BIONOMICS OF THE PITCHER PLANT MIDGE METRIOCNEMUS KNABI (COQUILLETT) (DIPTERA : CHIRONOMIDAE)

A

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ABSTRACT

BIONOMICS OF THE PITCHER PLANT MIDGE METRIOCNEMUS KNABI (COQUILLETT) (DIPTERA : CHIRONOMIDAE)

The phenology, diapause characteristics and ovarian development of Metriocnemus knabi (Coq.) were examined in Manitoba and Ontario specimens. Field studies showed no significant correlation between the size of the leaf and the number of larvae inhabiting it. Larval growth ceased between 5 - 10°C, and larvae overwintered in all stages except the first instar. Winter mortality was low, and seldom exceeded 10%. Eye development in the imago was used to determine pupal age. Adults emerged throughout the summer, and the sex ratio was about 50% ♂ : 50% ♀ . Laboratory experiments showed the life cycle to be 44 - 50 days at 26°C.

Photoperiodic differences in diapause induction and termination were shown between Manitoba larvae and the North Carolina larvae studied by Paris and Jenner (1959). Manitoba larvae entered and terminated diapause at a longer photoperiod than North Carolina larvae. The spring emergence was controlled by photoperiod, but modified by temperature.

Field-collected larvae supercooled to -15°C and withstood colder temperatures following acclimatization as natural field temperatures decreased. Larvae kept in leaves of Sarracenia purpurea survived -25°C for 10 days.

M. knabi developed an average of 134.4 ± 19.2 follicles and

the number of mature follicles was closely correlated to the female's size. Follicles began development during the prepupal stage and increased in size during the pupal and adult stages. All follicles matured synchronously 24 hours after the females emerged. No second cycle of follicles was observed.

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CHAPTER I

INTRODUCTION

Within the family Chironomidae there is a wide diversity in habitat, larval food, and larval and adult behaviour. Although most species prefer benthic habitats, members of the family may be found in all fresh water situations, and in brackish and saline waters as well. Size of the aquatic habitat varies from large lakes to small bodies of water existing for only one year.

Some species of this family oviposit in the boles of plants and tree holes if water is present for several months (Kitching 1969). Metriocnemus knabi Coquillett, a member of the subfamily Orthocladiinae, utilizes solely the fluid-filled pitcher leaves of Sarracenia purpurea Linn. as ovipositional and developmental sites. The larvae have never been found in a developmental site other than the pitcher leaves, and although the plant genus contains nine species in North America, M. knabi is associated only with S. purpurea. The significance of the insect's dependence upon this plant is reflected by the parallel distribution of plant and insects. A similar situation exists between Metriocnemus edwardsi and the California pitcher plant Darlingtonia californica.

Very little is known about M. knabi, as is true for most chironomids, and the abundance of this species in Manitoba provided an opportunity to study it in the field and in the laboratory. The purpose of this thesis is to study the phenology, diapause, cold-tolerance and ovarian development of M. knabi.

CHAPTER II

LITERATURE REVIEW OF METRIOCNEMUS KNABI

The genus Metriocnemus v.d. Wulp has a world wide distribution. The number of species is still undetermined, but includes more than thirty.

Members of the genus Metriocnemus prefer slow-flowing streams (Johannsen 1937), although a number are found in benthic situations (Dyson and Lloyd 1935). Very little is known about this genus, with only one species having received any attention. This species is Metriocnemus knabi, the pitcher plant chironomid.

The genus Metriocnemus was first recorded on the North American continent when Coquillett described M. knabi taken from the leaf-pitcher of Sarracenia purpurea in 1904. His description was based on two males and four females, and covered the adult characters only.

An earlier, confusing reference by Smith (1901) quotes a prior reference in which C.W. Johnson described Aedes fuscus Osten-Sacken taken from S. purpurea as a long, white, worm-like larva. Smith subsequently identified this larva as Chironomus sp.[1] One year later Smith (1902) stated Johnson's description applied to Culex pungens (= Wyeomyia smithii, Culicidae). Dyar (1902) described the mosquito Aedes fuscus with illustrations that bore no resemblance to any chironomid.

In 1905, Johannsen republished Coquillett's description of the adult, and added descriptions of the larvae and pupae, based on

1. Description approximated M. knabi.

specimens provided by Knab. In 1908 Johannsen corrected an error in his 1905 paper dealing with the shape of the proleg claw.

Knab (1905) described the life history of M. knabi, particularly the feeding habits of the larva, the unusual pupation in a gelatinous mass, and the running behaviour of the adult. He made no mention of mating behaviour, nor the manner of oviposition. Knab also described the larva and pupa, and compared the pupation to that of a European chironomid, Chironomus minutus Zett.

Malloch (1937) published a key to the Orthocladiinae in which M. knabi was joined by three other species of North American Metriocnemus, one of which was M. edwardsi.

In 1951, Jenner, working on photoperiodism, reared a small dipterous larva from Sarracenia purpurea which he tentatively identified as a member of the family Ceratopogonidae. As no records exist showing ceratopogonids as inquilines of pitcher plants, it is probable that the larvae were those of M. knabi. A subsequent paper by Paris and Jenner (1959) dealt with the photoperiodic responses of M. knabi, particularly with critical photoperiods for termination of diapause and effects of various light intensities.

A recent note by Buffington (1970) dealt with the cohabitation of S. purpurea by M. knabi and W. smithii but no new information was added on the biology of either species. Paterson (1971) found winter survival of M. knabi greater than 95% in New Brunswick.

CHAPTER III

MATERIALS AND METHODS

A number of basic methods were employed in the experiments described in later chapters. As the experiments were diverse, and differed in several variables, the methods outlined below form a basis for the individual experiments.

(1) Sampling sites

Four field locations were selected for collections of S. purpurea and M. knabi (Figure 1.).[1] These were located in the following areas:

(a) A bog at the Whiteshell Nuclear Research Establishment (F.I.G. Ecological Area) 7.8 miles north-west of Pinawa, Manitoba.

(b) A bog at Telford, Manitoba, North of Highway # 4, 7.3 miles east of Rennie, Manitoba.

(c) A bog and marsh area 9.8 miles north of The Pas, Manitoba: 0.5 mile east on the Radio Range Road.

(d) A mat of floating vegetation on the south shore of an unnamed lake 42 road miles south-east of Kenora, Ontario at mile 17 of the Mando camp logging road.

In the fall, microhabitats of S. purpurea within the bog were staked so that winter collections could be made. Colored stakes 1.5m high were used to mark groups of pitcher leaf rosettes.

1. For a detailed study of plants associated with S. purpurea in these bogs, see Evans (1971).

(2) Field Studies

(i) Ground Temperatures

To determine temperatures to which larvae were subjected in the field, temperature recordings were made at Pinawa using a Taylor continuous recording thermometer with a remote sensing probe . The probe was buried in the sphagnum at the level of larval hibernation (approximately 5 cm). Temperature records were collected for the period October 1, 1969 to October 8, 1970.

(ii) Emergence traps

Two types of emergence traps were used during the summers of 1969 and 1970. The first trap (Figure 2) was a cage used to cover one or more entire plants. It was used at Kenora, Telford and Pinawa.

The second trap was formed from two 130 cm lengths of 3.0 mm wire. The wires were shaped into a tall V, and fastened at right angles to each other at the apex. The wire loops formed the frame for a nylon stocking (Figure 3). The cage was anchored by forcing the free ends of the wire into the sphagnum. The nylon stocking was pulled down securely to prevent entry or loss of adults from the cage. These cages were also used to study oviposition in artificial containers.

(iii) Sampling

Leaves of S. purpurea were pulled intact from the stem, retained in a vertical position to avoid loss of fluid, then transported to the laboratory where the volume of each leaf was measured.

The volume of fluid varied from leaf to leaf and within each leaf depending on precipitation. To standardize measurements, the capacity of the pitcher was measured by filling it to the collar with water, then measuring the volume of water. This provided a measurement of maximum capacity. Although pitchers were seldom found containing the maximum amount of fluid, this measurement provided a standard means of comparison.

(3) Laboratory Studies.

(i) Rearing of Larvae

Leaves of S. purpurea were cut open to the base and the contents washed into a white bottomed pan with distilled water. The larvae were removed and examined under a dissecting microscope. Larval head capsules were measured at the widest point posterior to the eyes, and the larvae were sorted to stadium. Larvae used for experiments were placed (25 per pan) into clear plastic pans 15 cm diameter by 3 cm deep containing 175 ml distilled water (Figure 4).

The pans were covered to prevent escape by larvae. Chlorinated tap water had no apparent harmful effect on the larvae but distilled water was used to standardize conditions.

The larvae were fed two drops of a "soup" consisting of crushed turtle food and dried Daphnia in equal proportions with sufficient water to liquefy the mixture. This diet rarely fouled the water, and supplied the necessary components for growth and survival. Two drops of the dilute mixture twice weekly was sufficient food for 25

fourth instar larvae. If the medium was renewed twice weekly, survival to pupa was 95 + %.

The larvae were reared at a constant temperature of 26 ± 1.0°C, a temperature found to combine rapid growth with little or no mortality.

(ii) Equipment for photoperiodic studies

Larvae were reared under different photoperiodic regimes by placing them into specially designed "light boxes" (Figure 5). The plywood boxes measured 60 x 50 x 50 cm and were ventilated on four sides by a series of 2.5 cm holes. The holes were shielded by a hood of black mayfair cover paper to prevent light leaking into the box.

The lighting system consisted of a timer, a stepdown transformer to convert 110 volt line current to 12 volts, and a 20 ohm resistor wired in series to a "pea" bulb. The 6 volt "pea" bulb was used to eliminate light leakage between the boxes and reduce heat production. The light intensity at the floor was 1.5 foot candles.[1]

Paris and Jenner (1959) showed that larvae can perceive light intensities as low as 0.0025 foot candles. The intensity of 1.5 foot candles proved adequate to induce photoperiodic reactions in larvae, but was not strong enough to cause excessive avoidance reactions associated with negative phototaxis (cf. page 16). The light boxes were placed inside a large incubator[2] maintained at 26 ± 1.0°C.

Smaller light boxes were used in several experiments where space was limited. The wiring was identical to the larger boxes, but

1. Measured by Weston Illumination Meter, Model 756
2. Coldstream Incubator Model 153W

since the lights were closer to the pans, the light intensity was 5.0 foot candles. The increased light intensity had no apparent effect on the photoperiodic reactions of the larvae.

(iii) Cold Temperature Studies

(a) Equipment

A number of experiments were performed to determine the effect of sub-zero (^{o} C) temperatures on "conditioned" and "unconditioned" larvae. Conditioned larvae were those subjected to decreasing temperatures over a period of 20 days. Unconditioned larvae were placed directly from field temperatures into low or sub-zero temperatures.

Equipment used in these experiments included coolers, cold rooms and freezers set to maintain the desired temperatures $\pm$ 1.0^{o}C. Seven units were operated simultaneously providing temperatures of 5, -1, -5, -10, -15, -20, and -25^{o}C.

Each experimental group of larvae was placed into styrofoam containers measuring 10 cm diameter x 6 cm deep (#108 Poly-Maid containers). The larvae were covered with water to a depth of 1 cm. Each container was capped with a styrofoam lid.

Whole leaves of pitcher plants containing M. knabi were also subjected to sub-zero temperatures. The leaves provided very little insulation for the larvae, and freeze-drying caused high larval mortality. Drying was minimized when the leaves were wrapped with a 2 cm layer of wet sphagnum prior to freezing.

Supercooling levels in larvae were measured with a Honeywell Brown Electronikcontinuous recording thermometer equipped with a

copper-constantan contact (Figure 6). The larvae were air-dried at room temperature for 4 minutes to prevent freezing at 0°C which was initiated by surface moisture. They were then attached to the contact with a dab of silicon grease and placed in a gelatin capsule within a 3" vial stoppered with a cork. The vial was then placed inside a round-bottomed flask and lowered into a bath of dry ice and 95% ethyl alcohol. The rate of cooling was approximately 4°C per minute. The freezing temperature was taken as the minimum temperature prior to the release of the heat of ice crystallization.

(b) Conditioning

Conditioning of larvae to cold temperatures was undertaken to study the effects of acclimatization on supercooling and survival. Ten larvae were placed into each of 14 poly-foam containers as outlined above. All 14 containers were then placed into the 5°C cooler for 5 days after which 12 were removed, and placed into the -1°C cooler. After 5 days, 10 pans were removed and placed at -5°C. The process of elimination of two pans per temperature was continued to -10°C, after which 2 pans were placed directly into each of the lower temperatures. The larvae were permitted 24 hours for recovery after the water thawed.

(iv) Ovarian Development Studies

Field-collected fourth instar larvae were placed into rearing pans at 26°C and 15 hours light, fed and allowed to pupate. Female pupae were dissected in 0.7% saline at intervals following pupation, and examined for the total number of developing follicles, the number per ovary, the amount of yolk deposition, and the length of the maturing

follicles. Correlations between the size of the pupa and number of developing follicles were drawn using the distance from the most anterior portion of the antennal sheath to the distal edge of the wing sheath as a measure of pupal size.

Measurements of wing length and counts of follicles were taken of adult flies which had been killed in 75% alcohol and immediately dissected in 0.7% saline. Follicular measurements were taken at magnifications of 40x under a dissecting microscope.

CHAPTER IV

FIELD OBSERVATIONS

Field notes on the developmental stages were made at the Kenora, Telford, Pinawa and The Pas collection sites. Field conditions were simulated in laboratory experiments whenever possible to work out details of the bionomics and ecology of M. knabi in Manitoba. The experiments are not listed chronologically, but are grouped under the life stages of M. knabi.

(1) Egg

Members of the Orthocladiinae lay their eggs in water in gelatinous strings, irregular clumps in a gelatinous mass, or singly (Johannsen 1937). M. knabi usually oviposits singly within the pitcher leaf. Occasionally groups of 5 - 20 eggs may be observed in a gelatinous mass, or single eggs may be surrounded by jelly, but the majority lack the gelatinous protection. Enzymes within the fluid may be responsible for dissolution of the covering although females that oviposit in distilled water seldom produce the gelatinous covering.

Eggs were found in pitchers from June 11 to August 26, 1969 and from June 5 to September 7, 1970. Oviposition was continuous throughout the summer months, resulting in a mixed ratio of instars in fall. The eggs were laid in any pitcher containing more than 1 ml of fluid. The result during the early summer was often a young population of chironomids in leaves from the previous year. As the old leaves withered or were covered by sphagnum, and new leaves of the current year become increasing abundant, the number of eggs

deposited in old leaves decreased.

(i) Description

The minute, spindle-shaped eggs were usually found scattered over the surface of the pitcher debris. The yolk was granular, filling the non-embryonated egg completely. Freshly-laid eggs showed no yolk differentiation. Under low power magnification they appeared translucent, with a whitish yolk and a transparent chorion that permitted observation during embryonic development.

Eggs from a Pinawa collection (June 26, 1970) were measured and found to have a mean length of 0.309 ± 0.006 mm and mean diameter of 0.134 ± 0.008 mm. There was no significant difference in length or diameter of eggs collected from Pinawa, Telford and Kenora.

(ii) Fertility

The eggs were examined for embryonic development; eggs with insufficient or homogenous milky yolk 24 hours after oviposition did not develop. The yolk of fertile eggs had a granular appearance and filled the entire egg. Embryonic development was visible after 24 hours.

A series of newly-opened leaves were collected from Telford on July 29, 1970. Forty-two eggs were removed and examined for fertility. The eggs were separated and placed under a 16 hours light: 8 hours dark regime at 20°C. Twenty-seven of the eggs proved to be fertile and hatched, but 15 retained their milky appearance. Later collections of eggs confirmed the previous finding that not all eggs laid in the field are fertilized, but the percentage of infertile

eggs was closer to 10% than to 36% as occurred in the first sample. Non-embryonated eggs remained intact in the pitcher, and were seldom attacked by fungi.

Winter collections made in December and January revealed unhatched eggs that were intact 4 months after oviposition. Warming to 20°C under a 16L:8 D regime failed to initiate embryonic development in these eggs.

(2) Larvae

The small, worm-like larva has been adequately described by Knab (1905) and Johannsen (1905, 1937). The larvae from Pinawa, Telford, The Pas and Kenora pitchers did not differ significantly from these original descriptions.

Head capsule widths of the four larval instars were examined to determine variations among the four populations. Thirty specimens of each instar were measured at the widest point posterior to the eyes. The results are graphed in Figure 7. This figure shows the distinct separation of instars by the width of the head capsules. Means and standard deviations of the width were calculated, and are listed in Table 1.

A collection of larvae taken from The Pas on May 15, 1969 contained a large number of sightless larvae. A random sample of 96 larvae (second, third, and fourth instars) showed 18 (18.8%) to lack one or both geminate eyespots, or the pigments associated with them. The eyespots of other larvae were displaced laterally towards the base of the mandibles, or posteriorly to the last quarter of the head capsule. A subsequent collection (May 13, 1970) revealed only

Table I

Head capsule widths of preserved larvae of M. knabi, n = 30 per instar.

Location and Date	Instar							
	I		II		III		IV	
	Mean	S.D	Mean	S.D.	Mean	S.D.	Mean	S.D.
Pinawa, Man. July 15, 1969	0.085	0.003	0.117	0.006	0.187	0.007	0.274	0.012
Telford, Man. July 16, 1969	0.085	0.003	0.113	0.007	0.177	0.007	0.265	0.009
Kenora, Ont. July 16, 1969	0.085	0.003	0.116	0.008	0.179	0.008	0.273	0.012
The Pas, Man. June 15, 1969	----	----	0.106	0.007	0.171	0.009	0.288	0.010

1 eyeless fourth instar among 748 larvae. A further collection from The Pas (October 27, 1970) did not contain any larvae with ocular abnormalites. Only one other larva, a fourth instar found at Pinawa on May 11, 1970 had aberrant eyespots: it lacked the pigment of the right eye. All larvae were preserved in 70% - 75% ethyl alcohol, and no decomposition was evident.

(i) Location of the larvae in the leaf

The larvae are found primarily within the insect remains and debris at the bottom of the pitcher. During the summer the larvae may be found mining sediments at the debris-fluid interface, as well as climbing the pitcher walls 1 - 2 cm above the debris.

During the month of October all larvae crawl to the extreme bottom of the pitcher forming a compact, aggregate mass of mixed instars (Figure 8). The majority of the larvae have their head pointing downwards. The winter is spent in this location, the larvae encased in a thin film of ice and in close contact with other individuals.

(ii) Crawling behaviour

The larvae do not swim, and all movement is done in contact with the substrate. Fourth instar larvae frequently climb the pitcher walls up to and above the fluid level, but this behaviour does not appear to be associated with feeding or pupation, as second and third instars also perform the crawling behaviour. Lloyd and Turner (1936) showed that larval crawling of Metriocnemus longitarsus had no apparent cause. It was enhanced by oxygen depletion in the sewage water but continued despite favorable conditions of oxygen, food and habitat medium.

M. knabi also climbs the pitcher walls when the leaf is disturbed.

The larvae are negatively phototactic, moving away from a point source of light except in the prepupal stage (see Chapter V). Negative phototaxis is characteristic of all instars, and probably aids in food location. Larvae lacking eyespots fail to exhibit a phototaxis and their movement is without consistent direction.

(iii) Food

The larvae are scavengers, consuming primarily the flesh of the pitcher's victims. This saprozooic mode of nutrition (Torre-Bueno, 1962) is possibly augmented by the consumption of living bacteria and limited quantities of algae.

The larvae actively chew into soft-bodied carcasses such as caterpillars and may be seen feeding beneath the cuticle of the caterpillar. Insects with hard cuticles require partial decomposition of the intersegmental membranes by plant enzymes and bacteria before entry by M. knabi larvae. The larvae are also cannibalistic, consuming smaller larvae weakened by pathogens or immobile pupae during periods of food shortage. Starved larvae will spread their mandibles and aggressively attack other larvae that contact them. However, larvae in the field are seldom found with insufficient food, and aggressive behaviour rarely occurs.

(iv) Larval Populations

The numbers and stages of larvae in S. purpurea leaves vary greatly. To determine the number of larvae per leaf, as well as the ratio of instars, collections of leaves containing larvae were made at

Telford and Pinawa during the summer of 1969.

Linear regressions and correlation coefficient tests were performed on samples of 67 leaves and their larvae, taken at random from the Pinawa and Telford bogs during the summer of 1969. The results show the regression equation of $y = 0.303 (x) + 37.191$ and the correlation coefficient $r = 0.183$ for Pinawa leaf sizes (x) and larvae (y); and $y = 0.237 (x) + 18.969$, $r = 0.095$ for Telford leaf sizes (x) and larvae (y).

As the populations in the leaves were continually changing during the summer, another sample of leaves ($n = 58$) was taken from Pinawa during the winter of 1970 to acquire a population estimate when the larval numbers were static. Comparison of larval number (y) and leaf sizes (x) resulted in the regression equation $y = 0.320 (x) + 29.180$ and $r = 0.173$.

Snedecor (1946) states that the correlation coefficient $r = 0.183$ (Pinawa leaves) is not significant at a 95% confidence level when $n-2 = 65$. The level of significance for Telford ($r = 0.095$ at $n-2 = 65$) is also well beneath this significance level. The Pinawa winter data ($r = 0.173$ at $n-2 = 56$) is not significant as it is lower than Snedecor's value of 0.260.

These results show that there is no significant correlation between the size of the leaf and the number of larvae in it.

Larval growth at Pinawa and Telford was examined throughout the summer of 1969. Figures 9 and 10 show the frequency of each instar as a per cent of the total number of larvae examined for each of the collecting dates during the months of the field study. Some

difficulty was encountered in following the larval population development: (a) females emerged prior to the opening of new pitchers and showed no discrimination in oviposition sites, laying their eggs in leaves from the previous year (old pitchers). When the old pitchers decayed or were covered by sphagnum, they could not be collected. (b) Collecting emphasis was changed in early July to newly opened leaves with their correspondingly younger populations. Because the new leaves opened throughout the remaining summer, collections consisted of current year leaves of various ages. This resulted in a variety of instars at the time of collection.

(v) Dispersal potential

On June 26 and July 3, 1970, collections of week-old leaves containing less than 1.0 ml fluid were made at the Pinawa bog. Twenty-three leaves were examined, and the instars sorted. Thirty-one larvae were found, 35.5% of which were fourth instar larvae and 64.5% were either first or second instars. Since the leaves were open for less than one week the fourth instar larvae could not have matured in the pitchers.

Subsequent investigations showed that larvae crawled a short distance out of water and it was possible that the larvae crawled into the new pitchers. Two attempts were made to determine how far larvae move, but neither was successful. In the first one, larvae in leaves from the previous year were marked with Rhodamine B dye (Heron, 1968: Hamilton 1969), and new leaves in the same rosettes were examined for marked individuals. None was found. In the

second attempt to study dispersal, a square of sphagnum 10 cm deep was removed around a rosette of pitchers. The sphagnum was separated by hand and washed into a fine mesh seive. Only one fourth instar larva was recovered. No conclusion can be drawn, since the larva could have come from a decayed leaf in the rosette.

(vi) Larval diseases

Survival of larvae in the field appears to be very high, possibly as a result of small populations per leaf and the benefit of an isolated habitat. Only one pathogen was found infecting larvae, and only at the Kenora collection site. Some larvae contained globular white objects similar to microsporidian spore-bodies, in the ventral coelom of abdominal segments 5-7. Occurrence of these objects in the last abdominal segment was also common. Infected larvae generally contained twenty to thirty white spheres, the number increasing to more than forty in larger larvae. The diameters of 10 of the spheres were measured in each of 13 fourth instar larvae from a July 4, 1969 collection. The range was from 0.058 - 0.090 mm with a mean of 0.076 ± 0.007 mm. They appeared primarily in fourth instar larvae (7.5% of 187), but were also observed in third instar larvae (1.9% of 216).

The pathogen produced little if any mortality, as no dead larvae were found containing the white bodies. However growth rates were retarded and pupation was postponed. One infected fourth instar larva was found containing two fully chitinized spermathecae, structures that normally do not appear until the pupal stage.

Feeding habits of infected larvae did not change, but movement was hindered and infected larvae frequently fell prey to healthy larvae.

(vii) Winter survival

Collections of larvae were obtained from Pinawa and other locations during the winters of 1969 and 1970. After thawing for 24 hours at 20°C, leaves were cut open and the larvae examined for percent survival (Table 2). The larval mortality was very low in all cases, only exceeding 10% on one collection date (Pinawa : January 29, 1971). Larvae overwintered in the last three stages. Table 3 shows the ratio of instars overwintering during 1970 at Pinawa.

I conclude from these results that winter temperatures (5 - 6 months below 0°C) cause very little mortality in overwintering larvae of M. knabi at 50°N. Latitude.

(3) Pupa

Knab (1905) noted the gelatinous mass surrounding the pupa. The method of pupation does not differ in Canadian bogs. The pupal stage lasts approximately 48 - 60 hours under field conditions at Pinawa and Telford.

(4) Adult

(i) Emergence and Sex Ratio Studies

Adults were observed in the emergence cages from July 4, to August 17, 1969, and from June 12 to September 9, 1970. The largest emergence occurred at Kenora from June 26 to July 3, 1969.

Table 2

Winter mortality of M. knabi larvae

Location	Date	Number of larvae examined	Mortality	
			Number	%
Kenora, Ont.	May 3, 1970	465	24	5.16
The Pas, Man.	May 13, 1970	748	19	2.54
Pinawa, Man.	May 11, 1970	601	11	1.83
Pinawa, Man.	Nov. 27, 1970	904	8	0.89
Pinawa, Man.	Dec 12, 1970	625	45	7.20
Pinawa, Man.	Jan. 29, 1971	401	42	10.47

Table 3

Ratio of instars of larvae of M. knabi overwintering in S. purpurea leaves at Pinawa, Manitoba.

Date	Number of larvae	Instar (%)		
		II	III	IV
27.XI.70	904	28.2	32.6	39.2
12.XIII.70	635	22.3	32.6	45.1
29.I.71	401	21.2	42.6	36.2

Table 4 lists the sex ratios obtained from laboratory reared larvae taken in 1969 and 1970, and shows the ratio to be nearly 50% ♂ : 50% ♀ except for the four Pinawa collections. Pinawa collections reveal a greater number of females than males, the reason for which is unknown. In all rearings mortality was minimal.

(ii) Behaviour

Male adults were captured less frequently than the females as they flew more readily when disturbed. Their flight was usually a steep climb towards the sun, and because of their small size they were soon lost to the viewer. During the two summers of field observations I failed to observe M. knabi males closer than 10 cm to the females while they were on the ground, and swarming of males was not observed.

The female is reluctant to fly, and often requires prodding to induce flight. When the female does fly, the flights are short, seldom exceeding 15 cm, or the distance between the nearest leaves of a rosette. The female's flight is slow, approximately 4 - 10 cm per second, and if the distance is greater than 15 cm, twigs and grass leaves form momentary landing sites. The landing site chosen is usually horizontal or diagonal, and seldom exceeds a 45^{o} inclination. During this period the female is very active, running along the upper and lower surfaces of grass, down to the sphagnum, and even into crevices in the moss.

When the female lands on the lip, or the hood of a S. purpurea leaf, oviposition behaviour begins with active running, primarily over the lip. The female slows down and stops only when she is

Table 4

Sex ratios of M. knabi adults

Location	Date	Number of Adults	Percent	
			Male	Female
Telford	18.VII.69	14	42.9	57.1
Kenora	3.VII.69*	86	47.6	52.4
	30.X.69	49	49.0	51.0
	3.V.70	107	46.7	53.3
The Pas	13.V.70	75	44.0	56.0
	27.X.70	84	44.0	56.0
Pinawa	25.I.70	40	32.5	67.5
	29.VII.70	45	40.0	60.0
	12.XII.70	367	37.6	62.4
	20.I.71	164	35.4	64.6

* Adults caught in emergence cage

within the cup of the leaf. If fluid is present she stops on the inner surface of the leaf with her head in a vertical position, about 2 - 4 mm above the surface of the fluid (Figure 11). In this position she "stamps" her legs in a manner suggesting stationary running, rapidly moves her wings in short bursts, then pushes herself out backwards from the leaf surface with her abdomen arched downwards. After ovipositing she arises from the fluid surface, flies from the cup of the leaf, and begins the search for another leaf. Females displaying this behaviour always had sperm in their spermathecae.

Oviposition occurred in containers other than pitchers. On June 2,1969 nylon emergence cages (Figure 3) were set up at the Telford bog, and an assortment of shell vials and jars were placed in the cages to provide artificial oviposition sites. The vials and jars were allowed to fill with rainwater, and were left undisturbed for several weeks. On July 16, 1969 fifteen first instar larvae were found in a 4 ounce jar. No access to the jars was possible from the exterior of the cage, so the females must have mated and laid eggs within the cage.

The larvae were observed throughout the summer. Drowned victims (primarily ants) provided food for the larvae. At last observation on August 16, the larvae were in the fourth stadium.

CHAPTER V

LABORATORY OBSERVATIONS ON DEVELOPMENT AND BEHAVIOUR

Studies were made on the biology on M. knabi under controlled laboratory conditions. Only one previous study was made on this insect (Knab 1905), and this was limited to general observations of behaviour. Due to the unique habitat occupied by this insect, no comparable studies have been made, other than Kitching's (1969) work on tree-hole inquilines.

The purpose of this chapter is (1) to observe embryonic development and hatching behaviour; (2) determine the duration of the larval stage; (3) examine development of larvae at different temperatures; (4) record pupal metamorphosis and; (5) determine the adult life span. Most emphasis is on the larval stage and its development, and the response of the prepupal larva to light.

(1) Egg

(i) Embryonic development

Few eggs were available for study, and the exact time of their oviposition was not known. Egg collections made during July at Telford and Pinawa showed that eggs, when kept at 20°C, hatched after 5 days (range 4 - 6 days).

Abdominal segmentation and differentiation of the cephalic region were visible approximately 24 hours after oviposition. After 2 days there was a visible reduction in the volume of yolk, and rudimentary maxillae and mandibles were observed. By the third day

the prolegs had developed, and the eyes were visible. The entire external anatomy of the larva was visible after the fourth day and internal organization occurred during the next one or two days of development.

(ii) Hatching

Hatching was accomplished by a forceful exit involving active "crawling" motions, and pressure applied to the anterior portion of the egg by the larval head. The mandibles moved in a biting motion, but biting of the chorion was not observed. Davis (1966) doubts that biting is important in hatching. The egg chorion split longitudinally releasing the larva which immediately stretched to twice the length of the egg. Newly hatched larvae had a swelling of the first three abdominal segments that persisted at least 24 hours.

(2) Larva

(i) Development at 26 ± 0.5°C

A collection of 120 eggs was taken on August 29, 1970 from leaves at the Agassiz Forest Reserve, approximately 16 road miles southwest of the Pinawa collection site. The eggs were placed in rearing pans under long day photoperiods (more than 12 hours light) at 26 ± 0.5°C . Larvae that survived the first stadium (75 of 120) were observed during development. The larvae matured rapidly, and developed to the fourth stage in 22.2 ± 2.3 days. The period from egg to pupa was 36 - 40 days.

A second group of eggs from the same collection was placed

under a photoperiod of less than 12 hours light. The larvae (65 of 80) developed to the fourth stage in 20.8 ± 1.3 days, showing that photoperiod has no effect on the developmental rates of the first three instars.

On December 12, 1970, leaves from Pinawa were thawed, and 25 of the smallest second instar larvae were placed in each of six rearing pans under 15 hours light at 26 ± 0.5°C . The larvae developed to the fourth stage in 13.0 ± 0.9 days.

These results show that under conditions of sufficient food and warm temperature the combined egg and first larval stadia are approximately 9 - 11 days in duration: the combined second and third stadia are of 12 - 14 days duration and the fourth stadium lasts 14 - 20 days. Together with pupal development, emergence and oviposition (an additional 6 - 8 days) it is possible for M. knabi to produce a generation every 44 - 50 days.

(ii) Development at different constant temperatures

Temperature often plays a part in insect development by affecting the rates of growth (Davidson 1944). Early second instars reared under a 15L:9D photoperiod, developed at approximately the same rate in constant temperatures ranging from 20 - 28°C . When larvae were placed at 15°C, growth was retarded significantly. At 10°C very little development occurred, and at 5°C growth was inhibited. Larvae emptied their gut contents at 5°C, but did not feed.

An upper threshold for development of larvae was not examined in this study but it is likely only a few degrees above 30°C . At 30°C high mortality (50+ %) occurred in the fourth larval stadium.

(3) Pupa

(i) Phototactic response in the prepupal stage

During the latter period of the fourth stadium, approximately 2 - 3 days prior to pupation, the larva undergoes a number of anatomical and physiological changes. It ceases feeding, all gut contents are voided and the first three abdominal segments swell. Red patches of developing ommatidia may be seen beneath the head capsule posterior to the eyespots. This is the prepupal stage (Figure 12).

The behaviour of the larva also changes. Knab (1905) was the first to notice the perpendicular position of the pupa above the pitcher leaf fluid, with its head pointing upwards. In my studies, it was observed that larvae pupated in a similar position when the light was overhead. In the complete absence of light, larvae still crawled above the water to pupate, usually with their heads directed upwards. This suggested that a negative geotaxis was involved in pupation.

To confirm this, a pan of 25 fourth stage larvae collected from Pinawa, December 12, 1970, were placed in a light box at 20°C and 15 hours light. The pan was illuminated from beneath and to one side by a "pea" bulb. The top of the pan was covered by a black cloth to exclude light.

Eighteen pupae were formed within 30 days: 15 of which pupated close to the light (within 45° of a line drawn from the center of the pan to the light). This demonstrated a complete shift in the larval phototactic response from negative to positive. All had pupated above the water, but had their heads pointing directly to the light (downwards).

Thus, it appears a negative geotaxis (stronger than the

phototaxis) is involved in sending the larvae above the water; but a positive phototaxis is involved in orientation of the body.

(ii) Development of imaginal characters

As the imago develops within the transparent pupal cuticle, anatomical changes are clearly visible. The development and tanning of imaginal structures follow an orderly transformation in both the male and female.

To record the development of imaginal characters, 250 fourth stage larvae from a Pinawa collection made on January 29, 1971, were placed into rearing pans (25 per pan). The larvae were fed, kept at 28°C until the first larva pupated, and then all the pans were transferred to 21 - 22°C and 15 - 16 hours light. The prepupae were checked every 30 minutes and the time of pupation noted. The pupae were examined immediately, then at 1, 3.5, 6, 12 and 24 hours after pupation.

The prepupae secrete a gel just above the surface of the water, then remain almost immobile for 8 - 16 hours before pupating. Ecdysis of the larval exuviae occurs by a dorsal splitting of the thorax, followed by withdrawal of the head. This is in turn followed by an undulatory movement that results in shedding the larval exuviae posteriorly. The larval exuviae frequently remain in the gel.

During the first 6 hours in the pupal stage, major changes occur in the form and structure of the eye. Other changes of the body occur as well, but the changes in the eye are more easily defined.

Immediately after moulting the pupa is translucent to creamy in color. The imaginal terminalia are undifferentiated and the dorsal

paddles of newly formed pupae are cylindrical and rounded distally. The facets in the compound eye are slightly colored (orange to red) and the eye is halved dorso-ventrally by a thin red line of facets slightly darker than the others. No tanning is visible on any part of the cuticle.

After one hour, the red median line of facets expands ventrally to cover approximately 1/3 of the ventral half of the eye (Figure 13). The dorsal half of the compound eye remains light orange. The compound eye grows posteriorly as well, almost touching the larval eye. The paddles flatten, and the anterior margin of the wing becomes visible. After 3.5 hours the posterior margin of the wing is visible, the ventral half of the compound eye is a darker orange-red than the dorsal half, and tanning of the pleural and wing sclerites occurs.

After 6 hours tanning of the anterior margins of the pupal tergites and sternites occurs (Figure14). The spines on the posterior margin of the pupal tergites are visible (light brown), the ventral half of the compound eye is now red, and the dorsal portion orange-red. The posterior margin of the compound eye is rounded. The paddles become tanned and pigmentation appears in the imaginal genitalia and ultimate abdominal segment.

Twelve hours after pupation the eyes become bright red (Figure 15): the ventral half still appears darker. The posterior margin of the compound eye covers half of the larval eye, and the developing coxae and antennae show some pigmentation.

Twenty-four hours after pupation the eyes are deep red (Figure 16) and the larval eyes are incorporated into the compound eye. The terminal abdominal segments darken, and the developing legs are

are distinct, but unpigmented. In the male the hypopygium becomes defined, but remains translucent. The posterior margin of the postnotum and the ventral margin of the pre-episternum both show pigmentation. Some hairs and setae are visible on the abdomen and wings.

Beyond 24 hours the imaginal structures undergo only superficial changes; tanning is completed, and bristles, setae and body hairs become defined.

(iii) Emergence

The pupal stage lasts approximately 46 - 48 hours at 20°C but may last up to 8 days at 10°C. The pupa accumulates air beneath its cuticle during the final 60 - 90 minutes prior to emergence. Violent writhing motions by the pupa carry it to the upper surface of the gel where the thoracic cuticle splits dorsally to release the imago. Emergence is complete in 30-60 seconds.

An apparent periodicity of emergence occurs at 21°C and 15 hours light per day. Emergence is greatest approximately two hours after "sunrise" and shortly after "sunset". The nocturnal emergence is inferred from male specimens in which the antennal bristles become plumose approximately 6 hours after emergence. Males found at "sunrise" all had plumose antennae.

The pupal stage of M. knabi is similar to that of other Chironomidae and is the period of greatest anatomical reorganization. Some of the external changes are covered in the section above, and in Chapter VIII I will report on one of the internal changes, that of ovarian development.

(4) Adult

The adult ranges in length from 2.0 - 2.8 mm. Like other chironomids, it has reduced mouth parts and it has never been observed to feed.

Mating was never observed, and females kept in cages with large numbers of males never had sperm in their spermathecae. To induce swarming by males, and possibly mating, different cage sizes from a small cube cage (15 cm per side) up to a large cage (1.5 x 1.25 m sides and 2m height) were used. Various photoperiods including a dawn and a twilight period were used. These conditions, plus the addition of S. purpurea leaves, different relative humidities and different constant temperatures all failed to induce swarming or mating. As a result, all laboratory rearings of M. knabi had to be done with larvae collected from the field.

Flight activity by females was minimal. After emergence, females frequently remained beside their pupal exuviae for 8 - 10 hours if undisturbed. Occasionally the female did not move more than 1 cm from the pupal exuviae for periods up to 15 hours. The reduced activity of the female may minimize expenditure of energy while the egg follicles are maturing. As the female is autogenous and non-feeding, all energy used will draw on stored reserves. Consumption of these reserves may be detrimental to her potential fecundity.

Twenty-four hours after emergence females flew and ran actively, particularly in relative humidities of less than 70%.

Activity of the male was always more vigorous than the female. Males seldom remained beside the pupal exuviae longer than 3 hours.

They were strongly attracted to light and often moved towards a light source before the antennal bristles had become plumose. If released, they flew directly towards a source of light.

The adult life span is similar in both sexes. On August 10, 1970, 20 males and 20 females were reared from larvae collected at Pinawa July 23, 1970. The adults were placed separately into covered 2 dram vials containing a damp cotton pad and were kept at 20°C. The majority of the adults (72.5%) died within 5 days, but several females lived to the seventh day. Oviposition by virgin females did not occur in this situation, but some eggs were laid when water was substituted for the cotton.

The experiments in this chapter show that M. knabi may complete a life cycle in 44 - 50 days; that prepupal larvae exhibit phototaxis and geotaxis during pupation; and that female adults may live for 7 days after pupation.

Observations during pupal development show that external imaginal characteristics develop during the first 24 hours, and stage of ocular development may be used to determine the age of the pupae.

CHAPTER VI

PHOTOPERIODIC RESPONSES OF LARVAE

Daylength is one of the most reliable indicators of seasonal change in nature. As a result, many animals rely on daylength to initiate migratory, nesting or sexual behaviour, with their attendant physiological changes. Many insects depend on daylength for determinations of emergence, mating and oviposition times (de Wilde 1962, Beck 1968). In addition, the photoperiodic control of diapause is vital to many insects as it prepares the insect for a period of unfavorable weather conditions.

Within the past two decades, the greatest emphasis of photoperiodic research has been placed on induction and termination of diapause (Adkisson 1966). Only two studies of photoperiodic reactions in Chironomidae are known to the writer: Paris and Jenner (1959) described the photoperiodic reaction of M. knabi in North Carolina, and Engelmann and Shappirio (1965) described the effects of short day photoperiods on Chironomus tentans.

The purpose of my study was not to repeat Paris and Jenner's experiments, but to make a comparison between two widely separated populations (North Carolina 35°N; Pinawa, Man. 50°N). Because of the northern location of Manitoba larvae, the differences that occur, particularly in the photoperiods capable of inducing and terminating diapause, are substantial. The summer is much shorter at 50°N latitude and we know that seasonal temperatures are important in the termination of diapause in other insect larvae.

(1) Diapause Termination

A preliminary experiment on diapausing larvae showed that pupation was high (50%) during constant light (24L:0D), or constant darkness (0L:24D), but low (under 10%) when the photoperiod was 12L:12D. Another experiment showed that 9 hours to 13 hours light per day failed to produce pupation of larvae taken from Kenora in January, 1970.

Three experiments were then set up to determine the response of fourth instar larvae to long and short photoperiods and to different temperatures. To establish the general response of fourth instar larvae, larvae were collected at Kenora, May 3, 1970, sorted, and placed 25 apiece into each of 14 pans. Two pans were then placed in each of 7 light boxes at photoperiods of 0:4:8:12:16:20 and 24 hours light per day. The results, based on the percent of pupations among survivors after 46 days are shown in Figure 17 and Table 5.

To determine with more precision the exact photoperiod causing termination of diapause, another experiment was conducted using larvae collected from Kenora on May 10, 1970. Replicate pans, each with 20 larvae, were placed at 12.5; 13; 13.5;14;14.5;15 and 16 hours light per day. The results of this experiment are shown in Figure 18 and Table 6.

Temperature was previously shown to affect the developmental rates of M. knabi larvae (cf. page 28). To determine its effect on diapause termination, 24 fourth instar larvae from a Pinawa, January 29, 1971, collection were placed into each of 10 rearing pans. The larvae (n = 50 per temperature) were then subjected to temperatures of 5 , 10 , 15 , 20 , and 27°C under 15 hours photoperiod. The pupation

TABLE 5

Photoperiodic response of M. knabi collected from Kenora, May 3, 1970. Fourth instar larvae were reared to the pupa at 25°C. N= 50 per photoperiod.

Photoperiod	Number of pupations of survivors	Percent pupations of survivors
OL: 24D	33/34	97.0
4L : 20D	25/32	78.1
8L : 16D	16/42	38.1
12L : 12D	2/40	5.0
16L : 8D	12/14	85.8
20L : 4D	16/18	88.9
24 L: 0D	24/38	63.2

Table 6

Photoperiodic response of M. knabi collected from Kenora, May 3, 1970. Fourth instars were reared to the pupa at 25°C. N = 40 per photoperiod.

Photoperiod	Number of pupations of survivors	Percent pupations of survivors
12.5L : 11.5 D	0/27	0
13 L : 11D	0/38	0
13.5 L 10.5 D	0/19	0
14 L : 10 D	0/36	0
14.5 L : 9.5 D	15/29	51.7
15 L : 9D	24/33	72.7
16 L : 8D	18/26	69.2

results are graphed in Figure 19. Larvae maintained at 5°C did not pupate.

Discussion:

The first experiment shows that larvae pupate in a number of different photoperiods from 0 hours to 24 hours light per day with a decrease in pupal numbers as the periods of light and dark equalize. The second experiment shows the critical photoperiod for termination of diapause to lie between 14 and 14.5 hours. This differs from Paris and Jenner's (1959) data on North Carolina larvae. Their larvae responded (terminated diapause) to photoperiods of 12.5 to 13 hours light. The difference between the two populations is probably one of adaptation to different climatic conditions at different latitudes. At 50°N latitude a daylength of 14.5 hours (including civil twilight) is reached in Nature on April 17. Although the larvae are physiologically capable of responding to this daylength, the frozen or near-freezing pitcher fluid inhibits growth until sometime in May.

Figure 19 shows the pupation of larvae in different constant temperatures, with the rate of pupation decreasing with decreasing temperature. (The pupal stage lasts up to 8 days at 10°C, but at 5°C no growth or pupation occurs).

Field records of sphagnum temperatures at the Pinawa bog show that a weekly mean of 10°C is not reached until mid-May (Figure 20). Allowing 3 - 4 weeks for larval and pupal development, the adults should emerge in the first week of June. Field records from Pinawa and Telford confirm this time of emergence.

Paris and Jenner stated that larvae terminated diapause at a photoperiod shorter than the diapause-initiating photoperiod. They believed a physiological conditioning occurred during the winter, which prepared the insect for emergence at a shorter photoperiod. No evidence of conditioning was found in Manitoba and Ontario larvae which emerged in the field when the daylength was 17.5 - 18.0 hours; the limiting factor was undoubtedly the cold spring temperatures in the field. The spring emergence of adults is therefore controlled by photoperiod, but modified by temperature.

(2) Diapause Maintenance

The maintenance of larval diapause by M. knabi is solely governed by the daily photoperiod. In Manitoba, photoperiods between 9 - 13 hours light maintain diapause in larvae, but all others cause terminations of diapause in varying percentages of the population. Fourth instar larvae remain in diapause even when the temperature is favorable for development. Larvae subjected to these conditions still feed, but the rate of feeding is decreased, and the production of faecal matter is reduced. If disturbed, the activity of diapausing larvae is as vigorous as non-diapausing larvae, but they are generally less active.

Bradshaw (1969, 1970) showed the importance of food and photoperiod in diapause termination of Chaoborus americanus. Diapausing larvae kept at constant temperatures above 20°C occasionally resorb part of their fat body despite the availability of food, but mortality due to starvation is not significant. Paris and

Jenner (1959) showed that starved larvae terminate diapause almost as readily as fed larvae, so the availability of food in spring probably does not affect pupation. It probably does affect fecundity if the fat body (stored yolk reserve) is used prior to ovulation.

Paris and Jenner (1959) noted that fourth instar larvae kept for long periods under favorable temperatures but diapause-maintaining daylengths occasionally pupated. Manitoba and Ontario larvae react similarly.

(3) Diapause Induction

Preliminary experiments on larvae reared from the egg in temperatures favorable to development indicated that larvae can develop directly to the adult stage from the egg without an intervening diapause.

To examine the effects of different photoperiods on the induction of diapause in larvae reared from eggs, a series of 4 light boxes (0; 14; 15; and 18 hours light per day) were placed in a 26°C incubator. Twenty eggs from an August 29, 1970 Agassiz bog collection were placed in a rearing pan in each of the light boxes. The larvae that survived were observed, and the number of pupations after 60 days recorded. The results are tabulated in Table 7.

Table 7 shows that a critical photoperiod capable of inducing diapause in larvae occurs between the periods of 14 and 15 hours light per day. This photoperiod is similar to that which terminates diapause. Paris and Jenner (1959) showed that the photoperiod which induced diapause in North Carolina larvae occurred between 12 - 13

Table 7

Diapause induction of M. knabi larvae collected at Agassiz Forest Reserve, August 29, 1970. N=20 eggs per photoperiod. Experiment terminated after 60 days.

Photoperiod	Number of survivors pupating	Percent pupation of survivors
OL: 24D	7/13	53.8
14L: 10D	1/17	5.9
15L: 9D	16/16	100.0
18L: 6D	4/15	26.7

hours light per day. The difference in populations from North Carolina and Manitoba is apparent once again.

Table 7 also confirms Paris and Jenner's statement that direct development from the egg to the adult is possible under favorable photoperiods. Diapause is therefore not an obligatory stage.

The combined results of this chapter are illustrated in Figure 21. This graph compares the times of diapause termination and induction, and initial and final emergence between the Canadian and American populations. As was expected, the Manitoba and Ontario populations have a shorter season favorable to adults than the southern population. The shorter season has probably led to selection of those characters that are advantageous to survival, viz. diapause induction at a longer photoperiod. The last adult observed in the field in Manitoba was seen September 9 when the daylength was 14.5 hours. The temperature at the time was favorable for pupation (10 + ^{o}C-see Figure 20) but no adults were seen later than this date. Two weeks later the mean weekly ground temperature at the level of the larvae in pitcher leaves dropped below 10^{o}C.

Diapause could be beneficial in coordinating or synchronizing a mass emergence of adults in June. However, the larvae of M. knabi overwinter as second, third and fourth instars and emergence occurs throughout the entire summer. As a result, no single brood can be followed throughout development, and a synchronization of adult emergence is not apparent.

CHAPTER VII

LARVAL SUPERCOOLING AND COLD-TOLERANCE

Introduction

In temperate regions insects usually encounter freezing temperatures at some stage of their life cycles. To survive the adverse conditions they employ a number of physiological mechanisms which permit supercooling and/or tolerance of freezing. Many insects supercool to some extent with no adverse effects if the cold is of short duration: mortality due to freezing increases with time (Salt 1961).

Insects that are acclimatized to low non-freezing temperatures often supercool to lower temperatures than non-acclimatized individuals (Mellanby 1959: Somme 1968 a,b).

Little work has been done on acclimatization and supercooling in the families Culicidae and Chironomidae. Mellanby (1959) showed acclimatization to low temperatures increased the survival of Aedes aegypti in freezing temperatures, and Evans (1971) showed supercooling in larvae of Wyeomyia smithii. Anderson (1946) and Crisp and Lloyd (1954) showed that chironomid larvae overwinter in frozen substrates with no apparent damage. Scholander et al. (1953) showed that some arctic chironomid larvae which overwintered in frozen substrates did not supercool but froze slowly at $0^{\circ}C$.

The purpose of this chapter is to demonstrate that larvae of M. knabi supercool, and that the freezing point is depressed by acclimatization to low temperatures.

(1) Supercooling Temperatures of Larvae

To determine whether supercooling occurred in fourth-stage larvae, a dry ice-ethyl alcohol bath was prepared, and larvae were tested for freezing points as described in Chapter III.

Twenty-five larvae collected from Pinawa on September 4,1970 were frozen and the temperatures of freezing were recorded. Eleven froze at $0^{o}C$; the lowest freezing temperature was $-7.3^{o}C$, and the mean was $-2.7^{o}C \pm 2.1^{o}C$. During mid-winter thirty-five larvae taken from Pinawa (December 12, 1970) were frozen; the lowest freezing temperature was $-15.6^{o}C$. The mean was $-8.5^{o} \pm 4.7^{o}C$, with two modes: $0^{o}C$ (5 larvae) and $-11^{o}C$ (6 larvae).

These results show that fourth stage larvae supercool and larvae can withstand colder temperatures as natural (field) temperatures decrease.

(2) Survival in Cold Temperatures

(i) Non-acclimatized larvae

To examine the susceptibility of larvae in water to sub-zero (^{o}C) temperatures, 2 poly-foam containers holding 50ml water and 10 larvae were placed into each of the following temperatures: 5, -1, -5, -10, -15, -20, and $-25^{o}C$. The results after 10 hours are tabulated in Table 8. Larvae at 5^{o} and $-1^{o}C$ were examined after 5 days. Some mortality occurredin these two temperatures, but mortality was significant below $-5^{o}C$.

(ii) Acclimatized larvae

Larvae were taken from Pinawa on September 27, 1970, and sorted

Table 8

Survival of non-acclimatized larvae of M. knabi from Pinawa when subjected to cold temperatures for 10 hours. $\overline{N} = 20$ for each temperature.

Collection date	Instar	Percent survival after 10 hours						
		5°C*	-1°C*	-5°C	-10°C	-15°C	-20°C	-25°C
(a) 4.IX.70	IV	100	100	75	10	0	0	0
(b) 27.IX.70	III	100	95	70	30	10	0	0
(c) 27.IX.70	IV	100	100	75	25	5	0	0

* Percent survival after 5 days.

according to larval stage. Ten third instar larvae were placed in each of 14 poly-foam containers and acclimatized in a series of decreasing temperatures down to -10°C as described in Chapter III. Fourth instar larvae were acclimatized similarly. The mortality was calculated after 5 days exposure, and tabulated in Table 9 (a,b).

On October 29, 1970, 14 leaves of S. purpurea from Pinawa were conditioned as above while wrapped in wet sphagnum moss. The larvae were sorted after thawing and examined for mortality from the 5 days exposure. The results are listed in Table 9 (c, d, e).

Two conclusions may be drawn from Table 9. (a) Cold-acclimatized larvae can withstand colder temperatures than non-acclimatized larvae (compare with the Pinawa September 27, 1970 results in Table 8). This is probably the result of reduced nucleators in the gut (Salt 1961, 1970) as all the larvae had voided their gut contents. (b) Larvae acclimatize (cold-condition) under field conditions. Preliminary experiments had shown that the mortality level due to freezing in pitcher leaves did not differ from mortality in the poly-foam containers providing the leaves were wrapped in wet sphagnum. As a result, the enhanced survival of larvae collected at a later date, (October 29, 1970) then frozen into leaf fluid, is indicative of field acclimatization prior to the experimental conditioning.

(iii) Field acclimatized larvae

To determine whether progressive conditioning to cold temperature occurred during the winter, larvae in frozen pitcher leaves were collected from Pinawa on January 28, 1971. The leaves were immediately wrapped in wet sphagnum and placed in a freezer maintained at

Table 9

Survival of acclimatized larvae of M. knabi from Pinawa when subjected to cold temperatures for 5 days. Conditioning was begun at 5°C and lowered in 5° decrements every 5 days, to -10°C.

Date of collection	No.	Instar	Percent survival after 5 days						
			5°C	-1°C	-5°C	-10°C	-15°C	-20°C	-25°C
(a) 27.IX.70	20	III	90	100	90	65	70	35	15
(b) 27.IX.70	20	IV	100	100	85	50	50	5	0
(c) 29.X.70	*	II	100	100	100	100	62	83	22
(d) 29.X.70	*	III	100	100	100	100	82	80	17
(e) 29.X.70	*	IV	100	100	93	100	56	50	24

*Number of larvae was variable in each leaf

Table 10

Survival of larvae of M. knabi in leaves of S. purpurea when subjected to -25°C. Collection from Pinawa, Manitoba, Jan. 28,1971

Days	Instar II		Instar III		Instar IV	
	Number	%Survival	Number	%Survival	Number	%Survival
1	18	100	23	100	32	100
2	21	100	30	96.8	25	100
5	16	94	27	89	34	97.1
10	27	92.5	12	83.4	28	92.8
15	22	45.5	33	20.3	29	48.3
20	23	26.1	29	17.3	23	30.4

-25 ± 1°C. Two leaves were thawed per sampling date. The results were tabulated (Table 10) and graphed (Figure 22).

The results show survival by field-acclimatized larvae to be significantly higher than survival without acclimatization (Table 8) when the larvae are subjected to -25°C.

The results of this chapter show that some larvae of M. knabi are capable of withstanding sub-zero temperatures as low as -25°C for 10 days with little effect. The results also show that acclimatization to cold enhances survival of larvae in sub-zero temperatures.

CHAPTER VIII

EGG MATURATION AND OVARIAN DEVELOPMENT

Introduction

Studies of ovarian development in Diptera have primarily been undertaken on species of medical importance. Few detailed studies have been made on the Chironomidae since 1900 when Miall and Hammond outlined follicular development in Chironomus dorsalis. In recent years biomass and productivity experiments have shown the importance of chironomids to the aquatic food chain. Consequently, a few studies on reproductive potential of chironomids have been made in the last decade (Oliver 1968, 1971).

Most examinations of follicular development have been limited to the subfamily Chironominae due to the large size of the adults, availability, and the ease of culture. Few studies have been made on the Orthocladiinae, a poorly known subfamily.

This study was undertaken with three objectives in mind: (a) to determine the potential fecundity of M. knabi, (b) to correlate potential fecundity with the size of the female, and (c) to determine the rate of yolk deposition and follicular maturation.

(1) Potential Fecundity

The number of follicles matured by chironomids varies greatly. Chironomus plumosus matures and lays 1500-2000 eggs (Wensler and Rempel 1962), but Pseudodiamesa arctica lays only 140-230 eggs (Oliver 1968).

To determine the total number of follicles matured by M. knabi

(potential fecundity), developing follicles were counted in 46 pupae and 80 adults reared from a Pinawa collection (January, 1971). There were 128.3 ± 33.2 follicles per pupa and 134.4 ± 19.2 follicles per adult. This represented the number of follicles (in the first cycle) in which yolk deposition occurred.

It is doubtful that these numbers are representative of the total number of eggs deposited, as field-caught females had never laid their full complement of eggs at the time of capture. As mating was not successful in the laboratory, and virgin females could not be forced to oviposit, the number of eggs remaining in the ovaries of M. knabi at the time of natural death is unknown.

(2) Size-Fecundity Correlation

Variation in the number of follicles matured by a species is frequently correlated to the size of the female. The relationship is known for the Culicidae (Clements 1963) and the Simuliidae (Chutter 1970), but no comparable information is available for the orthocladine chironomids.

The size of the female pupa was measured as described in Chapter III, and the size correlated to the number of maturing follicles. This resulted in the regression equation $y = 310.66 (x) - 318.77$ with an r value of 0.546 ($n = 46$). Snedecor (1946) stated that this value was very significant (at the 1% level of confidence) for $n - 2 = 44$.

Wing length (from the arculus to the distal margin) was measured on 22 female adults and correlated to the number of developing follicles. This resulted in the regression equation $y = 286.78 (x) - 245.98$ with

an r value of 0.631, highly significant at the 1% level of confidence, using Snedecor's (1946) tables.

The two correlations drawn above show that the number of follicles matured is directly proportional to the size of the female. As all oviposited eggs are the same size (0.24mm), the relationship is probably due to the increased size of the fat body in larger females.

(3) Rate of Follicular Maturation

Studies of the rate of follicular maturation by chironomids are few in number. In most cases these examinations have been limited to the amount of yolk present in the follicles at emergence, and a mention of the female's age at the time of initial oviposition (Anderson and Hitchcock 1968).

Oliver (1968) studied the maturation of follicles in 9 species of arctic chironomids, using the amount of yolk deposited as a criterion of maturity, but he did not determine a rate of follicular maturation for the species.

To determine the rate of follicular maturation in pupae of M. knabi, a collection of fourth instar larvae taken from Pinawa, January 31, 1971, were reared at a constant temperature of 20°C. The exact time of pupation was noted, and the pupae were dissected at 1, 12 and 24 hours after pupation. The length of 20 follicles was measured in each of 46 pupae. The results are tabulated in Table 11 and graphed in Figure 23.

Adults reared from the same collection were dissected at precise times after emergence. The lengths of twenty follicles were measured in each of 68 adults, then tabulated in Table 12, and graphed

Table 11

Rate of follicular maturation in M. knabi pupae reared from a Pinawa, Manitoba collection Jan. 31, 1971. Twenty follicles measured in each pupa.

Age of pupa (hours)	No. of pupae	Mean follicular length (mm)	S.D.
1	16	.052	.003
12	15	.068	.008
24	15	.090	.007

Table 12

Rate of follicular maturation in M. knabi adults reared from a Pinawa, Manitoba collection Jan. 31, 1971. Twenty follicles measured in each adult.

Age of Female (hours)	No. Dissected	Mean follicular length (mm)	S.D.
1	19	0.133	.008
6	14	0.148	.008
8	1	0.162	------
12	13	0.187	.031
18	1	0.196	------
24	17	0.234	.007
36	2	0.242	.005
60	1	0.245	------

in Figure 23.

These results show that follicles of M. knabi reach maximum size approximately 24 hours after emergence, and implies that the female is capable of oviposition one day after emergence. This correlates with the time of increased activity of the female (Chapter V).

(4) Rate of Yolk Deposition

The rate of yolk deposition was noted concurrently with the rate of follicular maturation. The method used was similar to that used by Oliver (1968).

Females of M. knabi do not feed, and the yolk must be derived from the reserve stores of the fat body. Examination showed that deterioration of the fat body was progressive, and ended 24 hours after the female emerged. Activity by the female was minimal during this period, but yolk deposition occurred. All follicles matured simultaneously.

During the latter period of the fourth instar (prepupal stage) the follicles are spherical, and contain approximately 1/10 yolk. A spherical mass, probably a single nurse cell, as illustrated by Wulker and Gotz (1968) for Chironomus spp. larvae, is present in the proximal half of the developing follicle (Figure 24). Twelve hours after pupation the nurse cell is visible in both the maturing follicle and the penultimate follicle (Figure 25). The first cycle follicle contains about 1/3 yolk. Twenty-four hours after pupation the maturing follicle contains 3/4 yolk, and has doubled its length (Figure 26).

As the pupa matures, follicular growth and yolk deposition occur at a linear rate. However, this rate accelerates when the female

emerges, and yolk is deposited more rapidly. One hour after emergence the spherical follicles begin elongation and yolk is deposited in the penultimate follicle. The nurse cell is no longer visible, and yolk fills 9/10 of the follicle. After 12 hours (Figure 27) the spindle-shaped follicle undergoes only slight elongation to become a mature egg. Yolk occupies 1/3 of the penultimate follicle at this time.

The eggs attain maximum development at 24 hours (Figure 28) and the spherical penultimate follicles contain 1/2 yolk. Further yolk deposition is minimal in the penultimate follicles.

Two virgin females, one 36 and the other 60 hours of age showed no increase in the amount of yolk in either the penultimate follicle or the mature follicle. The 60 hour female however, had abnormal egg development, as some of the follicles were nearly spherical and larger than others. The majority of the follicles had less yolk than expected (only 4/5 yolk), but whether the incomplete yolk was a result of resorption, or a result of abnormal initial development is unknown.

A second cycle of eggs (the penultimate follicles) was not observed in any female. These follicles ceased development at a stage similar to the first cycle follicles of an 18 hour pupa.

This chapter shows that M. knabi has a mean reproductive potential of 130 eggs and that the actual number of eggs produced by a female is closely correlated to her size. The results also show that all follicles of the first cycle are mature approximately 24 hours after emergence.

CHAPTER IX

SUMMARY

1. Eggs of M. knabi were usually laid singly in pitcher leaves. In field collections, approximately 90% were fertile.
2. There was no significant correlation between the size of the leaf and the number of larvae inhabiting it. The width of the larval head capsules of each instar varied little between populations. The larvae were negatively phototactic, and saprozoic. Winter mortality of larvae was low, and seldom exceeded 10%.
3. Adults emerged throughout the summer and the sex ratio was about 50% ♂ : 50% ♀ .
4. Eggs of M. knabi hatched 4 - 6 days after oviposition. Hatching was preceded by crawling motions of the embryo, and by expansion of the body. This resulted in pressure being applied to the anterior portion of the egg.
5. When reared at 26°C, with sufficient food, M. knabi was able to complete one generation in 44 - 50 days. Larval development was retarded significantly at temperatures below 20°C, but mortality was high (50+%) at 30°C.
6. A negative geotaxis was displayed by the larvae but, as pupation approached, a positive phototaxis was displayed in the orientation of the pupae.
7. Imaginal characters were well defined in the pupa 24 hours after pupation. Changes in the eye were easily followed and these are described for 1, 6, 12, and 24 hours after pupation.

8. Emergence was greatest about 2 hours after sunrise, but some males emerged shortly after sunset. Males were active almost immediately, but females were inactive up to 15 hours after emergence.

9. Larvae from Manitoba (50°N) entered diapause in the field and in the laboratory at 14.5 - 15 hours light per day. Diapause was maintained between 9 - 13 hours light per day, and was terminated in the laboratory at 14 - 14.5 hours light per day. In early summer less than optimum temperatures in the field prevented emergence until the daylength was 17.5 - 18 hours light and the mean weekly ground temperature was above 10°C.

10. Field-collected fourth stage larvae supercooled to a minimum of -15°C. Larvae collected later in the season, after acclimatization to natural decreasing field temperatures had lower supercooling points than larvae collected earier in the season. Larvae are cold-hardy snd winter survival under natural conditions was very high, above 95%. Survival in the laboratory at -25°C for 10 days was also greater than 90%.

11. M. knabi developed an average of 134.4 ± 19.2 follicles and the number of mature follicles was closely correlated to the females size. Follicles began development during the prepupa and increased in size (lengthened) during the pupal and adult stages. All follicles matured synchronously 24 hours after emergence. No second cycle of follicles was observed.

Figure 1. Sampling sites.

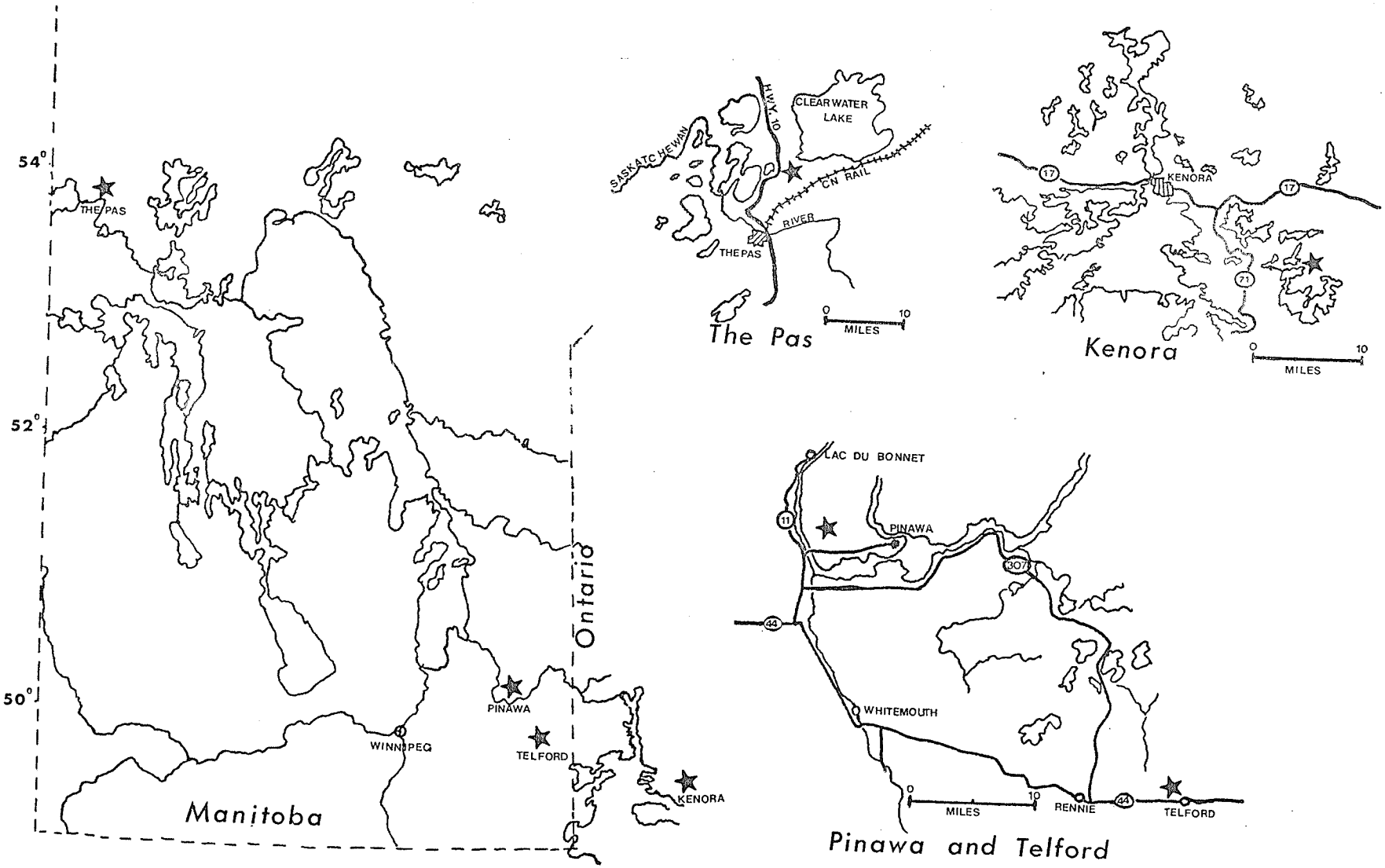

54°
52°
50°
THE PAS
PINAWA
WINNIPEG
TELFORD
KENORA
Manitoba
Ontario
SASKATCHEWAN
H.W.Y. 10
CLEAR WATER LAKE
CN RAIL
RIVER
THE PAS
0
10
MILES
The Pas
KENORA
17
71
0
10
MILES
Kenora
LAC DU BONNET
11
PINAWA
307
44
WHITEMOUTH
0
10
MILES
RENNIE
44
TELFORD
Pinawa and Telford

Figure 2. Pyramidal emergence cage showing removable upper container.

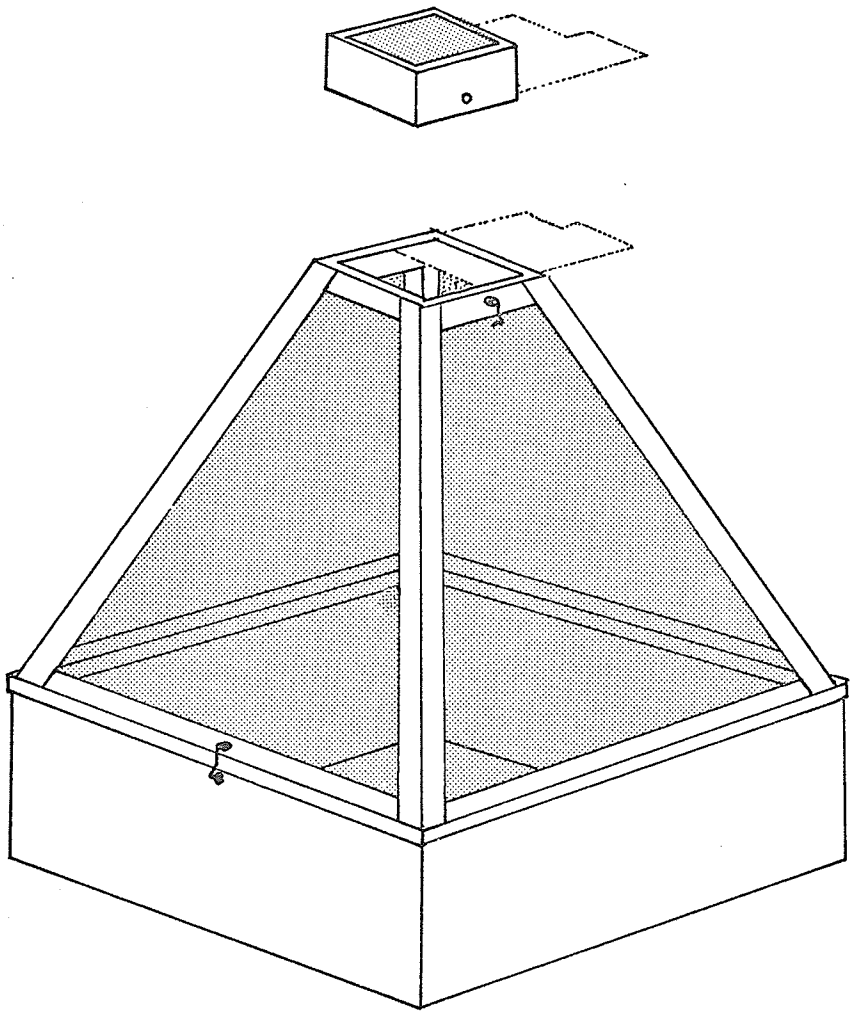


Figure 3. Stocking-type emergence cage used to cover individual leaves of *S. purpurea*.

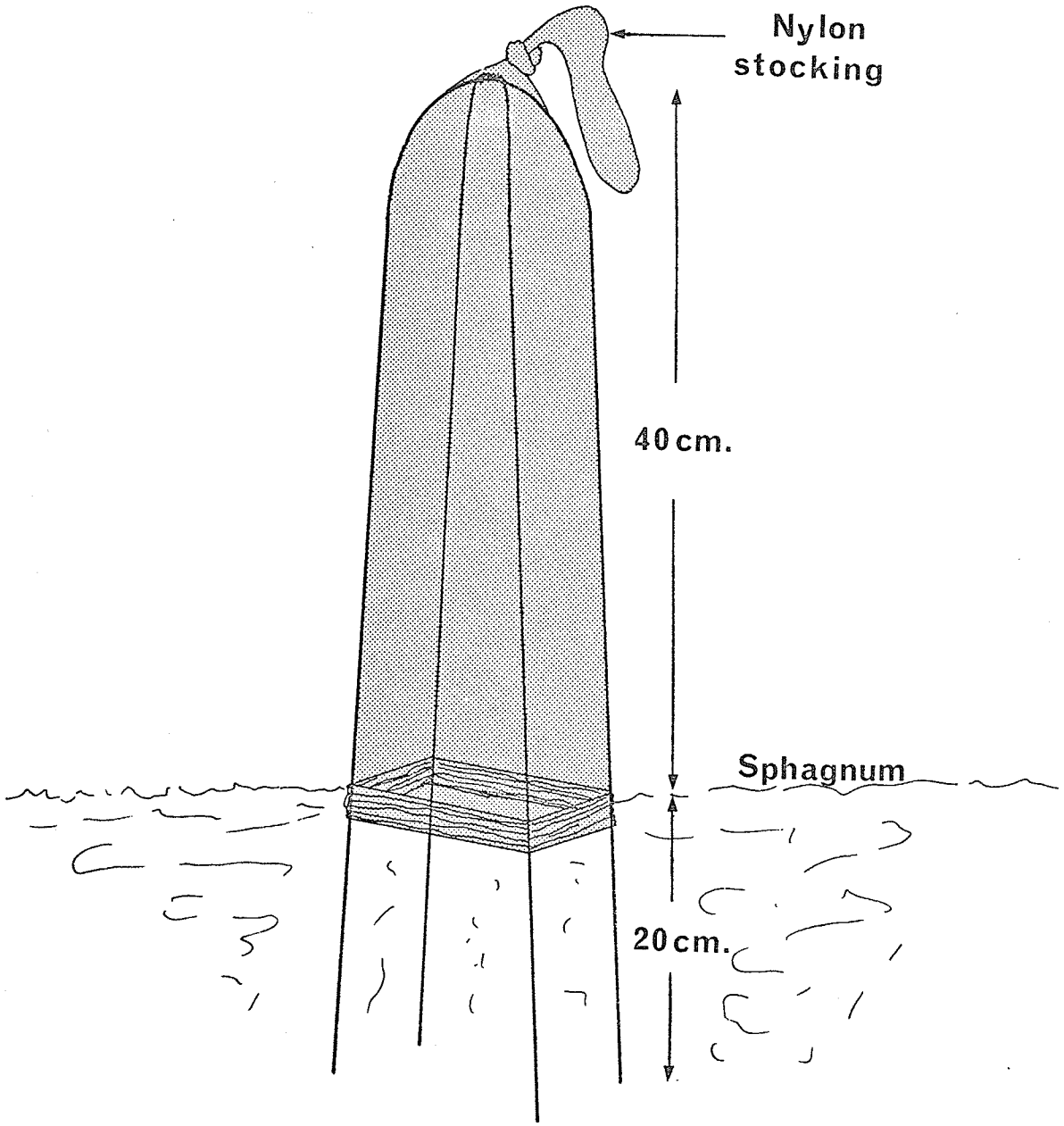

Nylon
stocking
40 cm.
Sphagnum
20 cm.

Figure 4. Equipment used for rearing larvae of M. knabi: clear plastic rearing pan, counter, food mixture, record sheets and pipettes are shown.

Figure 5. Light box used in photoperiodic studies on M. knabi larvae.

Figure 4

Figure 5

Figure 6. Equipment used in freezing experiments including recording thermometer, dry ice bath and copper-constantan electrode (arrow).

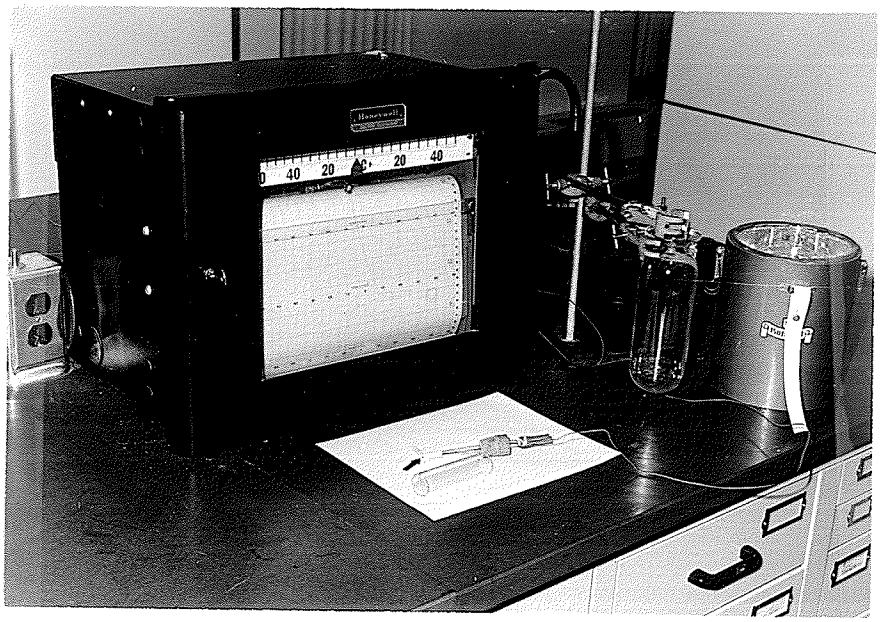


Figure 6

Figure 7. Head capsule widths of preserved larvae of M. knabi: 30 specimens measured per instar.

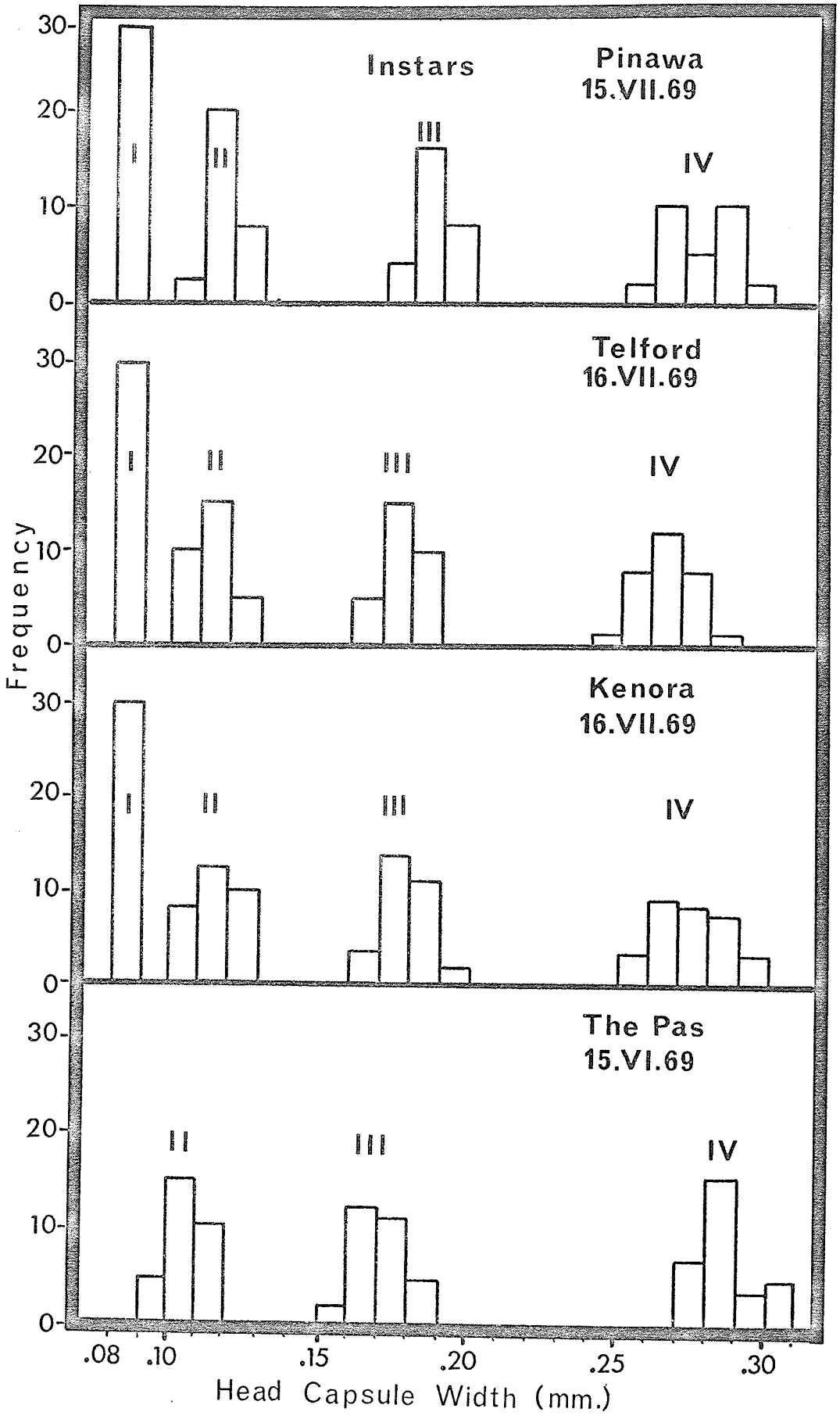

Instars
Pinawa
15.VII.69
Telford
16.VII.69
Kenora
16.VII.69
The Pas
15.VI.69
I
II
III
IV
Frequency
0
10
20
30
.08
.10
.15
.20
.25
.30
Head Capsule Width (mm.)

Figure 8. Winter location of larvae of M. knabi, showing the vertical positioning at the base of the leaf-cup of S. purpurea.

Figure 8

Figure 9. Frequency of each instar of *M. knabi* as a percent of the total number per collection date during 1969 at Pinawa, Manitoba.

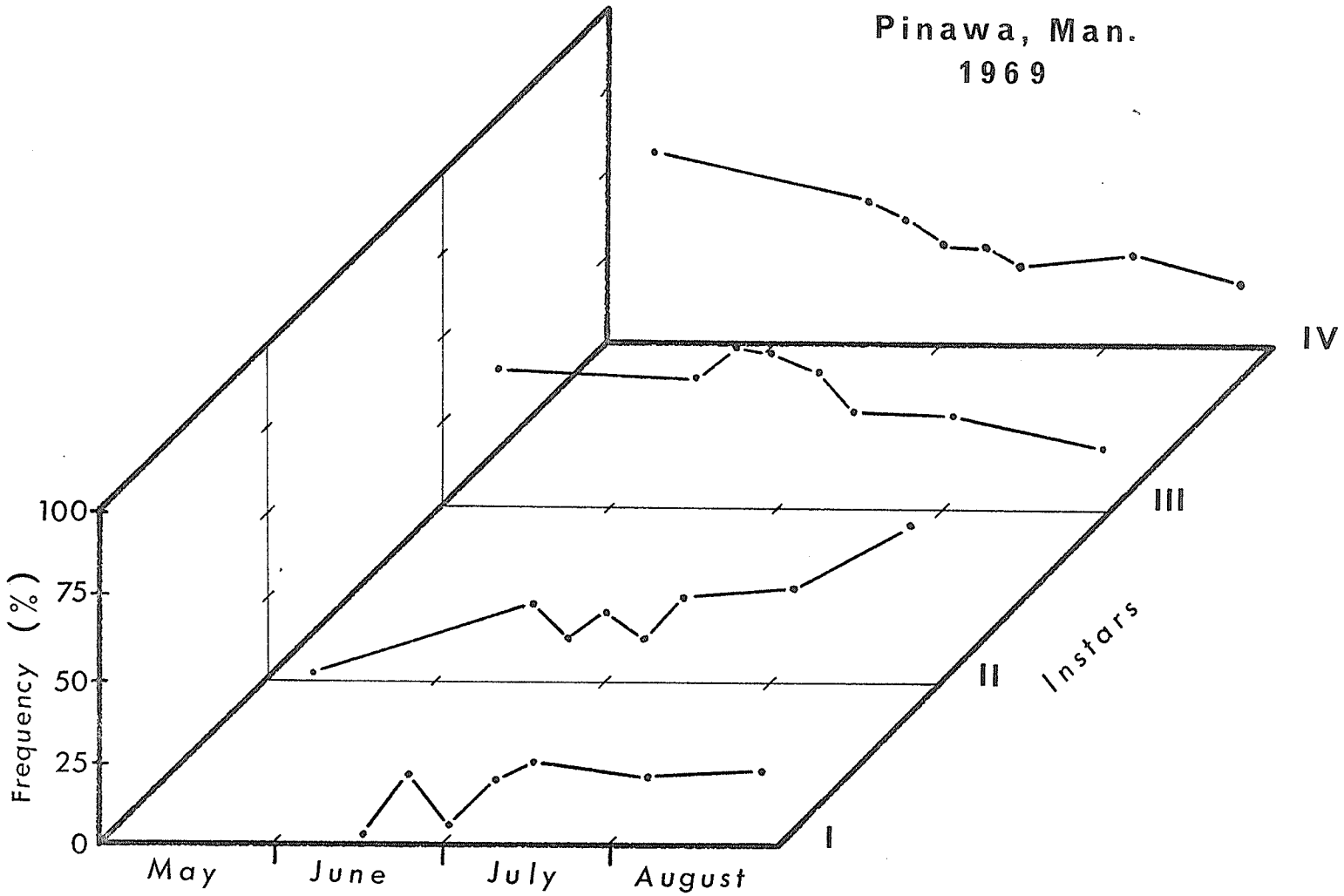

Pinawa, Man.
1969
IV
III
II
I
Instars
100
75
50
25
0
Frequency (%)
May
June
July
August

Figure 10. Frequency of each instar of M. knabi as a percent of the total number per collection date during 1969 at Telford, Manitoba.

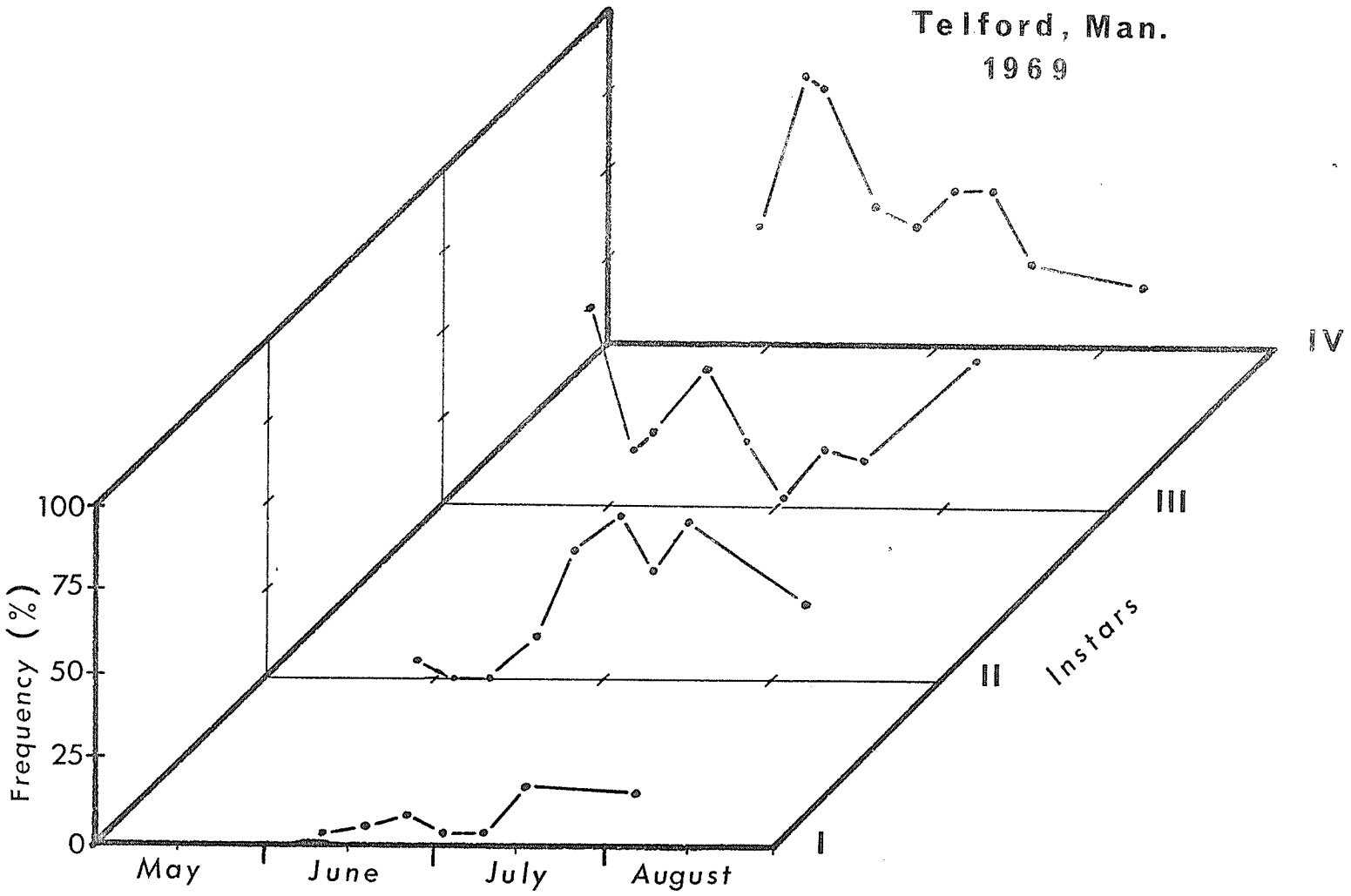

Telford, Man.
1969
Frequency (%)
100
75
50
25
0
May
June
July
August
I
II
III
IV
Instars

Figure 11. Female M. knabi at the ovipositional site.

Figure 12. Prepupal stage of M. knabi, showing swollen thorax, and developing ommatidia beneath the larval head capsule.

Figure 11

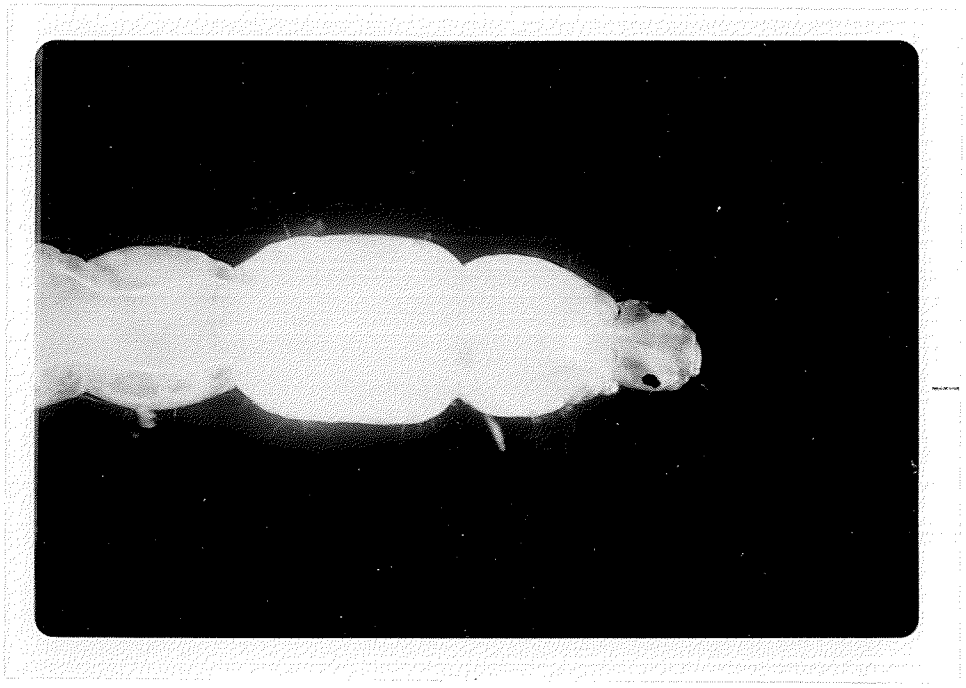


Figure 12

Figure 13.-16. Development of imaginal characters in pupae of M. knabi. Figure 13. 1 hr: Figure 14.- 6 hrs: Figure 15. 12 hrs: Figure 16.- 24 hrs.

Figure 13

Figure 14

Figure 15

Figure 16

Figure 17. Pupations of M. knabi under different photoperiods after 46 days. Larvae collected at Kenora, May 3, 1970.

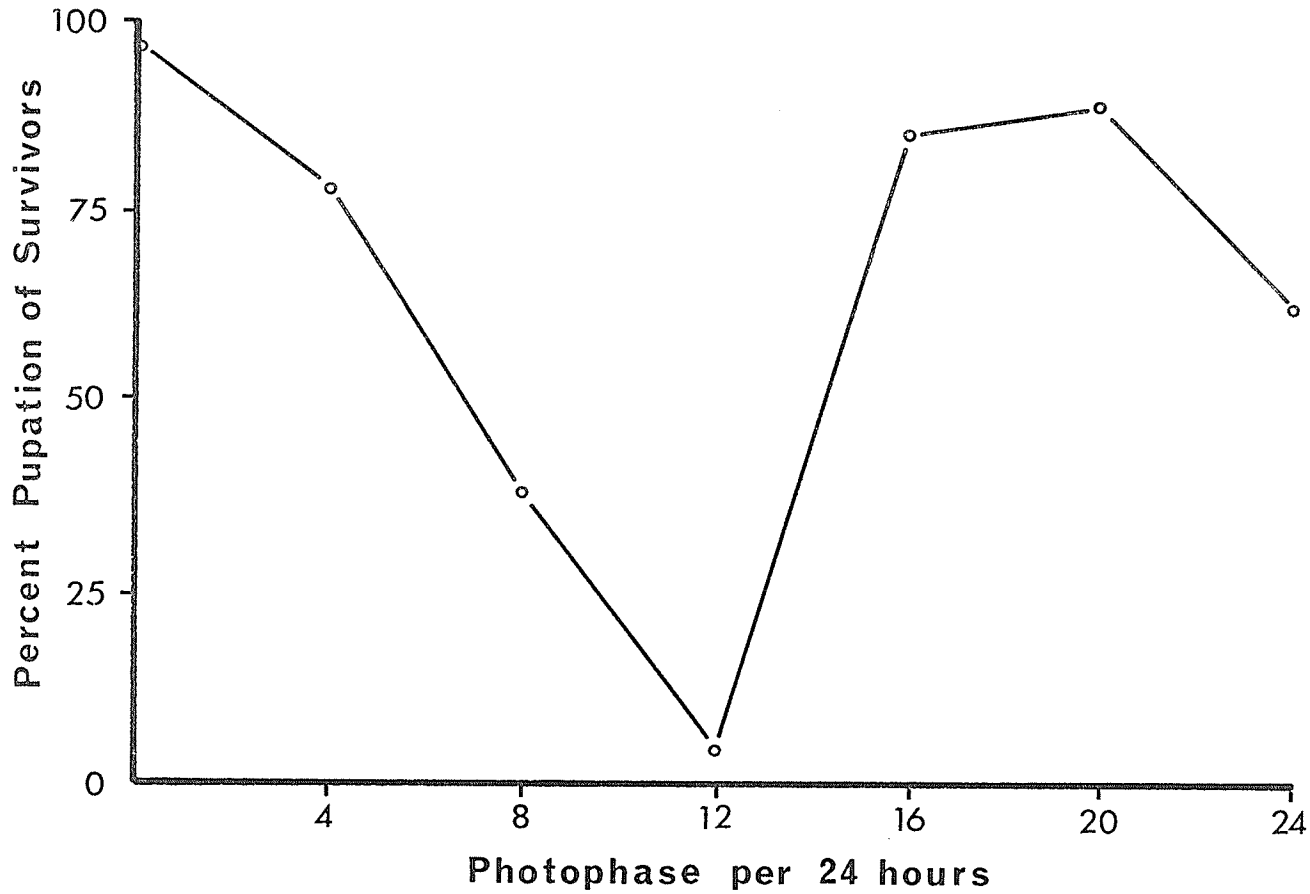

100
75
50
25
0
Percent Pupation of Survivors
4
8
12
16
20
24
Photophase per 24 hours

Figure 18. Pupations of M. knabi under different photoperiods after 46 days. Larvae collected at Kenora, May 10, 1970.

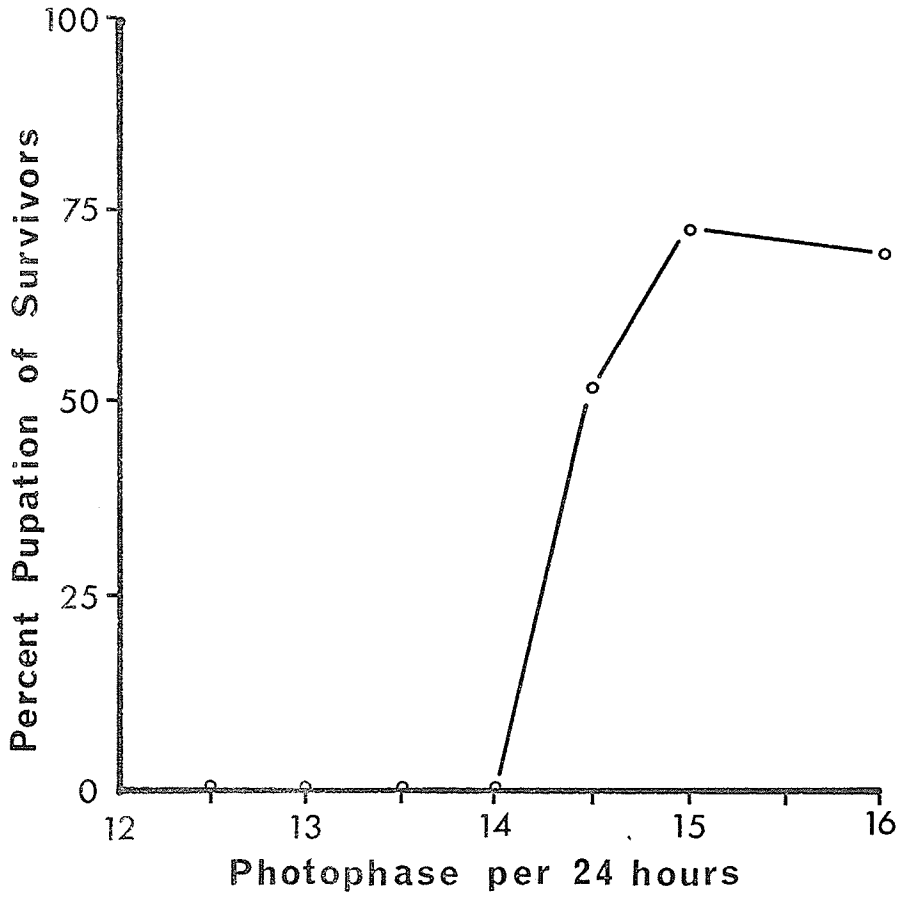

100
75
50
25
0
Percent Pupation of Survivors
12
13
14
15
16
Photophase per 24 hours

Figure 19. The effect of temperature on the rate of pupation of M. knabi collected from Pinawa, January 29, 1971 and placed under a 15L: 9D photoperiod.

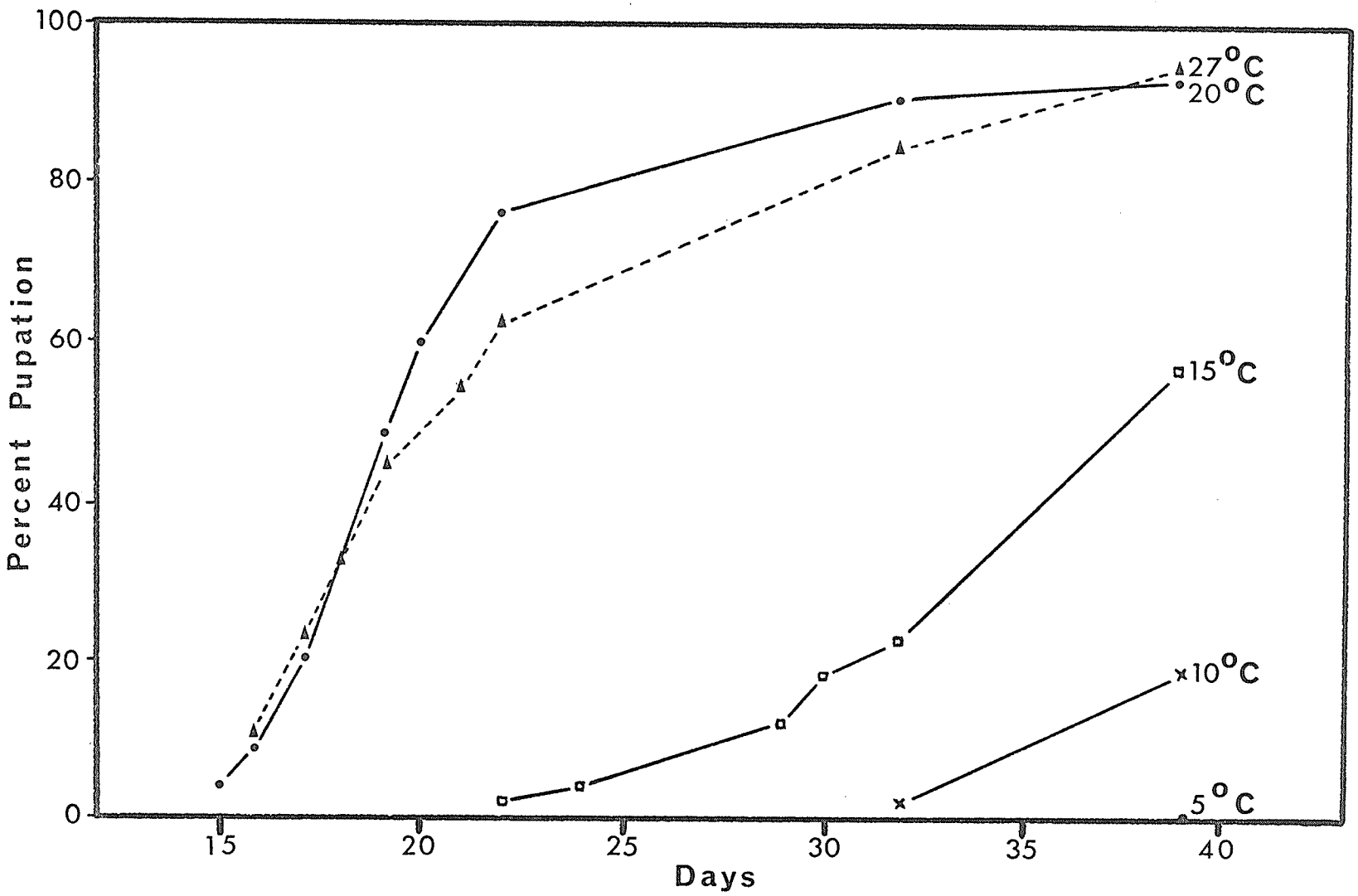

100
80
60
40
20
0
Percent Pupation
15
20
25
30
35
40
Days
27°C
20°C
15°C
10°C
5°C

Figure 20. Mean weekly sphagnum temperatures at the Pinawa, Manitoba bog from October 5, 1969 to October 10, 1970. Probe 5 cm deep in sphagnum.

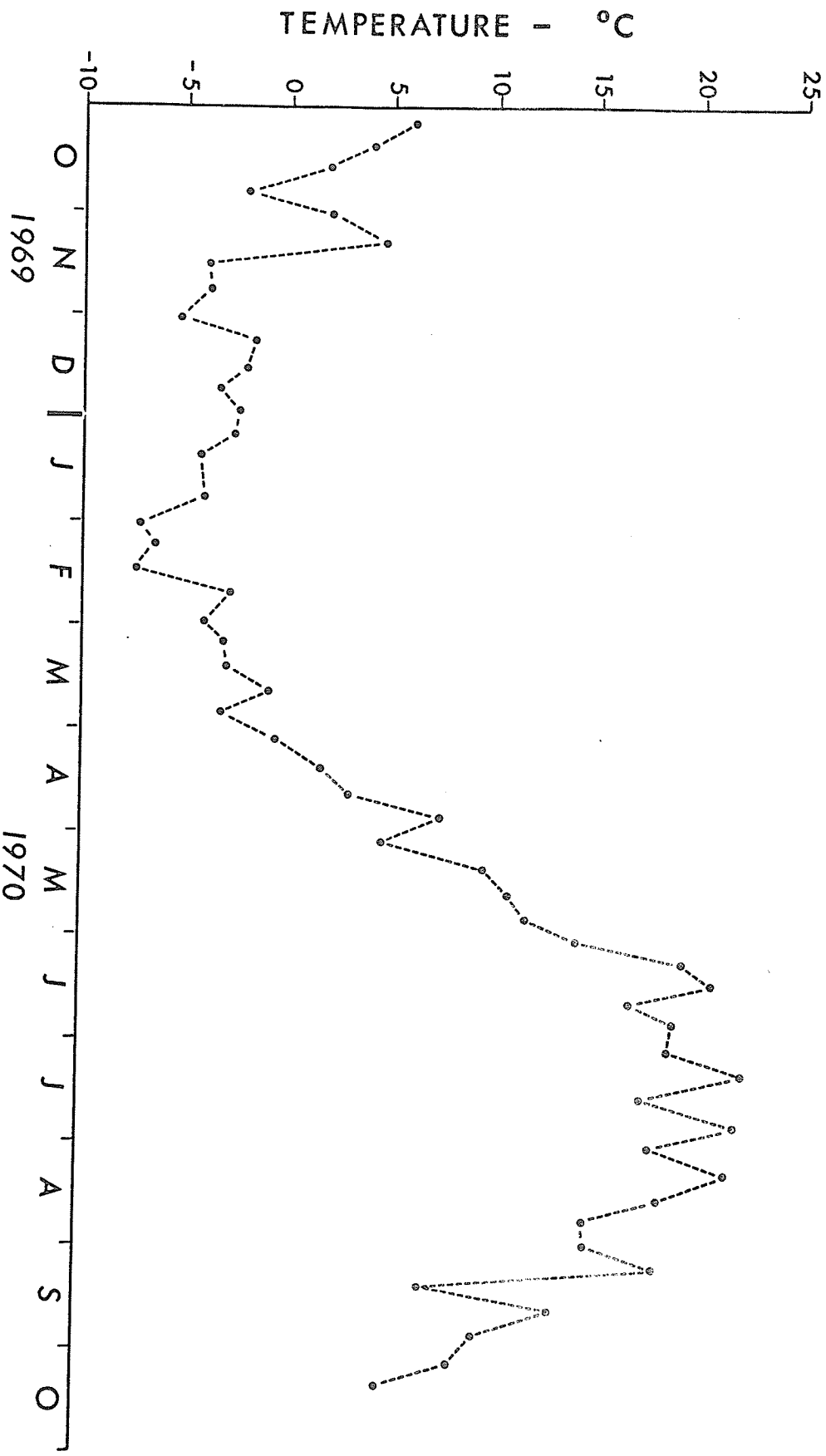

TEMPERATURE - °C
-10
-5
0
5
10
15
20
25
O
N
D
J
F
M
A
M
J
J
A
S
O
1969
1970

Figure 21. Comparison of diapause induction and termination dates between larvae from North Carolina (35°N) and Manitoba (50°N.). A - diapause termination of North Carolina larvae in the laboratory and field: B - diapause termination of Manitoba larvae in the laboratory: C - emergence of Manitoba adults: D - laboratory induction of diapause and cessation of emergence in Manitoba: E - field induction of diapause in North Carolina larvae.

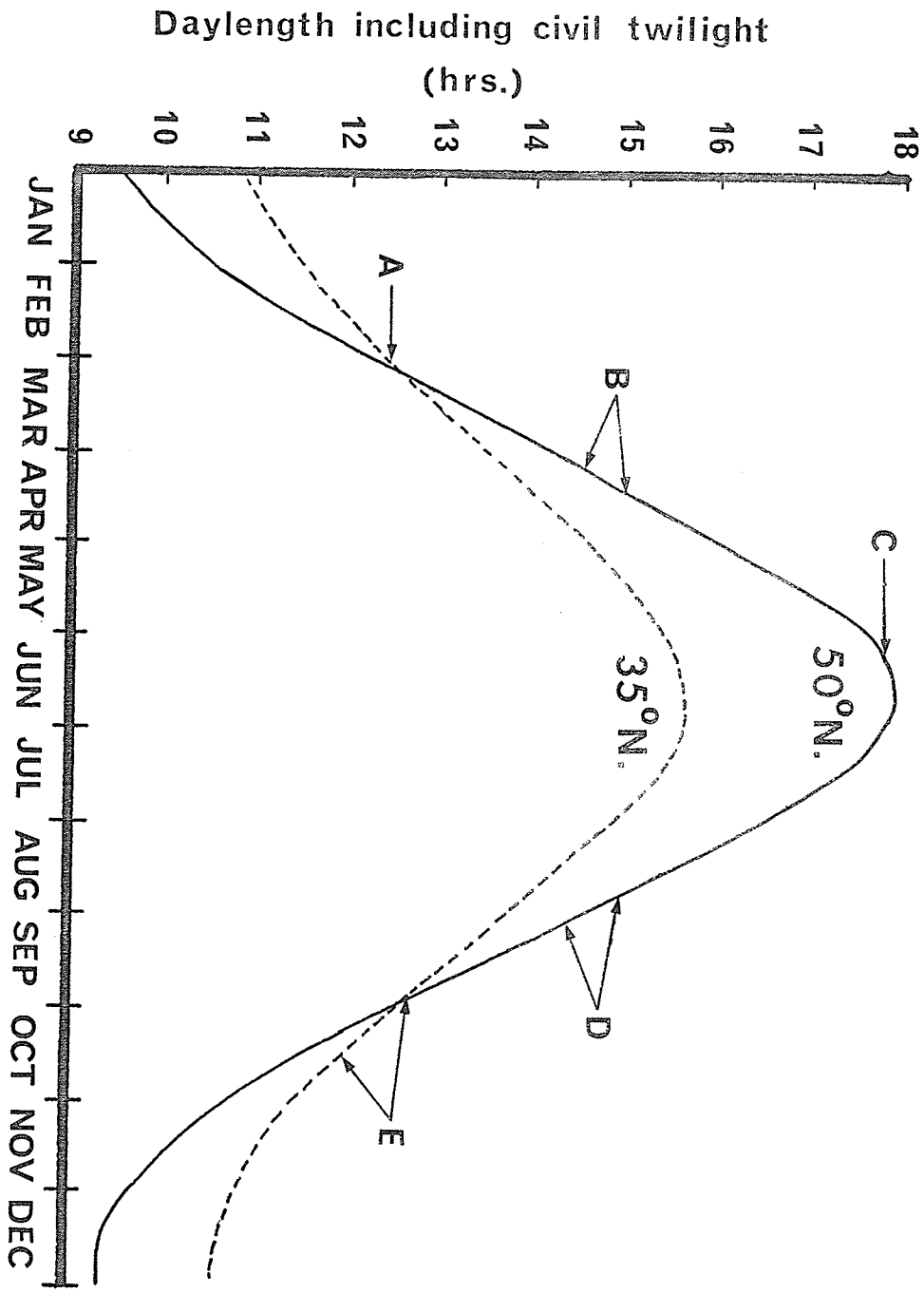

Daylength including civil twilight
(hrs.)
9
10
11
12
13
14
15
16
17
18
JAN FEB MAR APR MAY JUN JUL AUG SEP OCT NOV DEC
A
B
C
D
E
50°N.
35°N.

Figure 22. Survival of larvae of M. knabi in leaves of S. purpurea when subjected to -25°C. Collection from Pinawa, Manitoba, January 28, 1971.

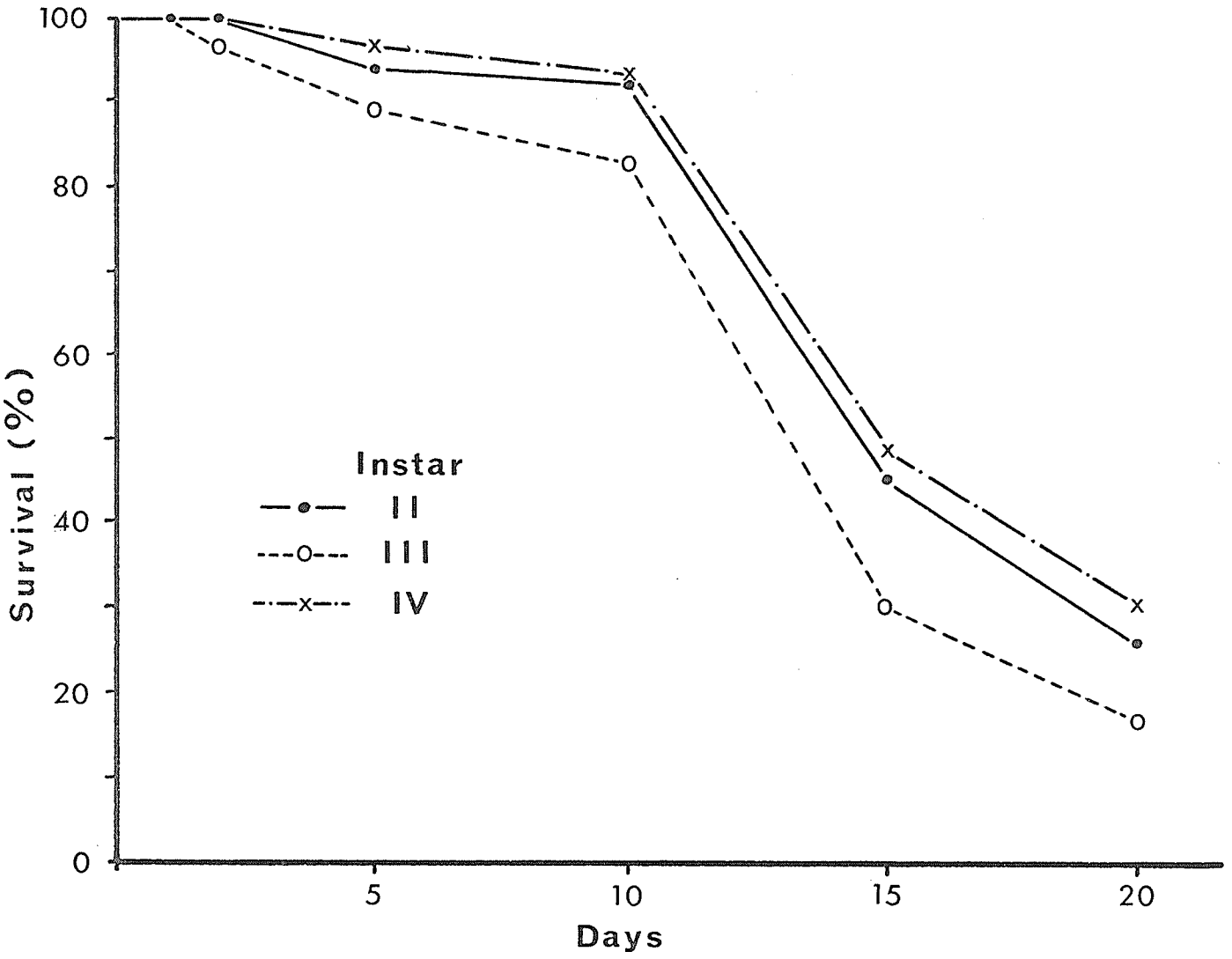

100
80
60
40
20
0
Survival (%)
Instar
II
III
IV
5
10
15
20
Days

Figure 23. Follicular development in M. knabi. Points represent mean follicular length per time interval. Lines represent one standard deviation. Circles represent single individuals.

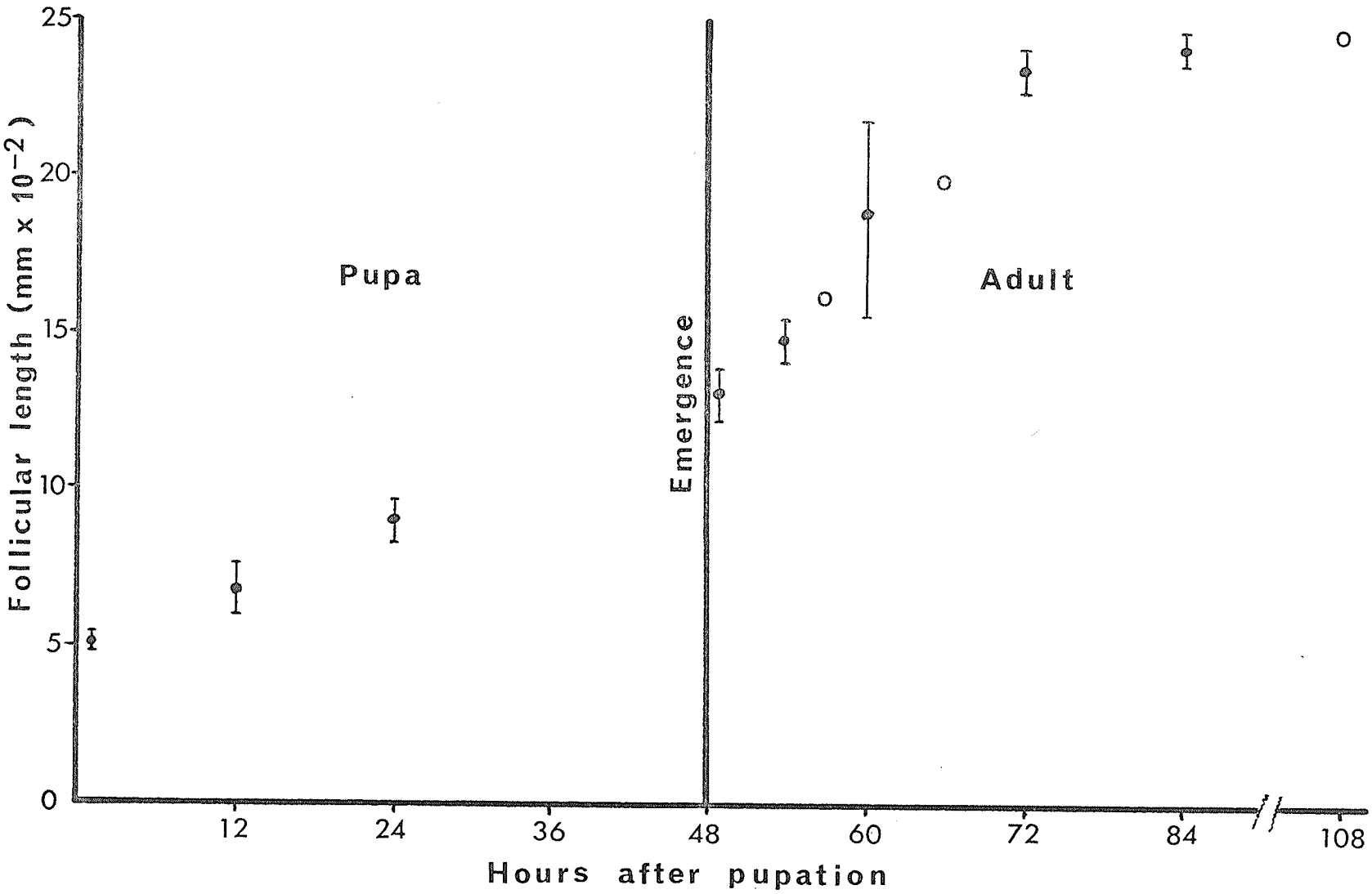
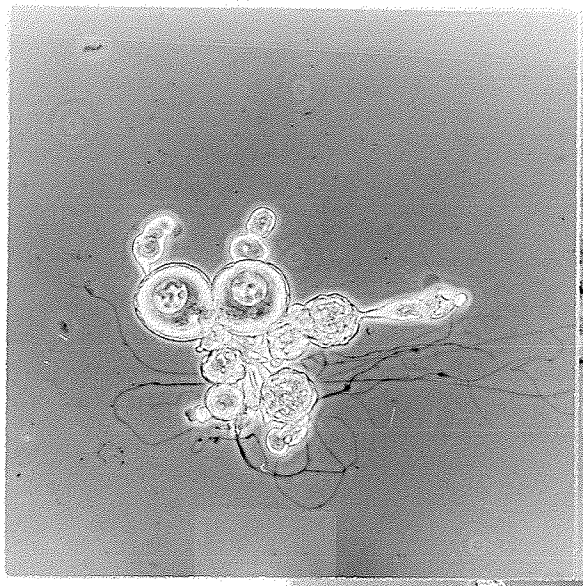
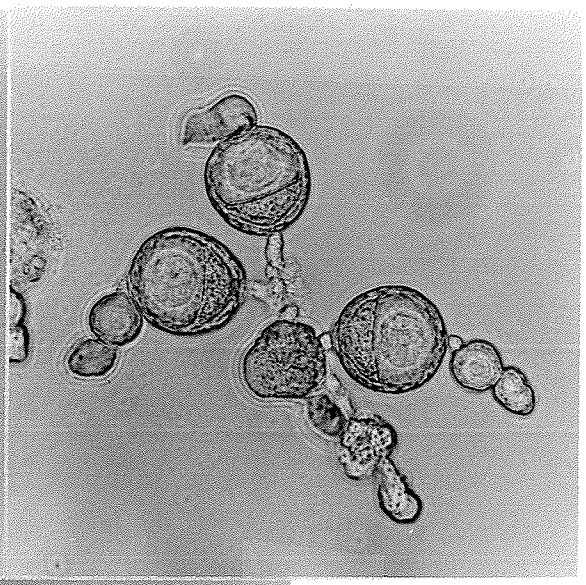

Follicular length (mm x 10^{-2})
25
20
15
10
5
0
Pupa
Emergence
Adult
12
24
36
48
60
72
84
108
Hours after pupation

Figure 24.- 26. Development of pupal follicles. Figure 24.- 1 hr (x200): Figure 25. 12 hr (x220): Figure 26.- 24 hr (x 240).

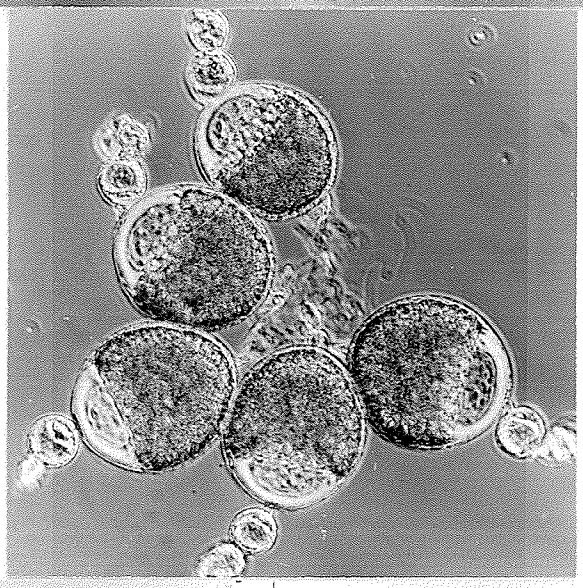
Figure 27-28. Development of adult follicles. Figure 27. - 1 hr (x 165): Figure 28.- 12 hrs (x 165).



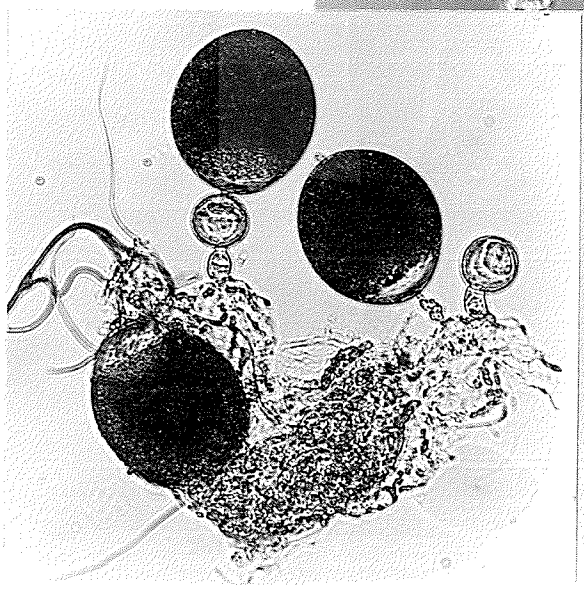
24



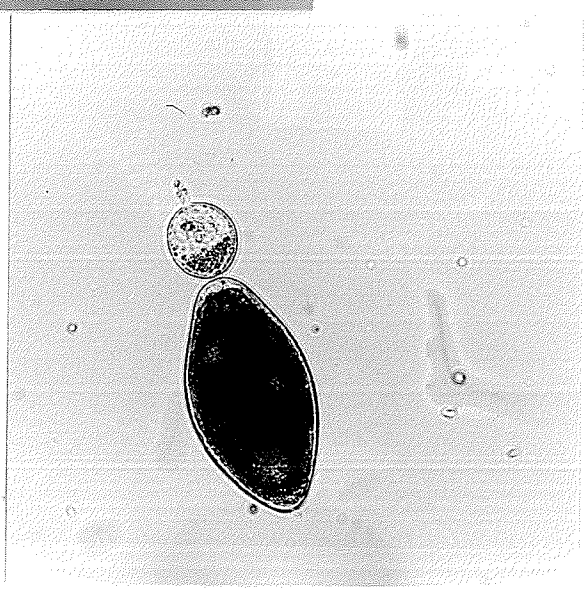
25



26



27



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LITERATURE CITED

Adkisson, P.L. 1966. Internal clocks and insect diapause. Nature 154(3746): 234-241.

Anderson, F.S. 1946. East Greenland lakes as habitats for chironomid larvae. Medd. Groenland 100: 5-65.

Anderson, J.F., and Hitchcock, S.W. 1968. Biology of Chironomus atrella in a tidal cove. Ann. Ent. Soc. Amer. 61: 1597-1603.

Beck, S.D. 1968. Environmental photoperiod and the programming of insect development. Chap. 9. In Evolution and Environment. New Haven and London. Yale Univ. Press. pp. 279-296.

Bradshaw, W.E. 1969. Major environmental factors inducing the termination of larval diapause in Chaoborus americanus. Johannsen (Diptera: Culicidae). Biol. Bull. 136: 2 - 8.

Bradshaw, W.E. 1970. Interaction of food and photoperiod in the termination of larval diapause in Chaoborus americanus (Diptera: Culicidae). Biol. Bull. 139: 476-484.

Buffington, J.D. 1970. Ecological considerations of the cohabitation of pitcher plants by Wyeomyia smithii and Metriocnemus knabi. Mosq. News. 30(1): 89-90.

Chutter, F.M. 1970. A preliminary study of factors influencing the number of oocytes present in newly emerged blackflies (Diptera: Simuliidae) in Ontario. Can. J. Zool: 48: 1389-1400.

Clements, A.N. 1963. The Physiology of Mosquitoes. MacMillan Co: New York. 393pp.

Crisp, G. and Lloyd, L. 1954. The community of insects in a patch of woodland mud. Trans. Roy. Ent. Soc. London 105: 269-313.

Coquillett, D.W. 1904. Several new Diptera from North America. Can. Ent. 36: 10-12.

Davidson, J. 1944. On the relationship between temperature and rate of development of insects at constant temperatures. J. Animal Ecol. 13: 26-38.

Davis, C.C. 1966. A study of the hatching process in aquatic invertebrates. Hydrobiologia 27: 196-207.

de Wilde, J. 1962. Photoperiodism in insects and mites. Ann. Rev. Ent. 7: 1-26.

Dyar, H.G. 1902. Illustrations of the larvae of North American Culicidae II. J.N.Yk. Ent. Soc. X: 194-201. (4 Plates)

Dyson, J.E.B. and Lloyd, L. 1936. The distribution of the early stages of Metriocnemus longitarsus Goet. Proc. Leeds Phil. and Lit. Soc. Sci. Sect. 3(3): 174-176.

Engelmann, W. and Shappirio , D.G. 1965. Photoperiodic control of the maintenance and termination of larval diapause in Chironomus tentans. Nature 207: 548-549.

Evans, K.W. 1971. Bionomics of the pitcher plant mosquito Wyeomyia smithii (Dipt; Culicidae). M.Sc. thesis. University of Manitoba.

Hamilton, A.L. 1969. A method of separating invertebrates from sediments using longwave ultraviolet light and fluorescent dyes. J. Fish. Res. Bd. Canada. 26: 1667-1672.

Heron, R.J. 1968. Vital dyes for behavioural and population studies of the larch sawfly Pristophora erichsonii (Hymenoptera: Tenthredinidae). Can. Ent. 100: 470-475.

Jenner, C.E. 1951. Photoperiodism in the fresh-water pulmonate snail, Lymnaea palustris. PhD. thesis, Harvard University, Cambridge Mass.

Johannsen, O.A. 1905. Aquatic Nematocerous Diptera II. Chironomidae. New York State Mus. Bull. 86: 76-331.

Johannsen, O.A. 1937. Aquatic Diptera. Pt. III. Chironomidae,: subfamilies Tanypodinae, Diamesinae and Orthocladiinae. Cornell Agr. Exp. Sta. Mem. 205: 1-84.

Kitching, R.L. 1969. Population studies of insects in water-filled tree-holes . J. Appl. Ecol. 6(3): 19p-20p.

Knab, F. 1905. A chironomid inhabitant of Sarracenia purpurea, Metriocnemus knabi Coq. J.N. Yk. Ent. Soc. 13: 69-73.

Lloyd, L.L. and Turner, J.N. 1936. The migrations of the larvae of Metriocnemus longitarsus Goet., (Chironomidae) in response to gravity and light. Proc. Leeds Phil. and Lit. Soc. Sci. Sect. 3(3): 177-188.

Malloch, J.R. 1914. The early stages of Metriocnemus lundbecki Johannsen. Ent. Soc. Wash. Proc. 16: 132-135.

Mellanby, K. 1959. Acclimatization affecting the position of the cold and heat death points of larvae of Aedes aegypti (L). Bull. Ent. Res. 50: 821-823.

Miall, L.C. and Hammond, A.R. 1900. The Harlequin Fly. Clarendon Press. Oxford. 196pp.

Oliver, D.R. 1968. Adaptations of arctic Chironomidae. Ann. Zool. Fenn. 5: 111-118.

Oliver, D.R. 1971. Life history of the Chironomidae. Ann. Rev. Ent. 16: 211-230.

Paris, O.H. and Jenner, C.E. 1959. Photoperiodic control of diapause in the pitcher-plant midge, Metriocnemus knabi. In: Photoperiodism and Related Phenomena in Plants and Animals. ed. R. B. Withrow, Am. Ass. Adv.Sci.55. Washington.903 pp.

Paterson, C.G. 1971. Overwintering ecology of the aquatic fauna associated with the pitcher plant Sarracenia purpurea L. Can. J. Zool.49: 1455-1459.

Salt, R.W. 1961. Principles of cold-hardiness. Ann. Rev. Ent. 6: 55-74.

Scholander, P.F., Flagg, W., Hock, R.J. and Irving, L. 1953. Studies on the physiology of frozen plants and animals in the arctic. J. Cell. Comp. Physiol. Suppl. 1: 42-56pp.

Smith, J.B. 1901. Some notes on the larval habits of Culex pungens. Entomol. News. XII: 153-157.

Smith, J.B. 1902. Life history of Aedes smithii Coq. J. N. Yk. Ent. Soc. X: 10-15.

Snedecor, G.W. 1946. Statistical Methods, 4th ed. Iowa State College Press.Ames, Iowa.534pp.

Somme, L. 1968 a. Akklimatisering hos insekter til hoye og lave temperaturer. (Acclimatization to high and low temperatures in insects). Eng. summary. Fauna (Oslo) 21(2): 98-107.

Somme, L. 1968b. Acclimation to low temperatures in Tribolium confusum Duval (Col.: Tenebrionidae). Norsk. Ent. Tidssk. 15(2): 134-136.

Torre-Bueno, J.R. 1962. Glossary of Entomology. Brooklyn. Ent. Soc. N. Yk. 336pp.

Wulker, W. and Gotz, P. 1968. Die Verwendung der Imaginalscheiben zur Bestimmung des Entwicklungszustandes von Chironomus-Larven (Dipt.). Z. Morphol. Tiere 62(4): 363-388.