*Odonatologica 13 (4): 529-550* *December 1, 1984*

# PHOTOPERIODIC CONTROL OF LARVAL DEVELOPMENT IN *LEUCORRHINIA DUBIA* (VANDER LINDEN): A COMPARISON BETWEEN POPULATIONS FROM NORTHERN AND SOUTHERN SWEDEN (ANISOPTERA: LIBELLULIDAE)

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*Received May 15, 1984 / Accepted June 3, 1984*

The larval photoperiodic responses at 15° and 20° C, and, to a limited extent, the larval development in the field, were examined in a population of *L. dubia* from N of the Arctic Circle. The results were compared with similar data from a S Swedish population. In the latter, the life cycle duration was 3 years; in the northern population it was probably longer. The basic effects of photoperiod on development were of the same type in both populations. Larvae in at least the 4 instars preceding the final one showed a simple photoperiodic reaction of the long-day type, and a hibernation diapause was induced by short days. A long-day diapause starting in the early part of the final instar, and a short-day diapause chiefly effective at a later interecdysis stage in the same instar, ensure that emergence is restricted to the spring, and that most larvae spend the last winter before emergence in a suitably advanced stage within the final instar. The remaining final instar development is stimulated by the long days during the spring. Larvae overwintering in pre-final instars normally enter diapause in the final instar. The differences between the populations are primarily related to the photoperiodic regime and the length of the summer in the respective areas. Compared to the southern population, the northern population displays a 4-6 hours longer critical daylength, a generally weaker diapause in the final instar, and somewhat different responses in the penultimate instar. Northern larvae often showed responses typical for short days at LD 21.5:2.5 and (in particular in the penultimate instar, and at 15° C) at continuous light. The induction of hibernation diapause in the penultimate instar is thus likely to occur early in the northern population, thereby restricting the entry into the final instar to the early part of the season. Thereafter, the weaker final instar diapause of the northern larvae permits them to spend their last winter in a more uniform and advanced state than the larvae in the southern population despite the shorter summers. This produces an early, brief emergence period believed to be essential for *L. dubia* in N Sweden. In the southern

population a later entry into the final instar is produced by the different response pattern of the penultimate instar: long days produce a slight prolongation of development, and the short-day (hibernation) diapause is less readily induced than in the preceding instars. When a potentially dangerous "last minute" entry into the final instar occurs in the autumn, it leads to overwintering in an early interecdysis stage and a certain delay in emergence, but it pays off by a shorter development than with an early induction of penultimate instar diapause.

## INTRODUCTION

In temperate latitudes, photoperiod is one of the key factors in the timing of important recurring events in the life cycle of many Odonata (CORBET, 1980; NORLING, 1984a). The interaction of the often complex photoperiodic responses and temperature permits a flexible adjustment of the life cycle to the variable conditions that may occur also within a small and climatically homogeneous area. In many insects, the photoperiodic responses of populations from different latitudes are known to be genetically adapted to the average local conditions, e.g. by different critical daylengths[1] (cf. BECK, 1980). In Odonata, the extent and expression of such a genetically based variation is little known (cf. NORLING, 1975, 1984a, 1984b).

*Leucorrhinia dubia* is a common and often dominant species at bog-pools and other similar, usually acid waters in Sweden. It appears to be as successful in the harsh climate of the northernmost spruce forest area as in southern Sweden. The seasonal regulation of a population in the latter area was presented in an earlier communication (NORLING, 1976) in which emphasis was placed on the responses of larvae in the final larval instar to photoperiod and temperature. For comparison, some of these results are included here, and some unpublished data from this population have been added.

The purpose of the present investigation was to examine and compare the photoperiodic responses of populations from climatically different areas, and thereby throw some light on genetically based traits in their life cycles adapting the populations to the conditions in the respective areas.

## MATERIAL AND METHODS

Most of the northern larvae were collected in the Sappisaasi area, 67° 50'N, 21° 40'E, north of Vittangi, in three water-bodies at approximately 335, 375 and 400 m above mean sea level (cf. NORLING, 1984b). The area is situated some 140 km north of the Arctic Circle and belongs to the

[1] The critical daylength (critical photoperiod) for a photoperiodic response can be defined as the daylength (photoperiod) at which there is a shift between two different modes of expression of this response (e.g. high and low incidences of diapause), characteristic for different ranges of photoperiod (cf. BECK, 1980). When the response to photoperiod is partly graded, as it frequently is in Odonata (e.g. NORLING, 1976), a critical daylength can be difficult to define exactly.

northernmost part of the boreal spruce forest area. Samples were taken on 16-18 June and 21-22 July 1972 with the sole purpose to obtain some life-history data. Collection dates of larvae used in the experiments are shown in Table I. Material for experiments as also collected in the Abisko area, 68° 20'N, 18° 50'E, in water-bodies situated in subalpine birch forest about 380 m amsl. All material of northern origin was subsequently treated as "one" population (N). The Abisko material was evenly dispersed among the experimental groups. No differences between Abisko and Sappissaasi larvae were detected.

Table I

Dates of collection, subsequent treatment, and timing of experiments for material used in the present investigation. Within each area (N = northern; S = southern) the place of collection is shown in parenthesis (V = Sappisaasi, Vittangi; Ab = Abisko). For the northern material, where larvae from different places were mixed in the experiments, the number of larvae used experimentally is also given

| Area | Collection date | | | Treatment before experiments | Start of experiments | |
|---|---|---|---|---|---|---|
| N | 16-17 Sept. | 1972 | (V; n=96) | 0-4° C; DD* | 10 Nov. | 1972 |
| | | | | | 1 Feb. | 1973 |
| | 13 June | 1973 | (V; n=33) | 4° C; DD | 17 Jan. | 1974 |
| | 17 June | 1973 | (Ab; n=13) | | | |
| | 17 July | 1974 | (V; n=40) | "Chilled" | 14 Jan. | 1975 |
| | 21 July | 1974 | (Ab; n=7) | until 25 July** | 21 Feb. | 1975 |
| | July | 1977 | (Ab; n=55)*** | 4° C; DD | 22 Sept. | 1977 |
| S | 12 Oct. | 1972 | (Fjällmossen) | 0-4° C; DD | 5 Dec. | 1972 |
| | 26+29 Oct. | 1973 | (Fjällmossen) | 4° C; DD | 30 Nov. | 1973 |
| | | | | | 25 Jan. | 1974 |
| | 8 Aug. | 1974 | (Fjällmossen) | Approx. natural photoperiod and temp. to 15 Aug.** | 12-15 Aug. | 1974 |
| | | | | | 3 March | 1975 |
| (S') | 14 Oct. | 1977 | (Holmeja) | 4° C; DD | 17 Oct. | 1977 |

* In reality occasionally interrupted darkness. — ** Thereafter placed in an environmental chamber with LD 6:18 and 14°C during the photophase and 7°C during the scotophase (for induction of diapause); transfer to 4°C and DD during November. — *** Collected by Mr Olle Hammerstedt. Almost all specimens that were preparing for ecdysis died during the cold storage, or shortly afterwards, whereas the larvae that had not reached apolysis at the time of collection remained healthy.

Material of southern origin was principally obtained from Fjällmossen, 58° 42'N, 16° 31'E, 84 m amsl (cf. NORLING, 1976, 1984b); but limited experiments were also performed on larvae from Holmeja, 55° 33'N, 13° 17'E (Tab. I). Reference to the southern (S) population normally means the Fjällmossen population. Sampling for life-history studies at the latter locality took place on 22

occasions during 1972 and 1973 (cf. NORLING, 1984b, fig. 1). Some climatic data from the two study areas are shown in Figure 1.

Collections were made with a net (diameter about 0.3 m) with 1x1 mm mesh size, separately supplemented with a small net (diameter 120 mm) with 0.2x0.7 mm mesh size sufficient to retain the smallest specimens. The net contents were either washed out in plastic trays or were placed on a plastic sheet for rapid examination for larger larvae. For further details on collection methods, cf. NORLING (1984b).

Difficult was experienced in separating *L. dubia* and *L. rubicunda* (L.). The latter species was common at the N localities and was the only other libellulid to be found there; however, *L. rubicunda* was relatively scarce at the S study site. The abdominal pigment pattern readily served to separate larvae with a head width exceeding 1.7 mm (i.e., the last 6 or 7 instars). Larvae with a head width of less than 1.0 mm (i.e. the first 4 or 5 instars, one of which is the prolarva) could nerver be positively identified to species, although some guesses were made from detailed size measurements (Fig. 2).

The head width of the last five instars was measured according to NORLING (1971), while smaller specimens, usually preserved in alcohol, were measured using an ocular grid in a microscope. The last 4 or 5 instars could be readily identified by means of their head width.

Experiments involved mainly larvae in the last five instars, referred to as F (final), F-1 (penultimate) and etc. to F-4. The treatment between collection and the start of the experiments is presented in Table I. For larvae stored at 4° C, the change to experimental temperatures was allowed to take at least 5 hours. The experiments were performed in insulated, light-proof boxes, each supplied with a white fluorescent lamp (Philips TL 4W 33), shaded to produce an even illumination of 100-200 lux at the bottom. The larvae were kept in separate compartments (usually c. 65x75 mm) in large trays provided with a moderate flow of constant-temperature tap water pumped from a central external source. Temperature fluctuations rarely exceeded ± 0.5° C.

The responses to photoperiod were studied mainly at 20° C: Both populations at LD 13:11 (13hr light: 11hr dark), 16:8, 19.3:4.7 and LL (continuous light); the S population at LD 17.7:6.3; and the N population at LD 21.5:2.5. The few larvae from Holmeja were examined at LD 12:12 and LL. At 15° C, the responses were examined at LD 13:11 (S population only), 16:8, 19.3:4.7 and LL (both populations). No more than eight experimental groups could be run simultaneously because of equipment limitations. Experiments were also limited by lack of space and the amount of material available. Each day the larvae were checked for ecdyses and were fed ad libitum with *Tubifex*. Larvae undergoing diapause were checked at 2- or 3-day intervals.

It was necessary to transfer F-1 larvae from one condition to another to study the responses in the F instar at photoperiod/temperature combinations unsuitable for development in earlier instars. The transfer was made at a time near ecdysis, which was judged by eye development (ELLER, 1963; NORLING, 1976). The remaining duration of the F-1 instar (here, instar = interecdysis period) was considered to be determined by the original photoperiod. A change of photoperiod probably does not affect development during the final stages of the moulting cycle. At a transfer between photoperiods at the same temperature, the total duration of the F-1 instar was thus estimated by adding this remaining duration to the period previously spent in the instar; however, at a transfer from 20° to 15° C, the remaining duration was halved before being added to the initial period, spent at 20° C. The rate of non-diapause development at 15° C is approximately half that at 20° C (Fig. 4h, I; NORLING, 1976).

The F instar was arbitrarily subdivided into five phases, defined by eye and wing development (cf. NORLING, 1976). In the experiments, the duration of these phases were estimated by regular observations.

## RESULTS

### OBSERVATIONS IN THE FIELD

The S population usually had a 3-year life-cycle. Emergence was concentrated to early June, oviposition occurred mainly June and early July, and eggs began to hatch during the first half of July (NORLING, 1976), and continued to hatch into August. The material from the N localities was insufficient for a reliable estimate

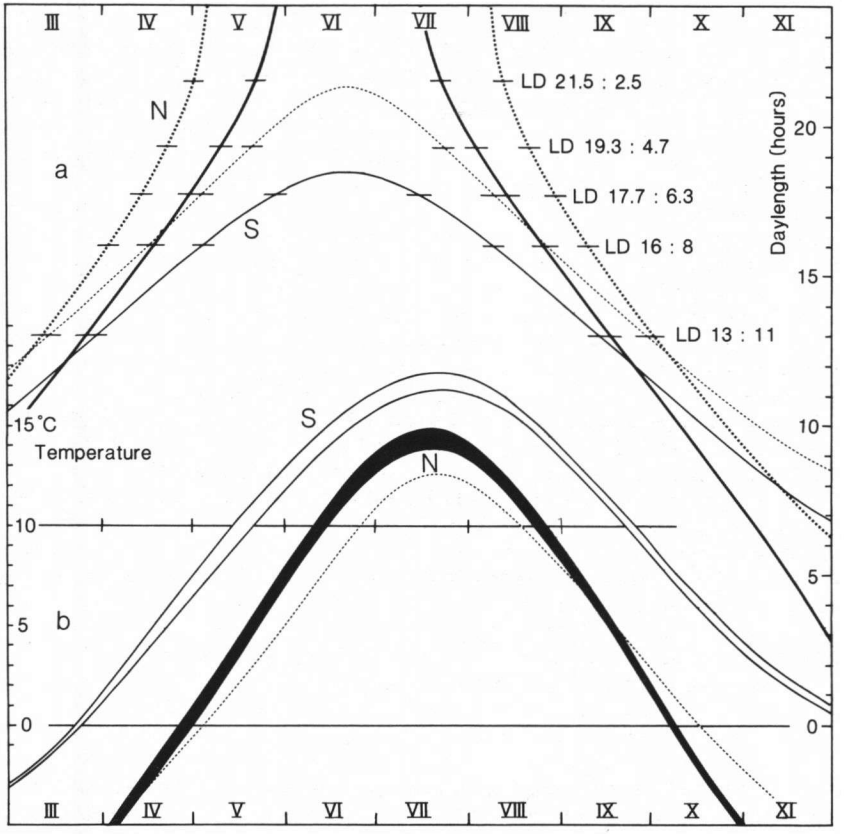


Fig. 1. Daylengths and mean air temperatures in the southern (S) and northern (N) study areas. The months from March to November are indicated on the abscissa. (a) Daylength, calculated from KALAJA (1958) for 58° 42'N and 67° 50'N. Solid lines: sunrise to sunset; broken lines: the same, with the periods of Civil Twilight added. Photoperiods used in the experiments are indicated on the curves (LD = light: dark periods). — (b) Air temperature, drawn from monthly reference normals calculated for the period 1931 to 1960 (S.M.H.I., 1973). The solid curves give the range between two stations within 30 km of the study sites in each area: Norrköping — Nyköping (S; white curve) and Vittangi — Lannavaara (N; black curve). The thin broken line representes Abisko.

of the duration of the life cycle; however, it appeared to be longer than three years. Emergence and hatching in the N area are treated below.

Two aspects of the pattern of development were similar in the N and S populations. The first winter after oviposition was spent in early larval instars (Fig. 2), and the winter before emergence was spent in the F instar. However, the stages of development during the first winter were generally earlier and less well documented for the N population than for the S population. During the study in the N area it seemed likely that the first winter was encountered in the third and fourth instars, but the numerous *L. rubicunda* larvae at the study sites make this conclusion uncertain (Fig. 2f). There also remained the possibility that part of the N population overwintered in the egg stage, although a cold-resistant egg stage is unknown within the genus *Leucorrhinia*. At the N localities, few larvae with a

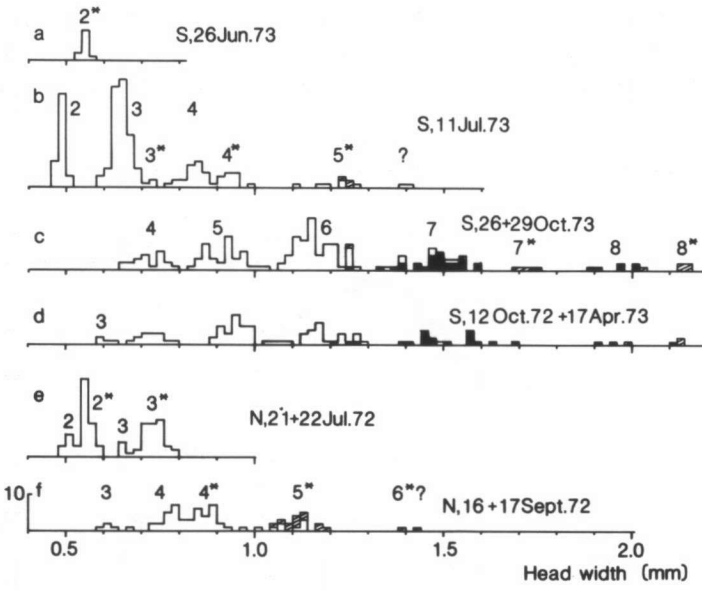


Fig. 2. Head width size-class frequency histograms of *Leucorrhinia* larvae from the S and N study sites, collected soon after the start of hatching (a, b, e), and after completion of their first growth season (c, d, f). Larvae that could not be identified to species are shown in white, *L. dubia* in black, and *L. rubicunda* with oblique lines. In (c) and (d), the largest of the fast-growing *rubicunda* larvae are too large to be included. The frequency peaks represent separate instars and are assigned instar numbers, with the prolarva equivalent to the first instar. The instar peaks suspected, or known, to represent *rubicunda* are indicated by an asterisc. Within each sample it is assumed that *rubicunda* larvae are slightly larger than *dubia* larvae in the same instar. Between July and autumn, there is also an intraspecific difference in the size of larvae with the same instar number, probably reflecting a reduced size increase per ecdysis in late-hatching and/or slow-growing specimens. Because of the collection methods, the samples are strongly biased towards the larger sizes.

head-width below 2 mm were obtained in the spring samples, and thus little information in this context was obtained.

In both N and S populations, larvae accumulated in the F instar during the summer. Before winter, development to a large extent continued up to phase 4, a stage not far from the initiation of the moulting cycle, and the winter was spent here (Fig. 3a). Larvae in the F instar overwintered in a more uniform and advanced stage in the N population than in the S population. The difference between the populations as regards the synchrony within the F instar appeared to be at least as great when observed shortly before the start of emergence of each as during winter (Fig. 3B). As F instar development during the spring is rapid, these observations suggest that the emergence period in the N area is both short and, in relation to the local climate, extremely early.

On 16-18 June 1972 emergence had not yet started at the N study sites, but the advanced state of development of the F instar larvae observed on this occasion (Fig. 3b), together with the warm weather during the rest of the month, indicated that emergence probably took place during the last 10 days of June, most likely

Table II

Number of larvae in the four last instars on different collection dates

| Date | | Instar | | | |
|---|---|---|---|---|---|
| | | F-3 | F-2 | F-1 | F |
| **S population** | | | | | |
| Main site | | | | | |
| 4 May | 1972 | | | | |
| 12 Oct. | | 61 | 51 | 2 | 65 |
| 17 Apr. | 1973 | | | | |
| 9 May | | | | | |
| 22 July | 1973 | 20 | 15 | 17 | 11 |
| 4 Aug. | | 32 | 24 | 16 | 18 |
| 17 Aug. | | 22 | 15 | 5 | 16 |
| 31 Aug. | | 14 | 16 | 8 | 23 |
| 14 Sept. | | 12 | 18 | 4 | 15 |
| 26+29 Oct. | 1973 | 42 | 87 | 8 | 99 |
| 2 May | 1974 | | | | |
| Pool adjacent to main site | | | | | |
| 4 May | 1972 | | | | |
| 12 Oct. | | 24 | 13 | 2 | 26 |
| 26+29 Oct. | 1973 | | | | |
| *Total overwintering* (12 Oct.-9 May) | | *127* | *151* | *12* | *190* |
| **N population** | | | | | |
| 16-18 June | 1972 | 25 | 48 | 65 | 29 |
| 16-17 Sept. | | 8 | 18 | 21 | 55 |
| 13+17 June | 1973 | 37 | 19 | 15 | 17 |
| *Total overwintering* | | *70* | *85* | *101* | *101* |

with the last individuals emerging in early July at one of the localities. Emergence in 1972 was thus no more than three weeks later than for the S populations, where it seemed to start during the very last days of May (cf. Fig. 3b). On June 1973, development in the F instar had barely started in the N area, indicating that emergence during 1973 may have started about one week later than in 1972. In the S area no difference in the start of emergence was detected between the two years.

A large number of F-1 instar larvae overwintered at all the N localities, which was not the situation at the S locality (Tab. II; Fig. 3b). The low frequency of F-1 instars found in the S population during the winter 1972-73 could be traced directly to the separation between the two year-class cohorts that hatched during 1970 and 1971, respectively. During 1973, the visible minimum between the corresponding 1971 and 1972 year-class cohorts disappeared early in the summer. Some larvae of the 1972 cohort certainly reached the F-3 instar by early July, and both year-classes are thus represented in the late summer samples shown in Table II. Here it is seen how the frequency of F-1 instars decreased during late summer, indicating that a formation of secondary cohorts was splitting the previous

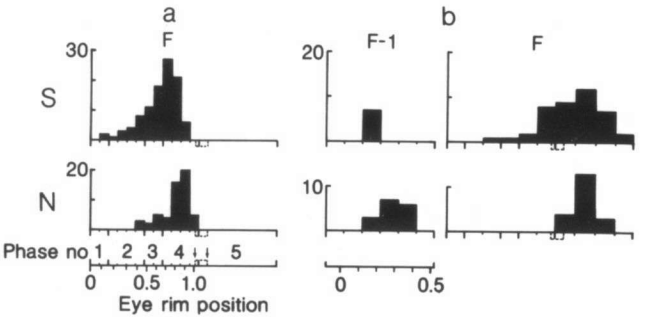


Fig. 3. Frequency histograms showing the interecdysis distribution of larvae from the S and N population in (a) the F instar during winter, and (b) the F-1 and F instars shortly before the beginning of emergence. For the F instar, the abscissa represents successive stages during rapid nondiapause development through the instar, from ecdysis (left) to emergence (right), which takes about 25 days at 20° C. The developmental phases used previously, and which are mainly defined by eye development (cf. NORLING, 1976, fig. 2), are indicated in the scale below (a) together with the relative position of the posterior eye rim (cf. Fig. 8). The phases have been subdivided into subunits of approximately equal duration at nondiapause development. In the field-measured material in (b), these units are twice as wide as in (a). The variation in the eye rim position at the start of phase 5, which is defined by wing development (corrugation of the costa), is indicated by arrows. The subdivision of phase 5 is largely based on the histolysis of the labium. For F-1 instar, the scale represents the interecdysis, mesial progression of the pigmented area of the developing F instar eye, from the cuticular eye rim (0) towards the median line (which equals 1.0). — S population: (a) Collection on 12 Oct. 1972 (n = 16), 17 Apr. 1973 (n = 5) and 26 + 29 Oct. 1973 (n = 80); (b) collection on 27 May 1973. The number of overwintered F-1 instars in (b) is remarkably high (cf. Tab. II). — N population: (a) Collection on 16-17 Sept. 1972; (b) collection on 16 June 1972 at the best-yielding site (site 1 in NORLING, 1984b).

continuum of instars. It appeared that growth was restricted in the F-2 instar, possibly also in the F-3 instar, during this time. However, the field observations did not provide further details about what happened. Winter collections at a few other southern localities indicated that a low number of overwintering F-1 instar larvae is a common feature in South Swedish populations.

In the S population, larvae overwintering in the F instar had mostly spent the previous winter in the F-2 and F-3 instars (NORLING, 1976, fig. 1). The total range for the penultimate overwintering probably was the F-1 to F-4 instars. The corresponding field data for the N population are extremely uncertain, but suggest that the penultimate winter was mainly spent in the F-1 and F-2 instars.

The start of the entry into the F instar by larvae that had overwintered in the F-1 instar appeared to be delayed in the S population, and did probably not take place until, or after, the end of the emergence period (NORLING, 1976). A similar delay was absent or less conspicuous in the N area, according to observations of eye development in the F-1 instar shortly before the start of the emergence period, a time when no ecdyses seemed to have taken place in later instars in any of the populations (Fig. 3b). There is no evidence in this study that any larvae emerged during the same season as they entered the F instar, although this probably can occur in South Swedish populations (NORLING, 1976, fig. 2).

## PHOTOPERIODIC RESPONSES IN THE F-4 TO F-1 INSTARS

In both populations the photoperiodic reaction was of the long-day type in all examined pre-final instars (F-4 to F-1). The typical response to short-day photoperiods (daylengths below the critical value for the population in question) was a distinctly prolonged development, a diapause (diapause is here defined as a prolongation of development that is not directly caused by adverse conditions; cf. BECK, 1980; NORLING, 1984b). In experiments started during November or December (e.g. Fig. 4i) the first ecdysis was usually delayed as a result of a previously induced diapause; however, larvae that had spent a sufficient time at winter temperatures usually moulted once soon after the start of the experiments, and then re-entered diapause (Fig. 4a, b, j,). In the latter situation the short-day diapause in southern F-1 instar larvae could fail (Fig. 4j). It was sometimes also noted that diapause in one instar during the experiments was followed by a more normal development in the next instar despite strongly diapause-inducing conditions (e.g. Fig. 4a, b, i). A similar partial "diapause refractoriness" has also been observed in *Aeshna viridis* Eversm. (NORLING, 1971, figs 6, 7).

Long-day photoperiods promoted development and most larvae rapidly entered the F instar (Fig. 4d, h, l). However, development of the southern F-1 instar larvae nearly always was slightly slower (22%) at long-day photoperiods (Fig. 5a, b) than at short-day or intermediate (near-critical) photoperiods if the short-day diapause failed (Fig. 5c; NORLING, 1976). At least a few northern

F-1 instar larvae entered diapause under any examined conditions (cf. below).

Clearly defined and fully comparable values for the critical photoperiods of the two populations were not obtained. However, the difference between the populations is considerable, as seen in the experiments at 20° C. In the S population LD 13:11 produced a short-day response whereas LD 19.3:4.7 and LL produced long-day responses (Fig. 4j, h), irrespective of season. LD 16:8 produced a distinct short-day response during late summer (Fig. 4f), and LD 17.7:6.3 produced a long day response during winter experiments only. LD 16:8 during winter and LD 17.7:6.3 during late summer produced varied responses (Fig. 4g, k), indicating proximity to the critical photoperiod. When LD 13:11 delayed the first ecdysis in an experiment begun on 30 November 1973, LD 16:8 did not. Overall, the winter experiments with southern larvae at LD 16:8 produced a response suggesting a delayed short-day effect of this photoperiod.

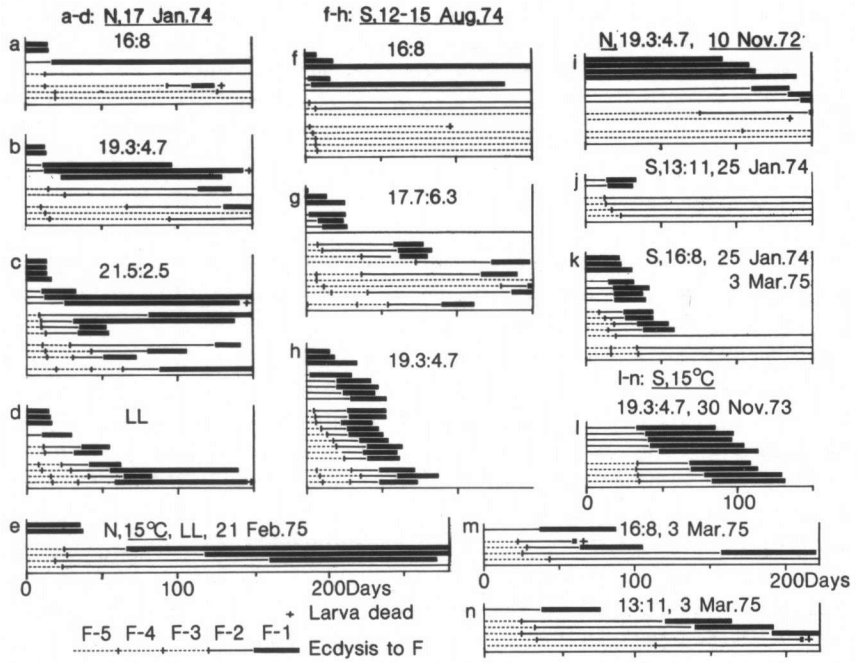


Fig. 4. Individual development of larvae in pre-final instars up to the entry into the F instar in some of the experiments, showing the different responses to various experimental conditions. Where possible, parallel experimental groups have been selected. The instars are indicated by differing lines according to the legend, and the ecdyses are shown as transverse bars, except between the F-2 and F-1 instars. Origin of larvae (S or N population), date on which experiment was begun, and photoperiod (light: dark periods) are shown. The temperature was 20° C unless otherwise stated (i.e. 15° C in e, l-n). The vertical zero line indicates the start of the experiment.

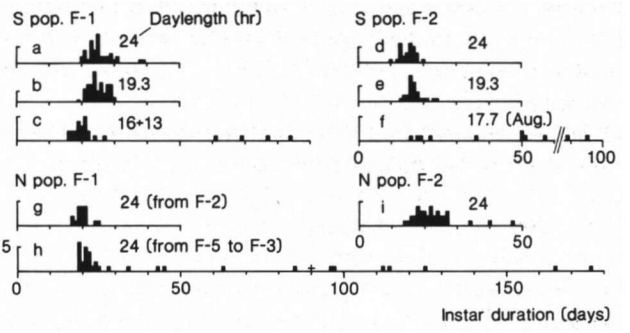


Fig. 5. Summary of the durations (interecdysis intervals) of the F-2 and F-1 instars in all experiments at long-day photoperiods and 20° C. For comparison, some results from other photoperiods are included for the S population (c, f). The bars on the ordinate indicate five specimens. A cross indicates the death of a larva. In order to minimize remaining effects of previous natural photoperiods, and to reduce the possible influence of an excessive time spent in the laboratory, only larvae that reached the respective instar between one week and five months after the start of the experiments are included. — Comments: (c) The four diapausing specimens shown all belong to LD 13:11. Two specimens in a more intense diapause have been omitted for reason of space. — (f) Limited to experiments begun near mid-August. — (g) The larvae were in the F-2 instar when experiments began. — (h) The larvae were in the F-5 (n = 1), F-4 (n = 12) and F-3 (n = 22) when experiments began.

The F-1 instar usually did not enter diapause at intermediate photoperiods.

Development of the N population, which was examined only in autumn and winter experiments (Tab. I), was prolonged at LD 13:11 and up to LD 19.3:4.7 (Fig. 4a, b, i). The only experiments performed at LD 21.5:2.5 produced varied responses (Fig. 4c), which appeared somewhat closer to a long-day response than the southern LD 17.7:6.3 late summer group (Fig. 4g). Even at LL the response of the N population was not completely of the long-day type: varied responses occurred regularly in the F-1 instar (Figs 4d, 5h). Nine out of the 35 larvae (26%), which were in the F-5 to F-3 instars at the beginning of the experiments, later showed a distinct prolongation of the development in the F-1 instar (duration more than 50 days) that was comparable to a short-day response. Slightly prolonged development was shown by three or four specimens; however, the majority of the larvae, including all those introduced in experimental conditions in the F-2 instar (Fig. 5g), showed a rapid development in the F-1 instar that was comparable to that of southern non-diapausing larvae at short-day or intermediate photoperiods (Fig. 5c). Also, the development in the F-2 instar often was slightly prolonged at LL (Fig. 5i) when compared to the S population at LD 19.3:4.7 and LL (Fig. 5d, e). The only similar responses in larvae of S origin occurred at LD 17.7:6.3 in late summer (Figs 4g, 4f). Generally, the LL experiments with northern larvae, like the LD 16:8 winter experiments with southern larvae, showed a delay in the onset of the short-day diapause (fig. 4d, k).

The small group of N larvae at LL, 15° C in Figure 4e apparently showed a typical short-day response in both the F-2 and F-1 instars (compere the S population at 15° C, Fig. 4 l-n). Similar results at LL, 15° C were obtained during the 1977 experiments with N larvae.

As noted above, larvae from the S population appeared to be less susceptible to induction of short-day diapause in the F-1 instar than in the immediately

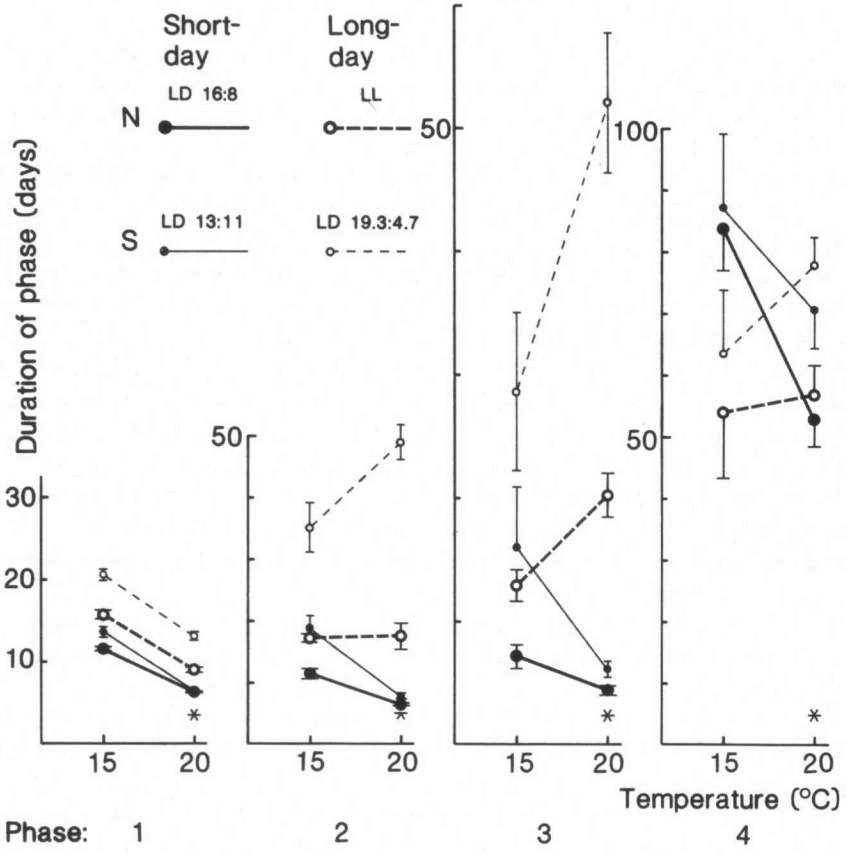


Fig. 6. Long-day and short-day responses in the first four phases of the F-instar at 15° and 20° C. The mean and one standard error of the duration of each phase are shown. The ordinates are adjusted so that the duration of each phase for rapid (nondiapause) development at 20° C is at approximately the same level (see asterisc). — N population: Experiments begun on 14 Jan., and on 21 Feb. 1975 at LL, 20° C, with larvae in the F-4 to F-2 instars. Transfer to the other experimental conditions took place when the ecdysis to the F instar was approaching. The number of larvae in each group was 8 or 9. — S population: Experiments begun on 30 Nov. 1973 at LD 19.3:4.7, 20° C, with larvae in the F-3 and F-2 instars. The design of the experiment was the same as for the N population. The number of larvae in each group varied between 6 and 9.

preceding instars (Fig. 4f, j, n). If the earlier instars showed a variable response, then the F-1 instar was of short duration (Fig. 4g, k, m). This may indicate a shorter critical daylength acting in the F-1 instar, In the N population the opposite occurred. The larvae appeared more prone to display the typical short-day response in the F-1 instar than in the preceding instars (Figs 4c, d, 5h, i).

## PHOTOPERIODIC RESPONSES IN THE FINAL INSTAR

When the F instar was reached during the experiments, larvae from both populations showed prolonged development (diapause) in this instar at any tested constant photoperiod. This applied also when the rare overwintering southern F-1 instars were used in the experiments (LD 16:8, n = 3; LD 17.7:6.3, n = 2; LD 19.3:4.7, n = 5; LL, n = 1). The diapause could be terminated or averted by a substantial increase in daylength, preferably as a transition from short-day to long-day photoperiods during the F instar or the late part of the F-1 instar (NORLING, 1976). Thus, the F instar displays a photoperiodic reaction of the short-day/long-day type. During diapause, the rate of development in phases 1 to 4 varied and was dependent on photoperiod and temperature (Figs 6, 7). The duration of phase 5 ( the time from the folding of the costal rib to emergence), which is a minimum estimate of the time between apolysis and emergence (cf. data in SCHALLER, 1960), was short and appeared to be dependent on temperature only in these experiments. Phase 5 usually took 10-11 days at 20° C and 19-22 days at 15° C. The change of the duration of phase 5 in response to temperature is similar to that observed for the duration of earlier instars (NORLING, 1976; cf. Fig. 4), and probably is characteristic of nondiapause larval development.

The basic F instar response pattern of the N population was similar to that of the S population (Fig. 6). At short-day photoperiods, the first three phases showed a weakly or moderately reduced rate of development, and the temperature response was approximately normal. At long-day photoperiods the reduction of the developmental rate was greater, the difference from the short-day response increasing from phase 1 to 3. In phase 2 and 3, the sensitivity to different photoperiods increased with temperature, and at long-day photoperiods a reversal of the temperature response partly occurred. The slowest development was generally found in phase 4, the duration of which varied relatively little between the groups.

Generally, development during diapause was faster in larvae of northern origin than in those from the S locality. Except in the short-day, 20° C groups, the difference between the populations was particularly great in phases 2 and 3, i.e. in the stages where the differential response to photoperiod within each population was at maximum. The critical photoperiod for the transition from the short-day to the long-day response differs greatly between the two populations (Fig. 7)

irrespective of the criteria used in the comparison.

Experiments using northern F instar larvae that were previously stored under cold conditions were carried out on four occasions. Most of the results are shown in Figure 8, where also some southern material is included; however, for more complete results for the S population, cf. NORLING (1976). All the larvae from both populations developed rapidly at LL, 20° C, after transfer from cold storage in the dark. LD 13:11 produced a distinctly prolonged development in larvae that were in early developmental phases when stored, whereas this photoperiod had a less pronounced effect on larvae in more advanced phases. The diapause to LD 13:11 of the N population was similar to, but possibly less intense than that of the S population. The N population showed a response at LD 19.3:4.7 that was similar to the response shown at LD 13:11, whereas the S population developed at a rate closer to that at LL (cf. also NORLING, 1976). The differential response to photoperiod in these experiments was normally detected after some 5-10 days observations of the rate of eye development; however, in one particularly well documented experiment (17 Oct. 1977), utilizing larvae in phases 1-3 from southernmost Sweden, the different effects of extreme photoperiods (LD 12:12 and LL, n= 7+7) were already evident after 3-5 days.

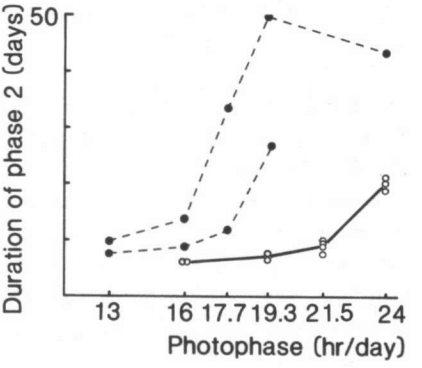


Fig. 7. Effect of different photoperiods on developmental time in phase 2 of the F instar. — N population (solid line): Experiment started on 17 Jan. 1974 in the F-1 instar. Each specimen is represented by a small circle. — S population (broken lines; from NORLING, 1976): — Upper line: responses at constant photoperiods; — Lower line: a decrease in daylength preceded the entry into the F instar.

These experiments also indicated a certain seasonal difference in the responses of the N population, as previously recorded for the S population (NORLING, 1976). The development was slightly slower in an early winter experiment (10 Nov. 1972) than in a later one (1 Feb. 1973), especially at short-day photoperiods. In the somewhat unusual 21 Feb. 1975 and 22 Sept. 1977 experiments (for pretreatment cf. Tab. I) an unusually high rate of development was found; however, at LL, 15° C (not shown in Fig. 8) one specimen out of six entered diapause in the 22 Sept. 1977 experiment.

The most advanced N specimens (eye rim position 0.7-0.9 in Fig.8) usually emerged only 1-2 days later at short-day photoperiods than at LL. An examination of wing and eye development data obtained from the 10 Nov. 1972 experiment indicated that the difference was caused by a shortened duration of

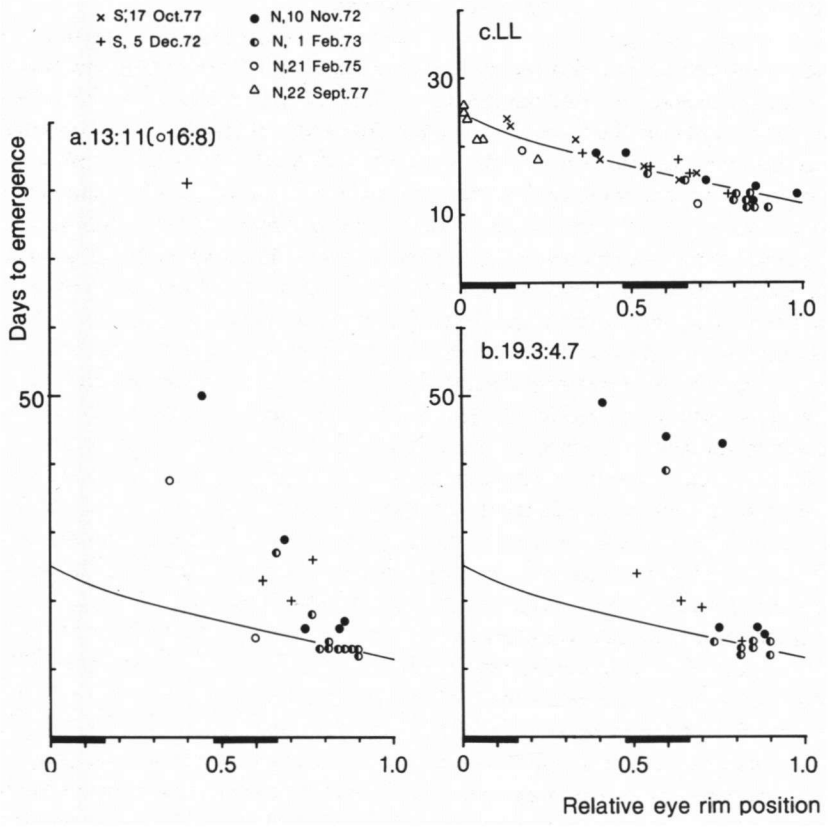


Fig. 8. Time to emergence for cold-treated F instar larvae in different phases of development at different photoperiods. Temperature 20° C. Origin of larvae (S', Holmeja; S, Fjällmossen; or N, Sappisaasi + Abisko) and the date on which each experiment was begun are shown in the legend. The pretreatment of the larvae is shown in Table I. The abscissa illustrates the developmental stage (phase) of the larvae at the start of the experiments by the position of the posterior eye rim in its progression from the mesial tip of the larval eye (0) to a muscle scar behind it (1.0; cf. fig. 2 in NORLING, 1976). Phases 1 and 3 are indicated by thickenings of the axis. The curve is a reference for nondiapause development, calculated from fig. 2 in NORLING (1976).

phase 5 at LL (i.e. 9 days at LL compared to 10-11 days at LD 13:11 and 19.3:4.7). This unexpected effect of photoperiod on phase 5 was confirmed in the 21 Feb. 1975, 22 Sept. 1977 (N material) and 17 Oct. 1977 (S material) experiments.

## DISCUSSION

The basic pattern of photoperiodic responses is similar for both populations, and it is considered to regulate development as follows (NORLING, 1876). Pre-final instars, at least the F-4 to F-1 instars, grow under long-day conditions, when temperatures are suitable. As the daylength decreases during late summer, preparation for overwintering in these instars starts by induction of diapause. The early season of the species is primarily determined by the responses to photoperiod and temperature in the F instar. Larvae that reach this early during the summer develop slowly because of the strong diapause-inducing effects of long days and high temperatures. During late summer, when the days are shorter, the rate of development in the early part of the F instar increases, and the larvae start to accumulate in a more advanced stage (phase 4, near the initiation of the moulting cycle), whereafter development appears to cease (hibernation diapause). Larvae entering the F instar late in the season have to overwinter in an early developmental phase, and during the next spring they emerge somewhat later than the main group. During the spring, long days stimulate development in the overwintering instars (except partly in the southern F-1 instar), assisted by the effects of previous exposure to low temperatures. Emergence is normally restricted to larvae that have overwintered in the F instar.

In the N population, the most important differences from the S population were (1) a 4-6 hours longer critical daylength for the examined responses, (2) a lower intensity of the diapause responses in the F instar, in particular in the earlier phases, (3) a different response pattern in the F-1 instar, and (4), as observed in the field, a more efficient synchronization of development before emergence. As discussed below, these differences can all be related to environmental conditions prevailing in the respective study areas, and they are also to a certain extent interrelated.

Compared to populations in S Sweden, the N population of *L. dubia* experiences shorter, usually cooler summers, longer, more severe winters, less predictable weather conditions, and extreme natural photoperiods (Fig. 1; WALLEN, 1965). The short summer of high latitudes is of particular importance as it restricts the amount of development that can take place within one season to a small fraction of the life cycle. Survival of the long winters must occur in a wide variety of developmental stages, and the cold-sensitive period centred around reproduction must be short enough to be completed in one season. *L. dubia* can overwinter as larvae in at least the third to the F (13-14th) instar (Fig. 2) but certainly not as adults or prolarvae (first instars), and probably not as eggs. Then, the following sequence of events must be short enough to occur within one summer: (1) completion of larval development and emergence, (2) adult maturation, mating and oviposition, (3) embryonic development and hatching of eggs and (4) development as far as to an instar cabable of overwintering, probably

the third instar.

In the N population, the compression of the summer season has brought the shortening of the first and last events to, or near, its limit. Most larvae spend the winter before emergence in the most advanced stage possible (Fig. 3), and young larvae probably overwinter in the third and fourth instars (Fig. 2). This at least appeared to be the situation in 1972, when temperatures in the N area were exceptionally high during late June and early July, i.e. during maturation and egg development. In more normal years, the first winter may perhaps also be spent as second instars, or possibly even as eggs; however, there are no indications of the latter.

The adult and the egg stages were not examined in the present study; therefore, their possible contribution to adaptations to subarctic conditions is unknown. STEINER (1948) in Germany and PAJUNEN (1962) in Finland give adult maturation periods of 14 and 10-15 days, respectively, for females, and slightly less for males. Maturation can be shortened to about a week during very favourable weather (PAJUNEN, 1962). The average length of the male resproductive period was estimated to be 17 days (PAJUNEN, 1962). PRENN (1930) in Austria reported a 3 week duration of the egg stage, but egg development in *L. dubia* is probably strongly temperature dependent as in the North American *L. intacta* (Hag.) (DEACON, 1975).

Tentatively, the minimum time from emergence of adults to hatching of eggs under normal summer conditions is about 30-35 days, which approximately agrees with the observations in the present study. However, periods of cold and bad weather prolong the maturation period and egg development, restrict the reproductive activities and increase the mortality. In the N area, such periods are often prominent, and during a cool summer, successful reproduction may thus be at stake. Also during relatively normal years, the period of successful reproduction for a substantial proportion of the northern adult population might be curtailed by the onset of cold conditions in the sense that part of their offspring does not reach a resistant stage before winter. Unless an egg diapause is present, even a slight delay of the emergence may thus decrease the expected reproductive success significantly in the N area. This would explain the extreme commitment to early emergence observed in the N population, for example reflected by the extremely advanced overwintering stage within the F instar.

A conspicuous feature of the light conditions in the N area is the long period of continuous daylight around the summer solstice (Fig. 1). For most insects the effective length of the day (photophase) probably includes the periods of Civil Twilight (BECK, 1980). If this is true for *L. dubia* under the conditions of the northern summer, the population at Sappisaasi experiences continuous light for a period of about 100 days, extending into early August. Accordingly, extremely long critical daylengths are expected in this population. In fact, any tested photoperiod failed to produce an altogether typical long-day response in

northern larvae, and the overall pattern of development at LL was often similar to that of southern larvae at near-critical photoperiods (LD 16:8 and 17.7:6.3; Figs 4, 5, ).

In the F-1 and F-2 instars of the N population, a typical hibernation diapause was noted at LL, 15° C (Fig. 4e). At LL, 20° C, this response became restricted to the F-1 instar, and it occurred only in some larvae that reached this instar two or more ecdyses after the start of the experiments (Fig. 5h). These short-day responses at LL appear to be connected with an extremely long critical daylength, which interacts with temperature. Apparently photoperiod alone is a partly insufficient source of temporal information in this population, at least for the induction of hibernation diapause in the above mentioned instars. The delay in the onset of short-day diapause at LL, 20° C (Fig. 4d), and the similar results for the S population at LD 16:8 (Fig. 4k), may be explained by a slow increase in critical daylength after the overwintering. Also the diapause-promoting effect of 15° C at LL in northern larvae (Fig. 4e; cf. the S population, Fig. 4m, n) might be partly connected with such an increase, since the lower temperature prolongs development, and diapause can be induced after fewer moults. During autumn and winter, the critical daylength appears to be depressed by the action of short days and low temperatures, as indicated by the seasonally different responses to near-critical photoperiods in the S population (Fig. 4f, g, k). A similar decrease in critical daylength during the cold season has been reported by DEACON (1975) in *Leucorrhinia intacta* and by SAWCHYN (1972) in two *Coenagrion* species.

Larval diapause is hormonally regulated, and normally delays the events that trigger the moulting cycle (CHIPPENDALE, 1977). The latter consists of apolysis, secretion of new cuticle, and ecdysis. Once started, the moulting cycle is considered to be beyond strict hormonal control (LOCKE, 1976). During prolonged exposure to temperatures below the threshold for completion of this cycle, tentatively near 7° C, mortality is high in larvae trapped between apolysis and ecdysis by the low temperature, whereas larvae in the early phase of the instar survive (U. Norling, unpublished observations on *Aeshna* species; cf. also footnote in Tab. I). If a diapause is not generally involved in this low-temperature survival, which is yet unproven, these observations may have an important bearing on the evolution of hibernation in Odonata (NORLING, 1984a). At any rate, a hibernation diapause ensures that the winter is encountered in an early interecdysis stage, and the initiation of diapause should start at a time when the moulting cycle, including its hormonal inception, can still be completed in larvae that just have escaped diapause induction.

In the N area, there is a definitive, often sudden temperature drop during September, after which no development can take place. If apolysis occurs about half-way through the instar, as in the F-1 instar of *Aeshna cyanea* (Müll.) (SCHALLER, 1960), the moulting cycle in the F-1 instar of *L. dubia* can be estimated to require 3 weeks at 15° C, and more at lower temperatures. With

allowance for the process of diapause induction, it seems likely that diapause inducing conditions have to start at continuous light to ensure that the winter is encountered in a suitable state. In the S population, diapause in the late pre-final instars starts during August (compare Figs 1 and 4f-h), which in this respect should be approximately equivalent to July in the N area (Fig. 1).

Also some responses in northern F instar larvae may be related to a critical photoperiod close to LL. The rate of prolonged development (intensity of diapause) in early phases at LL was similar to that at near-critical photoperiods in the S population (Fig. 7). At any rate, this weak long-day diapause is appropriate in the N area, where it enables most larvae to reach phase 4 before winter despite extreme long-day conditions and a short summer. On the other hand, a weak summer diapause of the northern type in South Sweden might produce some autumn emergence when the F instar is entered early.

At short-day photoperiods and 20° C, the difference in F instar (prolonged) development between the populations was minor; however, at 15° C, a temperature more usual during short-day conditions in the field, the difference was greater, except in phase 4 (Fig. 6). The difference may be fundamental, giving the N population a higher potential for synchronization in phase 4.

Exposure to shorter days during autumn sensitizes the larvae in the F instar to long-day stimulation of development (NORLING, 1976). Probably, low temperatures can have similar effects as short days in this respect (cf. Fig. 8c, the 22 Sept. 1977 experiment; INGRAM, 1975). Prolonged exposure to low temperatures also increases the ability of the larvae to begin rapid development as soon as the temperature becomes favourable, even at short-day photoperiods. After a long period of cold storage of F instar larvae in late phase 4, the hormonal signal to start the moulting cycle is probably triggered almost immediately upon return to higher temperature. Less advanced F instar larvae react conspicuously to photoperiod and appear to show critical daylengths similar to those for other developmental events (Fig. 8; NORLING, 1976, fig. 5) Overall, the most rapid development in cold treated F instar larvae was observed in the N population, which may indicate a greater intrinsic capacity of these larvae to start and complete development rapidly during the spring.

In the N population, the F-1 instar showed a greater propensity to enter hibernation diapause than the earlier instars, which possibly is related to a longer duration of the moulting cycle in this instar. However, in the S population the F-1 instar entered hibernation diapause less readily than the F-2 and F-3 instars, and the F-1 instar also displayed an incipient long-day diapause. As discussed below, this difference between the populations can be related to seasonal regulation and the difference in the length of the summer between the study areas.

The complex of responses involved in seasonal regulation is, through selection, affected by the interaction of (1) the expected reproductive success as a function of the data of emergence, (2) the likelihood for winter survival as a function of the

timing of the induction of hibernation diapause, and (3) mortality, and other detrimental effects of protracted development, as a function of the time spent in the larval stage. In the N population, the observed physiological properties clearly aim at the earliest possible emergence, at the expense of a shorter developmental time. An early hibernation diapause in the F-1 instar (1) provides a safe strategy for survival of the winter, but it also (2) prevents late entries into the F instar, and (3) produces a certain accumulation of larvae in the F-1 instar during the late part of the season. The result is a concentration of the entries into the F instar towards the early part of the season. During the rest of the season, the responses of the F instar ensure a good synchronization in phase 4, thereby producing a gap, or frequency minimum, in the early part of the F instar. This development pattern restricts emergence to an extremely early, brief period.

In the S population, the responses are in reality similar, but they are displaced towards earlier developmental stages, which reflects the longer summers and a weaker selection for early emergence. The safest overwintering strategy (earliest induction of diapause), and the accumulation associated with it, occurs in the F-2 and F-3 instars, while the responses in the F-1 instar foreshadow those in the early part of the F instar. Rapid F-1 instar development at relatively short days permits late entries into the F instar. These properties create a frequency minimum in the F-1 instar during winter, which is comparable to the gap in the early F instar seen in the N population. The low frequency of overwintering F-1 instars automatically reduces the number of early entries into the F instar, and the rudimentary long-day diapause of the F-1 instar produces an additional delay in the attainment of the F instar during the early and middle part of the summer. Overall, entries into the F instar in the S population thus tend to become concentrated towards the later part of the season. This timing of the last larval ecdysis, together with the stronger F instar diapause, make the S population to spend the last winter in a less uniform and less advanced state of development than the N population (Fig. 3a). In Sweden it appears that also larvae overwintering in the F-1 instar may sometimes be stimulated by long-day conditions during the spring, quickly pass through the F instar, and emerge after the main group (NORLING, 1976, Fig. 2; for Austria, cf. also PRENN, 1930), although this was not observed in the examined population. Depending on the fate of overwintering F-1 instar larvae (emergence, or long-day diapause, respectively), a late ecdysis into the F instar either improves synchrony, or shortens the larval life at a moderate cost in synchronization of emergence. These advantages probably compensate for a reduced likelihood of winter survival, associated with a late induction of hibernation diapause. A late ecdysis in slightly smaller larvae serves no purpose, and an early induction of diapause is the best strategy.

The only other European *Leucorrhinia* species that reaches subarctic areas is the closely related *rubicunda*. Observations in the field suggest that this species

grows more rapidly (Fig. 2), but otherwise has a life cycle similar to that of *dubia*, and it has the same kind of synchronization within the F instar. However, *L. rubicunda* emerges earlier, and may react more rapidly to the increase in temperature during the spring (PAJUNEN, 1962). The earlier emergence may explain its greater success in such climatically harsh areas as the Scandinavian montane birch forest 70-80 km north of Vittangi, at Abisko and in northern Norway (U. Norling, unpublished observations).

The North American *L. intacta*, which has been studied in an area with warmer summers than in Scandinavia (Ontario, Canada, 43° 32'N; DEACON, 1975), is another interesting comparison. It is early, as are the European *Leucorrhinia* species, but an early, synchronized emergence appears less important than in the *L. dubia* populations. The F instar holds a less unique position in the fast-growing, univoltine *L. intacta*, and the winter before emergence can be spent in several instars. For overwintering, the F instar is reached only during the late summer and early autumn beacause of a long-day induced diapause in the F-1 instar. The development in the early part of the F instar at short-day photoperiods is significantly slower than in *L. dubia*, and the winter is therefore spent in approximately phase 1 and 2 (DEACON, 1975, and pers. comm.).

The pattern of development in the *L. intacta* population represents a continuation of the climatically related trend seen between the N and S populations of *L. dubia*. Responses, which are entirely, or mainly, restricted to the F instar in the latter populations, are here largely extended to earlier instars, and overwintering F instar larvae are in the very beginning of the instar. With the tropical origin of Odonata borne in mind, the life cycle pattern of *L. intacta* can probably be regarded as the most primitive one. The patterns of development in the northern species and populations are then secondary developments, suitable for survival near the northern limit for dragonfly existence, or for phenological specialization.

## ACKNOWLEDGEMENTS

I wish to thank Professor PER BRINCK and Dr KEN DEACON for valuable criticism and corrections in the preliminary draft of the manuscript, and Mrs JANE HOLMQUIST and Dr BJÖRN NAGELL for helpful suggestions during its early preparation.

## REFERENCES

BECK, S.D., 1980. *Insect photoperiodism.* Second edition. Academic Press, New York.

CHIPPENDALE, G.M., 1977. Hormonal regulation of larval diapause. *A. Rev. Ent.* 22: 121-138.

CORBET, P.S., 1980. Biology of Odonata. *A. Rev. Ent.* 25: 189-217.

DEACON, K.J. *The seasonal regulation of Leucorrhinia intacta Hagen (Odonata: Libellulidae).* M. Sc. Thesis, Univ. Waterloo, Waterloo, Canada.

ELLER, J.C., 1963. *Seasonal regulation in Pachydiplax longipennis (Burmeister) (Odonata: Libellulidae).* Ph. D. Thesis, Univ. North Carolina, Chapel Hill.

INGRAM, B.R., 1975. Diapause termination in two species of damselflies. *J. Insect Physiol.* 21: 1909-1916.

KALAJA, P., 1958. Die Zeiten von Sonnenschein, Dämmerung und Dunkelheit in verschiedenen Breiten. *Veröff. finn. Geodät. Inst.* 49: 1-63.

LOCKE, M., 1976. The role of plasma membrane plaques and Golgi complex vesicles in cuticle deposition during the moult/intermoult cycle. *In:* H.R. Hepburn [Ed.], The insect integument, pp. 237-258. Elsevier, Amsterdam.

NORLING, U., 1971. The life history and seasonal regulation of Aeshna viridis Eversm. in southern Sweden (Odonata). *Entomol. scand.* 2: 170-190.

NORLING, U., 1975. Livscykler hos svenska Odonater. *Entomologen* 4: 1-14.

NORLING, U., 1976. Seasonal regulation in Leucorrhinia dubia (Vander Linden) (Anisoptera: Libellulidae). *Odonatologica* 5: 245-263.

NORLING, U., 1984a. Life-history patterns in the northern expansion of dragonflies. *Adv. Odonatol.* 2. (In press).

NORLING, U., 1984b. The life cycle and larval photoperiodic responses of Coenagrion hastulatum (Charpentier) in two climatically different areas (Zygoptera: Coenagrionidae). *Odonatologica* 13: 429-449.

PAJUNEN, V.I., 1962. Studies on the population ecology of Leucorrhinia dubia v.d. Lind. (Odon., Libellulidae). *Annls zool. Soc. "Vanamo"* 24: 1-79.

PRENN, F., 1930. Aus der Nordtiroler Libellenfauna III. Zur Biologie von Leucorrhinia dubia v.d. L. *Verh. zool.-bot. Ges. Wien* 79: 283-296.

S.M.H.I. (Sveriges Meteorologiska och Hydrologiska Institut), 1973. *Medeltemperaturer 1931--1960, Referensnormaler.*

SAWCHYN, W.W., 1972. *Environmental controls in the seasonal succession and synchronization of development in some pond species of damselflies (Odonata: Zygoptera).* Ph. D. thesis, Univ. Saskatchewan, Saskatoon.

SCHALLER, F., 1960. Étude du développement postembryonnaire d'Aeschna cyanea Müll. *Annls Sci. nat.* (Zool.) 2: 751-868.

STEINER, H., 1948. Die Bindung der Hochmoorlibelle Leucorrhinia dubia Vand. an ihren Biotop. *Zool. Jb.*, Syst. 78: 65-96.

WALLÉN, C.C., 1965. Medeltemperaturens variabilitet. *In:* Atlas över Sverige, pp. 33-34, Stockholm.