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# ECOPHYSIOLOGICAL STUDIES ON INVERTEBRATE DIAPAUSE

**Gillian Telfer**

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A thesis presented in candidature for the degree of Master of Science

Department of Biological Sciences

University of Durham

April 1997



- 5 MAR 1998

*"The study of insect dormancies is a study of misstatements and misunderstandings"*

Jungreis (1978)

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This is for little Rosie.

I certify that all material in this thesis which is not my own work has been identified and that no material is included for which a degree has previously been conferred upon me.

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## Summary

Diapause performs two main functions in the life-cycle of an insect. In addition to protecting the organism from adverse seasonal conditions it synchronises active stages of development with optimal conditions for growth. In most temperate species studied photoperiod and temperature are the two principal zeitgebers involved in insect periodicity and diapause expression. Generally insects respond to either quantitative changes in day-length or according to a fixed critical threshold of light. The rate of diapause development is controlled primarily by temperature, as numerous studies have demonstrated that chilling hastens termination of dormancy in a large number of autumnal-hibernal diapausing insects. This study focused on the effects of photoperiod and temperature on a number of diapausing insect species found around County Durham. In the crane fly *Tipula subnodicornis*, larvae subjected to a critical regime equal to or below L:D 12:12 pupated significantly later than individuals kept on a regime of L:D 14:10 and above. In the carabid beetle *Nebria salina*, long-day suppressed ovarian maturation whereas short-day favoured egg development. Male reproductive development occurred irrespective of the applied photoperiod. In *Tipula fusca* and *Coleophora alticolella*, chilling hastened development towards hatching and adult emergence, respectively. Intraspecific variation in response to the combined effects of chilling and photoperiod was observed in *C. alticolella* and the relevance of such findings in the field were discussed.

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## Chapter 1

### 1.1 Introduction

#### 1.1.1 Invertebrate diapause

A widely accepted definition of diapause was outlined by Tauber *et al.* (1986) who state that:

"...diapause... [is] a neurohormonally mediated, dynamic state of low metabolic activity. Associated with this are reduced morphogenesis, increased resistance to environmental extremes and altered or reduced behavioural activity" (Tauber *et al.* 1986).

One important characteristic of diapause is its anticipatory nature that involves specific physiological preparations in response to environmental cues occurring before the onset of, for example, extreme conditions of temperature, drought and low abundance of food. Another key feature is that dormancy does not end until the unfavourable conditions have passed, therefore distinguishing it from non-adaptive torpor which is manifested in direct response to sub-lethal conditions. Diapause is generally regarded to perform two main functions in the life cycle of an insect: (i) to prepare the insect for oncoming unsuitable environmental conditions, thus increasing the chances of survival and (ii) to synchronise the life cycle so that stages of active development will take place under optimal conditions for growth. This function is more obvious in insects that have a short imaginal life (Andrewartha, 1952; Danilevskii, 1965). At present photoperiod and temperature are regarded as the two principal environmental zeitgebers involved in insect periodicity.

To fully understand this aspect of insect phenology substantial work has been carried out on the influence of temperature and photoperiod on diapause. With respect to photoperiod, until relatively recently the majority of studies had focused on diapause-induction and diapause-termination in Lepidoptera (Danilevskii, 1965). In 1976 Tauber & Tauber published a review on the subject of insect seasonality in which they re-evaluated the widely-accepted but generally unfounded concepts held at the time on the influence of natural stimuli on diapause-maintenance and termination (Tauber & Tauber, 1976). Until then very few field-related studies had researched the effect of photoperiod throughout the course of diapause and the mechanisms by which insects responded to naturally changing photoperiods were therefore unclear. By exposing

diapausing larvae collected in the field to both stationary and changing daylengths two main photoperiodic responses were proposed: graded vs. critical response (Tauber & Tauber, 1976);

#### *Graded response*

Insects possessing this type of photoperiodic reception perceive and respond to the absolute duration of actual day-length. For example, in autumn-diapausing species diapause is maintained by the actual duration of the decreasing photoperiod. Sensitivity to photoperiod either gradually diminishes with time or it remains throughout the course of diapause, ultimately playing a role in diapause-termination. In the lacewing *Chrysopa carnea*, diapause development is decelerated (and therefore diapause is maintained) by the decreasing autumn photoperiod. With the passage of time sensitivity to day-length is progressively lost, eventually reaching a point where photoperiod does not influence diapause termination. In a related species, *Chrysopa downesi*, the decreasing day-length in the field acts to maintain diapause, with diapause development accelerated and eventually terminated by increasing photoperiod (Tauber & Tauber 1973a, b, 1976).

#### *Critical photoperiod (or "all-or-none" response)*

This type of response occurs in insects that measure long day-length against short day-length with respect to a critical photoperiodic threshold. Autumn-diapausing insects exposed to daylengths below the defined threshold will remain in diapause while those exposed to a regime above the threshold will terminate dormancy. In summer-diapausing insects the diapause is generally induced and maintained by long photoperiod and terminated in response to short autumn day-lengths (Tauber & Tauber, 1976). As in insects who respond to the absolute duration of day-length, sensitivity to photoperiod either persists throughout the diapause or progressively diminishes. In the mosquito *Wyeomia smithii* (Smith & Brust, 1971) and the green lacewing *Meleoma signoretti* (Tauber & Tauber, 1975) short photoperiod induces dormancy and sensitivity to day-length continues throughout the course of diapause. In both species long photoperiod terminates dormancy. In *Chrysopa harrisii* (Tauber & Tauber, 1974) and the grass spider *Agelena limbata* (Kurihara *et al.* 1979) short day-length acts to maintain diapause in its early stages, but ultimately photoperiod is not involved in its termination. In this case either the diapause runs its full course and ends spontaneously, or a second diapause-maintaining factor such as temperature will be dominant.

As photoperiod and temperature are the major environmental cues involved in diapause expression in temperate insects, the present work aimed to investigate the influence of such factors in insects found around County Durham. The effect of day-length on the number of days to hatching and pupation in two species of crane fly, *Tipula fusca* and *Tipula subnodicornis* was studied, while in the carabid beetle *Nebria salina*, adults were exposed to differing regimes of day-length to determine the effect of photoperiod on gonad maturation. As numerous studies have demonstrated that a large number of autumnal-hibernal diapausing insects will terminate diapause following a period of chilling, the effect of temperature and day-length on adult emergence in diapausing *Coleophora alticolella* was also investigated.

## Chapter 2

### 2.1 Introduction

#### 2.1.1 *Tipula fusca* and *Tipula subnodicornis*: taxonomy, distribution and general life cycle

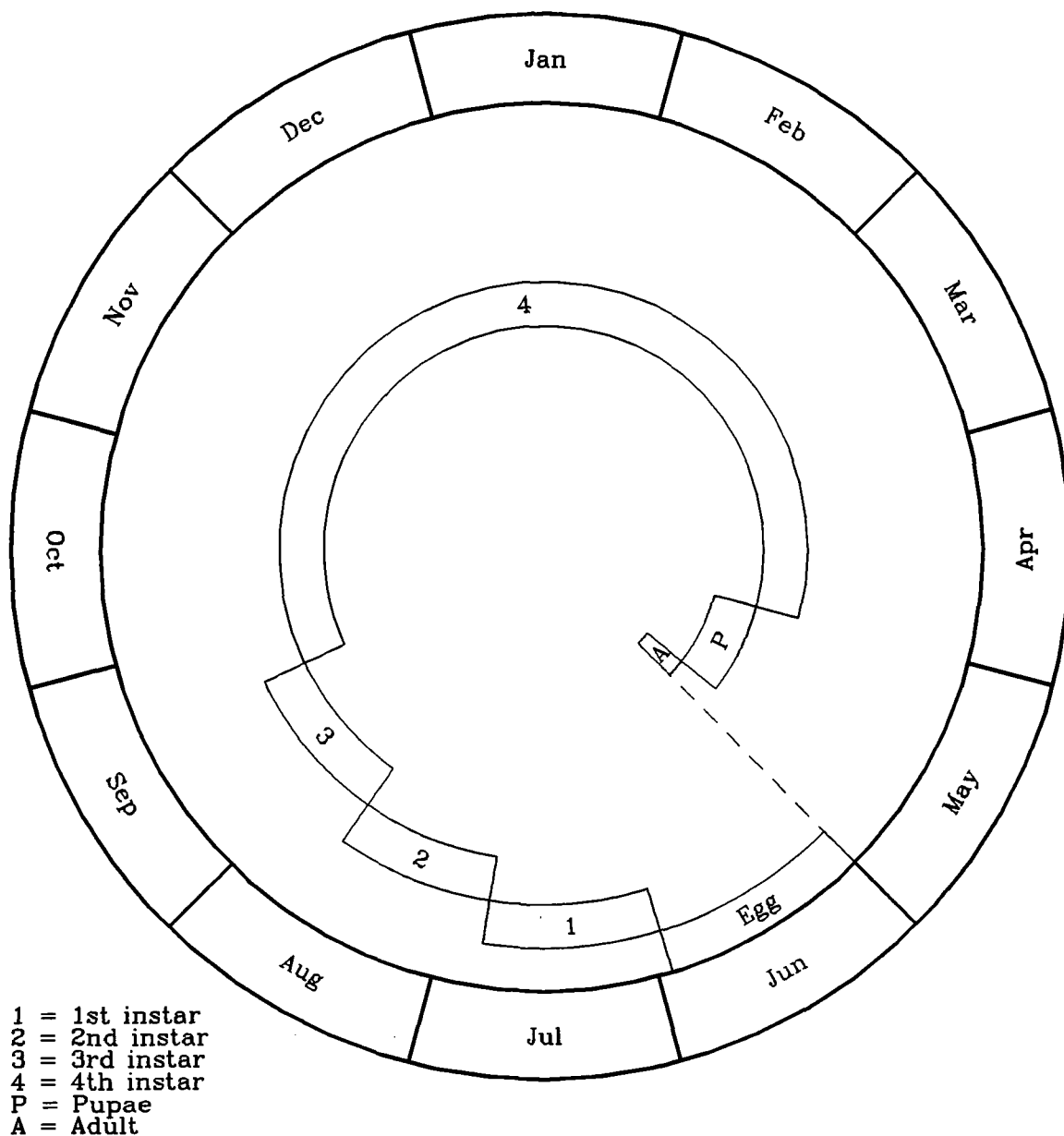
*Tipula fusca* (Diptera: Tipulidae, = *T. czizeki* de Jong) is a univoltine cranefly that has an egg diapause and a late Oct./Nov. flight period (Pritchard, 1983). This species predominantly inhabits the wet grassland areas of north-east Europe such as Great Britain, Norway and northern Italy (Lindner, 1980).

*Tipula subnodicornis* Zetterstedt (Diptera: Tipulidae) is an upland peat dweller found abundantly on moorland dominated by *Juncus squarrosus* and *Eriophorum vaginatum*. In Europe it has a widespread northern distribution, commonly found in Finland (Mannheims, 1954), the Faroe Isles (Lindner, 1980), Norway, Sweden and Lapland (Tjeder, 1955). In Great Britain the general life cycle and moorland distribution was outlined in the Pennines by Coulson (1962), while Freeman (1964) gave details for the New Forest, Hampshire.

Coulson (1962) described in detail the annual life cycle and development of *T. subnodicornis* on the Moor House Nature Reserve, Cumbria (National Grid Reference NY 758329). This species shows a highly synchronised period of spring adult emergence, peaking between mid-May and mid-June. Mark recapture tests indicate that adult life expectancy is between 48-22 hours for males and 32-15 hours for females (Coulson, 1962). Following emergence the adults mate and within three hours approximately 50% of eggs are deposited within the first three centimetres of the upper peat litter, rising to 90% after 10 hours. The egg stage lasts approximately three weeks in the field and precedes four larval stadia, all of which are active feeding. Stadia one to three last around three to four weeks and by mid-September the majority of larvae have entered instar four, which is approximately 32 weeks long and includes the diapause stage. Highest rates of growth are observed in the early instars, while the greatest part of larval development occurs between July and December. Pupae are observed in the field around the beginning of May, preceding adult emergence by around twenty days. Figure 1 represents the annual life-cycle of *T. subnodicornis* at Moor House (taken from Coulson, 1962).



Fig 1: Life cycle of *Tipula subnodicornis* at Moor House Nature Reserve  
(taken from Coulson, 1962)



### 2.1.2 Photoperiodic response in *Tipula subnodicornis*

In a study on the life-cycles of the two common moorland crane-flies *Tipula subnodicornis* Zetterstedt and *Molophilus ater* Meigen over a series of different altitudes in the Pennines, synchronisation of the spring adult emergence was found to be dependent upon the insertion of a winter diapause (Coulson *et al.* 1976). Both species when placed on a short day-length (L:D 6:18) at 10°C entered diapause. While *M. ater* could be kept indefinitely in diapause on this regime, all of the *T. subnodicornis* larvae eventually pupated. Both species when taken from the field and tested at different intervals throughout the winter showed a decreasing response to photoperiod (Coulson *et al.* 1976).

Further work on *T. subnodicornis* demonstrated that the winter diapause could be broken in its early stages by long photoperiod, as larvae subjected to long day-lengths pupated significantly earlier than subjects kept on a short-day regime (Butterfield, 1976). From these findings however it was not clear how the crane-flies actually perceived day-length: for example, whether the insects responded to either a critical threshold of light or to the actual value of the applied photoperiod, as had been reported in the summer-diapausing crane-fly *Tipula pagana* (Butterfield, 1976).

### 2.1.3 Aims of the present work

The aim of the present study was to investigate the effect of photoperiod on the number of days to hatching in chilled diapausing *T. fusca* eggs. To determine the photoperiodic response of diapausing *T. subnodicornis* larvae, individual groups were exposed to different regimes of day-length and the number of days to pupation under each regime was measured and compared under controlled conditions of temperature.

## **2.2 Methods**

### **2.2.1 Sample site: Chapel Fell**

Chapel Fell (National Grid Reference NY 863349) is located in the Northern Pennines, 6 km south-east of St. John's Chapel. It is predominantly covered by blanket peat and three main types of vegetation: *Agrost-Festucetum*, *Eriophoretum* and Fen and flush, all of which are heavily grazed (Rawes & Welch, 1964). *Eriophorum vaginatum* is the most dominant species, covering approximately 60% of Chapel Fell, while *Juncus* spp. constitute approximately 10% of the total vegetation type.

### **2.2.2 *Tipula fusca*: method of collection and experimental regimes**

Newly emerged *T. fusca* adults were collected from Chapel Fell in October 1994 and stored in jars lined with moist filter paper in the laboratory. Deposited eggs were placed on a short-day regime (L:D 6:18) at 1°C until 13th January 1995. Two equal groups containing approximately 500 eggs ( $\pm 5$  eggs) were prepared and incubated at 10°C in constant-temperature cabinets ( $\pm 0.5^\circ\text{C}$ ) on a regime of either L:D 18:6 or L:D 6:18. The eggs were checked daily to monitor the date of hatching. Throughout the experiment, regular inspections were made on the state of the filter paper; for example, extra water was added if conditions were deemed too dry and clean filter paper replaced any with observable signs of fungal infection.

### **2.2.3 *Tipula subnodicornis*: larval extraction (Berlese method)**

Twenty peat cores measuring 17.5 x 17.5 cm were removed from Chapel Fell on 29th November 1994. In the laboratory, each core was placed on a circular mesh-grid and heated by a 100 watt light bulb. The general principle of this technique is that with time, the soil is warmed throughout and any invertebrates present in the core will move away from the desiccating heat, ultimately falling into jars lined with damp tissue paper. The whole process was completed within one week.

### **2.2.4 *Tipula subnodicornis*: experimental regimes**

Extracted larvae were stored in moist peat containing *Eriophorum vaginatum* and kept at 5°C in constant-temperature rooms ( $\pm 0.5^\circ\text{C}$ ) on a short photoperiod until 5th December 1994. The larvae were then divided into groups of 45 (three groups of 15 larvae) and subjected to the following light regimes at 10°C:

**Table 2.1: Range of light regimes used to determine the effect of photoperiod on the number of days to pupation in final instar *Tipula subnodicornis* larvae**

Group	Photoperiodic regime (L:D)
A	8:16
B	10:14
C	12:12
D	14:10
E	16:18
F	18:6

The two photoperiodic extremes used in the experiment, that is L:D 8:16 and L:D 18:6 corresponded approximately to the natural daylengths associated with the winter solstice and the spring emergence period at Chapel Fell, respectively. Additional photoperiods represented the intermediate light regimes generally recorded between the two seasons. Throughout the experiment the condition of the peat was regularly inspected and kept moist. Fresh vegetation such as leafy liverworts and grass were supplied on a twice-weekly basis. The samples were checked daily and the number of days to pupation in each regime was recorded.

## 2.3 Results

### 2.3.1 Effect of photoperiod on the number of days to hatching in *Tipula fusca* eggs

Table 2.2 represents the number of days to hatching in chilled *T. fusca* eggs exposed to long and short photoperiod at 10°C. On 2nd February 1995 (20 days after chilling), 115 first-instar larvae emerged on the long-day regime. Thereafter, a further nine eggs hatched over the next six days. On 15th February (day 33), 105 hatchings were recorded in the group subjected to short day-lengths. An additional 10 eggs hatched the following day (day 34). The remaining eggs were found to be non-viable following dissection on 25th February.

The mean number of days to hatching on the long and short photoperiod was 20.15 days (s.d. =  $\pm 0.68$ ) and 33.09 days (s.d. =  $\pm 0.28$ ), respectively. When compared, the number of days to emergence was significantly lower in the eggs subjected to day-lengths of L:D 18:6 ( $z = 193.81$ ,  $P < 0.01$ ), indicating that the rate of development towards hatching was faster under this regime.

**Table 2.2 - Effect of long and short photoperiod on the number of days to hatching in chilled *T. fusca* eggs incubated at 10°C**

Group (L:D)	No. of days to hatching	No. of eggs hatched	% hatched
18:6	20	115	22
18:6	21	6	1
18:6	23	2	<1
18:6	26	1	<1
6:18	33	105	21
6:18	34	10	2

### 2.3.2 Effect of photoperiod on the number of days to pupation in final instar *T. subnodicornis* larvae

Table 2.3 represents the effect of photoperiod on the number and percentage of larvae surviving to pupation. The distribution of the number of days to pupation between the groups are shown in Figures 2 to 7. Mean number of days to emergence is represented in Figure 8.

On 9th January 1995 (day 36) two and seven larvae respectively pupated on the L:D 16:8 and L:D 18:6 regimes (Figs. 2 and 3). With the exception of one individual pupating on day 61, all the pupations occurred over a three-week period. Maximum numbers of pupations did not exceed five and seven per day, respectively. In the group subjected to day-lengths of L:D 14:10, pupae were not recorded until 23rd January (day 50) (Fig. 4). Under this regime, pupations continued over the next two and a half weeks and did not exceed two per day. On both the L:D 12:12 and L:D 14:10 photoperiods, pupae were first observed on 13th February (day 66), continuing thereafter until 1st March (day 92) and 14th March (day 100), respectively (Figs. 5 and 6). Maximum number of pupations did not exceed two and three per day in these groups, respectively. Only five larvae pupated on the L:D 8:16 regime during the period 27th February (day 84) to 14th March (day 100).

With respect to the mean number of days to pupation, one-way ANOVA followed by Tukey-HSD and Sheffe indicated that larvae exposed to daylengths equal to or below L:D 12:12 pupated significantly later than individuals exposed to photoperiods of L:D 14:10 and above ( $F_{5,91}=130.71$ ,  $18:6=16:8 \neq 14:10 \neq 12:12=10:14=8:16$ ,  $P<0.0001$ ). Therefore the rate of development towards pupation appeared to be dependant on a critical threshold of light, in this case a regime of L:D 12:12.

**Table 2.3: Effect of photoperiod on mortality and numbers pupating in final instar *T. subnodicornis* larvae at 10°C**

<b><u>L:D</u></b>	<b><u>Nos. pupating (% pupating)</u></b>		<b><u>No. of deaths (% mortality)</u></b>	
18:6	26	(57.8%)	19	(42.2%)
16:8	25	(55.6%)	20	(44.4%)
14:10	10	(22.2%)	35	(77.8%)
12:12	11	(24.4%)	34	(75.6%)
10:14	20	(44.4%)	25	(56.6%)
8:16	5	(11.1%)	40	(88.9%)

Fig. 2: Distribution of days to pupation in fourth instar *Tipula subnodicornis* larvae at L:D 18:6, 10°C

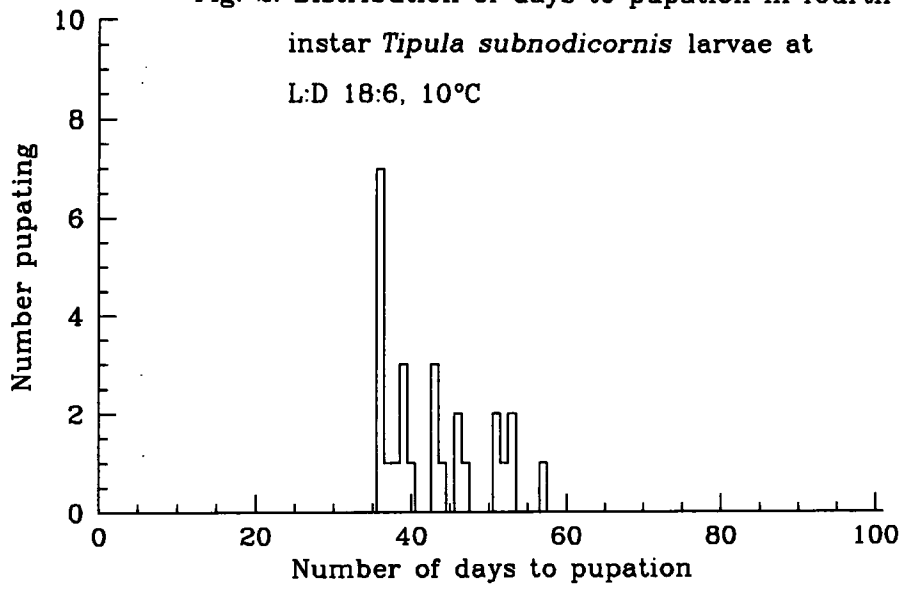


Fig. 3: Distribution of days to pupation in fourth instar *Tipula subnodicornis* larvae at L:D 16:8, 10°C

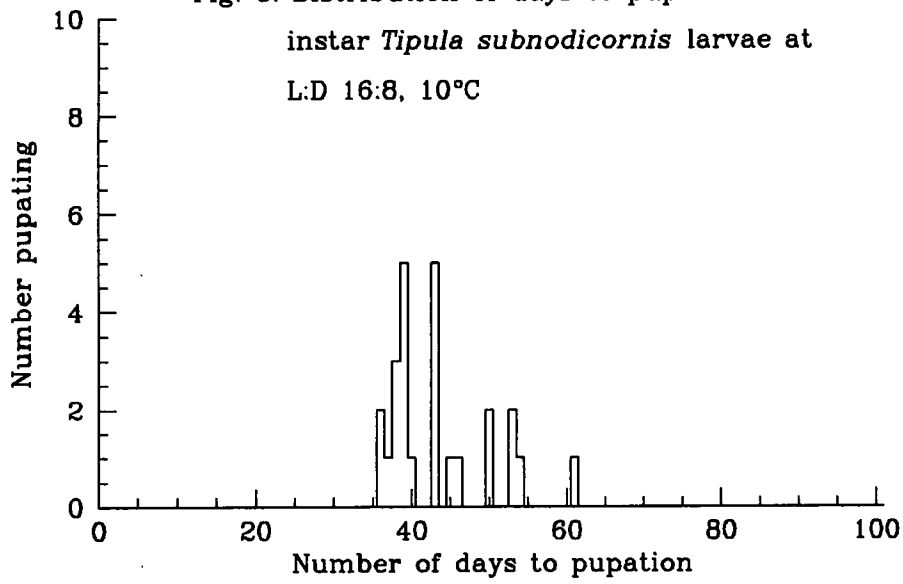


Fig. 4: Distribution of days to pupation in fourth instar *Tipula subnodicornis* larvae at L:D 14:10, 10°C

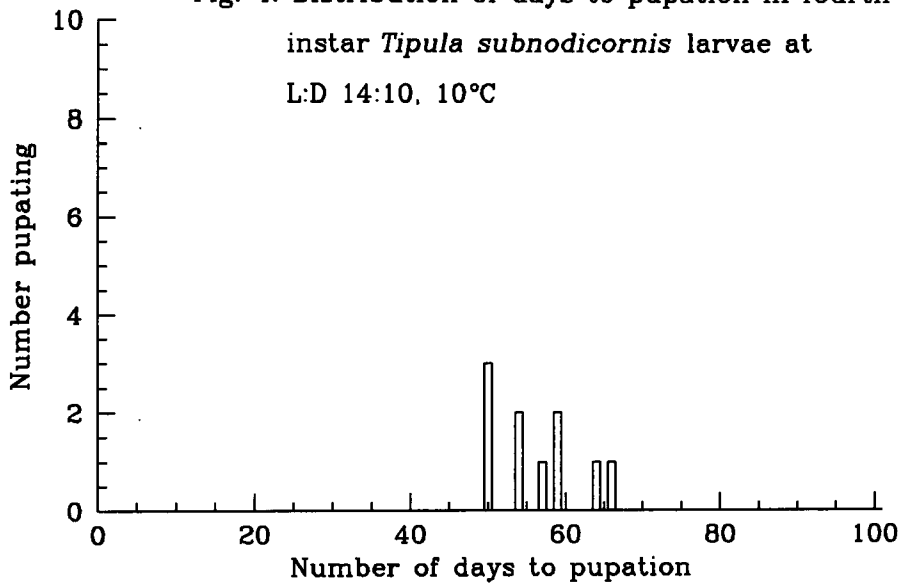


Fig. 5: Distribution of days to pupation in fourth instar *Tipula subnodicornis* larvae at L:D 12:12, 10°C

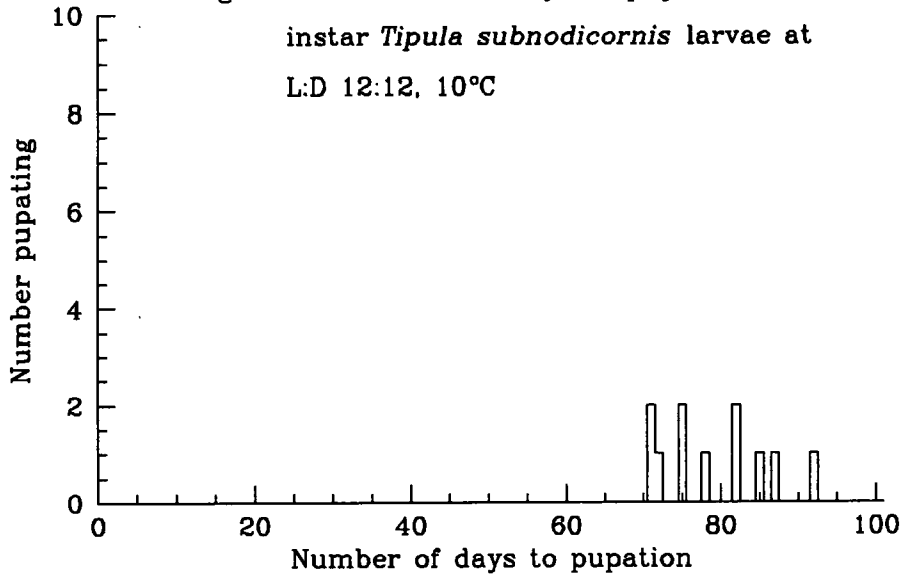




Fig. 6: Distribution of days to pupation in fourth instar *Tipula subnodicornis* larvae at L:D 10:14, 10°C

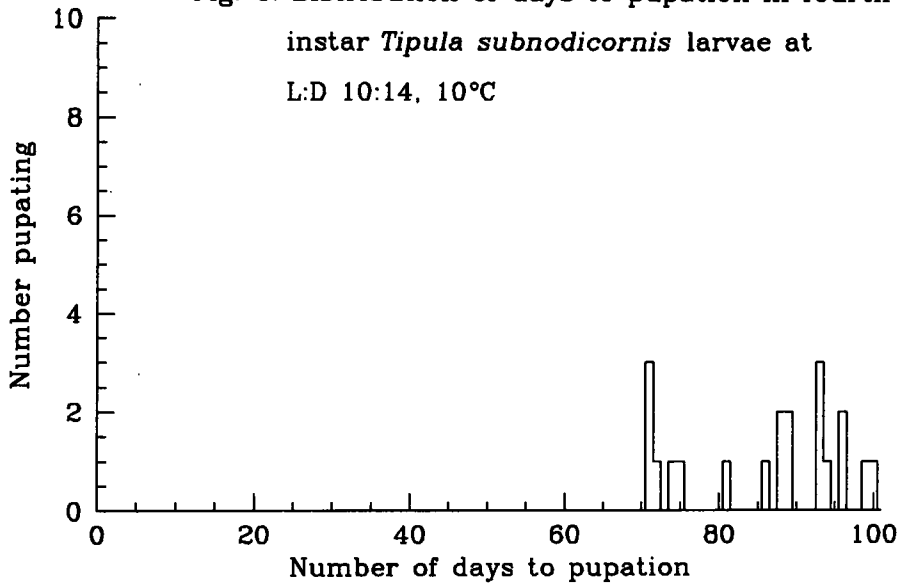


Fig. 7: Distribution of days to pupation in fourth instar *Tipula subnodicornis* larvae at L:D 8:16, 10°C

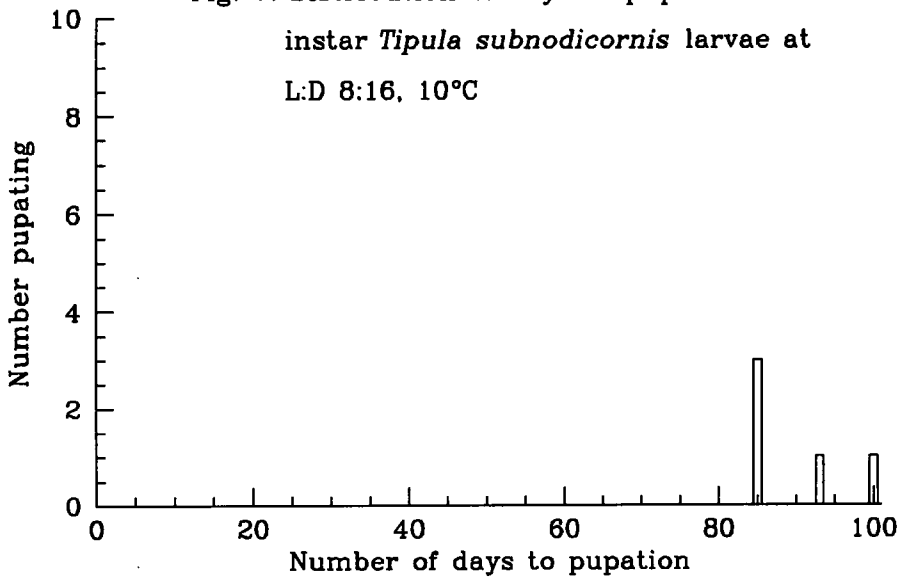
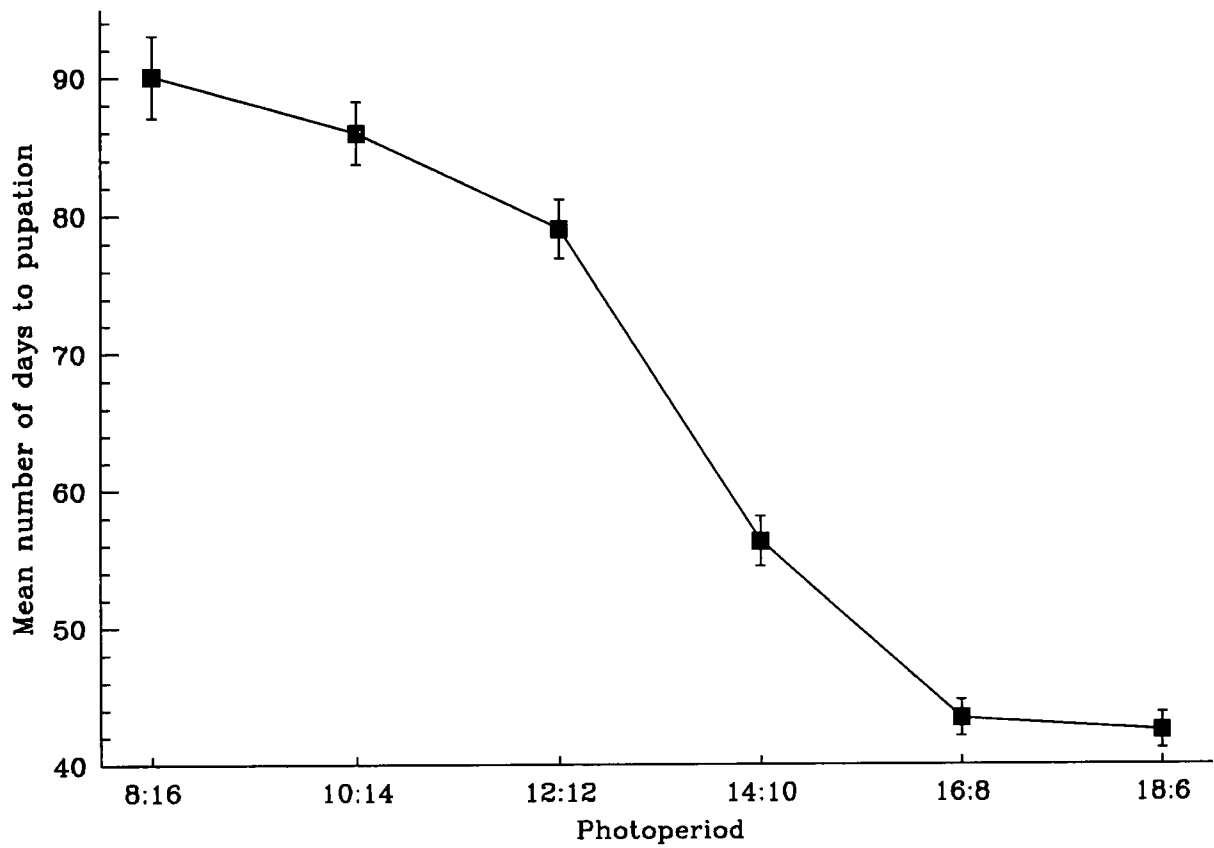


Fig. 8: Effect of photoperiod on the mean number of days to pupation in fourth instar *Tipula subnodicornis* larvae at 10°C.



### 2.3.3 Effect of photoperiod on larval mortality in *Tipula subnodicornis*

Table 2.3 represents the number and proportion of larvae dying in each regime. The lowest mortalities, 42.2% and 44.4%, were recorded in the L:D 18:6 and L:D 16:8 photoperiodic groups. Significantly higher rates were obtained in the L:D 14:10, L:D 12:12 and L:D 6:18 regimes, with 77.8%, 75.6% and 88.9% mortality recorded ( $\chi^2 = 75.43$ , d.f. = 5,  $p < 0.01$ ). As individuals on the short-day regime took approximately twice as long to pupate than those placed on a long-day, such high death rates could have resulted from factors such as depletion of energy reserves and desiccation.

Only 10 individuals survived to pupation on the L:D 14:10 regime (72.8% mortality). In this group progressive blackening of the cuticle was observed in seven larvae shortly before their death, and the origin of this discolouration was investigated. Two possibilities were considered: firstly, on his study of *T. subnodicornis* from Moor House, Coulson (1962) described a contagious fungal infection which caused widespread infection and death within seven days following introduction to the laboratory. Secondly, Butterfield (PhD thesis, 1973) noted that blackening of the cuticle resulted from accidental puncturing of the epidermis by forceps during larval examination. As there was not a widespread infection and because blackened larvae survived for longer than seven days in the laboratory, it was concluded that accidental cuticle damage was the primary cause of death.

## 2.4 Discussion

In temperate regions photoperiod is the most reliable indicator of prevailing seasonal change and consequently many insect species have evolved the ability to perceive and respond to changes in day-length in order to regulate their life-cycles. One of the major strategies used to maintain seasonality is the inclusion of a diapause stage at some point during the insect's development. In the majority of temperate insects studied, photoperiod is the major environmental cue inducing diapause, with maintenance of dormancy normally controlled by differing responses to day-length and altered thresholds of temperature (Tauber *et al.* 1986). With respect to photoperiod, studies of autumnal-hibernal diapausing insects have demonstrated that a large number lose their sensitivity to day-length as diapause progresses, to a point where the dormancy spontaneously terminates. Thereafter, these insects are held in quiescence by the intervening low temperatures of winter until spring, when temperatures rise to a level that favours development. This type of response was demonstrated in the crane fly *Tipula subnodicornis* as larvae taken from the field in February and subjected to long or short photoperiod at 10°C did not differ significantly when the mean dates of pupation were compared (Butterfield, 1976). In this species the rate of pupal development towards adult emergence was found to be temperature-dependent, as the mean number of days to emergence was increased from 20 days at 10°C to 33 days at 7°C (Butterfield, 1973). Later studies demonstrated that the diapause in *T. subnodicornis* could be broken in its early stages by long photoperiod, as larvae taken from the field in mid-December and exposed to photoperiods simulating summer daylengths at 10°C pupated 40 days earlier than individuals placed on a short regime (Butterfield, 1976). However from these findings it was not clear how the crane flies responded to changes in day-length: for example whether perception was according to a fixed critical threshold of light or if the response was quantitative and graded according to the actual value of the applied photoperiod (Tauber & Tauber, 1976). Therefore, the object of the present study was to determine the photoperiodic response of diapausing *T. subnodicornis* larvae when placed under differing light regimes at 10°C.

Pupation occurred in all of the photoperiodic regimes applied. However when compared, the number of days to pupation differed greatly between the groups. Larvae exposed to photoperiodic regimes equal to or lower than L:D 12:12 pupated significantly later than individuals kept on a regime of L:D 14:10 and over. In agreement with Butterfield (1976), subjects reared under short-day conditions (L:D 6:18) took twice as long to pupate than those placed on a long-day regime (L:D 18:6).

It would therefore appear that *T. subnodicornis*, in the early stages of diapause respond to a critical threshold of light. Photoperiods of L:D 14:10 and over will hasten development towards pupation while daylengths equal to or below L:D 12:12 will restrain development. It is suggested that in the field short photoperiod would slow down development towards pupation in the winter until the spring when temperatures would rise above the developmental threshold.

In a study on the development of overwintering *Tipula pagana* eggs, rate of development and timing of hatching were lower and less synchronous than in *T. subnodicornis* and *Molophilus ater* Meigen (Butterfield & Coulson, 1987; Coulson *et al.* 1987). Greater hatching synchrony at 10°C was achieved if the eggs were pre-chilled at 5°C and 1°C, or exposed to long photoperiod a few days before hatching. This indicated that the eggs could be divided into two types and classified according to whether they: (i) hatched with a moderate degree of synchrony shortly after completing development (for example, within the first ten days following initial hatching) or (ii) hatched asynchronously, producing a long-tailed pattern of emergence.

In the present study the effect of photoperiod on the hatching of chilled diapausing *Tipula fusca* eggs was investigated. When compared, the number of days to hatching was lower in the eggs kept on the long-day regime than on the short photoperiod. However under both photoperiods hatching was synchronous, with 91% and 93% of the total number of viable eggs hatching within the first 24 hours following initial emergence. In addition, the mean hatching dates between the groups differed by only 13 days. As unchilled *T. fusca* eggs normally take approximately three months longer to hatch and show less synchrony (Coulson, pers. comm.) it appears that chilling, rather than long-day itself has a greater effect on development towards hatching and synchronisation of emergence. The slight difference in the number of days taken to hatch between the groups could have resulted however from the additional warming of the eggs by the lamps on the long-day regime (subsequently measured by thermal hydrography to be +0.5°C).

## Chapter 3

### 3.1 Introduction

#### 3.1.1 *Nebria salina*: general distribution and life-cycle

From field studies on the general life cycle of the carabid *Nebria salina* it appears that this beetle is an atypical autumn breeder as defined by Larsson (1939). In Britain it is a 'non-wet' species commonly found on well-drained upland heaths below 1900 ft. (Houston, PhD Thesis 1980). The life cycle of *N. salina* is atypical in that it follows an annual bimodal peak of activity that is separated by a period of summer diapause. Throughout Britain, differences are found with respect to the timing of adult emergence: immature beetles are present at Chapel Fell, Northern England between late May and mid-June and around mid-March in Anglesey, Wales (Gilbert, 1958). The teneral adults are voracious eaters and accumulate large fat reserves after a short period of two to three weeks of intense activity in the field. Thereafter the beetles disappear under nearby logs and stones where they enter and remain in a state of aestival diapause until the resolution of activity in mid-August. Activity rates peak in mid-September with the timing of autumnal breeding. First, second and third instar larvae are found in their greatest numbers around September, November and January respectively.

#### 3.1.2 Diapause and gonad maturation in Carabidae: a review

Classification of Carabidae according to breeding type was first described by Larsson (1939) who distinguished between two groups of beetles that bred either in the spring or in the autumn. From his work on Fenno-Scandinavian carabids, Lindroth (1945) noted the apparent existence of unstable reproductive periods and grouped species in a slightly different way, by placing the emphasis on whether the carabids overwintered in the larval or adult form. Using either method of classification, beetles that bred in the spring generally produced summer larvae that hibernated as adults and those that bred in the autumn produced offspring that overwintered as larvae (Larsson, 1939; Lindroth, 1945).

This simple method of classification proved however to be unsatisfactory following the discovery of carabid species with life cycles atypical of the groups outlined by Larsson (1939) and Lindroth (1945). In a study on the timing of breeding in carabid species

commonly found in the Netherlands, a continuous sequence of reproduction was reported between early spring and late autumn (Den Boer *et al.* 1989). Out of 68 species studied, seven were winter breeders, some bred twice in one year while others reproduced throughout most of the year without major interruption. The majority of beetles hibernated after reproduction and bred again over successive years. Only one species, *Nebria brevicollis*, appeared to be semelparous. Classification according to the hibernating form and timing of breeding was therefore not sufficient as many of the species overwintered as both larvae and adults, with reproduction not restricted to spring and autumn.

From his work of annual rhythms in temperate carabids, Thiele (1977) distinguished Carabidae into five distinct groups (Table 3.1):

**Table 3.1: Types of annual rhythms found in temperate carabid species (Thiele, 1977)**

<u>Type</u>	<u>Example</u>
1. Spring breeders: summer larvae/adult hibernators.	<i>Pterostichus rhaeticus</i>
2. Species with winter larvae that reproduce in the summer or autumn, but do not have an adult dormancy.	<i>Calathus erratus</i>
3. Species with winter larvae, spring adult emergence and aestival diapause prior to reproduction in the autumn.	<i>Leistus terminatus</i>
4. Species with 'flexible' reproductive periods where breeding is not restricted to either spring or autumn and larval development is not hindered by season.	<i>Abax ater</i>
5. Species with developmental time greater than one year.	<i>Abax ovalis</i>

Reviewing the above classification Den Boer *et al.* (1989) concluded that the majority of carabid species found in Western Europe belonged to types one to three. Species characteristic of type four followed a life cycle that perhaps resulted from environmental heterogeneity. The fifth type included species that delayed reproduction until the year following adult emergence often as a result of a change in latitude, as exemplified by *Carabus problematicus* whose life-cycle is annual at 100 a.s.l. near

Durham and biennial at 300m a.s.l. in the Pennines (Butterfield, 1986). To add to the debate it was suggested that a distinction could therefore be made according to when the beetles developed in the field, making a division into either summer developers or winter developers (Den Boer *et al.* 1989).

The carabid *Nebria brevicollis*, described above as being virtually semelparous, is one of the six species of *Nebria* currently inhabiting the British Isles. The general life cycle of this beetle has been outlined in Britain (Davies, 1953; Gilbert, 1958; Williams, 1959; Greenslade, 1964) and elsewhere in Europe (Larsson, 1939; Lindroth, 1945; Van der Drift 1951). Its life-cycle is very similar to *N. salina* in that the adults emerge in spring and are active for approximately two to three weeks before entering a period of inactivity that lasts until the autumn. In England pupation, emergence and the short burst of pre-diapause activity occurs approximately three weeks earlier than in Scotland (Penney, 1969). Synchronisation of the breeding season and subsequent appearance of larvae is achieved by the increasing of the diapause period by a similar length of time. Throughout Britain, a span of approximately three to four months therefore exists between adult emergence and breeding.

Early studies of the life-cycle of *N. brevicollis* indicated that interpolated between the two major events of spring emergence and autumn breeding was an aestival diapause that was involved in the arrest of gonad maturation. Existence of such a reproductive diapause was confirmed experimentally following the dissection and examination of the sexual organs of male and female *N. brevicollis* trapped throughout the year. Specimens caught in May showed no signs of differentiation, whereas those trapped in late August were in a state of reproductive development (Penney, 1969).

Ganagarajah (1965) divided the life cycle of adult *N. brevicollis* into 3 basic stages; spring emergence (which contained the pre-diapause period), summer diapause and autumn breeding. Examining the role of the neuro-endocrine complex and its relationship to reproduction, he described the basic stages of development found in both sexes of *Nebria* during each period. In early pre-diapause, both male and female gonads were undeveloped. In the males, the testes did not start to develop until the end of the pre-diapause period, enlarging to their maximum size at the beginning of the summer diapause. Sperm however was not present until mid-August. In the females, enlargement of the ovaries, characterised by ovariole differentiation, was not observed until after the diapause had ended. By autumn the beetles had resumed activity and the timing of breeding was marked by the presence of spermatophores in the spermatheca and mature terminal oöcytes in the ovaries. Final maturation of the ovaries rapidly



occurred after the resolution of activity in the autumn with maturation of the males shortly preceding that of the females (Ganagarajah, 1964; Penney 1969).

### 3.1.3 Factors affecting growth and development in *Nebria*

Until the breeding experiments of Penney (1969) and Thiele (1969) no studies had been carried out on the gonad maturation of aestivating carabids, and the roles played by factors such as photoperiod and temperature on development were unclear. The change from long photoperiod to short photoperiod generally associated with summer inactivity and autumn activity was initially believed to be an environmental cue for autumn breeders to activate gonad maturation in late summer/early autumn (Thiele 1969). In spring breeders the reverse was thought to be the case, with the change from short to long day-length initiating growth. From a study on the factors controlling dormancy in *N. brevicollis* and *Patrobus atrorufus* Stroem, the aestival diapause was clearly shown to be dependent upon photoperiod (Thiele, 1969). In both species, short-days accelerated gonad maturation in females whereas long-days suppressed growth; individuals subjected to light regimes of L:D 6:18 developed their ovaries while those reared under conditions of L:D 18:6 did not. Length of exposure to photoperiod was also found to be a critical factor, with ovarian maturation in *P. atrorufus* only occurring in beetles previously subjected to short day conditions for one month. In *N. brevicollis*, females required two to three months to fully develop eggs. In both species however, maturation of the males took place almost irrespective of the applied photoperiod. These results were confirmed by Penney (1969) on her work with *N. brevicollis*: females exposed to short day-lengths for four weeks were immature whereas beetles subjected to similar regimes for seven weeks showed signs of ovarian development. Again, male maturation took place irrespective of the applied photoperiod (Penney, 1969).

In other species of Carabidae, the photoperiodic effect is not as 'straightforward' as documented in *N. brevicollis*. For example, growth in some beetles is dependent on a variety of factors such as temperature and/or exposure to different combinations of photoperiod. In the spring breeders *Pterostichus rhaeticus* and *P. oblongopunctatus*, ovarian development is under a 'two-step' photoperiodic control where growth is only initiated after exposure to sufficient periods of short-day followed by long-day (Thiele 1966, 1968a, 1971; Ferenz 1977<sup>1</sup>). In both species, previtellogenesis is activated by short-day whereas vitellogenesis is bound to long-day. Eggs will not be produced if the beetles are reared in either short or long-day conditions alone. Male development is also controlled primarily by photoperiod: short-days will promote the formation of

spermiozeugma, while long-days will suppress development, reversibly (Ferenz, 1975<sup>2</sup>). This short-day/long-day effect has only been demonstrated in a few species with gonad maturation dependent upon a narrow critical range of temperature (10°-15°C) (Müller, 1960; Strübing, 1963; Thiele, 1975).

#### 3.1.4 Aims of the present study

The effect of photoperiod on the gonad maturation of *N. salina* has not been fully investigated and therefore the mechanisms involved in its control are unclear. Current evidence indicates that the diapause is maintained by long photoperiod and is involved in the synchronisation of the breeding period throughout Britain (Butterfield, 1996). In the present work diapausing male and female adults were subjected to different regimes of day-length to determine the effect of photoperiod on sexual development. Nearing the end of the experiment we also noted the night/day activity of the beetles previously exposed to two months of experimental light regimes to determine whether photoperiod had significantly affected behaviour. Conflicting theories have been suggested as to why such an atypical life cycle is followed by this species and the photoperiodic effect determined was discussed.

<sup>1,2</sup> Incorrectly identified as *Pterostichus nigrita* (Martin Luff, pers. comm.)

## 3.2 Methodology

### 3.2.1 Sample site and method of collection

Hamsterley Forest (National Grid Ref.: NZ0530), area 2020 ha, is owned and managed by the English Forestry Commission. Located to the east of the Pennines and between the rivers Tees and Wear it has an altitude range varying between 150m-420m a.s.l. Sitka spruce (*Picea sitchensis*) and the native Scots Pine (*Pinus sylvestris*) are the predominant tree types in the Forest.

Collection of invertebrates took place at Hamsterley between 20th May 1995 and 31st June 1995. The initial sample site was a heather-covered clear fell area (Plate A). Method of collection was by pit-fall trapping using empty disposable drinking cups (measuring 6.5cm x 9cm) sunk rim-deep into the soil. This method was reviewed by Greenslade (1964) and acts as a basic trap for terrestrial arthropods. Fifteen traps were laid and emptied every two to three days. Unfortunately the site gave poor catches of *Nebria salina*; at the time of collection the area was dominated by *Carabus problematicus* and *Necrophorus* spp. that tended to either kill or severely mutilate the smaller trapped beetles. Further investigation located higher numbers of beetles sheltering under stones beside a nearby dirt track (Plate B). On the turning of the stones the beetles were very still and in some cases found in aggregates of up to three. Collection was subsequently carried out in this area by hand.

The beetles were transferred to the laboratory in large jars containing soil and vegetation taken from the surrounding area and identified and sexed. As *N. salina* and *N. brevicollis* possess similar morphologies, special care was made to distinguish between the two species during identification. Males and females were kept separate until the start of the experiment in fish tanks (measuring 30 x 20 x 20cm) containing peat, stones and vegetation on a long photoperiod at 15°C. Throughout captivity the beetles were fed on fresh earthworms (four large diced worms to each batch of approximately 15 beetles every two days) or when unavailable, diced mealworms equivalent in respect of quantity. Any uneaten material was removed.



**Plate 1: Hamsterley Forest Site A**



**Plate 2: Hamsterley Forest Site B**

### 3.2.2 Experimental regimes

To investigate the effect of photoperiod on the reproductive maturation of adult *N. salina* six different experimental regimes were used (Table 3.2);

**Table 3.2: Effect of photoperiod on the reproductive maturation of adult *Nebria salina*: experimental regimes**

Group	Exposure time	Temperature	Photoperiod (L:D)	No. of males	No. of females
A	1 month	15°C	18:6	18	14
B	1 month	15°C	12:12	17	13
C	1 month	15°C	6:18	17	14
D	2 months	15°C	18:6	18	14
E	2 months	15°C	12:12	17	14
F	2 months	15°C	6:18	17	14

Each batch comprised of approximately equal numbers of males and females as the presence of a male has been demonstrated to greatly influence egg production and viability (Nelemans, 1987). Groups A to C and D to E were killed by ether on 1st August 1995 and 1st September 1995, respectively and stored in alcohol.

### 3.2.3 Characterisation of reproductive condition

Female *N. salina* have two polytrophic ovaries with each ovary containing on average approximately 22 ovarioles. The male testes are rectangular in structure that lie in the abdominal cavity. Development of the testes is characterised by the gradual filling of the abdominal region by testicular mass from the apex to the pronotum.

According to Houston (1980) qualitative measurements of the reproductive maturity of beetles can be made according to the following criteria;

#### ***Teneral***

Includes recently emerged, immature beetles that have a softened cuticle and, in some cases, are not fully developed in colour. Females possess translucent ovaries that are very small and do not show any signs of development. In males the testes and vesiculae seminales are small and under-developed. In both sexes a full crop and large

fat reserves are normally observed in beetles caught after the initial burst of activity following emergence (Penney, 1969).

### ***Developing***

In the females the ovaries are enlarged and contain developing oöcytes. In the males the testes and vesiculae seminales are markedly larger than in the teneral adults. Fat reserves are moderate to large.

### ***Mature***

The abdomen of the female is distended due to the presence of eggs in the uterus. In the males the testes and vesiculae seminales are also very large. Fat reserves are low to moderate.

### ***Spent***

The ovaries and testes are very small and translucent. In the females a well developed network of trachea is often visible and corpora lutea are loosely held together. Fat reserves are low.

In the present study the maximum width of the ovary and ovarioles was measured in the females and the amount of abdomen covered with testicular mass and maximum width of testes was recorded in the males. Mature females were easily identified by the presence of eggs whereas male development was classified according to the following criteria (adapted from Luff, 1973);

Immature:  $\leq 3.6$  mm of abdomen filled by testes.

Developing:  $\leq 5.5$  mm of abdomen filled by testes.

Mature:  $\geq 5.5$  mm of abdomen filled by testes.

In previous studies on *N. brevicollis* combined regimes of long-day and high temperatures (20°C) prevented gonad maturation (Thiele, 1969). As there was a limitation with the number of beetles collected for the study, 15°C was chosen to be the incubation temperature as this appeared to be an adequate temperature for development in autumn breeders.

### **3.2.4 Effect of photoperiod on daily activity in *Nebria salina***

On 31st August 1995 the effect of photoperiod on the daily activity of Groups D to F was studied. Daily rhythmicity of carabids has been intensively investigated in the field

and in the laboratory using a variety of simple and specialised techniques such as field traps and actographs under both natural and artificial light regimes. A comprehensive review of this subject was outlined by Thiele & Weber (1968). As we aimed to investigate activity during the night a methodology had to be devised that would allow recordings to be made in periods of darkness. A method was tested whereby the dorsal surfaces of the beetles were painted with a coloured chalk solution ('EBT' Series Soluble Toner, Fiesta daylight fluorescent colours manufactured by Swada (London) Ltd.). This chalk was highly visible under a standard 'Black Light' lamp. Using a fine paintbrush, females were covered in pink chalk and males in blue. On 31st August, for one day only, the number and sex of active beetles were recorded every 15 minutes during the following time periods;

1. 6-9 a.m.: the last three hours of darkness for all three photoperiodic groups.
2. 3-4 p.m.: the first hour of darkness for L:D 6:18 group
3. 9-10 p.m.: the first hour of darkness for L:D 12:12 group.

### 3.3 Results

#### 3.3.1 Effect of photoperiod on ovarian maturation in *N. salina* after one month

Plate 3 represents the stages of development attained in female *N. salina* following exposure to experimental light regimes for a period of either one or two months.

In females exposed to day-lengths of L:D 18:6 for one month, the ovaries were clearly undeveloped and did not show any signs of differentiation. Fat content was very high; on removal of the abdominal membrane large amounts of fat globules were released. Developing oöcytes were observed in one and two beetles from the L:D 6:18 and L:D 12:12 groups, respectively. The ovaries of these beetles appeared to be substantially larger and creamier in colour in comparison to the L:D 18:6 females. Fat content was comparatively lower.

Mean ovary widths after exposure to the experimental light regimes for one month are shown in Figure 9. In the L:D 18:6, L:D 12:12 and L:D 6:18 beetles, mean measurements were 0.58mm (s.d. =  $\pm 0.067$ ), 0.69mm (s.d. =  $\pm 0.070$ ) and 0.84mm (s.d. =  $\pm 0.085$ ) respectively. Mean ovariole widths are represented in Figure 10 and was 0.044mm (s.d. =  $\pm 0.011$ ), 0.087mm (s.d. =  $\pm 0.022$ ) and 0.107mm (s.d. =  $\pm 0.018$ ) respectively. One-way ANOVA followed by Tukey-HSD and Scheffe indicated that there were significant differences with respect to both mean ovary and ovariole width ( $F_{2,29}=33.9661$  and  $F_{2,34}=43.3934$  respectively,  $p<0.0001$ , 18:6 $\neq$ 12:12 $\neq$ 6:18), indicating that females exposed to daylengths of L:D 18:6 for one month were immature whereas individuals subjected to regimes of L:D 12:12 and L:D 6:18 were in a state of reproductive development.

#### 3.3.2 Effect of photoperiod on ovarian maturation in *N. salina* after two months

Beetles exposed to long daylengths for two months did not show any further signs of development and appeared to be at a similar stage of development as reached in the beetles exposed to similar conditions for one month. Mean ovary and ovariole width was 0.62mm (s.d. =  $\pm 0.054$ ) and 0.044mm (s.d. =  $\pm 0.011$ ) respectively.



Fig. 9: Effect of photoperiod on mean ovary width (mm) after one month at 15°C

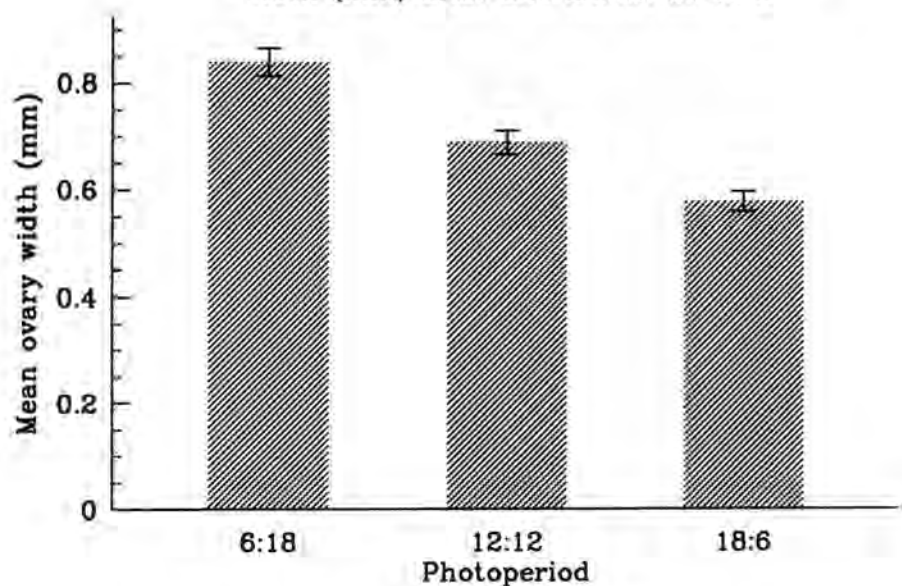
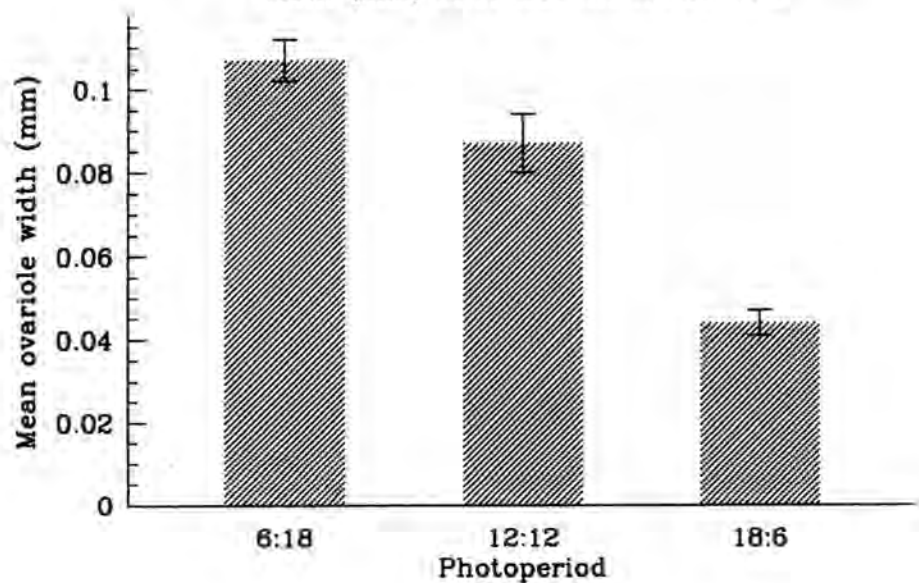


Fig. 10: Effect of photoperiod on mean ovariole width (mm) after one month at 15°C



**Immature**



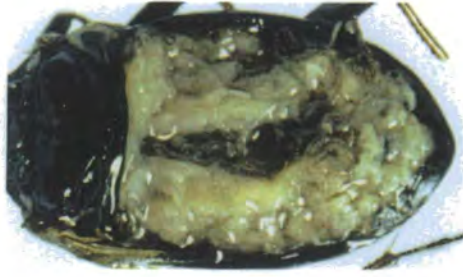
**L:D 18:6  
1 and 2 months**

**Developing**



**L:D 12:12  
1 month**

**Developing**



**L:D 6:18  
1 month**

**Mature**



**L:D 12:12 and  
6:18 2 months**

**Plate 3: Stages of reproductive maturation in female *Nebria salina* after exposure to experimental light regimes for either one or two months**

Females exposed to photoperiods of L:D 12:12 and L:D 6:18 for two months had distended abdomens due to the presence of eggs. Eggs were packed together in groups of either one or two, were yellow in colour and individually measured 2-3mm in length. The mean number of eggs (16) on the L:D 12:12 regime did not differ significantly from the mean number (10) on the L:D 6:18 regime ( $t_{17} = 1.89$ ,  $p > 0.05$ ). Four beetles from the L:D 12:12 group failed to reach maturation; in these individuals the mean width of the ovaries and ovarioles was 0.925 mm and 0.125 mm, respectively. All had clearly visible oöcytes indicating that they were in a state of reproductive development.

### 3.3.3 Effect of photoperiod on male maturation in *N. salina* after one month

Plate 4 represents the stages of development attained in the males after one month. The mean length of abdomen covered by testes in the L:D 18:6, L:D 12:12 and L:D 6:18 groups was 3.77 mm (s.d. =  $\pm 0.709$ ), 4.92 mm (s.d. =  $\pm 0.344$ ) and 5.275 mm (s.d. =  $\pm 0.391$ ) respectively (see Figure 11), while mean width of testes was 0.200 mm (s.d. =  $\pm 0.05$ ), 0.233 mm (s.d. =  $\pm 0.024$ ) and 0.248 mm (s.d. =  $\pm 0.023$ ), respectively (Figure 12). In the beetles exposed to a regime of L:D 18:6, seven males were classified as immature and eleven were in a state of reproductive development. With the exception of three individuals who had appeared to reach maturation on the L:D 6:18 regime, all the beetles subjected to day-lengths of L:D 12:12 and L:D 6:18 were classified as developing. The testes of all of the males examined appeared to be extremely spongy in texture and quite undifferentiated. Fat content was moderate.

With respect to the amount of abdomen covered by testes and mean testes width, one-way ANOVA followed by Tukey-HSD indicated that males exposed to regimes of L:D 6:18 and L:D 12:12 were significantly more developed than beetles placed on daylengths of L:D 18:6 ( $F_{2,47} = 41.1$  and  $F_{2,47} = 7.64$ ,  $p < 0.0001$ ,  $18:6 \neq 12:12 = 6:18$ ).

### 3.3.4 Effect of photoperiod on male maturation in *N. salina* after two months

Mean figures are shown in Figures 13 and 14. In the L:D 18:6 group six beetles were classified as immature and 12 as developing. Mean width of testes was 0.138 mm (s.d. =  $\pm 0.019$ ) and mean length of abdomen covered by testes was 3.83 mm (s.d. =  $\pm 0.39$ ). With the exception of one and two males respectively, all of the beetles exposed to regimes of L:D 6:18 and L:D 12:12 were in a state of development. Mean length of abdomen covered by testes and testes width was 4.35 mm (s.d. =  $\pm 0.623$ ) and 4.02mm (s.d. =  $\pm 0.0554$ ) respectively, 0.151 mm (s.d. =  $\pm 0.021$ ) and 0.129 mm (s.d. =  $\pm 0.001$ ), respectively.

Fig. 11: Effect of photoperiod on length of abdomen covered by testes (mm) after one month at 15°C

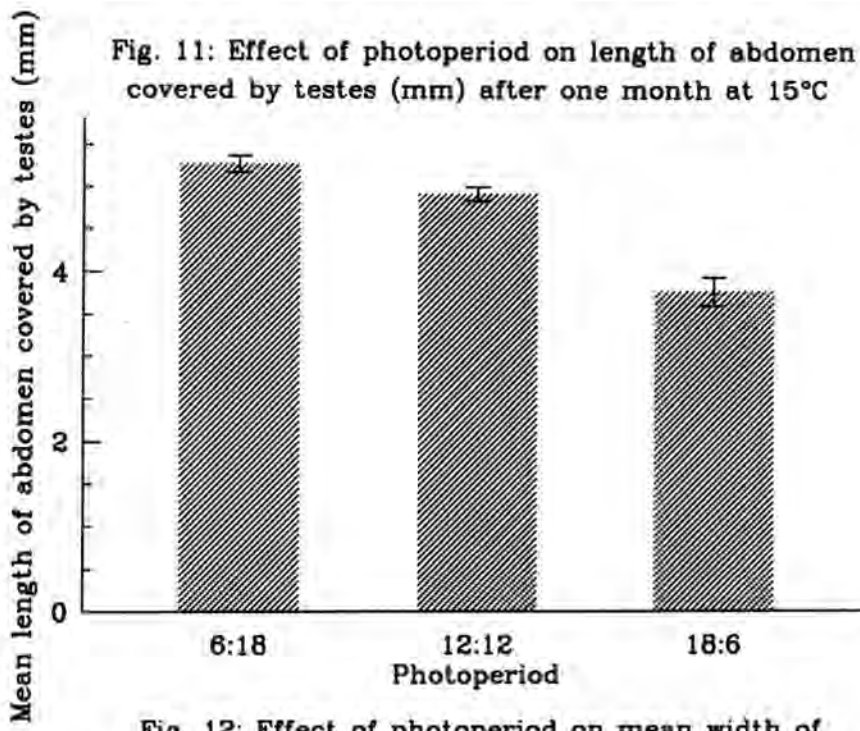
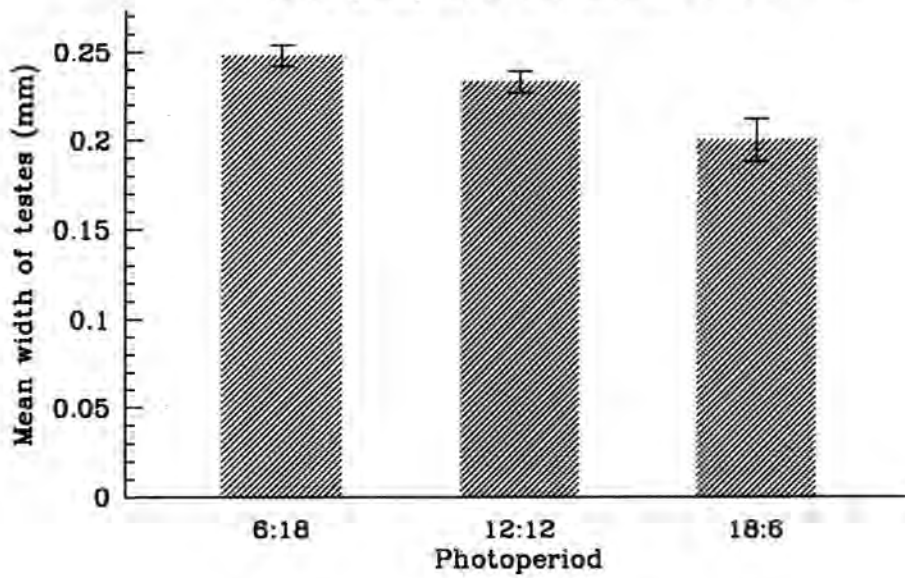
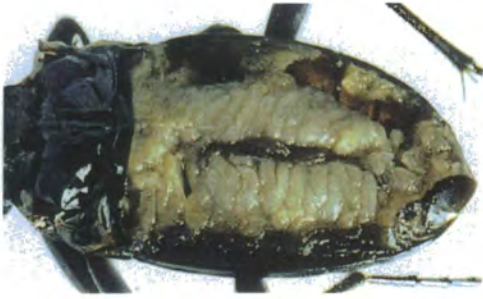


Fig. 12: Effect of photoperiod on mean width of testes (mm) after one month at 15°C





L:D 18:6  
1 month



L:D 12:12  
1 month



L:D 6:18  
1 month



L:D 18:6  
2 months



L:D 12:12  
2 months



L:D 6:18  
2 months

Plate 4: Stages of reproductive maturation in male *Nebria salina* after exposure to experimental light regimes for either one or two months

Fig. 13: Effect of photoperiod on length of abdomen covered by testes (mm) after two months at 15°C

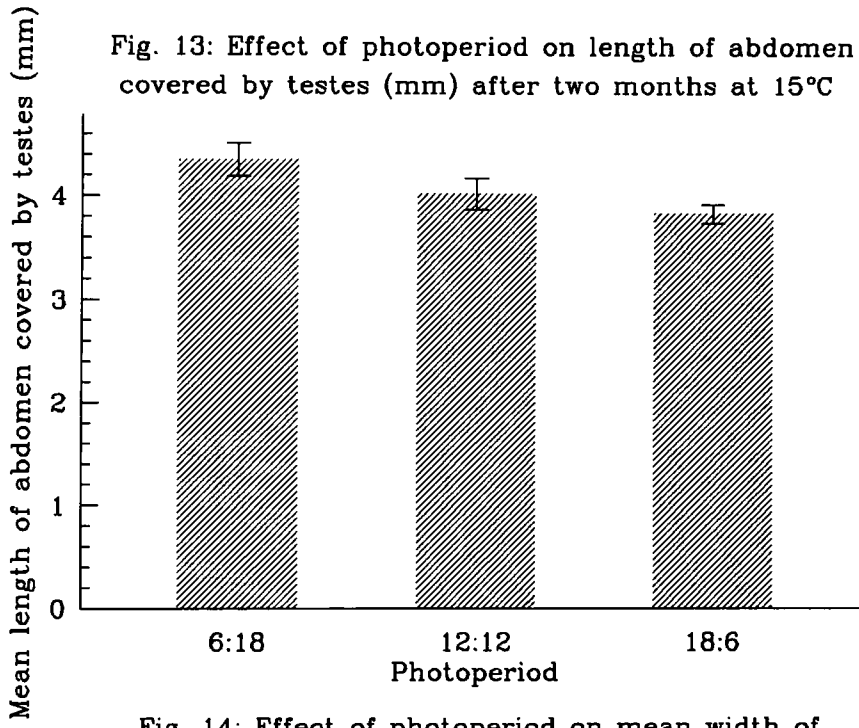
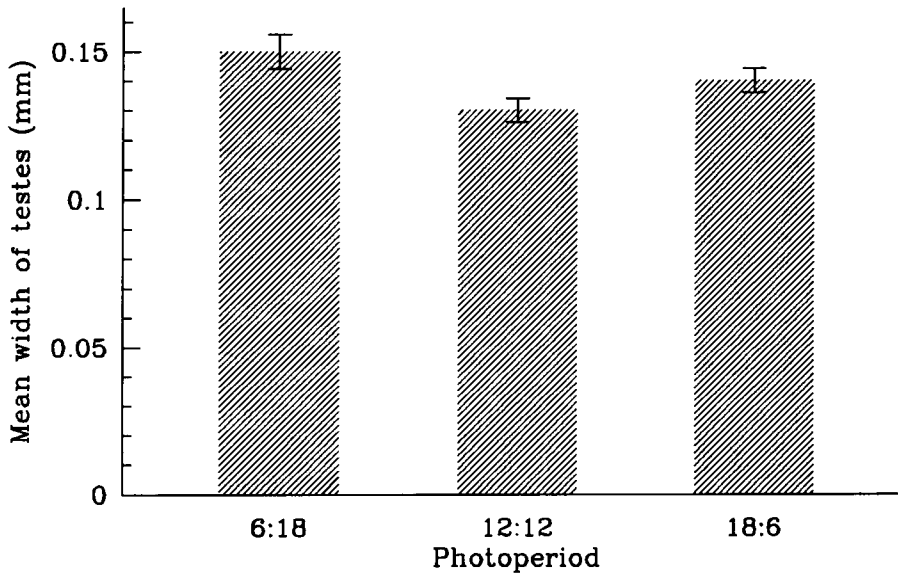


Fig. 14: Effect of photoperiod on mean width of testes (mm) after two months at 15°C



One-way ANOVA and Tukey-HSD indicated that the L:D 6:18 males were significantly more developed than the beetles exposed to L:D 12:12 and L:D 18:6 with respect to both sets of criteria measured in the study ( $F_{2,42} = 4.167$  and  $6.414$  respectively,  $p < 0.05$ , 6:18≠12:12=18:6).

On closer inspection, the testes of the males exposed to the experimental photoperiods for two months appeared to be harder in texture and more differentiated in form than in the beetles subjected to similar conditions for one month. The organs were clearly comprised of bundles of individual tubes measuring approximately 0.0075mm in width.

### 3.3.5 Effect of photoperiod on activity in *N. salina* after two months

Figures 15 to 17 represents the number of active male and female beetles following exposure to the experimental light regimes for two months. In all three groups activity was generally restricted to periods of darkness, and consequently the highest rates were observed in beetles exposed to photoperiods of L:D 6:18 ( $\chi^2 = 13.08$ ,  $p < 0.01$ ). In the first and third observational periods the maximum number of active beetles did not differ significantly between the L:D 12:12 and L:D 6:18 regimes ( $\chi^2 = 0.167$  and  $0.111$ ,  $p > 0.05$ ). A binomial test carried out to compare the activity rates between the sexes during the first observational period indicated that the males were significantly more active than the females in all of the three photoperiodic groups ( $p < 0.01$ ).

Fig. 15: Effect of L:D 18:6 on activity in *Nebria salina* after two months at 15°C.

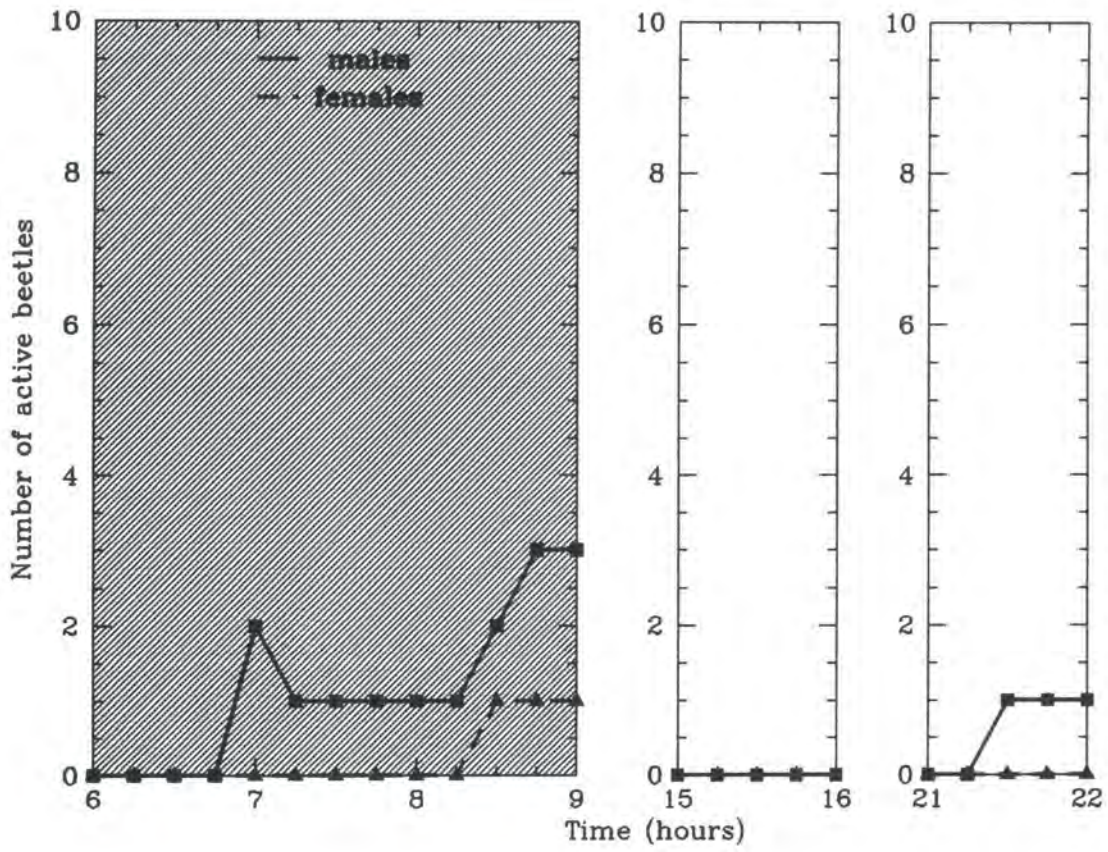




Fig. 16: Effect of L:D 12:12 on activity in *Nebria salina* after two months at 15°C.

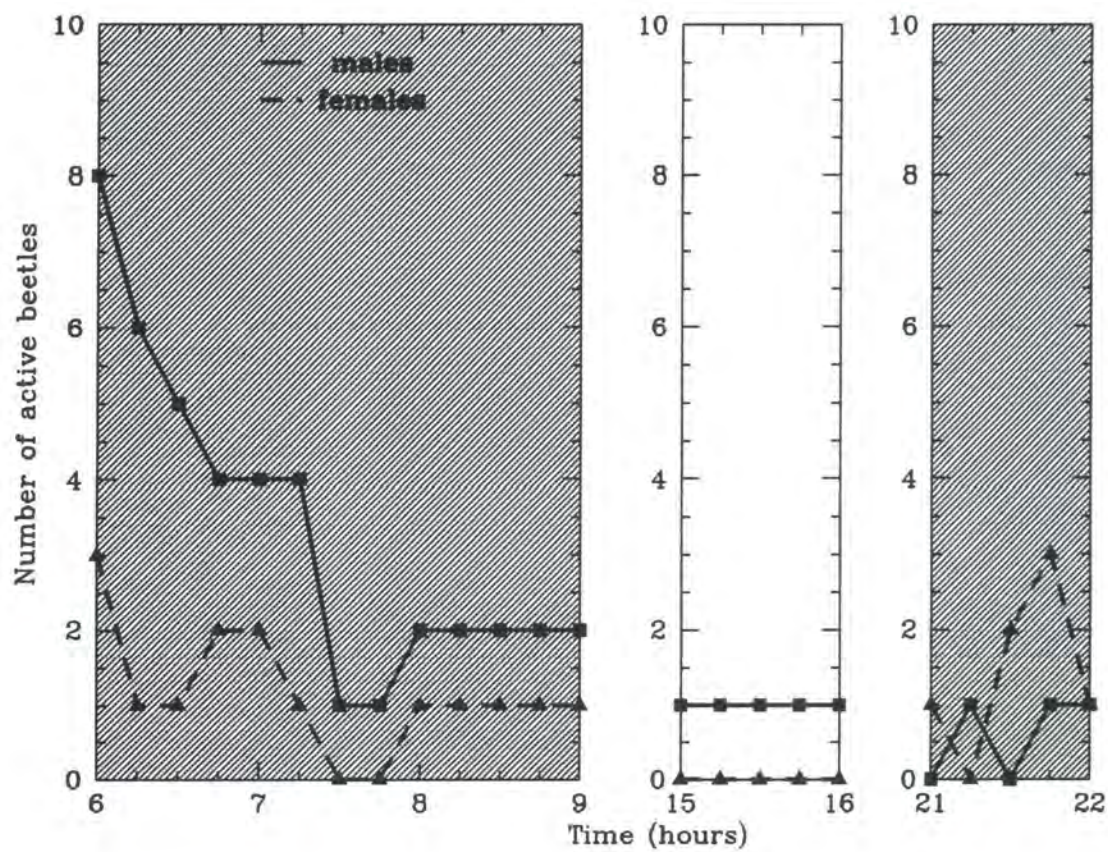
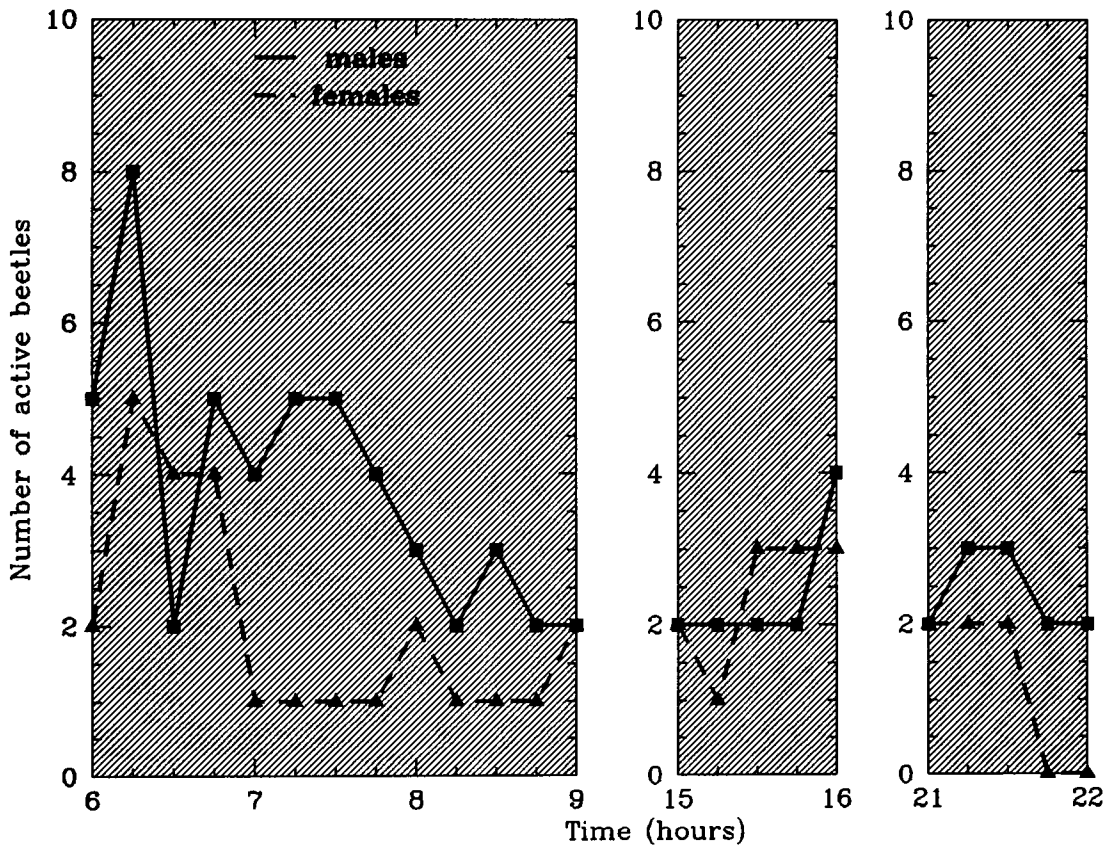


Fig. 17: Effect of L:D 6:18 on activity in *Nebria salina* after two months at 15°C.



### 3.4 Discussion

The aim of the present work was to determine the photoperiodic effect on gonad maturation in the carabid beetle *Nebria salina*. It was clearly demonstrated that ovarian development was dependent upon sufficient exposure to short day-length (L:D 12:12 and under). Signs of ovarian maturation, that is, visible formation of oöcytes were observed four weeks after continued exposure to short day-lengths while fully mature eggs were formed after eight weeks. Light regimes simulating summer daylengths prevented ovarian development. This agrees with the timing of reproduction in the field, which takes place approximately two to three months after the summer solstice. After four weeks, the proportion of the abdomen occupied by testes was smaller in the beetles exposed to L:D 18:6 than in individuals kept on a regime of L:D 6:18. Both sets of measurements showed apparent regression in beetles after eight weeks when compared with individuals killed after four weeks. The testes of these beetles however were clearly more differentiated, therefore bringing into question the validity of assessing maturity by the criteria used in the study. It would perhaps have been more appropriate to have assessed maturation by another factor such as the presence or non-presence of sperm.

During the present work the diurnal activity of beetles previously subjected to experimental photoperiods for two months was measured. Activity was mainly confined to periods of darkness, indicating that the species is nocturnal. This mode of behaviour complies with the general finding that autumn breeders with overwintering larvae tend to be nocturnal. Such insects are "moisture-requiring" and consequently are active at night to avoid the desiccating effects of strong sunlight (Thiele & Weber 1968). In this study, significantly lower levels of activity were recorded in individuals previously exposed to two months of long-day. Following dissection, these beetles were found to be sexually undeveloped. As decreased metabolism and reproductive immaturity are two physiological characteristics generally associated with the diapause syndrome, it was concluded that the beetles were still in diapause. High rates of activity as observed in the developing adults were correlated with the post-diapause behaviour normally observed in the field around September.

Various theories have been put forth to account for the presence of the diapause in aestivating beetles. Gilbert (1958) suggested that the diapause period enabled insects to develop their gonads. Later studies on *N. brevicollis* however demonstrated that maturation was attained after the diapause had ended, with males reaching sexual maturity two to three weeks before females (Ganagarajah, 1965; Penney, 1969).

Greenslade (1965) viewed the period of diapause as placing the organism 'in phase' with the correct breeding time. If breeding were to take place in the spring, the environmental conditions normally associated with summer would prove to be disastrous for larval development. The insertion of the summer diapause would therefore delay breeding until such a time when the subsequent larvae would develop under optimal conditions for growth. For example, Thiele (1969) demonstrated that temperatures of +2-4°C were obligatory for development of first and second instar *N. brevicollis* larvae. He concluded that the relatively high temperatures of summer would be "intolerable" for development and that "a mechanism preventing reproduction at this time, when temperatures are too high for the development of...larvae is indispensable. This mechanism is the arrest of ovarian maturation of the females resulting in an aestivation parapause" (Thiele, 1969).

Andrewartha (1952) suggested that the aestival diapause would ensure that active stages of development were timed with abundance of food. This was also supported by Masaki (1980) on his review of summer diapausers. In the codling moth *Cydia pomonella* species inhabiting the more southern, warmer regions enter winter diapause as first generation larvae. The insertion of the diapause appears to be related to the fruiting of an apple host-plant which occurs over a relatively short period of time (Sheldeshova, 1962). In *Hypera variabilis* the time to oviposition is significantly affected by the quality of food, as individuals fed on young, tender lucern plants oviposit earlier than those fed on older plants (Masaki, 1980). In *N. brevicollis* a correlation was found between activity in the field and abundance of microarthropods (Evans, 1955; Penney, 1969). Numbers of Collembola and Acarini were found to peak in June and September, the two months generally associated with adult emergence and breeding. Microarthropod levels however fell by 75% during July and August, when the carabids were in diapause. It was therefore suggested that the aestival diapause protected the beetle during periods of low food availability, with resumption of activity timed with the second annual peak of microarthropods in September. It was interesting to note that at Hamsterley Forest the timing of adult emergence of *N. salina* coincided with the dominance of Collembola and Acari species (Bolton, MSc thesis, 1995).

Butterfield (1996) suggested that carabid life-cycles may also be greatly affected by the quality of the mandibles. Houston (1981) clearly demonstrated that reproductive success in *Carabus problematicus* was directly related to mandible quality. Females possessing extremely worn mandibles were unable to feed properly and consequently could not produce mature eggs. Full reproductive power however was resumed when

the beetles were artificially fed on rabbit liver (Houston, 1981). As adult carabids generally live for a relatively long time during which they have to compete for food, it would therefore be advantageous for the individual to have well developed, sharp mandibles. As fecundity is dependent upon successful foraging, possessing mandibles of extremely good quality following adult emergence and during breeding would therefore be of prime importance. If breeding did not occur until several months after emergence, then the presence of a diapause stage would prevent wear.

The function of the diapause in *N. salina* therefore appears to be two-fold. Firstly, synchrony of the breeding period is achieved in beetles throughout Britain following exposure to short photoperiod experienced after the summer solstice. Regardless of geographical distribution, breeding is timed so that temperature-dependent offspring will develop under suitable environmental conditions for growth. As adult emergence is also temperature-dependent, species inhabiting the more southern regions of Britain will emerge earlier, but will extend the diapause period to compensate for this. Secondly, dormancy enables the beetles to conserve the quality of their mandibles, as diapausing beetles rest in conditions when food supply is low, thereby conserving mandible use and increasing the chances of survival.

## Chapter 4

### 4.1 Introduction

#### 4.1.1 Life-cycle of *Coleophora alticolella*

*Coleophora alticolella* (Family Coleophoridae) is a small tineoid moth whose larvae feed mainly on the seeds of Juncaceae (Richards and Clapham, 1941; Jordan, 1958). The life-cycle of this species was described in detail by Jordan (1958) who along with Reay (1964) and Welch (1965) studied its biology along the higher parts of its latitudinal distribution. It is fairly widespread throughout the British Isles, found at sea-level around the Cumbrian coast to 600m a.s.l. in the Pennines. The ecology of *Juncus squarrosus*, the moor-rush on which it predominantly feeds, has been studied extensively by Welch (1965, 1967), while Pearsall (1950) outlined the relationship between the plant and the moth.

*C. alticolella* follows an annual life-cycle comprising of four larval instars. Adults emerge around late May or early June, coinciding with the flowering of *Juncus* species and immediately mate. Jordan (1958) reported that newly emerged females contain on average around 10 to 20 eggs appropriately sized for laying. If many oviposition sites are available a single egg is laid on a developing inflorescence. (With increasing altitude the general decrease in plant abundance will result in multiple eggs being deposited within one capsule.) The majority of eggs are laid between the adjacent florets of the developing inflorescence often before the initiation of seed capsule development around June/July. Eggs take approximately three weeks to hatch and give rise to first instar larvae which burrow through the seed capsule and pericarp to the contained seeds on which they feed, remaining there until either the third or early fourth instar. Under ideal conditions for development larval stages one to three are completed within six weeks, with fourth instar larvae appearing around early August (Jordan 1958). At this time the moth weaves a white papery case which is impervious to water (Sich 1926) and protects the organism from dangers such as waterlogging (Jordan 1958) and desiccation (Randall, PhD Thesis 1980).

Throughout development an individual larva will consume on average 2.3 seed capsules and will continue to feed until approximately three weeks after the production of the papery case (Jordan 1958). In early autumn feeding ends and the majority of larvae migrate to the leaf-litter where they overwinter as final instar larvae. (The remaining

non-migrated larvae, perhaps parasitized, remain bound to the food-plant.) Pupation takes place in the following spring and shortly precedes adult emergence.

#### **4.1.2 Diapause development and 'characteristic reactions' of the diapause stages to temperature**

For many years the role of temperature and photoperiod throughout the course of diapause has proved to be a challenging area of study. In many species dormancy is maintained by altered responses to photoperiod and/or temperature that either persists throughout diapause or is progressively lost. Either way, the movement towards diapause termination is generally referred to as 'diapause development' (Tauber *et al.* 1986).

Early studies showed that in certain hibernial-diapausing insects diapause development could be hastened by exposing the organism to low winter-like temperatures (= "chilling"), with rapid development observed following transference to higher incubation temperatures. The effect of chilling on diapause development was first observed by Duclaux (1869) in the silkworm *Bombyx mori*. Larvae kept at room temperature remained in diapause for more than one year, but eventually perished. Silkworms chilled for 40 days and then transferred to higher temperatures hatched very soon thereafter. Muroga (1951) confirmed that embryonic development in *B. mori* was dependent upon exposure to a sufficient period of low temperature. On his work on the effect of temperature on reactivation, 90% emergence was obtained in larvae previously chilled for 60-70 days at 5°C before incubation. Similar proportions of emergence were produced in groups subjected to post-treatment temperatures of 2.5°C and 12°C by extending the length of chilling to 80 and 100 days, respectively (Muroga, 1951). The relationship between temperature and diapause-termination in this species therefore appeared to be non-linear in that there existed a definite optimum and limits of temperature for reactivation.

The process of reactivation was therefore held for many years to be a unique and characteristic feature of diapause development, with chilling required for the termination of hibernial-diapause and ultimately the synchronisation of post-diapause development. However this assumption was over-generalised, and recent research has indicated that chilling is not a prerequisite in at least 25 species (Hodek & Hodková, 1988). In these insects activation appears to be heavily influenced by factors such as photoperiod, by increase in temperature alone (rather than by chilling itself), or exposure to high temperature.

### **4.1.3 Aims of the present work**

As it appears that *C. alticolella* enter autumnal-hibernal diapause, the aim of the present work was to investigate the effect of temperature and photoperiod on diapause development. Subjects were chilled at different low temperatures for a period of either one or two months and then transferred to different regimes of photoperiod and temperature. Aspects such as the number of days to emergence and numbers emerging in all of the groups were recorded and compared.



## 4.2 Methodology

### 4.2.1 Sample site: Chapel Fell

Samples of the heath rush *Juncus squarrosus* containing visible larval cases of *Coleophora alticolella* were collected from Chapel Fell (National Grid Reference: NY 758329) on 5th October 1994 and stored in the laboratory on a regime of L:D 6:18 at 5°C. On 22nd February 1995, approximately 1,500 larval cases were separated from the *Juncus* head florets and stored in petri dishes lined with moist filter paper. Unbroken florets were added to the dishes to provide a food source for the developing larvae. Twelve groups each containing approximately 125 larvae were chilled for either one or two months on a specific regime as shown in Table 4.1. Following chilling, that is on either 22nd March or 22nd April, the samples were divided again into 4 groups containing approximately 30-35 larval cases and subjected to either 10° or 15°, long or short photoperiod. An inspection was carried out every one or two days and the date and number of emerging adults were recorded. To produce accurate mortality data any unemerged *Coleophora* cases were dissected and examined at the end of the experiment.

**Table 4.1: Effect of temperature and photoperiod on adult emergence in *Coleophora alticolella*: experimental regimes**

<u>Pre-treatment</u>	<u>Duration of Pre-treatment</u>	<u>Post-treatment Photoperiod</u>	<u>Post-treatment Temperature</u>
5°C Constant darkness	1 & 2 months	L:D 18:6 & 6:18	10° & 15° C
1°C Constant darkness	1 & 2 months	L:D 18:6 & 6:18	10° & 15° C
-1°C Constant darkness	1 & 2 months	L:D 18:6 & 6:18	10° & 15° C
-5°C Constant darkness	1 & 2 months	L:D 18:6 & 6:18	10° & 15° C
-5°C L:D 18:6	1 & 2 months	L:D 18:6 & 6:18	10° & 15° C
-5°C L:D 6:18	1 & 2 months	L:D 18:6 & 6:18	10° & 15° C

## 4.3 Results

### 4.3.1 Effect of photoperiod and temperature on the timing of adult emergence in *Coleophora alticolella*

The range of days over which emergence occurred in all of the regimes is shown in Figure 18. The distribution of days to adult emergence is shown in Figures 19 to 26.

#### *Larvae subjected to one month chilling*

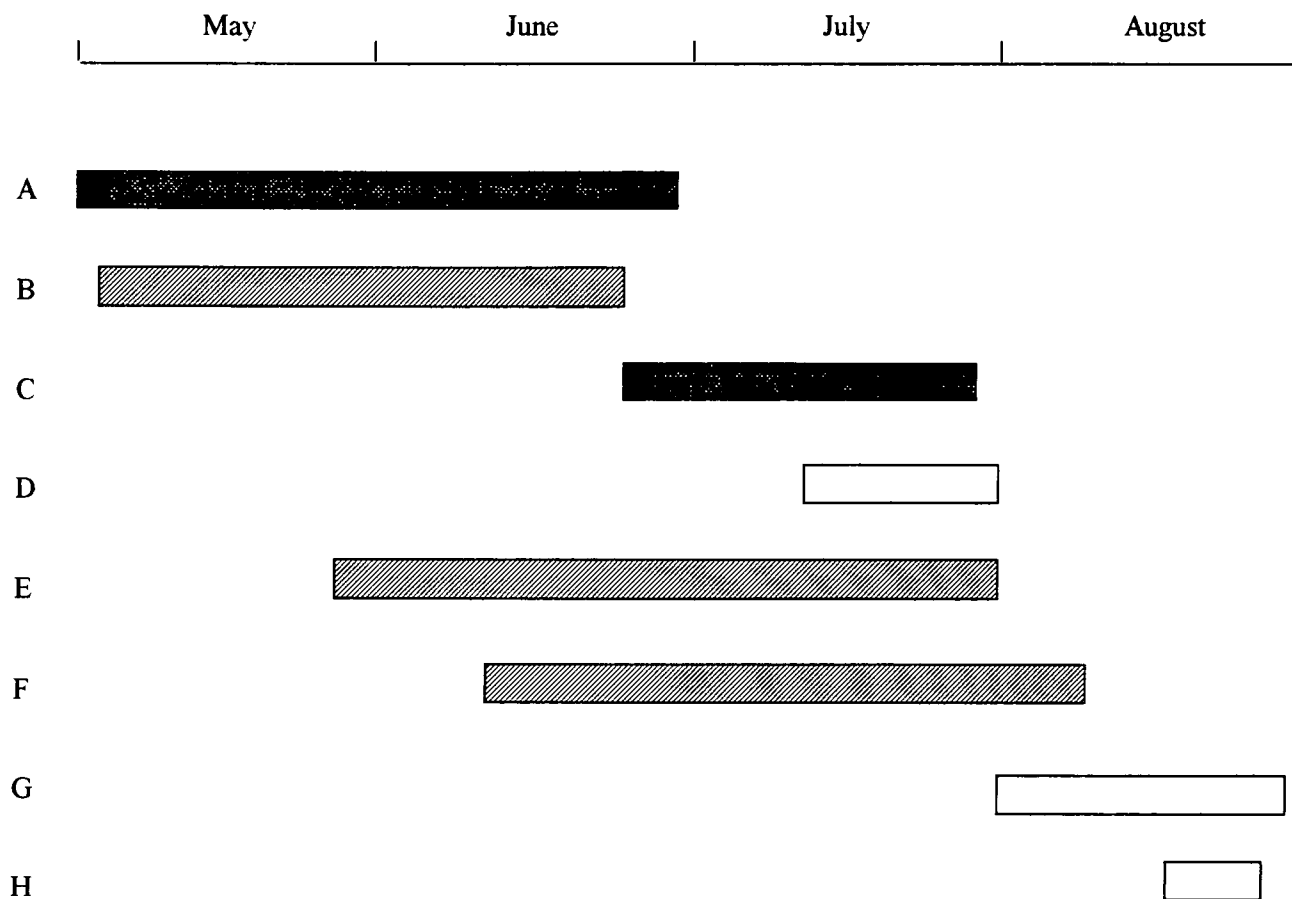
In larvae subjected to one month chilling followed by either long or short photoperiod at 15°C, initial emergence was recorded on 1st and 3rd May 1995, respectively (41 and 43 days after the end of the chilling treatment). In both groups the majority of adults emerged within the first 20-25 days following emergence. Thereafter, numbers emerging trailed-off considerably but continued until 26th and 21st June, respectively. Using a non-parametric chi-squared test for independent samples, the median date of emergence recorded in the two groups did not differ significantly ( $\chi^2 = 0.98$ ,  $p > 0.05$ ). In the moths transferred to 10°C conditions initial emergence on both the long and short-day regimes occurred on 21st June and 11th June and continued until 21st and 24th July, respectively.

#### *Larvae subjected to two months chilling*

A similar pattern of emergence was observed in the larval groups subjected to low temperatures for two months. On the long-day regime at 15°C, adults were first recorded on 30th May, 39 days after chilling. Initial emergence was noted 11 days later on the short-day regime. As found in the groups chilled for one month, emergence trailed-off and when compared the median dates of emergence did not differ significantly ( $\chi^2 = 1.04$ ,  $p > 0.05$ ). Eight individuals on the long-day regime at 10°C emerged during the period of 24th July (day 94) to 29th August (day 130), while only two adults emerged on the short-day regime (on days 116 and 125).

The time taken to emerge following exposure to the chilling pre-treatments was significantly lower in the larval groups previously chilled for two months ( $\chi^2 = 18.00$ ,  $p < 0.05$ ), indicating that the length of the chilling treatment significantly affected larval emergence.

Fig. 18: Range of days over which *C. alticollela* emerged as adults following exposure to differing regimes of temperature and photoperiod.



A	15°C	L:D	18:6	1 month chilling
B	15°C	L:D	6:18	1 month chilling
C	10°C	L:D	18:6	1 month chilling
D	10°C	L:D	6:18	1 month chilling
E	15°C	L:D	18:6	2 months chilling
F	15°C	L:D	6:18	2 months chilling
G	10°C	L:D	18:6	2 months chilling
H	10°C	L:D	6:18	2 months chilling

≥ 40 adults emerged  
 ≥ 10 < 40 adults emerged  
 < 10 adults emerged

Fig. 19: Number of days to emergence in *Coleophora alticolella* at L:D  
 18:6, 15°C after 1 months chilling

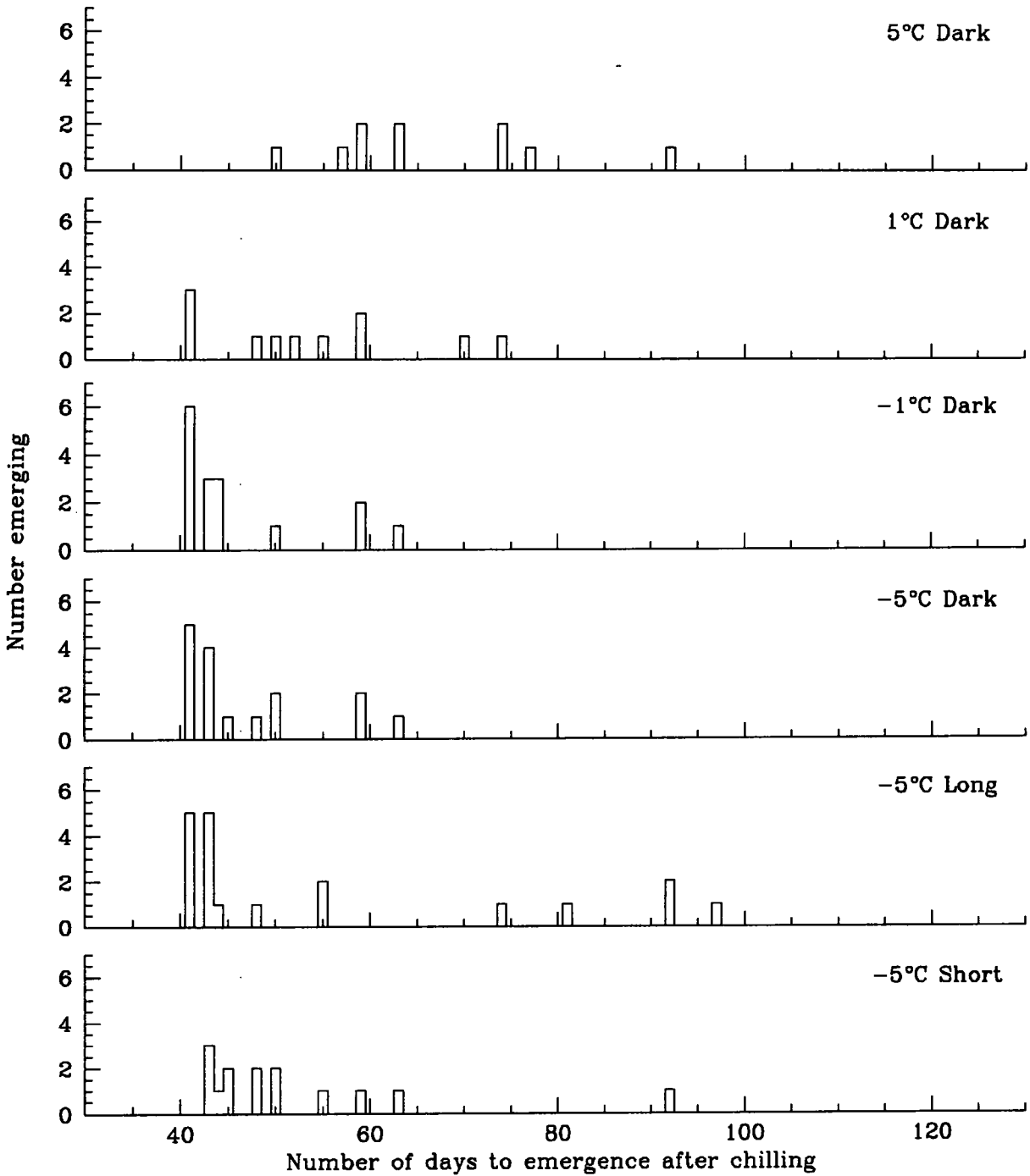


Fig. 20: Number of days to emergence in *Coleophora alticolella* at L:D  
6:18, 15°C after 1 months chilling

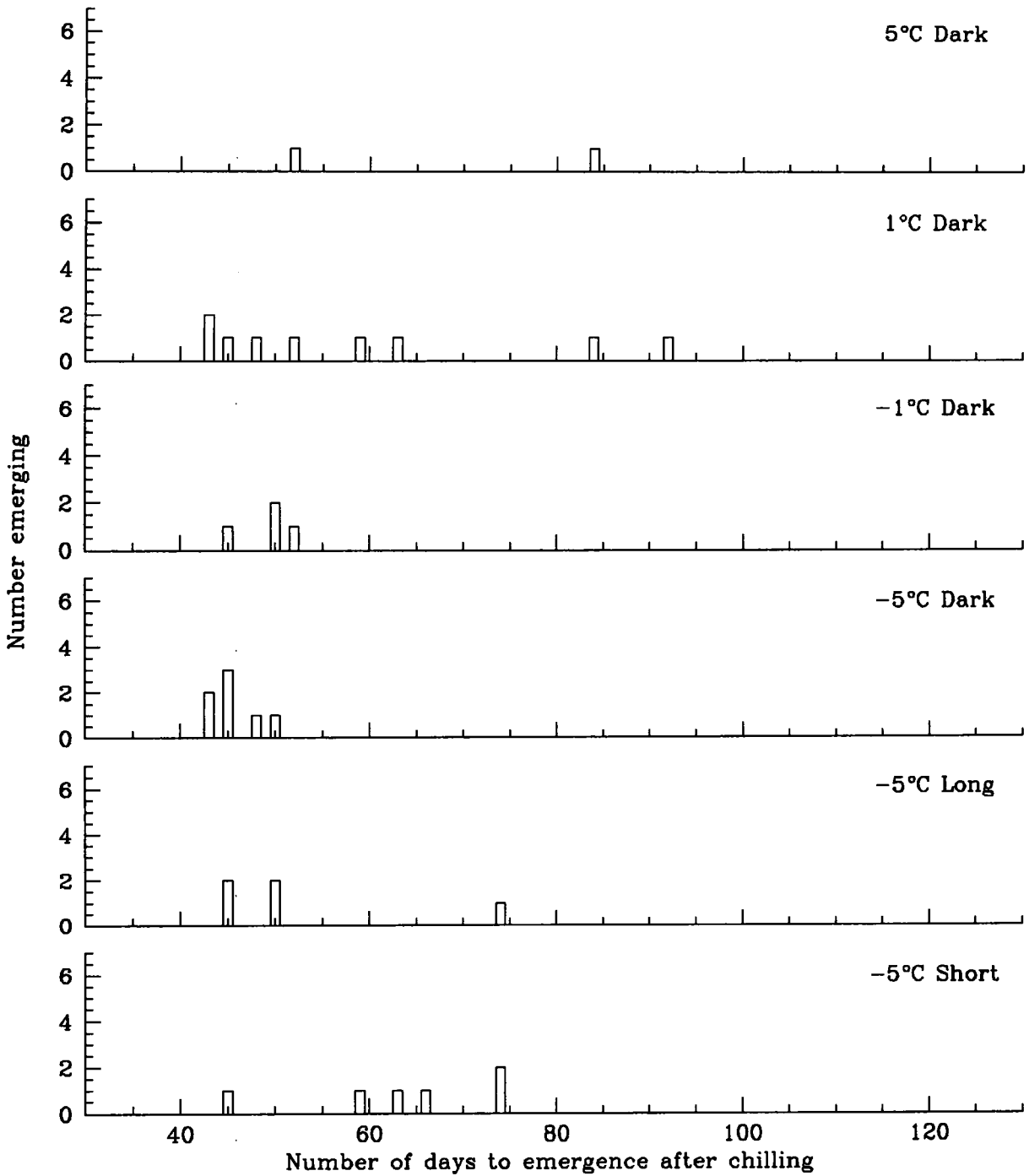


Fig. 21: Number of days to emergence in *Coleophora alticolella* at L:D  
18:6, 10°C after 1 months chilling

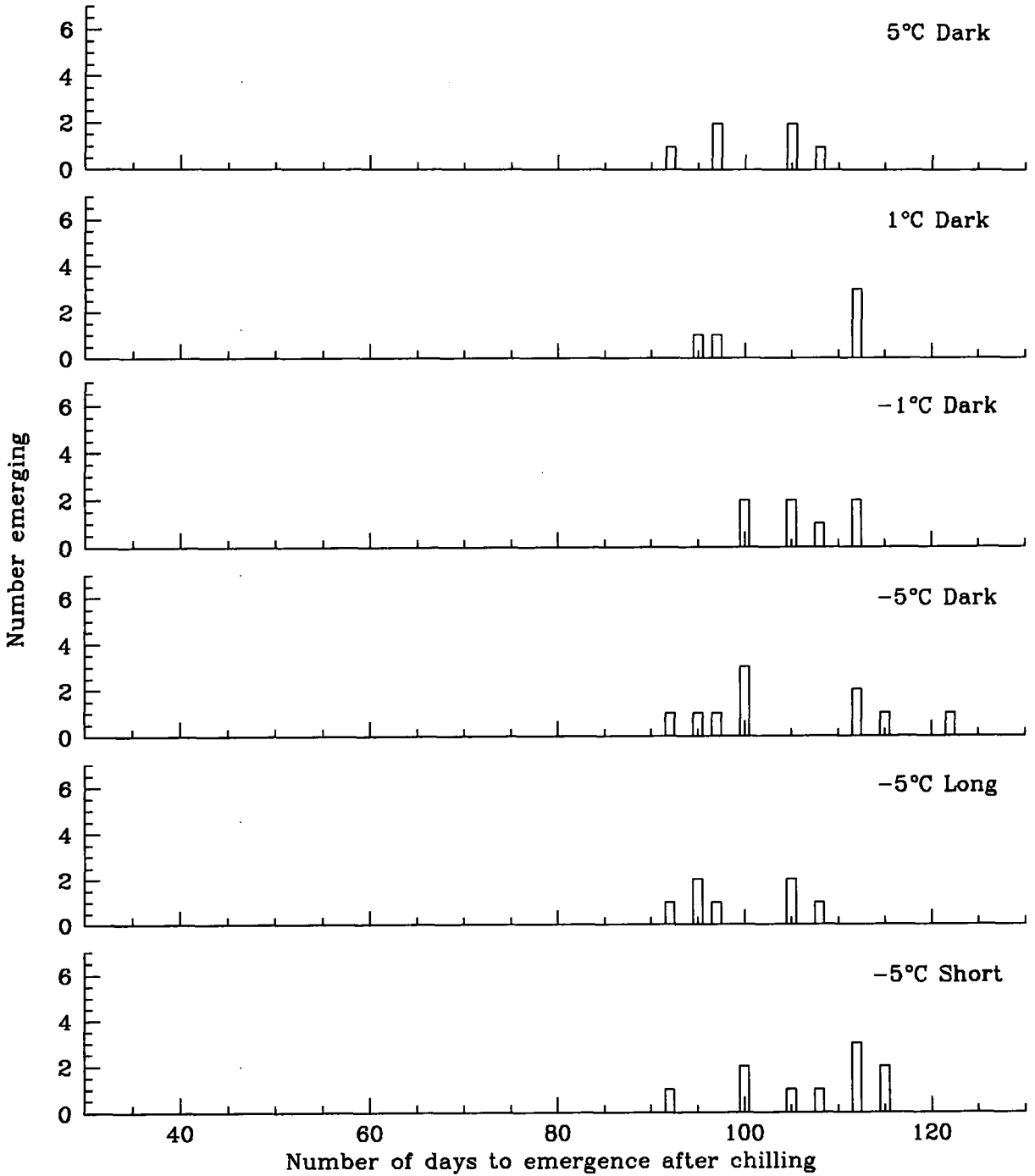


Fig. 22: Number of days to emergence in *Coleophora alticolella* at L:D  
6:18, 10°C after 1 months chilling

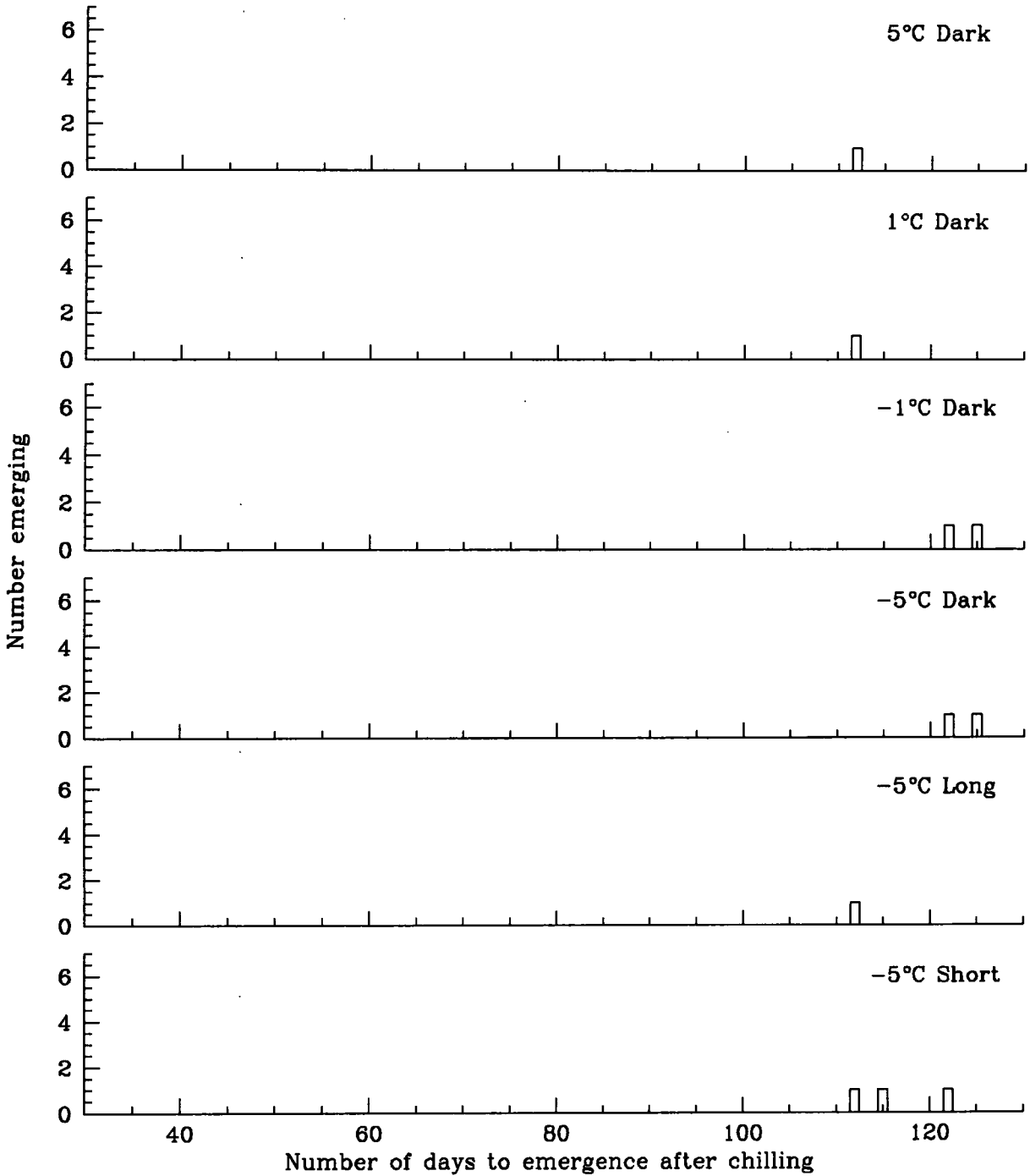


Fig. 23: Number of days to emergence in *Coleophora alticolella* at L:D  
 18:6, 15°C after 2 months chilling

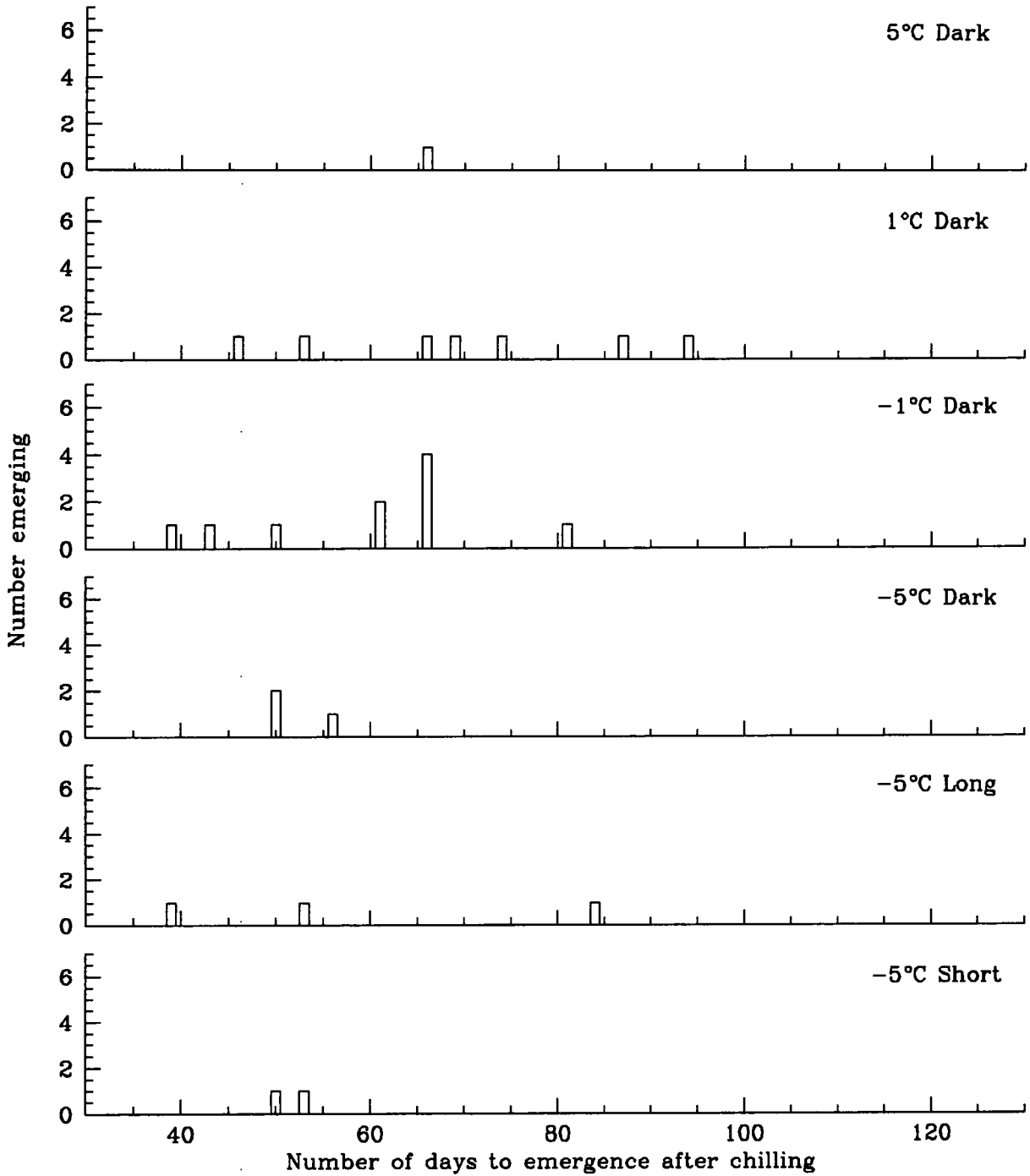




Fig. 24: Number of days to emergence in *Coleophora alticolella* at L:D  
6:18, 15°C after 2 months chilling

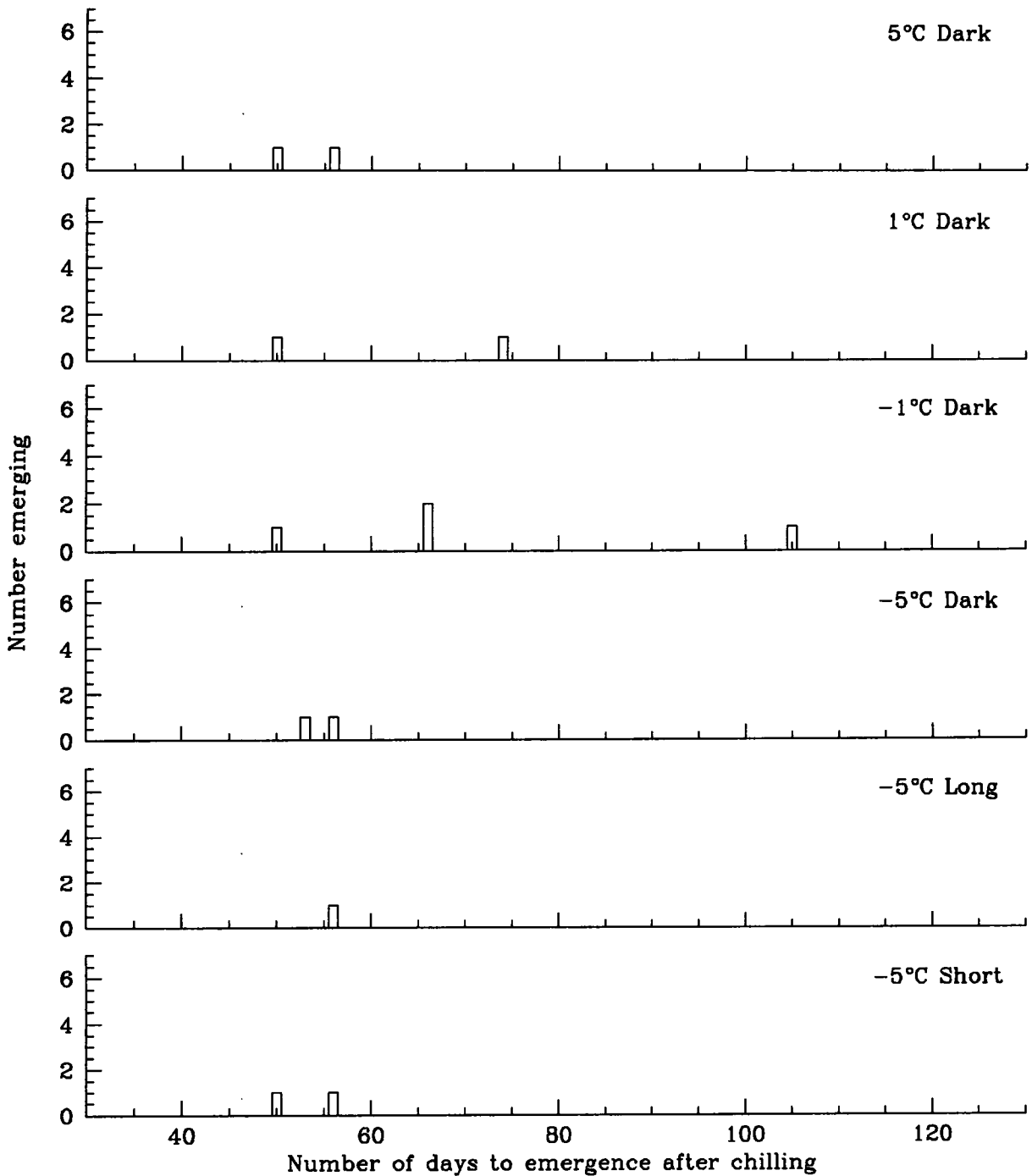


Fig. 25: Number of days to emergence in *Coleophora alticolella* at L:D  
18:6, 10°C after 2 months chilling

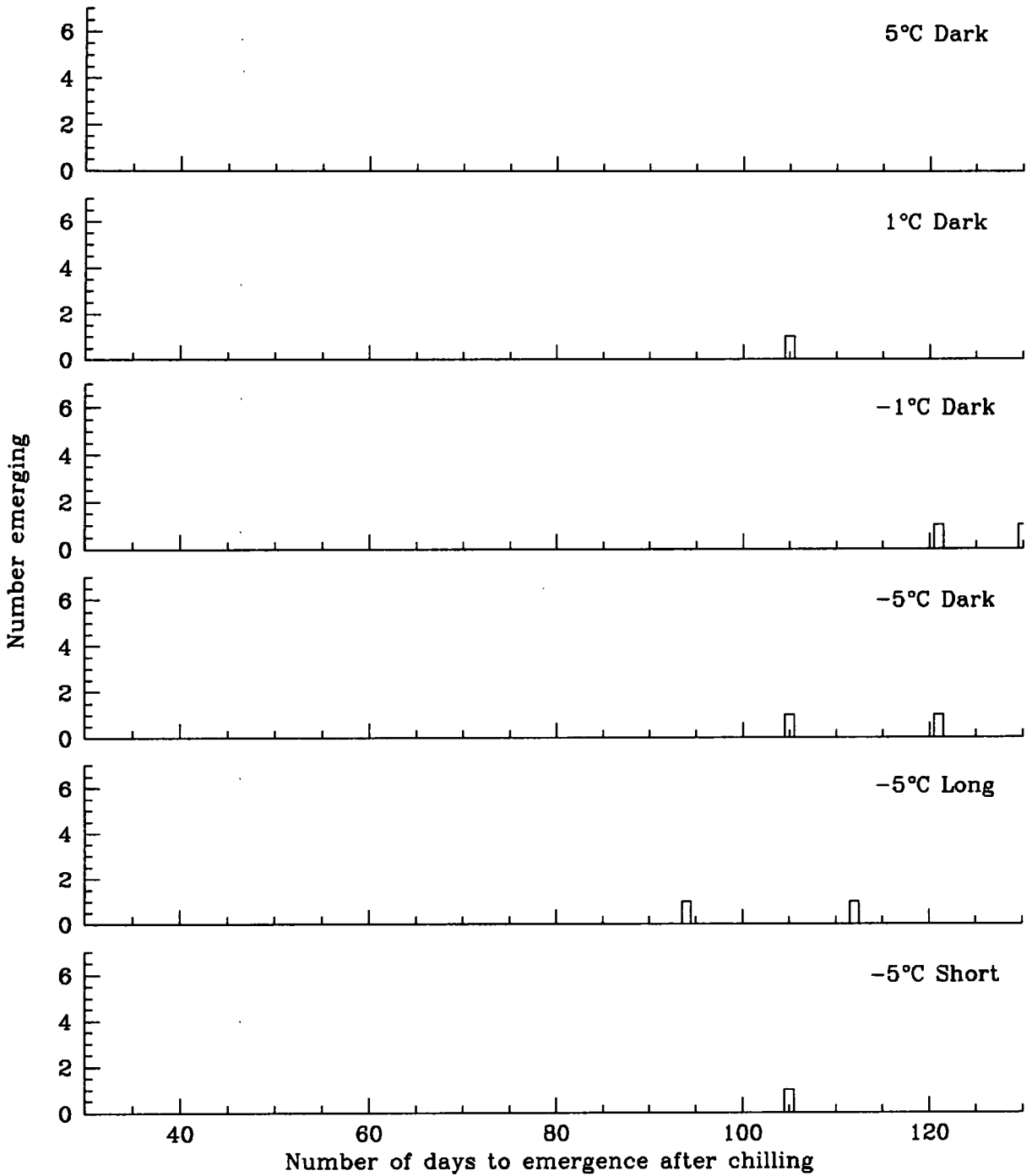
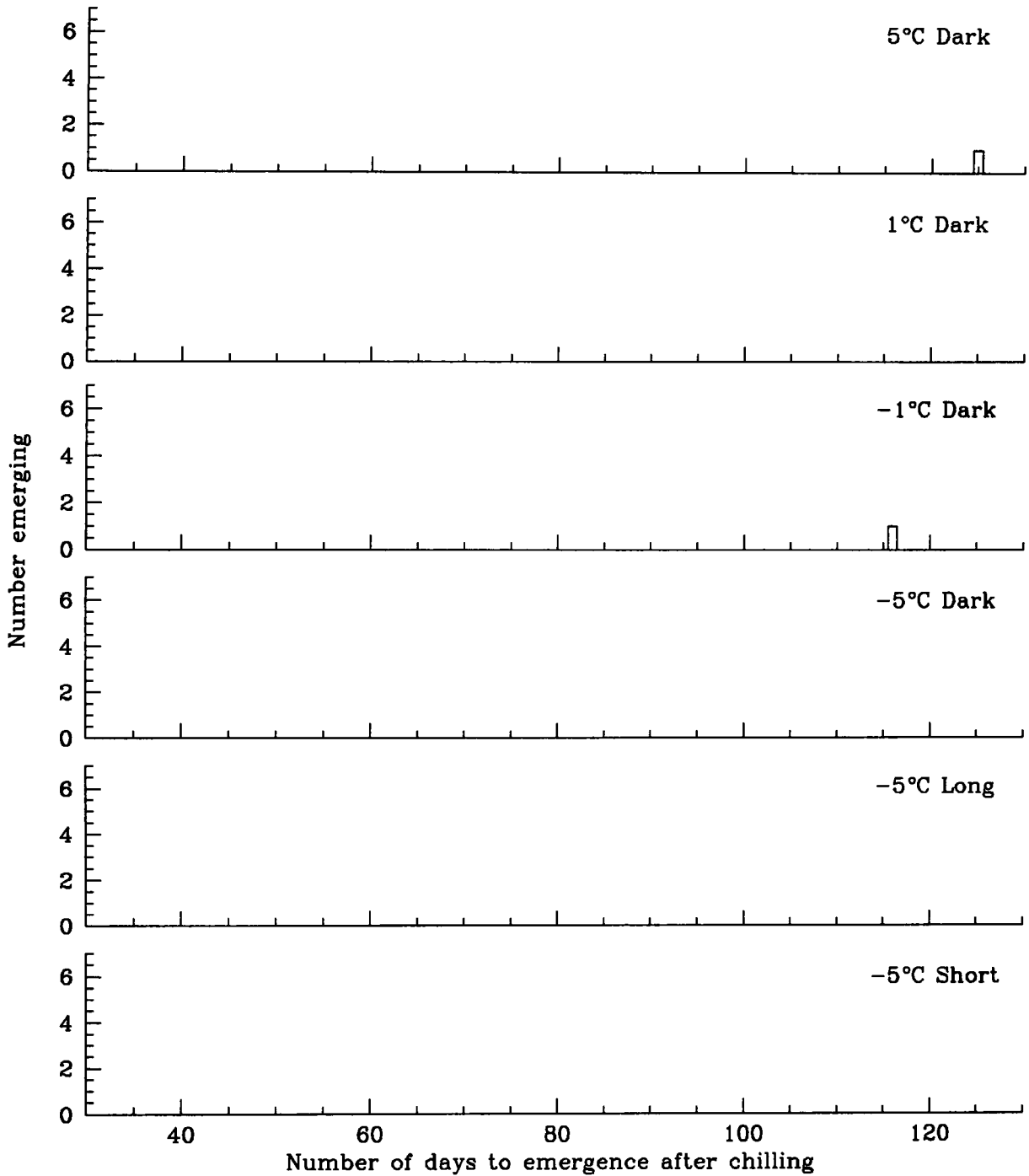


Fig. 26: Number of days to emergence in *Coleophora alticolella* at L:D  
6:18, 10°C after 2 months chilling



### 4.3.2 Effect of photoperiod and temperature on numbers emerging in *Coleophora alticolella*

The number of adults emerging in all of the groups are shown in Tables 4.3 and 4.4. A preliminary analysis of variance carried out to test the significance of each treatment on the actual numbers emerging indicated that the pre-treatment temperatures and photoperiods did not significantly affect the number of larvae emerging ( $F_{3,39} = 1.347$ ,  $p > 0.05$  and  $F_{2,39} = 0.088$ ,  $p > 0.05$  respectively) suggesting that (a) larvae exposed to chilling temperatures ranging from  $-5$  to  $+5^{\circ}\text{C}$  did not differ significantly with respect to numbers emerging and (b) larvae did not respond to photoperiod during chilling (Table 4.2). Therefore the data were combined and comparisons made on the effect of the post-chilling photoperiodic and temperature regimes.

The length of the chilling treatment, the post-chilling photoperiods and the incubation temperatures were found to significantly affect the number of adults emerging ( $F_{1,39} = 16.7$ ,  $50.18$  and  $8.08$  respectively,  $p < 0.0001$ ). With respect to the number of days to emergence, the combination of both long photoperiod and  $15^{\circ}\text{C}$  was more effective than short-photoperiod at  $10^{\circ}\text{C}$ . In addition, larvae kept at  $10^{\circ}\text{C}$  took twice as long to emerge than individuals kept at  $15^{\circ}\text{C}$ , irrespective of the length of the chilling period and photoperiodic regime.

**Table 4.2: Analysis of variance on the numbers of *Coleophora alticolella* emerging in all treatment groups**

<u>Source of variation</u>	<u>Sum of squares</u>	<u>Degrees of freedom</u>	<u>Mean square</u>	<u>F- value</u>	<u>Significance of F</u>
Main Effects	711.083	8	88.885	9.907	.000
Post-photo.	450.188	1	450.188	50.179	.000
Post-temp.	72.521	1	72.521	8.083	.007
Time of chill	150.521	1	150.521	16.777	.000
Pre-photo.	1.583	2	0.792	0.088	.916
Pre-temp.	36.250	3	12.083	1.347	.273
Explained	711.083	8	88.885	9.907	.000
Residual	349.896	39	8.972		
Total	1060.979	47	22.574		

### 4.3.3 Non-emerged *C. alticolella*

As the proportion of emerging moths was low we investigated this aspect further by dissecting all of the remaining larval cases. On 1st October 1995 all the cases were examined and the number of dead and alive larvae were recorded in Tables 4.3 and 4.4. Chi-square analyses carried out testing the significance of the various regimes on mortality and emergence are shown in Tables 4.5 to 4.8.

When compared, mortality was found to vary significantly between the different regimes ( $\chi^2 = 147.9$ , d.f. = 7,  $p < 0.001$ ). The highest mortalities, 74.3% and 71.4%, were respectively recorded in the 15°C long and short-day (2 months chilling) groups. By contrast, samples previously chilled for one month and exposed to similar post-chilling regimes contained the fewest numbers of dead larvae (26.2% and 32.6%, respectively). Similar mortality rates were obtained in the groups kept on a short or long-day at 10°C (Figure 4.5). When compared, photoperiod did not appear to affect mortality in the two 10°C and 15°C chilling regimes, indicating that significant larval death perhaps resulted from dehydration following exposure to a prolonged period of chilling.

The number of larvae emerging compared to the number of larvae not emerging was also found to differ significantly between the groups ( $\chi^2 = 171.4$ , d.f. = 7,  $p < 0.001$ ). The highest numbers emerged on the 15°C long (1 and 2 months chilling) and 10°C long (1 month chilling) regimes with 61.0%, 55.3% and 45% of adults emerging (Table 4.6). The lowest rates were recorded on the 15°C short-day (1 and 2 months chilling), 10°C short-day (1 month chilling) and 10°C long-day (2 months chilling) regimes, with 25.4%, 26%, 8.3% and 7.8% emergence recorded, respectively. Only two individuals (2.2%) previously chilled for two months emerged on the short-day regime at 10°C. With the exception of the 10°C (2 months chilling) groups, long photoperiod appeared to significantly favour adult emergence (Table 4.8).

Following dissection fully formed, dead unemerged adults were only found in the larval cases of groups previously kept at 10°C (Tables 4.3 and 4.4). These findings therefore suggest that exposing larvae to a combination of 10°C and short photoperiod is sufficient to keep the organism alive. However under such conditions, larvae are less likely to emerge than those kept on a long-day regime at 15°C.

**Table 4.3: Examination of *Coleophora alticolella* subjected to 1 month chilling pre-treatment followed by**

**(a) long photoperiod at 15°C**

	Original no. of larvae	Nos. emerged as adults	No. larvae still alive in case	No. larvae dead in case	No. of dead larvae parasitized in case*
5°C Dark	33	10	11	12	0
1°C Dark	32	11	8	9	4
-1°C Dark	33	16	12	3	2
-5°C Dark	34	16	13	2	3
-5°C Long	32	19	7	4	2
-5°C Short	27	14	4	4	5

(\* = Additional to number of non-parasitized dead larvae remaining in pupal case)

**(b) short photoperiod at 15°C**

	Original no. of larvae	Nos. emerged as adults	No. larvae still alive in case	No. larvae dead in case	No. of dead larvae parasitized in case*
5°C Dark	31	2	18	6	5
1°C Dark	33	9	15	9	0
-1°C Dark	32	4	19	9	0
-5°C Dark	33	7	12	12	2
-5°C Long	32	5	17	8	2
-5°C Short	32	6	16	8	2

**(c) long photoperiod at 10°C**

	Original no. of larvae	Nos. emerged as adults	No. larvae still alive in case	No. larvae dead in case †	No. of dead larvae parasitized in case*
5°C Dark	30	6	13	6 (5)	0
1°C Dark	33	5	12	10 (4)	2
-1°C Dark	30	7	11	10 (2)	0
-5°C Dark	28	10	4	8 (2)	4
-5°C Long	30	7	8	12 (3)	0
-5°C Short	28	10	7	3 (6)	2

**(d) short photoperiod at 10°C**

	Original no. of larvae	Nos. emerged as adults	No. larvae still alive in case	No. larvae dead in case †	No. of dead larvae parasitized in case*
5°C Dark	31	1	11	13 (6)	0
1°C Dark	35	1	18	13 (3)	0
-1°C Dark	32	2	19	6 (5)	0
-5°C Dark	33	0	17	10 (5)	1
-5°C Long	31	1	18	7 (4)	1
-5°C Short	22	3	5	9 (2)	3

(†) = Dead adults found in larval case. Additional figures.

**Table 4.4: Examination of *Coleophora alticolella* subjected to 2 months chilling pre-treatment followed by:**

**(a) long photoperiod at 15°C**

	Original no. of larvae	Nos. emerged as adults	No. larvae still alive in case	No. larvae dead in case	No. of dead larvae parasitized in case*
5°C Dark	33	1	4	26	2
1°C Dark	32	7	6	18	1
-1°C Dark	31	10	6	10	5
-5°C Dark	33	3	1	29	0
-5°C Long	28	3	4	18	3
-5°C Short	26	2	0	23	1

**(b) short photoperiod at 15°C**

	Original no. of larvae	Nos. emerged as adults	No. larvae still alive in case	No. larvae dead in case	No. of dead larvae parasitized in case*
5°C Dark	28	2	4	21	1
1°C Dark	32	2	9	17	4
-1°C Dark	29	4	6	16	3
-5°C Dark	32	2	10	16	4
-5°C Long	29	1	4	23	1
-5°C Short	25	2	4	17	2

**(c) long photoperiod at 10°C**

	Original no. of larvae	Nos. emerged as adults	No. larvae still alive in case	No. larvae dead in case †	No. of dead larvae parasitized in case*
5°C Dark	30	0	18	7 (4)	1
1°C Dark	29	1	16	11 (1)	0
-1°C Dark	30	2	15	9 (4)	0
-5°C Dark	32	2	18	10 (2)	0
-5°C Long	30	2	14	14	0
-5°C Short	25	1	13	11	0

**(d) short photoperiod at 10°C**

	Original no. of larvae	Nos. emerged as adults	No. larvae still alive in case	No. larvae dead in case †	No. of dead larvae parasitized in case*
5°C Dark	33	1	19	10 (3)	0
1°C Dark	33	0	16	9 (8)	0
-1°C Dark	32	1	20	6 (5)	0
-5°C Dark	31	0	14	16 (1)	0
-5°C Long	27	0	10	14 (3)	0
-5°C Short	25	0	8	17	0

**Table 4.5 - Chi-square analysis: numbers alive against numbers dead**

Group (Months Chilled)	Nos. Alive (%)	Expected	Nos. Dead (%)	Expected	Total
L:D 18:6 15°C (1)	141 (73.8%)	98.6	50 (26.2%)	92.4	191
L:D 6:18 15°C (1)	130 (67.4%)	99.7	63 (32.6%)	93.3	193
L:D 18:6 15°C (2)	47 (25.7%)	94.5	136 (74.3%)	88.5	183
L:D 6:18 15°C (2)	50 (28.6%)	90.4	125 (71.4%)	84.6	175
L:D 18:6 10°C (1)	100 (55.9%)	92.4	79 (44.1%)	86.6	179
L:D 6:18 10°C (1)	96 (52.2%)	95.0	88 (47.8%)	89.0	184
L:D 18:6 10°C (2)	102 (58.0%)	90.9	74 (42.0%)	85.1	176
L:D 6:18 10°C (2)	89 (49.2%)	93.5	92 (50.8%)	87.5	181
<b>Total</b>	<b>755</b>		<b>707</b>		<b>1462</b>

Using the formula:  $\chi^2 = \Sigma(O-E)^2/E$

$$\chi^2 = \underline{147.9}, \text{ d.f.} = 7, p < 0.001$$

**Table 4.6 - Chi-square analysis: numbers emerged against numbers alive but unemerged**

Group (Months Chilled)	Nos. Alive (%)	Expected	Nos. Dead (%)	Expected	Total
L:D 18:6 15°C (1)	86 (61.0%)	41.3	55 (39.0%)	99.7	141
L:D 6:18 15°C (1)	33 (25.4%)	38.1	97 (74.6%)	91.9	130
L:D 18:6 15°C (2)	26 (55.3%)	13.8	21 (44.7%)	33.2	47
L:D 6:18 15°C (2)	13 (26.0%)	14.6	37 (74.0%)	35.4	50
L:D 18:6 10°C (1)	45 (45.0%)	29.3	55 (55.0%)	70.7	100
L:D 6:18 10°C (1)	8 ( 8.3%)	28.1	88 (91.7%)	67.9	96
L:D 18:6 10°C (2)	8 ( 7.8%)	29.9	94 (92.2%)	72.1	102
L:D 6:18 10°C (2)	2 ( 2.2%)	26.1	87 (97.8%)	62.9	89
<b>Total</b>	<b>221</b>		<b>534</b>		<b>755</b>

Using the formula:  $\chi^2 = \Sigma(O-E)^2/E$

$$\chi^2 = \underline{171.4}, \text{ d.f.} = 7, p < 0.001$$



**Table 4.7: 2 x 2 Contingency tables comparing the effect of photoperiod and temperature on mortality**

**15°C**

<b>1 Month Chilling</b>	<b>Alive</b>	<b>Dead</b>		<b>2 Months Chilling</b>	<b>Alive</b>	<b>Dead</b>	
L:D 18:6	141	50	191	L:D 18:6	47	136	183
L:D 6:18	130	63	193	L:D 6:18	50	125	175
	271	113	384		97	261	358

**10°C**

<b>1 Month Chilling</b>	<b>Alive</b>	<b>Dead</b>		<b>2 Months Chilling</b>	<b>Alive</b>	<b>Dead</b>	
L:D 18:6	100	79	179	L:D 18:6	102	74	176
L:D 6:18	96	88	184	L:D 6:18	89	92	181
	196	167	363		191	166	357

Using the formula:

$$\chi^2 = [n(|ad-bc| - \frac{1}{2}n)^2] / (a+b)(c+d)(a+c)(b+d)$$

where n = number of larvae in both samples  
a = number of alive larvae in L:D 18:6 group  
b = number of dead larvae in L:D 18:6 group  
c = number of alive larvae in L:D 6:18 group  
d = number of dead larvae in L:D 6:18 group

<b>Group</b>	<b><math>\chi^2</math></b>	
15°C 1 month	1.63, d.f. = 1, p>0.95	Non-significant
15°C 2 months	0.25, d.f. = 1, p>0.95	Non-significant
10°C 1 month	0.36, d.f. = 1, p>0.95	Non-significant
10°C 2 months	2.43, d.f. = 1, p>0.95	Non-significant

**Table 4.8: 2 x 2 Contingency tables comparing the effect of photoperiod and temperature on emergence**

**15°C**

<b>1 Month Chilling</b>	<b>Emerged</b>	<b>Non-Emerged</b>		<b>2 Months Chilling</b>	<b>Emerged</b>	<b>Non-Emerged</b>	
L:D 18:6	86	55	141	L:D 18:6	26	21	47
L:D 6:18	33	97	130	L:D 6:18	13	37	50
	119	152	271		39	58	97

**10°C**

<b>1 Month Chilling</b>	<b>Emerged</b>	<b>Non-Emerged</b>		<b>2 Months Chilling</b>	<b>Emerged</b>	<b>Non-Emerged</b>	
L:D 18:6	45	55	100	L:D 18:6	8	94	102
L:D 6:18	8	88	96	L:D 6:18	2	87	89
	53	143	196		10	181	191

Using the formula:

$$\chi^2 = [n(|ad-bc| - \frac{1}{2}n)^2] / (a+b)(c+d)(a+c)(b+d)$$

where n = number of larvae in both samples

- a = number of alive emerged larvae in L:D 18:6 group
- b = number of alive unemerged larvae in L:D 18:6 group
- c = number of alive emerged larvae in L:D 6:18 group
- d = number of alive unemerged larvae in L:D 6:18 group

<b>Group</b>	$\chi^2$	
15°C 1 month	33.40, d.f. = 1, p<0.01	Significant
15°C 2 months	7.49, d.f. = 1, p<0.01	Significant
10°C 1 month	31.55, d.f. = 1, p<0.01	Significant
10°C 2 months	1.98, d.f. = 1, p>0.95	Non-Significant

#### 4.4 Discussion

Following induction, the duration of a diapause is normally dependant upon three factors. First, the more intense the diapause, the greater is the length of time generally required to resume development and consequently the diapause will be of a longer duration than one of a lower intensity. The conditions experienced throughout diapause development also act to regulate how fast a diapause of given intensity will proceed and ultimately, how long it will be before 'developmental competency' will be resumed. Finally, post-diapause development will only take place under favourable conditions experienced after the cessation of diapause development. With respect to the above, the present work demonstrated that in *Coleophora alticolella*: (i) there was apparent variation in either diapause response or intensity within the population as larvae exposed to the same experimental regimes either emerged as adults or remained as larvae in the larval case, (ii) that chilling ( $-5^{\circ}\text{C}$  to  $+5^{\circ}\text{C}$ ) favoured completion of diapause development and (iii) that a post-chilling temperature of  $15^{\circ}\text{C}$  was more favourable than  $10^{\circ}\text{C}$  with lower mortality and earlier emergence. To consider the relevance of these findings in the field, we must first look at each aspect separately.

Intraspecific variation can arise from two general situations. Differences within strains may result from spatial separation, where organisms are under the influence of different selective forces. This is especially prominent with changes in climate according to latitude which occurs, for example in species whose temperate strains exhibit a diapause while their tropical relations do not (Danilvesky, 1965; Lees, 1968). It is not necessarily the case that the latter group do not possess the ability to enter diapause; rather, these insects either show a reduced level of diapause intensity or will enter diapause if exposed to environmental conditions generally associated with temperate areas (Denlinger, 1986). In the ground cricket *Pteronemobius fascipes* individuals from the more northern, temperate areas enter hibernial diapause as eggs. In both the southern subtropical and tropical populations the diapause is generally not expressed. However a 10% diapause is observed which can be increased to 70% if selection for the egg diapause is continued for 15 generations (Masaki 1978).

Differences in emergence times and male-female responses respectively exemplify asymmetrical and bimodal variations within a species. In the silkmoth *Hyalophora cecropia* adults first emerge around mid to late May. In the same population a second group of moths emerge approximately three to four weeks later. In both groups, the males will always emerge earlier than the females (Sternburg & Waldbauer, 1969; 1978). This sexual difference may result from the fact that greater energy is required

to maintain ripe eggs than is required to maintain sperm and consequently the female diapause is generally more intense and of a longer duration than the males. In the phantom midge *Chaoborus americanus*, the pattern of emergence at 25°C in the laboratory is bimodal and separated by around 10 to 20 days. In this insect, the later emerging group contains comparatively smaller and paler larvae (Bradshaw, 1973).

With respect to the factors influencing diapause duration and post-diapause development, research has shown for many years that in a large number of temperate hibernal-diapausing insects a period of chilling will hasten the completion of diapause development. The findings of the present work indicated that chilling temperatures of -5°C to +5°C favoured diapause development in overwintering *C. alticolella*. The temperature of the post-chilling regime also appeared to be an important factor, with 15°C being the optimum temperature for development towards pupation and adult emergence. Unemerged, dead adults were only found in the pupal cases of groups exposed to post-chilling temperatures of 10°C, indicating that there was a temperature threshold for adult emergence. This was extremely surprising however in a moth that is found above 500m a.s.l. in the northern Pennines.

In the present work it is also suggested that either the overwintering larvae do not respond to photoperiod during the chilling treatment (and therefore daylength does not appear to influence diapause development or the termination of diapause), or that response to photoperiod follows exposure to a minimum length of chilling, not attained in this study. As all of the larvae had been kept at 5°C for approximately four months before the start of the experiment, the latter suggestion seems unlikely to be the case.

In *C. alticolella* emergence was found to take place, albeit to differing extents, under all of the experimental regimes. In both *Ephestia cantella* (Bell & Bowley, 1980) and *Plodia interpunctella* (Bell, 1982) diapause can also be completed under both long (diapause-averting) and short (diapause-promoting) photoperiods at 25°C and 20°C. In both species an order exists however with respect to 'stimulatory effect', with the greatest influence on emergence found under 25°C long-day conditions followed by (in order) 25°C short-day, 20°C long-day and 20°C short-day. This is similar to the pattern observed in the present work for both chilling groups in *C. alticolella* (15°C long followed by 15°C short, 10°C long and 10°C short).

It is generally considered that variations in diapause response and intensity in a given population acts to provide a "fail-safe" mechanism in areas of unpredictable climatic conditions (Danks, 1987). Theoretically, bimodal or discontinuous patterns of

emergence can serve to protect the population by ensuring that some insects survive unfavourable and unexpected periods of, for example, adverse weather conditions. Survival of individuals with a weak diapause response would bring about heterogeneity within the population, which would in turn favour the continuation of the species in harsh areas such as Chapel Fell.

Care must be taken when interpreting the results obtained in the present work. Although non-chilled *C. alticolella* do not emerge in high numbers (Fielding, unpublished) there are other factors that may influence diapause development. For example, the low temperatures used in the pre-treatment period perhaps only served as a contrast to the later post-treatment temperatures, and in themselves did not hasten diapause development. Also the stage at which both treatments were given may have affected development. For example in the heteropteran *Pyrhocoris apterus*, eight weeks exposure to 5°C in 10.5 week old females nymphs was more effective than a similar chilling treatment in 2.5 week old larvae (Hodek, 1983). Future studies should therefore be carried out on the thermal and photoperiodic responses of naturally-overwintering insects collected from the field throughout the course of dormancy. In addition, it would have been very interesting to have recorded the sex of the emerged adults to determine whether there were any male-female differences.

One criticism of this study was that the continuing conditions of fixed temperature and photoperiod did not take into account the changes in temperature that would occur in the field. Insect response to a natural regime cannot be replicated in cultures kept at constant temperature. Ideally, future work should therefore include the use of diurnally fluctuating regimes of temperature and perhaps changing photoperiod simulating those associated with seasonal change.

## Chapter 5

### 5.1 General discussion

The primary factor regulating growth in a poikilotherm is temperature. Consequently insects, in particular species that inhabit temperate areas, have devised various strategies to synchronise their life cycles with season in order to avoid adverse weather conditions. Such adaptations come in the form of insect displacement or migration, where the insect temporarily moves from an unfavourable to a more favourable environment, or dormancy where the insect undergoes "facultative or obligatory inhibition or retardation in development occurring during...ontogenesis" (Müller, 1970; Thiele, 1977). Other strategies which protect the insect from adverse conditions while maintaining seasonality include the change from an annual to a biennial life-cycle with change in altitude as found in the ground beetle *Carabus problematicus* (Houston, 1981) or the inclusion of an extra larval instar in direct response to high temperatures in the tussock moth *Dasychira pudibunda* (L.) (Geyspitz and Zarankina, 1963).

The aim of the present study was to investigate one type of dormancy, generally referred to as 'diapause', in a number of insect species found around County Durham. The most widely-accepted definition of the term was outlined by Tauber *et al.* (1986). As photoperiod is the primary environmental cue influencing insect periodicity in temperate regions, the effect of light regimes on diapause was the focus of the investigation. In the majority of insect species that have been studied, perception and response to photoperiod is according to a fixed critical threshold of light. In many autumnal-hibernal diapausing insects, individuals respond to short day-lengths by entering dormancy. Diapause terminates spontaneously around mid-winter when the low winter temperatures intervene and enforce a period of quiescence that lasts until the following spring when temperatures rise to a level that allows post-diapause development. In summer diapausing insects dormancy is often initiated and maintained by long photoperiod and ended in response to the shorter autumn day-lengths (Tauber *et al.* 1986). The present study therefore aimed to investigate the photoperiodic response of a winter diapausing species (*Tipula subnodicornis* larvae) and a summer diapausing species (adult *Nebria salina*) when placed under different regimes of light. As temperature has been demonstrated to modify rate of diapause development in some species the effects of chilling and photoperiod on diapausing *Tipula fusca* eggs and fourth instar *Coleophora alticolella* were also investigated.

From the results obtained in this study, diapause was clearly shown to be a multi-faceted and species-specific phenomenon with maintenance and termination in particular varying in intensity and form. For example, whereas long day-length significantly hastened development towards pupation in *T. subnodicornis* it prevented ovarian maturation in *N. salina*. Obvious changes found to have occurred in the diapausing insects were characteristic of the 'diapause syndrome', for example the sequestering of fats, shelter-seeking behaviour and the manufacturing of protective ancillary structures such as larval cases. In the present study low levels of activity were recorded in *N. salina* previously exposed to long-day conditions for two months. High activity however was measured in adults kept on photoperiods of L:D 12:12 and L:D 6:18 for a similar length of time. As low activity is a feature common to diapausing insects it was suggested that these insects were still in diapause. High beetle activity was correlated with post-diapause behaviour normally observed in the field around September. This was confirmed after the dissection and examination of the reproductive organs.

Following diapause induction there follows a period of diapause-maintenance during which insects are sensitive to environmental cues. Two main responses to diapause-maintaining factors were found in insects (Tauber & Tauber, 1976). First, as exemplified by *T. subnodicornis*, includes species that progressively lose their response to diapause-maintaining factors as diapause progresses, with dormancy spontaneously terminating sometime during late autumn or winter (Butterfield, 1976). The second type of response, in which insects remain sensitive to such factors throughout the entirety of diapause was shown in *N. salina*, as exposure to short photoperiod appeared to be the major factor involved in diapause-termination in this species.

The development that occurs in an insect that leads to the completion of diapause is generally referred to as 'diapause development', the rate of which is heavily influenced by factors such as temperature and photoperiod. This study clearly demonstrated that in *T. subnodicornis* long photoperiods of L:D 14:10 and over will break diapause in its early stages, while short photoperiods will restrain development towards pupation. As pre-pupal development and pupation is temperature-dependent it is suggested that larvae in the field will be prevented from pupating in the winter if temperatures were to rise above the developmental threshold. In *N. salina* short day-lengths of L:D 12:12 and under favoured reproductive maturation while long photoperiods prevented development. A prolonged exposure to low-temperatures allowed completion of diapause development in fourth instar *C. alticolella* and synchronised hatching in *T.*

*fusca* eggs. In both species photoperiod did not appear to play a major role in diapause development.

One interesting aspect of the present work was the apparent existence of differences in diapause response or intensity in *C. alticolella*. Intraspecific variation can arise from genetic differences found between strains or results from exposure to different selective forces experienced before or after the onset of dormancy. This finding emphasised the fact that diapause, while protecting the individual insect from unfavourable conditions acts to protect the population as a whole from potential catastrophes such as drought or failure of flowering of the food-plant. This is especially important in temperate areas where the climate is fairly unpredictable. Increasing the range of days over which emergence takes place would therefore reduce the chances of extinction if such natural catastrophes were to take place. In terms of energy-cost to the population, having such 'extreme' individuals is negligible and therefore 'spreading' is relatively common. As pointed out by Danks (1987) "very few insect populations, so to speak, have all their eggs in one temporal basket". Future work should therefore focus on such patterns of variation as this might give us a greater insight into the evolutionary nature of the diapause response and could, for instance, help us forecast changes in the population dynamics of invertebrates especially with the increasing threat of global warming.



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**Table I: Effect of photoperiod on date and numbers pupating in *Tipula subnodicornis***

Date	8:16	10:14	12:12	14:10	16:8	18:6
9.1	*	*	*	*	2	7
10.1	*	*	*	*	1	1
11.1	*	*	*	*	3	1
12.1	*	*	*	*	5	3
13.1	*	*	*	*	1	1
16.1	*	*	*	*	5	3
17.1	*	*	*	*	*	1
18.1	*	*	*	*	1	*
19.1	*	*	*	*	1	2
20.1	*	*	*	*	*	1
23.1	*	*	*	3	2	*
24.1	*	*	*	*	*	2
25.1	*	*	*	*	*	1
26.1	*	*	*	*	2	2
27.1	*	*	*	2	1	*
30.1	*	*	*	1	*	1
31.1	*	*	*	*	*	*
1.2	*	*	*	2	*	*
2.2	*	*	*	*	*	*
3.2	*	*	*	*	1	*
6.2	*	*	*	1	*	*
7.2	*	*	*	*	*	*
8.2	*	*	*	1	*	*
9.2	*	*	*	*	*	*
10.2	*	*	*	*	*	*
13.2	*	3	2	*	*	*
14.2	*	1	1	*	*	*
15.2	*	*	*	*	*	*
16.2	*	1	*	*	*	*
17.2	*	1	2	*	*	*
20.2	*	*	1	*	*	*
21.2	*	*	*	*	*	*
22.2	*	*	*	*	*	*
23.2	*	1	*	*	*	*
24.2	*	*	2	*	*	*



27.2	3	*	1	*	*	*
28.2	*	1	*	*	*	*
1.3	*	*	1	*	*	*
2.3	*	2	*	*	*	*
3.3	*	2	*	*	*	*
6.3	*	*	1	*	*	*
7.3	1	3	*	*	*	*
8.3	*	1	*	*	*	*
9.3	*	*	*	*	*	*
10.3	*	2	*	*	*	*
13.3	*	1	*	*	*	*
14.3	1	1	*	*	*	*

**Table II: Measurement of ovary and ovariole at widest point in female *N. salina* after exposure to one month experimental photoperiods at 15°C.**

L:D 18:6		L:D 12:12		L:D 6:18	
Ovary (mm)	Ovariole (mm)	Ovary (mm)	Ovariole (mm)	Ovary (mm)	Ovariole (mm)
A1: 0.600	0.0375	B1: 0.75	0.0875	C1: 0.925	0.1250
A2: 0.500	0.0375	B2: 0.65	0.0875	C2: 0.800	0.1000
A3: -	-	B3: 0.75	0.1250	C3: 0.750	0.0875
A4: 0.525	0.0375	B4: 0.725	0.0875	C4: -	-
A5: 0.650	0.0500	B5: -	0.1000	C5: 0.875	0.1250
A6: 0.550	0.0375	B6: 0.625	0.0750	C6: 0.900	0.1250
A7: 0.600	0.0500	B7: 0.775	0.0875	C7: -	0.0875
A8: 0.700	0.0625	B8: -	-	C8: 0.800	0.1125
A9: 0.675	0.0500	B9: 0.750	0.0875	C9: -	0.1125
A10: 0.600	0.0500	B10: 0.625	0.1125*	C10: 1.000	0.1375*
A11: 0.525	0.0375	B11: 0.575	0.0375	C11: 0.875	0.0875
A12: 0.500	0.0625	B12: 0.675	0.1000*	C12: 0.750	0.0875
A13: 0.550	0.0375	B13: -	0.0750	C13: 0.750	0.0875
A14: -	0.0250			C14: -	0.1125

\* = Developing oocytes seen in ovarioles

- = Ruptured ovary following removal of abdominal membrane; if distinguishable ovariole measurements were made.

**Table III: Measurement of ovary and ovariole at widest point, and if present number of eggs in female *N. salina* after exposure to two months experimental photoperiods at 15°C.**

L:D 18:6		L:D 12:12		L:D 6:18	
Ovary width (mm)	Ovariole width (mm)	Number of eggs or ovary and ovariole width (mm)		Number of eggs or ovary and ovariole width (mm)	
A1: 0.650	0.0875	B1:	Eggs - 29	C1:	Eggs - 22
A2: 0.600	0.0500	B2:	Eggs - 18	C2:	Eggs - 16
A3: 0.700	0.0875	B3:	Eggs - 17	C3:	Eggs - 4
A4: 0.550	0.0375	B4:	Eggs - 26	C4:	Eggs - 2
A5: 0.575	0.0750	B5:	Eggs - 7	C5:	Eggs - 11
A6: -	-	B6:	0.900 0.1125*	C6:	Eggs - 13
A7: 0.625	0.075	B7:	0.875 0.1375	C7:	Eggs - 7
A8: -	-	B8:	Eggs - 10	C8:	Eggs - 15
A9: -	-	B9:	Eggs - 16	C9:	Eggs - 6
		B10:	1.000 0.1250*	C10:	Eggs - 6
		B11:	- 0.1250*	C11:	Eggs - 7
		B12:	Eggs - 6		

- = Ruptured ovary following removal of abdominal membrane; if distinguishable ovariole measurements were made.

**Table IV: Measurement of length of abdomen covered by testes and mean testes width at widest point (mm) in male *N. salina* after exposure to one month L:D 18:6 photoperiod at 15°C.**

	Penney (1969)		Luff (1973)	
	Length of abdomen (mm)	Length of abdomen covered with testes(mm)	Width of testes at widest point (mm)	Stage of development
A1	7.4	3.2	0.125	Immature
A2	7.5	4.0	0.250	Developing
A3	7.0	2.7	0.125	Immature
A4	7.7	5.3	0.250	Developing
A5	7.2	3.0	0.175	Immature
A6	7.6	4.0	0.175	Developing
A7	7.5	4.5	0.250	Developing
A8	7.2	3.7	0.200	Developing
A9	7.2	3.0	0.125	Immature
A10	7.0	3.9	0.200	Developing
A11	7.5	3.0	0.125	Immature
A12	8.0	5.0	0.250	Developing
A13	7.2	3.2	0.200	Immature
A14	7.3	3.7	0.250	Developing
A15	7.4	4.0	0.200	Developing
A16	7.3	4.0	0.250	Developing
A17	7.2	3.2	0.125	Immature
A18	7.8	4.0	0.250	Developing

**Table V: Measurement of length of abdomen covered by testes and mean testes width at widest point (mm) in male *N. salina* after exposure to one month L:D 12:12 photoperiod at 15°C.**

	Length of abdomen (mm)	Length of abdomen covered with testes (mm)	Width of testes at widest point (mm)	Stage of development
B1	7.8	5.2	0.225	Developing
B2	7.6	5.5	0.250	Developing
B3	7.3	4.9	0.263	Developing
B4	7.8	5.1	0.263	Developing
B5	7.5	5.4	0.238	Developing
B6	7.2	4.5	0.213	Developing
B7	7.1	4.6	0.238	Developing
B8	7.3	4.7	0.213	Developing
B9	7.9	5.1	0.188	Developing
B10	7.6	4.5	0.263	Developing
B11	7.7	5.0	0.225	Developing
B12	7.5	5.0	0.200	Developing
B13	7.5	5.4	0.263	Developing
B14	7.4	4.7	0.238	Developing
B15	7.0	4.6	0.225	Developing
B16	7.6	4.5	0.200	Developing

**Table VI: Measurement of length of abdomen covered by testes and mean testes width at widest point (mm) in male *N. salina* after exposure to one month L:D 6:18 photoperiod at 15°C.**

	Length of abdomen (mm)	Length of abdomen covered with testes (mm)	Width of testes at widest point (mm)	Stage of development
C1	7.6	6.0	0.275	Mature
C2	7.4	4.9	0.238	Developing
C3	7.3	4.9	0.238	Developing
C4	7.7	5.5	0.250	Developing
C5	7.5	5.3	0.250	Developing
C6	7.2	5.2	0.250	Developing
C7	7.4	5.3	0.263	Developing
C8	7.4	5.8	0.238	Mature
C9	7.6	5.7	0.250	Mature
C10	7.6	5.1	0.250	Developing
C11	7.9	4.9	0.213	Developing
C12	7.5	5.4	0.263	Developing
C13	7.9	5.6	0.250	Developing
C14	7.4	4.5	0.188	Developing
C15	7.5	5.3	0.263	Developing
C16	7.2	5.0	0.288	Developing

**Table VII: Measurement of length of abdomen covered by testes and mean testes width at widest point (mm) in male *N. salina* after exposure to two months L:D 18:6 photoperiod at 15°C.**

	Length of abdomen (mm)	Length of abdomen covered with testes (mm)	Width of testes at widest point (mm)	Stage of development
D1	7.2	4.0	0.125	Developing
D2	7.1	3.9	0.125	Developing
D3	7.3	3.2	0.125	Immature
D4	7.1	4.0	0.138	Developing
D5	7.6	4.2	0.125	Developing
D6	6.8	3.0	0.125	Immature
D7	7.3	3.9	0.125	Developing
D8	6.8	4.4	0.150	Developing
D9	6.8	3.8	0.125	Developing
D10	7.5	3.6	0.125	Immature
D11	7.7	4.3	0.125	Developing
D12	6.9	4.2	0.150	Developing
D13	7.3	3.6	0.150	Immature
D14	7.5	3.7	0.150	Immature
D15	6.8	3.2	0.138	Immature
D16	7.0	4.1	0.188	Developing
D17	7.1	4.0	0.150	Developing
D18	6.4	3.8	0.150	Developing

**Table VIII: Measurement of length of abdomen covered by testes and mean testes width at widest point (mm) in male *N. salina* after exposure to two months L:D 12:12 photoperiod at 15°C.**

	Length of abdomen (mm)	Length of abdomen covered with testes (mm)	Width of testes at widest point (mm)	Stage of development
E1	7.2	3.6	0.113	Developing
E2	7.3	4.0	0.125	Developing
E3	7.3	4.6	0.125	Developing
E4	7.0	3.4	0.113	Immature
E5	7.5	3.0	0.125	Immature
E6	7.8	4.9	0.138	Developing
E7	7.5	4.7	0.150	Developing
E8	7.0	3.6	0.125	Developing
E9	7.5	4.1	0.125	Developing
E10	7.6	4.0	0.138	Developing
E11	7.3	4.4	0.150	Developing
E12	7.2	4.5	0.125	Developing
E13	7.3	4.0	0.125	Developing
E14	7.0	3.5	0.125	Developing



**Table IX: Measurement of length of abdomen covered by testes and mean testes width at widest point (mm) in male *N. salina* after exposure to two months L:D 6:18 photoperiod at 15°C.**

	Length of abdomen (mm)	Length of abdomen covered with testes (mm)	Width of testes at widest point (mm)	Stage of development
F1	7.4	3.7	0.163	Developing
F2	7.1	4.8	0.125	Developing
F3	8.0	5.1	0.175	Developing
F4	7.3	4.0	0.125	Developing
F5	7.6	4.8	0.200	Developing
F6	7.3	3.8	0.125	Developing
F7	7.5	5.2	0.150	Developing
F8	7.7	3.5	0.163	Immature
F9	7.1	4.6	0.163	Developing
F10	7.2	3.9	0.150	Developing
F11	6.7	4.0	0.125	Developing
F12	7.4	5.5	0.163	Developing
F13	7.0	4.5	0.163	Developing
F14	7.3	3.7	0.150	Developing
F15	6.3	4.2	0.138	Developing

**Table Xa : Effect of photoperiod on activity in *Nebria salina***

**L:D 18:6**

<b>Time</b>	<b>Males</b>	<b>Females</b>	<b>Total</b>
06.00	0	0	0
06.15	0	0	0
06.30	0	0	0
06.45	0	0	0
07.00	2	0	2
07.15	1	0	1
07.30	0	0	0
07.45	0	0	0
08.00	1	0	1
08.15	1	0	1
08.30	2	1	3
08.45	3	1	4
09.00	3	1	4
15.00	0	0	0
15.15	0	0	0
15.30	0	0	0
15.45	0	0	0
14.00	0	0	0
21.00	0	0	0
21.15	0	0	0
21.30	1	0	1
21.45	1	0	1
22.00	1	0	0

**Table Xb. Effect of photoperiod on activity in *Nebria salina***

**L:D 12:12**

<b>Time</b>	<b>Males</b>	<b>Females</b>	<b>Total</b>
06.00	8	3	11
06.15	6	1	7
06.30	5	1	6
06.45	4	2	6
07.00	4	2	6
07.15	4	1	5
07.30	1	0	1
07.45	1	0	1
08.00	2	1	3
08.15	2	1	3
08.30	2	1	3
08.45	2	1	3
09.00	2	1	3
15.00	1	0	1
15.15	1	0	1
15.30	1	0	1
15.45	1	0	1
16.00	1	0	1
21.00	0	1	1
21.15	1	0	1
21.30	0	2	2
21.45	1	3	4
22.00	1	1	1

**Table Xc. Effect of photoperiod on activity in *Nebria salina***

**L:D 6:18**

<b>Time</b>	<b>Males</b>	<b>Females</b>	<b>Total</b>
06.00	5	2	7
06.15	8	5	13
06.30	2	4	6
06.45	5	4	9
07.00	4	1	5
07.15	5	1	6
07.30	5	1	6
07.45	4	1	5
08.00	3	2	5
08.15	3	1	4
08.30	3	1	4
08.45	4	1	5
09.00	4	2	6
15.00	2	2	4
15.15	2	1	3
15.30	2	3	5
15.45	2	3	5
14.00	4	3	7
21.00	2	2	4
21.15	3	2	5
21.30	3	2	5
21.45	2	0	2
22.00	2	0	2

**Table XI a. Emergence of *Coleophora alticolella* exposed to 15°C L:D 18:6 after 1 month chilling pretreatment**

Group	Date										
	1/5	3/5	4/5	5/5	8/5	10/5	12/5	15/5	17/5	19/5	23/5
5° Dark	*	*	*	*	*	1	1	1	1	1	1
5° Dark	*	*	*	*	*	*	*	*	1	3	4
1° Dark	*	*	*	*	1	2	3	3	3	5	5
1° Dark	3	3	3	3	3	3	3	4	4	4	4
-1°Dark	2	3	4	4	4	5	5	5	5	6	6
-1°Dark	4	6	8	8	8	8	8	8	8	9	10
-5°Short	*	*	1	1	1	1	1	2	2	2	2
-5°Short	*	3	3	5	7	9	9	9	9	10	11
-5°Long	2	4	4	4	4	4	4	6	6	6	6
-5°Long	3	6	7	7	8	8	8	8	8	8	8
-5°Dark	4	8	8	9	10	11	11	11	11	11	11
-5°Dark	1	1	1	1	1	2	2	2	2	4	5
	26/5	30/5	3/6	6/6	10/6	13/6	16/6	21/6	26/6	29/6	
5° Dark	2	2	3	3	3	3	3	4	4	4	
5° Dark	4	4	5	6	6	6	6	6	6	6	
1° Dark	5	6	6	6	6	6	6	6	6	6	
1° Dark	4	4	5	5	5	5	5	5	5	5	
-1°Dark	6	6	6	6	6	6	6	6	6	6	
-1°Dark	10	10	10	10	10	10	10	10	10	10	
-5°Short	2	2	2	2	2	2	2	2	2	2	
-5°Short	11	11	11	11	11	11	11	12	12	12	
-5°Long	6	6	6	6	7	7	7	8	9	9	
-5°Long	8	8	9	9	9	9	9	10	10	10	
-5°Dark	11	11	11	11	11	11	11	11	11	11	
-5°Dark	5	5	5	5	5	5	5	5	5	5	

**Table XI b. Emergence of *Coleophora alticolella* exposed to 15°C L:D 6:18 after 1 month chilling pretreatment**

Group	Date											
	1/5	3/5	5/5	8/5	10/5	12/5	15/5	17/5	19/5	23/5	26/5	30/5
5° Dark	*	*	*	*	*	1	1	1	1	1	1	1
5° Dark	*	*	*	*	*	*	*	*	*	*	*	*
1° Dark	*	1	2	3	3	4	4	4	5	6	6	6
1° Dark	*	1	1	1	1	1	1	1	1	1	1	1
-1°Dark	*	*	*	*	1	2	2	2	2	2	2	2
-1°Dark	*	*	1	1	2	2	2	2	2	2	2	2
-5°Short	*	*	1	1	1	1	1	1	1	1	1	1
-5°Short	*	*	*	*	*	*	*	*	1	2	3	3
-5°Long	*	*	1	1	2	2	2	2	2	2	2	2
-5°Long	*	*	1	1	2	2	2	2	2	2	2	2
-5°Dark	*	1	3	4	4	4	4	4	4	4	4	4
-5°Dark	*	1	2	2	3	3	3	3	3	3	3	3
	3/6	6/6	10/6	13/6	16/6	21/6						
5° Dark	1	1	1	2	2	2						
5° Dark	*	*	*	*	*	*						
1° Dark	6	6	6	7	7	7						
1° Dark	1	1	1	1	1	2						
-1°Dark	2	2	2	2	2	2						
-1°Dark	2	2	2	2	2	2						
-5°Short	2	2	2	2	2	2						
-5°Short	4	4	4	4	4	4						
-5°Long	3	3	3	3	3	3						
-5°Long	2	2	2	2	2	2						
-5°Dark	4	4	4	4	4	4						
-5°Dark	3	3	3	3	3	3						

**Table XI c. Emergence of *Coleophora alticolella* exposed to 10°C L:D 18:6 after 1 month chilling pretreatment**

Group	Date									
	21/6	24/6	26/6	29/6	4/7	7/7	11/7	14/7	17/7	21/7
5° Dark	*	*	*	*	1	2	2	2	2	2
5° Dark	1	1	3	3	4	4	4	4	4	4
1° Dark	*	*	1	1	1	1	4	4	4	4
1° Dark	*	1	1	1	1	1	1	1	1	1
-1°Dark	*	*	*	1	3	4	4	4	4	4
-1°Dark	*	*	*	1	1	1	3	3	3	3
-5°Short	*	*	*	1	2	2	3	4	4	4
-5°Short	1	1	1	2	2	3	5	6	6	6
-5°Long	1	2	2	2	3	3	3	3	3	3
-5°Long	*	1	2	2	3	4	4	4	4	4
-5°Dark	1	2	2	3	3	3	3	4	4	4
-5°Dark	*	*	1	3	3	3	5	5	5	6

**Table XI d. Emergence of *Coleophora alticolella* exposed to 10°C L:D 18:6 after 2 months chilling pretreatment**

Group	Date					
	24/7	4/8	11/8	15/8	21/8	29/8
5° Dark	*	*	*	*	*	*
5° Dark	*	*	*	*	*	*
1° Dark	*	1	1	1	1	1
1° Dark	*	*	*	*	*	*
-1°Dark	*	*	*	*	1	1
-1°Dark	*	*	*	*	*	1
-5°Short	*	1	1	1	1	1
-5°Short	*	*	*	*	*	*
-5°Long	1	1	1	1	1	1
-5°Long	*	*	1	1	1	1
-5°Dark	*	1	1	1	2	2
-5°Dark	*	*	*	*	*	*

**Table XI e. Emergence of *Coleophora alticolella* exposed to 15°C L:D 18:6 after 2 months chilling pretreatment**

Group	Date										
	23/5	26/5	30/5	3/6	6/6	10/6	13/6	16/6	21/6	26/6	29/6
5° Dark	*	*	*	*	*	*	*	*	*	*	*
5° Dark	*	*	*	*	*	*	*	*	*	1	1
1° Dark	*	*	*	*	1	1	1	1	1	2	2
1° Dark	*	*	*	*	*	*	1	1	1	1	2
-1°Dark	*	*	1	1	1	2	2	2	3	5	5
-1°Dark	*	*	*	1	1	1	1	1	2	4	4
-5°Short	*	*	*	*	*	1	2	2	2	2	2
-5°Short	*	*	*	*	*	*	*	*	*	*	*
-5°Long	*	*	1	1	1	1	2	2	2	2	2
-5°Long	*	*	*	*	*	*	*	*	*	*	*
-5°Dark	*	*	*	*	*	1	1	2	2	2	2
-5°Dark	*	*	*	*	*	1	1	1	1	1	1

Group	Date						
	4/7	7/7	11/7	14/7	17/7	21/7	24/7
5° Dark	*	*	*	*	*	*	*
5° Dark	1	1	1	1	1	1	1
1° Dark	3	3	3	3	3	3	4
1° Dark	2	2	2	2	3	3	3
-1°Dark	5	5	6	6	6	6	6
-1°Dark	4	4	4	4	4	4	4
-5°Short	2	2	2	2	2	2	2
-5°Short	*	*	*	*	*	*	*
-5°Long	2	2	2	2	2	2	2
-5°Long	*	*	*	1	1	1	1
-5°Dark	2	2	2	2	2	2	2
-5°Dark	1	1	1	1	1	1	1



**Table XI f. Emergence of *Coleophora alticolella* exposed to 15°C L:D 6:18 after 2 months chilling pretreatment**

Group	Date											
	10/6	13/6	16/6	21/6	26/6	29/6	4/7	7/7	11/7	14/7	17/7	21/7
5° Dark	1	1	2	2	2	2	2	2	2	2	2	2
5° Dark	*	*	*	*	*	*	*	*	*	*	*	*
1° Dark	1	1	1	1	1	1	2	2	2	2	2	2
1° Dark	*	*	*	*	*	*	*	*	*	*	*	*
-1°Dark	1	1	1	1	2	2	2	2	2	2	2	2
-1°Dark	*	*	*	*	1	1	1	1	1	1	1	1
-5°Short	1	1	1	1	1	1	1	1	1	1	1	1
-5°Short	*	*	1	1	1	1	1	1	1	1	1	1
-5°Long	*	*	1	1	1	1	1	1	1	1	1	1
-5°Long	*	*	*	*	*	*	*	*	*	*	*	*
-5°Dark	*	1	1	1	1	1	1	1	1	1	1	1
-5°Dark	*	*	1	1	1	1	1	1	1	1	1	1

Group	Date	
	24/7	4/8
5° Dark	2	2
5° Dark	*	*
1° Dark	2	2
1° Dark	*	*
-1°Dark	2	2
-1°Dark	1	2
-5°Short	1	1
-5°Short	1	1
-5°Long	1	1
-5°Long	*	*
-5°Dark	1	1
-5°Dark	1	1

**Table XI g. Emergence of *Coleophora alticolella* exposed to 10°C L:D 6:18 after 1 month chilling pretreatment**

Group	Date				
	11/7	14/7	17/7	21/7	24/7
5° Dark	*	*	*	*	*
5° Dark	*	*	*	1	1
1° Dark	*	*	*	1	1
1° Dark	*	*	*	*	*
-1°Dark	*	*	*	*	*
-1°Dark	*	*	*	1	2
-5°Short	1	1	1	2	2
-5°Short	*	1	1	1	1
-5°Long	*	*	*	*	*
-5°Long	1	1	1	1	1
-5°Dark	*	*	*	*	*
-5°Dark	*	*	*	*	*

**Table XI h. Emergence of *Coleophora alticolella* exposed to 10°C L:D 6:18 after 2 months chilling pretreatment**

Group	Date	
	15/8	24/8
5° Dark	*	*
5° Dark	*	1
1° Dark	*	*
1° Dark	*	*
-1°Dark	1	1
-1°Dark	*	*
-5°Short	*	*
-5°Short	*	*
-5°Long	*	*
-5°Long	*	*
-5°Dark	*	*
-5°Dark	*	*

### Median tests

#### 15°C Long (1 month) and 15°C Short (1 month)

Combined median = 48, let  $\alpha = 0.1$

	Long	Short	
No. of scores exceeding c.m	39	19	58
No. of scores not "	47	14	61
	86	33	119

$$\begin{aligned}\chi^2 &= \frac{N(|AD-BC|-N/2)^2}{(A+B)(C+D)(A+C)(B+D)} \\ &= \frac{119(287.5)^2}{10,040,844} \\ &= \underline{0.98}\end{aligned}$$

Probability of occurrence under  $H_0$  for  $\chi^2 = 0.98$  with d.f. = 1 is  $p < 0.5(0.5) = p < 0.25$  for a one-tailed test. If  $p \leq \alpha$ , reject  $H_0$

$\therefore$  Accept  $H_0$ : two samples come from population with same median

-----

#### 15°C Long (2 months) and 15°C Short (2 months)

Combined median = 56, let  $\alpha = 0.1$

	Long	Short	
No. of scores exceeding c.m	14	4	18
No. of scores not "	12	9	21
	26	13	39

$$\begin{aligned}\chi^2 &= \frac{N(|AD-BC|-N/2)^2}{(A+B)(C+D)(A+C)(B+D)} \\ &= \frac{39(58.5)^2}{127,764} \\ &= \underline{1.04}\end{aligned}$$

Probability of occurrence under  $H_0$  for  $\chi^2 = 1.04$  with d.f. = 1 is  $p < 0.5(0.5) = p < 0.25$  for a one-tailed test. If  $p \leq \alpha$ , reject  $H_0$

$\therefore$  Accept  $H_0$ : two samples come from population with same median

**15°C (1 month) and 15°C (2 months)**

Combined median = 50, let  $\alpha = 0.1$

	Long	Short	
No. of scores exceeding c.m	46	31	77
No. of scores not "	73	8	81
	119	39	158

$$\begin{aligned}\chi^2 &= \frac{N(|AD-BC|-N/2)^2}{(A+B)(C+D)(A+C)(B+D)} \\ &= \frac{158(1816)^2}{28,945,917} \\ &= \underline{18.00}\end{aligned}$$

Probability of occurrence under  $H_0$  for  $\chi^2 = 18.00$  with d.f. = 1 is  $p < 0.5(0.001)$   
 $= p < 0.0005$  for a one-tailed test. If  $p \leq \alpha$ , reject  $H_0$

**∴ Reject  $H_0$ : two samples do not come from population with same median**



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