmal laminae of the females and used these in the preparation of his keys. The sculpturing of these plates on the dorsal surface of the thorax must complement the shape of the male claspers in order that tandem position might be achieved. Males were often seen attempting to achieve tandem position with females, apparently of another species, then flying away when the attempt failed.

Kormondy (1959) explained the successful coexistence of three closely related species of Tetragoneuria in a similar manner: "Barring physiological or genetic factors or differences in behavior, it would seem, therefore, that morphological incongruities between the conformation of the female head and the terminal abdominal appendages of the male effect isolation by preventing the male of one species from grasping the female of another".

4.5 Synchronization of development

Although emergence pattern was not measured in this study by direct count, the rate of disappearance of nymphs suggested a high degree of synchrony of emergence. It was not, however, directly comparable to that

described by Corbet (1954) for 'spring species'. Corbet and Corbet (1958) noted that in Anax imperator, a 'spring species', 50% of the annual population emerged within 3 days and 90% within 10 days. On the other hand, in Aeshna cyanea, a 'summer species', 50% emergence was achieved only after 25 days. Emergence was certainly more synchronized in all species studied here than in A. cyanea. In all instances emergence was complete within three weeks, one-half the total time required by Anax imperator (Corbet, 1954).

None of the species in this study meet the other requirements established for 'spring species'. Corbet (1954) described 'spring species' as overwintering in the final instar. At most, only about 10% of the Type A species were in this instar during the winter. The remainder were generally in the penultimate and antepenultimate instars. The Type B and C species overwintered in the egg stage. All seven species would, by this definition, be classified as 'summer species'. It is clear from this investigation that the species studied fit neither category of Corbet's classification.

Gower and Kormondy (1963), Eller (1964) and Benke (1969) had similar difficulty in interpreting their results in terms of Corbet's scheme. Their conclusions might best be summarized by the statement made by Gower and Kormondy for Lestes rectangularis: "The emergence of rectangularis is not marked by the degree of synchronization demonstrated by A. imperator, yet the onset is not so gradual as that described for a summer species." Benke (1969) proposed that nearctic odonates be classified as either 'synchronous' or 'asynchronous' depending on their pattern of emergence. The usefulness of Benke's proposal is questionable since he does not precisely define these terms.

It is suggested that Corbet's classification scheme be modified in order to accommodate the North American species. Since his categories of 'spring' and 'summer' species carry obvious seasonal connotations, they should be used to distinguish those species emerging in the spring from those emerging

in the summer. Corbet's concept of 'spring species' should perhaps be re-defined to include Odonata which emerge only in the spring regardless of whether they are univoltine or semivoltine and whether they diapause in the final instar or several instars. Summer species should include those species emerging in the summer after overwintering either in the nymphal or egg stage. Both 'spring' and 'summer' categories should be further subdivided to account for species emerging synchronously and those emerging asynchronously. Further, the temporal limitations defining synchronous emergence established by Corbet (1958) from data on Anax imperator should perhaps be relaxed from 90% emergence to 50% emergence in the first 10 days. This would allow species such as Lestes rectangularis (Gower and Kormondy, 1963), Pachydiplax longipennis (Eller, 1964) and the species in this study to be described as synchronously emerging species. Asynchronous species will have reached 50% emergence after 25 days from the beginning of emergence as in Aeshna cyanea (Corbet and Corbet, 1958). Under this new scheme Anax imperator (Corbet, 1954), Pachydiplax longipennis (Eller, 1964), Tetragoneuria cynosura (Lutz, 1963) and the Type A species in this study would fall into the category of synchronous spring species. Lestes rectangularis (Gower and Kormondy, 1963), the 'synchronous' species described by Benke (1969), and the Type B and C species in this study could be classified as synchronous summer species. Lestes sponsa (Corbet, 1956a) may perhaps be classified as an asynchronous spring species. Aeshna cyanea (Corbet and Corbet, 1958), Sympetrum striolatum (Corbet, 1956a), and several species of Zygoptera described by Kormondy and Gower (1965) are examples of asynchronous summer species.

This scheme introduces a problem in classifying those species whose emergence straddles the spring and summer seasons or those species whose emergence period may fluctuate from one season to another depending on environmental variables, as shown by Kormondy and Gower (1965). Certain modifications may be necessary in this regard.

A principal consequence of synchronized emergence in Odonata often mentioned but seldom demonstrated is to insure synchronous sexual maturation (Corbet et al., 1960; Lutz, 1968). This phase, in order to be accomplished in a relatively short time, requires that all specimens be in a similar state of preparedness for emergence. In this study the synchronous hatching and nymphal development of the Type A, B, and C species insured that mating and oviposition would take place at the peak of population density with minimum overlap between species Types.

A second function of synchronized emergence may be related to the availability of food for the newly emerged adults. It was noted that emergence in the Type A species of damselflies closely corresponded with mass emergence of Chaoborus and chironomid adults. These were observed to be the main food of the maturing damselfly adults. The emergence of Type B and Type C adults was not associated with mass emergence of any particular insects.

A hitherto uninvestigated role of synchronized nymphal development and emergence may be to permit optimal utilization of emergence sites and areas of a habitat that are rich in food for the rapidly developing nymphs. It appears more than a coincidence that the species Types are so uniformly temporally separated during the spring and summer months. Eggs of the Type B species hatch when Type A species are nearing completion of nymphal development. The Type C species hatch when the Type A are emerging and the Type B species are half way through their development. Finally, the new generation of Type A species hatches when the Type B are emerging and the Type C are completing their nymphal development. This provides a three phase succession of mature nymphs in the shallow, weedy shore areas where rapid development and emergence occurs. A close synchrony in nymphal development within a Type appears essential to protect the species from inter- and intraspecific

predation where older, larger nymphs might be tempted to feed on the smaller individuals in a crowded habitat. Temporal separation of the species Types minimizes inter-Type predation because young nymphs do not reach vulnerable size until species of the preceding Type are about to emerge.

A most important feature of the life cycles of the damselflies studied evidently has been the evolution of mechanisms which synchronize and integrate the development of the various species in such a way as to prevent inter- and intraspecific predation within Types on the one hand and to permit maximum use of a restrictive habitat and food supply on the other. The latter has been made possible by a seasonal succession with a minimum of inter-Type interaction. This efficient association of several species has been achieved through a complex interaction among inherent control mechanisms involving diapause and differential rates of development and cyclic seasonal qualities of temperature and photoperiod.

5. SUMMARY AND CONCLUSIONS

1. A study was made of the environmental control of seasonal succession and synchronization of development in seven pond-dwelling species of damselflies (Odonata: Zygoptera) in central Saskatchewan.
2. Regular sampling in the field, and laboratory experiments during 1969, 1970, and 1971 provided the data for the study.
3. The seven species of damselflies were separated into three categories based on the developmental stage in which they overwintered. Type A species overwintered as nymphs. Type B as eggs in the late eye spot stage of embryonic development, and Type C as eggs in the pre-blastokinesis stage of embryonic development. Type A species included Coenagrion angulatum, C. resolutum and Enallagma boreale; Lestes disjunctus disjunctus, L. unguiculatus and L. dryas represented the Type B category; Lestes congener had Type C characteristics.
4. A distinct seasonal succession of the three species Types was found. The Type A species overwintered mainly in the last three nymphal instars. Emergence took place during late May and early June. Oviposition occurred during June and July. The eggs hatched within three weeks of oviposition and nymphs developed during the remainder of the summer. The eggs of the Type B species hatched during the first week of May. Emergence began in the first week of July, oviposition two weeks later. Embryonic development to the stage in which the species overwinter was completed by the end of August. The eggs of the Type C species hatched during the last week of May. Nymphal development was completed by mid July. Oviposition began during the second week of August and continued into late September. Pre-diapause embryonic development was completed within ten days of oviposition.

5. A high degree of synchrony was observed in the emerging population of all three species Types. Generally, peak emergence occurred within ten days of the appearance of the first adults.
6. A diapause, whose induction was influenced by low temperature and short photoperiod, occurred in penultimate and final instar nymphs of the Type A species. Diapause prevented emergence in the fall.
7. Diapause development is accelerated by low overwintering temperature. The temperature controlled phase was terminated by January. Short photoperiod prolongs developmental arrest in the penultimate and final instars in the fall. This inhibition disappeared from the penultimate instar by January but continued in the final instar till spring. However, the threshold photoperiod became lower as winter progressed. The threshold photoperiod for development ranges from 12 to 14 hours.
8. Synchronization of emergence in the Type A species is accomplished by diapause, differences in thermal growth coefficients in different instars, and a threshold emergence temperature higher than that for nymphal development.
9. Eggs of the Type B species enter a diapause when embryonic development is essentially complete. Diapause development is initially influenced mainly by temperature (Phase I) and later by photoperiod (Phase II).
10. Phase I of diapause development occurs in the field during September and October and prevents hatching when environmental conditions are otherwise suitable. It occurs most rapidly at about 10°C and appears to be accelerated by long photoperiods.
11. Phase II of diapause development prevents hatching following completion of Phase I of diapause development in October. Short photoperiod maintains the diapause condition. Phase II of diapause development was

terminated in January, after which the eggs were prevented from hatching by the winter conditions. The threshold photoperiod for termination of Phase II of diapause development is approximately 12 hours.

12. Hatching of eggs in the Type B species will occur only if the eggs are in contact with water following completion of diapause development.
13. Synchronization of emergence in the Type B species is accomplished by the combined effect of the two phases of diapause in maintaining the eggs in identical stages of embryonic development during the winter, by synchronous initiation of the hatching process by wetting of the eggs in the spring, and by rapid nymphal development.
14. Eggs of the Type C species enter a diapause when embryonic development approaches blastokinesis. Diapause development is under the influence of temperature only.
15. Diapause development occurs most rapidly at 10°C. It was terminated in the field in November and early December.
16. Winter conditions prevent eggs from hatching after completion of diapause development.
17. Post-diapause embryonic development and hatching will take place only if the eggs are in contact with water.
18. Embryonic development takes place at all temperatures between 0°C and 26.5°C. Hatching will not occur below 4.5°C; embryonic development can cease at the late eye spot stage to await suitable hatching temperatures.
19. Synchrony in emergence of the Type C species is produced through diapause which retains the eggs in identical stages of embryonic

development in the fall, by simultaneous wetting of the eggs in the spring, by different development and hatching thresholds, and by rapid nymphal development in the spring.

20. Temporal separation of species Types and synchronization of development and emergence within Types enables the species to reach sexual maturity at the same time, to emerge in synchrony with prey insects, and to use shallow habitats with little interaction between nymphs of different species Types.

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APPENDIX

Appendix A. Seasonal distribution and relative abundance of damselfly nymphs in the shallow portion of the study pond.

Collecting date	No. of specimens							
	C. angulatum	C. resolutum	E. boreale	L. dryas	L. disjunctus	L. unguiculatus	L. congener	Total
1.5.70*	7							7
7.5.70*	90		20					110
14.5.70	83	39						122
20.5.70	136	66						202
28.5.70	132	30						162
6.6.70	141	18			7	3		169
12.6.70	17	8			5	2		32
18.6.70	22	14			67	74	4	181
22.6.70		1			60	99	20	180
26.6.70		1		1	19	38	15	74
2.7.70					18	26	70	114
8.7.70					75	54	120	249
15.7.70					13	5	169	187
21.7.70	1				1	1	178	181
30.7.70	214	31			1		34	280
6.8.70	296	51	7				10	364
14.8.70	113	20	1					134
20.8.70	291	158	5					454
27.8.70	80	34						114
4.9.70	256	44	3					303
10.9.70	139	29	3					171
17.9.70	252	74	18					344
23.9.70	67	17	1					85
1.10.70	79	5	3					87
14.10.70	121	85						206
20.10.70	140	91						231
26.10.70	67	60						127
3.11.70	53	31						84
11.1.71	213	81						294
23.2.71	278	68						346
21.4.71	75	8						83
30.4.71	134	15						149
6.5.71	137	13						150
13.5.71	147	25						172
20.5.71	92	13						105
27.5.71	69	11						80
3.6.71	161	21		1	16	9		208

Appendix A. Continued

Collecting date	No. of specimens							
	C. angulatum	C. resolutum	E. boreale	L. dryas	L. disjunctus	L. unguiculatus	L. congener	Total
10.6.71	31	6		2	20	10		69
18.6.71	10	4			26	52	10	102
24.6.71					8	21	29	58
1.7.71	3				31	31	70	135
9.7.71					25	23	33	81
15.7.71	1				14	30	34	79
22.7.71	156	47			1	5	29	238
29.7.71	49	33					43	125

*Collected in deep portion of study pond.

Appendix B. Seasonal changes in age structure of C. angulatum nymphs collected in the shallow portion of the study pond.

Collecting date	Total Specimens	Instar								
		F	P	A	A-1	A-2	A-3	A-4	A-5	A-6
1.5.70	7	-	5	2	-	-	-	-	-	-
7.5.70	90	2	47	33	8	-	-	-	-	-
14.5.70	83	33	43	6	1	-	-	-	-	-
20.5.70	136	132	4	-	-	-	-	-	-	-
28.5.70	132	132	-	-	-	-	-	-	-	-
6.6.70	141	141	-	-	-	-	-	-	-	-
12.6.70	17	17	-	-	-	-	-	-	-	-
18.6.70	22	22	-	-	-	-	-	-	-	-
22.6.70	0	-	-	-	-	-	-	-	-	-
26.6.70	0	-	-	-	-	-	-	-	-	-
2.7.70	0	-	-	-	-	-	-	-	-	-
8.7.70	0	-	-	-	-	-	-	-	-	-
15.7.70	0	-	-	-	-	-	-	-	-	-
21.7.70	1	-	-	-	-	-	-	-	1	-
30.7.70	214	-	-	-	-	5	19	68	96	26
6.8.70	296	-	-	-	2	21	98	105	57	13
14.8.70	113	-	-	5	5	29	31	23	16	4
20.8.70	291	-	-	17	47	117	70	31	7	2
27.8.70	80	-	2	21	25	19	13	-	-	-
4.9.70	256	-	26	74	64	58	24	9	1	-
10.9.70	139	1	24	18	52	30	11	3	-	-
17.9.70	252	3	23	26	100	71	14	5	-	-
23.9.70	67	2	22	12	21	8	2	-	-	-
1.10.70	79	15	43	13	7	1	-	-	-	-
14.10.70	121	33	62	21	5	-	-	-	-	-
20.10.70	140	28	81	23	6	2	-	-	-	-
26.10.70	67	16	38	11	1	1	-	-	-	-
3.11.70	53	13	30	9	1	-	-	-	-	-
11.1.71	213	42	115	52	4	-	-	-	-	-
23.2.71	278	92	144	36	5	1	-	-	-	-
21.4.71	75	18	45	8	3	1	-	-	-	-
30.4.71	134	28	83	19	4	-	-	-	-	-
6.5.71	137	19	90	21	6	1	-	-	-	-
13.5.71	147	41	76	30	-	-	-	-	-	-
20.5.71	92	80	12	-	-	-	-	-	-	-
27.5.71	69	64	5	-	-	-	-	-	-	-
3.6.71	161	161	-	-	-	-	-	-	-	-
10.6.71	31	31	-	-	-	-	-	-	-	-
18.6.71	10	10	-	-	-	-	-	-	-	-
24.6.71	0	-	-	-	-	-	-	-	-	-
1.7.71	3	3	-	-	-	-	-	-	-	-
9.7.71	0	-	-	-	-	-	-	-	-	-
15.7.71	1	-	-	-	-	-	-	-	1	-
22.7.71	156	-	-	-	-	2	13	72	53	16
29.7.71	49	-	-	-	1	4	11	17	10	6

Appendix C. Seasonal changes in age structure of C. resolutum nymphs collected in the shallow portion of the study pond.

Collecting date	Total Specimens	Instar								
		F	P	A	A-1	A-2	A-3	A-4	A-5	A-6
1.5.70	0	-	-	-	-	-	-	-	-	-
7.5.70	0	-	-	-	-	-	-	-	-	-
14.5.70	39	9	21	8	-	1	-	-	-	-
20.5.70	66	20	46	-	-	-	-	-	-	-
28.5.70	30	26	4	-	-	-	-	-	-	-
6.6.70	18	18	-	-	-	-	-	-	-	-
12.6.70	8	8	-	-	-	-	-	-	-	-
18.6.70	13	13	-	-	-	-	-	-	-	-
22.6.70	1	1	-	-	-	-	-	-	-	-
26.6.70	1	1	-	-	-	-	-	-	-	-
2.7.70	0	-	-	-	-	-	-	-	-	-
8.7.70	0	-	-	-	-	-	-	-	-	-
15.7.70	0	-	-	-	-	-	-	-	-	-
21.7.70	0	-	-	-	-	-	-	-	-	-
30.7.70	31	-	-	-	1	5	6	4	13	2
6.8.70	51	-	-	3	9	21	10	4	4	-
14.8.70	20	-	-	-	-	14	3	3	-	-
20.8.70	158	-	4	16	45	36	36	17	4	-
27.8.70	34	-	6	13	4	11	-	-	-	-
4.9.70	44	-	6	15	6	16	1	-	-	-
10.9.70	29	-	10	10	4	5	-	-	-	-
17.9.70	74	1	18	16	26	13	-	-	-	-
23.9.70	17	-	7	3	6	1	-	-	-	-
1.10.70	5	2	3	-	-	-	-	-	-	-
14.10.70	85	10	67	7	1	-	-	-	-	-
20.10.70	91	9	64	15	3	-	-	-	-	-
26.10.70	60	8	44	7	1	-	-	-	-	-
3.10.70	31	4	21	6	-	-	-	-	-	-
11.1.71	81	14	59	8	-	-	-	-	-	-
23.2.71	68	7	52	9	-	-	-	-	-	-
21.4.71	8	2	6	-	-	-	-	-	-	-
30.4.71	15	3	8	4	-	-	-	-	-	-
6.5.71	13	1	11	1	-	-	-	-	-	-
13.5.71	25	1	21	3	-	-	-	-	-	-
20.5.71	13	3	9	1	-	-	-	-	-	-
27.5.71	11	8	3	-	-	-	-	-	-	-
3.6.71	21	19	2	-	-	-	-	-	-	-
10.6.71	6	6	-	-	-	-	-	-	-	-
18.6.71	4	4	-	-	-	-	-	-	-	-
24.6.71	0	-	-	-	-	-	-	-	-	-
1.7.71	0	-	-	-	-	-	-	-	-	-
9.7.71	0	-	-	-	-	-	-	-	-	-
15.7.71	0	-	-	-	-	-	-	-	-	-
22.7.71	47	-	-	-	2	3	7	4	29	2
29.7.71	33	-	-	-	5	11	13	3	1	-

Appendix D. Seasonal changes in age structure of *E. boreale* nymphs collected in the study pond.

Collecting date	Total Specimens	Instar								
		F	P	A	A-1	A-2	A-3	A-4	A-5	A-6
29.4.69*	14	5	7	1	1	–	–	–	–	–
6.5.69	27	12	10	3	1	1	–	–	–	–
20.5.69	8	5	3	–	–	–	–	–	–	–
27.5.69	7	6	1	–	–	–	–	–	–	–
3.6.69	6	5	1	–	–	–	–	–	–	–
9.6.69	7	6	1	–	–	–	–	–	–	–
20.6.69	2	2	–	–	–	–	–	–	–	–
8.7.69	1	1	–	–	–	–	–	–	–	–
14.7.69	0	–	–	–	–	–	–	–	–	–
31.7.69	89	–	–	–	2	50	20	16	1	–
7.8.69	114	–	–	6	31	33	26	11	6	1
14.8.69	73	–	4	17	15	11	21	5	–	–
20.8.69	52	–	8	9	12	7	15	1	–	–
28.8.69	63	–	6	6	7	12	16	14	2	–
5.9.69	37	–	3	2	2	8	19	3	–	–
12.9.69	36	2	5	6	1	8	9	5	–	–
19.9.69	23	3	4	1	1	3	9	2	–	–
28.9.69	5	1	–	–	–	4	–	–	–	–
1.5.70	0	–	–	–	–	–	–	–	–	–
7.5.70	20	16	4	–	–	–	–	–	–	–
14.5.70**	0	–	–	–	–	–	–	–	–	–
20.5.70	0	–	–	–	–	–	–	–	–	–
28.5.70	0	–	–	–	–	–	–	–	–	–
6.6.70	0	–	–	–	–	–	–	–	–	–
12.6.70	0	–	–	–	–	–	–	–	–	–
18.6.70	0	–	–	–	–	–	–	–	–	–
22.6.70	0	1	–	–	–	–	–	–	–	–
26.6.70	0	1	–	–	–	–	–	–	–	–
2.7.70	0	–	–	–	–	–	–	–	–	–
8.7.70	0	–	–	–	–	–	–	–	–	–
15.7.70	0	–	–	–	–	–	–	–	–	–
21.7.70	0	–	–	–	–	–	–	–	–	–
30.7.70	0	–	–	–	–	–	–	–	–	–
6.8.70	7	–	–	–	2	3	–	1	1	–
14.8.70	1	–	–	–	–	1	–	–	–	–
20.8.70	5	–	–	2	2	1	–	–	–	–
27.8.70	0	–	–	–	–	–	–	–	–	–
4.9.70	3	–	–	–	1	1	–	1	–	–
10.9.70	3	–	–	–	–	1	1	1	–	–
17.9.70	18	1	1	5	6	4	1	–	–	–
23.9.70	0	–	–	–	–	–	–	–	–	–
1.10.70	4	3	1	–	–	–	–	–	–	–
14.10.70	0	–	–	–	–	–	–	–	–	–

Appendix D. Continued ...

Collecting date	Total Specimens	Instar								
		F	P	A	A-1	A-2	A-3	A-4	A-5	A-6
20.10.70	0	-	-	-	-	-	-	-	-	-
26.10.70	0	-	-	-	-	-	-	-	-	-
3.11.70	0	-	-	-	-	-	-	-	-	-
11.1.71	0	-	-	-	-	-	-	-	-	-

*Samples taken in deep portion of the study pond.
**Sampling area changed. All subsequent sampling done in shallow marshy portion of the study pond.

Appendix E. Seasonal changes in age structure of L. disjunctus nymphs collected in the shallow portion of the study pond.

Collecting date	Total Specimens	Instar						
		F	P	A	A-1	A-2	A-3	A-4
28.5.70	0	-	-	-	-	-	-	-
6.6.70	7	-	-	-	3	4	-	-
12.6.70	5	-	-	1	4	-	-	-
18.6.70	67	-	20	31	16	-	-	-
22.6.70	60	1	48	8	3	-	-	-
26.6.70	19	14	4	1	-	-	-	-
2.7.70	18	16	2	-	-	-	-	-
8.7.70	75	75	-	-	-	-	-	-
15.7.70	13	13	-	-	-	-	-	-
21.7.70	1	1	-	-	-	-	-	-
30.7.70	0	-	-	-	-	-	-	-
27.5.71	0	-	-	-	-	-	-	-
3.6.71	16	-	-	-	-	-	16	-
10.6.71	20	-	-	1	7	-	12	-
18.6.71	26	-	10	11	5	-	-	-
24.6.71	8	3	5	-	-	-	-	-
1.7.71	31	23	8	-	-	-	-	-
9.7.71	25	25	-	-	-	-	-	-
15.7.71	14	14	-	-	-	-	-	-
22.7.71	1	1	-	-	-	-	-	-
29.7.71	0	-	-	-	-	-	-	-

Appendix F. Seasonal changes in age structure of L. unguiculatus nymphs collected in the shallow portion of the study pond.

Collecting date	Total Specimens	Instar						
		F	P	A	A-1	A-2	A-3	A-4
28.5.70	0	-	-	-	-	-	-	-
6.6.70	3	-	-	-	-	-	3	-
12.6.70	2	-	-	-	1	1	-	-
18.6.70	74	-	18	43	13	-	-	-
22.6.70	99	2	76	19	2	-	-	-
26.6.70	38	21	15	2	-	-	-	-
2.7.70	26	26	-	-	-	-	-	-
8.7.70	54	54	-	-	-	-	-	-
15.7.70	5	5	-	-	-	-	-	-
21.7.70	1	1	-	-	-	-	-	-
30.7.70	0	-	-	-	-	-	-	-
27.5.71	0	-	-	-	-	-	-	-
3.6.71	9	-	-	-	-	-	9	-
10.6.71	10	-	-	-	3	7	-	-
18.6.71	52	-	11	31	10	-	-	-
24.6.71	21	5	11	4	1	-	-	-
1.7.71	31	18	12	1	-	-	-	-
9.7.71	23	23	-	-	-	-	-	-
15.7.71	30	30	-	-	-	-	-	-
22.7.71	5	5	-	-	-	-	-	-
29.7.71	0	-	-	-	-	-	-	-

Appendix G. Seasonal changes in age structure of L. congener nymphs collected in the shallow portion of the study pond.

Collecting date	Total Specimens	Instar					
		F	P	A	A-1	A-2	A-3
12.6.70	0	-	-	-	-	-	-
18.6.70	4	-	-	-	4	-	-
22.6.70	20	-	-	2	16	1	1
26.6.70	15	-	-	9	6	-	-
2.7.70	70	-	33	34	1	2	-
8.7.70	120	25	86	6	3	-	-
15.7.70	169	144	24	1	-	-	-
21.7.70	178	170	8	-	-	-	-
30.7.70	34	34	-	-	-	-	-
6.8.70	11	11	-	-	-	-	-
14.8.70	-	-	-	-	-	-	-
10.6.71	0	-	-	-	-	-	-
18.6.71	10	-	-	1	-	9	-
24.6.71	29	-	1	13	8	5	2
1.7.71	70	-	13	17	32	8	-
9.7.71	33	3	21	9	-	-	-
15.7.71	34	21	12	1	-	-	-
22.7.71	29	29	-	-	-	-	-
29.7.71	43	43	-	-	-	-	-

Appendix H. Seasonal changes in development rate (Days) of C. angulatum nymphs reared at 21°C.

		Collecting Date			
		Oct. 14	Jan. 11	Feb. 22	May 8
Final stadium at 8 hr pp.	N	2	6	3	-
	$\bar{X}$	86	43.7	44.3	-
	95% C.L.	-	±18.72	±54.91	-
Final stadium at 16½ hr pp.	N	11	6	3	-
	$\bar{X}$	29.5	22.0	17.3	-
	95% C.L.	1.72	1.75	2.88	-
Penultimate stadium at 8 hr pp.	N	10	12	4	13
	$\bar{X}$	65.6	17.2	22.0	10.8
	95% C.L.	±13.74	± 2.44	± 4.77	± 1.05
Penultimate stadium at 16½ hr pp.	N	15	15	12	14
	$\bar{X}$	23.7	17.7	14.0	11.9
	95% C.L.	± 1.95	± 1.61	± 1.08	± 3.35

Appendix I. Effect of photoperiod on duration of final stadium (Days) in nymphs of C. angulatum collected in the final instar and reared at 21°C.

Collecting date		Photoperiod (Hours)				
		8	10	12	14	16½
11.1.71	N	6	-	10	8	6
	$\bar{X}$	43.7	-	43.6	27.8	22.0
	95% C.L.	±18.72	-	±11.10	± 7.10	± 1.75
22.2.71	N	3	3	8	-	3
	$\bar{X}$	44.3	39.7	22.8	-	17.3
	95% C.L.	±54.91	±34.90	± 1.54	-	± 2.88

Appendix J. Effect of photoperiod on duration of final stadium (Days) in nymphs of C. angulatum collected in the penultimate instar January 11, 1971, and reared at 21°C.

	Photoperiod (Hours)				
	8	10	12	14	16½
N	8	13	15	11	15
$\bar{X}$	68.5	46.8	35.3	24.4	18.0
95% C.L.	±15.87	±14.56	± 6.24	± 2.65	± 1.03

Appendix K. Effect of temperature on stadium duration (Days) in nymphs of C. angulatum collected in the antepenultimate, penultimate, and final instars March 10, 1971, and reared under a 12 hour photoperiod.

A. Collected in Antepenultimate instar.

Stadium		Temperature °C 26.5	21	16
Antepenultimate	N	10	10	9
	$\bar{X}$	11.2	14.2	17.1
	95% C.L.	± 2.26	± 1.06	± 3.44
Penultimate	N	5	7	5
	$\bar{X}$	50.6	28.6	26.4
	95% C.L.	±17.79	±21.24	±10.76
Final	N	4	6	4
	$\bar{X}$	18.3	18.0	28.8
	95% C.L.	±16.89	± 3.75	± 2.09

B. Collected in Penultimate instar.

Penultimate	N	8	10	10
	$\bar{X}$	17.0	19.6	51.2
	95% C.L.	± 4.18	± 4.63	±22.60
Final	N	6	8	8
	$\bar{X}$	34.7	29.0	43.8
	95% C.L.	± 7.71	±12.20	±13.92

C. Collected in Final instar.

Final	N	9	8	8
	$\bar{X}$	17.3	22.8	41.0
	95% C.L.	± 6.17	± 1.53	±14.84

Appendix L. Seasonal changes in development rate (Days) of C. resolutum nymphs reared at 21°C.

		Collecting Date		
		Oct. 14	Jan. 11	Feb. 22
Final stadium at 8 hr pp.	N	3	2	-
	$\bar{X}$	81.3	56.0	-
	95% C.L.	±28.72	-	-
Final stadium at 16½ hr pp.	N	5	2	2
	$\bar{X}$	46.0	22.0	20.0
	95% C.L.	± 7.70	-	-
Penultimate stadium at 8 hr pp.	N	6	5	-
	$\bar{X}$	78.8	15.2	-
	95% C.L.	±15.91	± 2.97	-
Penultimate stadium at 16½ hr pp.	N	9	5	6
	$\bar{X}$	43.1	12.2	14.7
	95% C.L.	± 3.86	± 1.36	± 4.36

Appendix M. Effect of photoperiod on duration (Days) of final stadium in nymphs of C. resolutum collected in the final instar January 11, 1971, and reared at 21°C.

Specimen No.	Photoperiod (Hours)			
	8	12	14	16½
1	23	81	17	21
2	89	21	15	23
$\bar{X}$	56.0	51.0	16.0	22.0

Appendix N. Effect of photoperiod on stadium duration (Days) in nymphs of C. resolutum collected in the penultimate instar January 11, 1971, and reared at 21°C.

Stadium		Photoperiod (Hours) 8	10	12	14	16½
Penultimate	N	5	9	9	10	5
	$\bar{X}$	15.2	12.6*	21.2	17.0	12.2
	95% C.L.	± 2.97	± 2.74	± 1.20	± 3.82	± 1.36
Final	N	4	7	10	8	5
	$\bar{X}$	112.5	45.7	47.0	20.3	17.6
	95% C.L.	±34.60	±25.60	±17.67	± 4.84	± 1.25

*Inadvertently exposed to a long photoperiod for two days.

Appendix O. Effect of temperature on stadium duration (Days) in nymphs of C. resolutum collected in the penultimate and final instars March 10, 1971, and reared under a 12 hour photoperiod.

A. Collected in penultimate instar.

Stadium		Temperature °C 26.5	21	16
Penultimate	N	6	8	8
	$\bar{X}$	17.3	17.5	22.8
	95% C.L.	±10.18	± 3.07	± 3.99
Final	N	4	7	6
	$\bar{X}$	38.8	16.9	38.8
	95% C.L.	±25.92	± 2.11	± 2.16

B. Collected in final instar.

Specimen No.	26.5	21	16
1	22	22	34
2	-	22	32
$\bar{X}$	22.0	22.0	33.0