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SEASONAL REGULATION IN *LEUCORRHINIA DUBIA* (VANDER LINDEN) (ANISOPTERA: *LIBELLULIDAE*)*

U. NORLING

Department of Systematics, Institute of Zoology, University of Lund, Helgonavägen 3, S-223 62 Lund, Sweden

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The effects of photoperiod and temperature on the rate of larval development in *L. dubia* were investigated on a population in S. Sweden. In the field, life-cycle duration was generally 3 years. Eleven combinations of photoperiod and temperature were tested experimentally. The growth of later larval instars (except the final one) was retarded at LD 13:11 at all temperatures (15°, 20° and 25°C), while LD 19.3:4.7 and LL produced a higher growth rate which was positively correlated with temperature. Experiments performed at 20°C and LD 16:8 and 17.7:6.3 produced different results at different times of the year. A very weak long-day-induced delay occurred in the penultimate instar. The larvae spent their last winter in the final instar. When this instar was reached in constant or decreasing day-lengths, the subsequent development was retarded. During the first third of the instar, the degree of the retardation strongly increased with photoperiod in the interval LD 13:11 to 19.3:4.7 and the difference between long- and short-day responses increased with temperature from 15° to 25°C. The degree of developmental arrest in a certain photoperiod was also dependent on previous changes in photoperiod. During the middle period of the instar, development was extremely slow in most groups. The last part of the final instar was of short duration and was unaffected by light conditions. These final-instar reactions are most probably instrumental in achieving a high degree of synchronization in an advanced stage within the instar by retarding the most advanced larvae more strongly than less advanced ones. In the summer (late June, July) long days retard the development in the final instar more than in earlier instars, later on (late Aug., Sept.) short days retard mid-instar development much more than early-instar development, causing an accumulation of larvae in early mid-

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instar. Temperature affects the effectiveness of the mechanism. The latest larvae to reach the final instar (mid-Sept.) are slowed down by low temperatures and hibernate in early intermoult stages. An increase in day-length during the final (or late penultimate) instar could stimulate development and rapidly lead to emergence. This happened in spring, when previous exposure to low temperatures supported this reaction. The advanced starting position within the final instar is considered to be an important contribution to the earliness of the emergence (early June).

INTRODUCTION

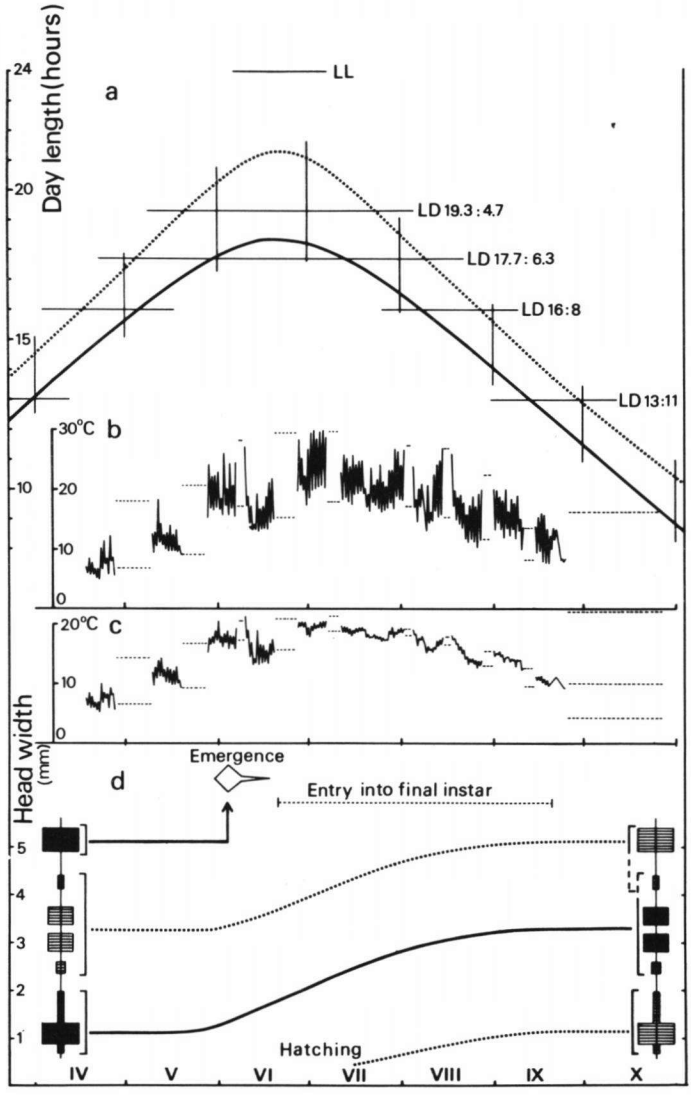
Photoperiod plays an important role in the seasonal regulation of development in many Odonata in temperate regions. The photoperiodic responses involved are apparently the same in many species, forming a two-stage reaction (cf. LUTZ & JENNER, 1964; INGRAM, 1971; NORLING, 1971, 1975), the principles of which may be very widespread (ZASLAVSKY, 1972). Typically, both long- and short-day conditions will finally retard growth, and a period of short days followed by long days is necessary for the rapid completion of development. Variations of this theme give the different species much of their peculiar life-history patterns (cf. NORLING, 1975). Temperature is also most important in the seasonal regulation (CORBET, 1962; LUTZ, 1968, 1974a, 1974b; INGRAM, 1971; NORLING, 1971; SAWCHYN, 1972).

The purpose of the present paper is to analyse the influence of photoperiod and temperature on development in the later larval instars and its consequences for the seasonal regulation in *Leucorrhinia dubia* (Vand. Lind.), a common species in boggy areas throughout Scandinavia. It is a typical "spring species" (CORBET et al., 1960; PAJUNEN, 1962; NORLING, 1975) according to the ecological classification of British Odonata proposed by CORBET (1954), i.e. most or all larvae spend the last winter in the final instar and the emergence occurs early and is well synchronized. In "summer species", there is no concentration in the final instar during the last winter. The emergence is usually later and less synchronized.

Fig. 1. Photoperiod, temperature, and life-cycle of *Leucorrhinia dubia* at Fjällmossen, 58°43′N, 16°31′E. Scale on abscissa in all cases: Months. – (a) Photoperiod. Full line: sunrise to sunset; – Broken line: the same, but civil twilight added. – Photoperiods used experimentally are shown as horizontal lines. The changes of months are indicated through vertical lines. – (b, c) Water temperature, recorded in 1973. b: 5 cm below surface; – c: before 11 July, 45 cm below surface, later at bottom. – Maxima and minima, mostly daily, are shown. During breaks of registration the maximum and minimum for the period are shown as broken lines. – (d) Life cycle, based on field work carried out in 1972 and 1973. At the sides, the approximate size distribution of the larvae during winter is shown, adjacent age groups marked with different patterns. The last five instars are drawn separately, while the smaller larvae are combined. The periods of emergence and entry into the final instar in 1973 are indicated. The diagram is schematic.

MATERIAL AND METHODS

The life-cycle of *L. dubia* was studied in 1972 and 1973 by sampling larvae in two bog pools at Fjällmossen, 58°43′ N, 16°31′ E, about 20 km ENE Norrköping, Sweden. Sampling was carried out from April to October, usually at intervals of 2-3 weeks, using a net with at least 1 mm mesh. The net contents



were washed out in light plastic trays. Very small larvae were preserved in alcohol, while larvae in the last six instars were measured in the field (head width) and subsequently returned to the pool if not required for experiments. Intra-instar development was noted for the two last instars (cf. below).

In 1973, the water temperature was recorded at two depths, 5 cm and 45 cm, by means of a Lamprecht thermograph placed near the centre of the pool (Fig. 1). From 11 July the lower sensor was placed on the bottom because of decreasing depth.

Larvae removed for experimental studies in late October 1972 and 1973 were kept at 0-4°C until the start of experiments in November to January. The photoperiod was not strictly controlled during this period, which was mainly spent in darkness. Acclimatization to experimental temperatures was always allowed to take several hours. Larvae collected in August-September 1973 and on 8 August 1974 were kept in approximately natural conditions as regards temperature and photoperiod (including civil twilight) until the start of the experiments two (1973) or six (1974) days after capture. Some larvae from the August 1974 sample were placed in a climate chamber in a light-dark period of 6 to 18 hours (LD 6:18 = 6 hours day and 18 hours night) and a temperature varying between 7° (night) and 14°C (day) until November. They were later treated as the October samples (Tab. II, W 74).

Experiments were made on larvae in the five last instars, which will be designated by the last letters of the alphabet: V, W, X, Y and Z. They were kept in light-proof boxes, each containing a 4 W white fluorescent lamp, giving 100-200 lux. Otherwise the equipment was as described by NORLING (1971). Three constant temperatures, 15°, 20° and 25° (± 0.5°C), and five photoperiods, LD 13:11, 16:8, 17.7:6.3, 19.3:4.7 and LL (continuous light), were used. No more than eight simultaneous combinations at two temperatures were feasible. The following combinations were never used: LD 16:8 and 17.7:6.3 at 15°C and LD 17.7:6.3 and LL at 25°C. Due to the short-day retardation of development in smaller larvae, short-day effects on final instar development usually had to be studied on larvae first reared under long-day conditions, mostly LD 19.3:4.7 at 20°C, or on those collected shortly before their entry into the final instar. The larvae were usually observed every day, and food, almost exclusively live *Tubifex*, was always available to them.

The development of adult structures was used to subdivide the final instar into five phases. Eye expansion during development was studied at a magnification of 20 X by estimating the position of the eye rim in relation to reference points on the head, viz. the median suture, muscle scars and the adjacent parts of the larval eye margin (Fig. 2). These observations were made at various intervals, usually 1-14 days, depending on rate of development. Notes on similar eye changes in the Y instar were mainly confined to the field work. The sudden folding of the costal rib within the wing-sheaths was taken as the event separ-

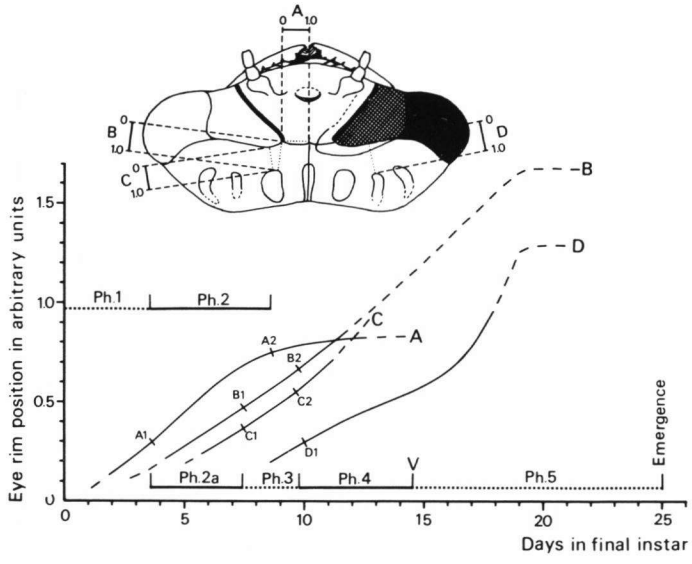


Fig. 2. Compound eye expansion of a single final instar larva at 20°C and highly stimulated development. The larva was collected in the penulatimate instar in May 1974 some 15 km S Fjällmossen, and was kept at LD 17.7:6.3. The curves illustrate how the pigmented eye rim migrates from the larval eye rim towards the median line (A) or towards the muscle scars shown in the figure (B, C, D). In each case, the distance between the reference points is taken as one unit. The rim of the developing adult eye is shown in the right side of the head at a late phase 2 stage. The larval eye is black and cross-hatched. The subdivision of the instar into five phases ("ph.") and the points on the curves delimiting them are shown. The folding of the costal rib within the wing-sheaths (V) separates phase 4 and 5.

ating the two last phases. For some larvae, reliable observations of eye development were difficult to make at this stage. Unclear reference points (in particular the muscle scars) and variations in judgement of these and of certain proportions might sometimes have produced differences between non-simultaneous experiments.

It is desirable to use more than one of the criteria illustrated in Figure 2, when estimating developmental stages. Then it should be noted that the experimental rate of development influences the relations between certain criteria (cf. p. 251).

The experimental duration of each phase except the last one was estimated with the help of interpolation between the eye rim position values obtained from the separate observations. The data are presented as means ($\bar{X}$) with their standard error (SE) except for the last phase. The significances of differences were tested using the Mann-Whitney U-test since the conditions for a t-test were not always fulfilled.

RESULTS

THE LIFE-CYCLE IN THE FIELD

The life-cycle is schematically shown in Figure 1. The flight period extended from the last days of May to mid-August. Mating and oviposition took place mainly in June and early July. In 1973 the eggs started to hatch in the first half of July. The young larvae grew slowly, and most of them were still very small when entering their first hibernation. Moulting activity was mainly restricted to June, July and August, at least in medium-sized larvae. The larvae usually reached the final instar in their third summer, the first ones at about mid-summer, the majority in July and early August, and the very last ones in the second half of September (data from 1973). In the final instar the development continued at a reduced rate, with hibernation occurring in a fairly advanced intermoult stage, in about 50% of the larvae in the early part of phase 4. Hibernation in phase 1 and the early part of phase 2 was rare. The most advanced larvae seemed to reach phase 2 in mid-July or a little later, phase 3 in mid-August and phase 4 in late August. No visible development occurred from October to April.

In the following spring the final instar larvae gathered in the shallow water and most of them emerged very early in June after a period of rapid development. The last few individuals to attain the final instar in the autumn hibernated in less advanced intermoult stages and seemed to emerge somewhat later than the majority. The few hibernating Y larvae apparently reached the final instar in late June, indicating a delayed development in the Y instar.

PHOTOPERIODIC RESPONSES IN INSTARS EARLIER THAN FINAL

Instars ranging between V and Y showed a photoperiodic reaction of the long-day type: LD 13:11 nearly always induced a very strong developmental arrest at all temperatures, perhaps weaker at 15°C. LD 19.3:4.7 and LL promoted more rapid development, the rate of which was temperature dependent. Penultimate instar duration at LD 19.3:4.7 was 53.8 ± 2.7 (n = 9), 24.3 ± 0.3 (n = 52) and 18.9 ± 0.8 (n = 15) days ($\bar{X}$ ± SE) at 15°, 20° and 25° respectively. The duration of earlier instars was usually shorter. When development was arrested at 20°C, the normal instar duration was several months. The short-day arrest appeared to fail more often in the Y instar than in earlier instars and it may also have been somewhat less intense in the former stage.

At intermediate photoperiods, LD 16:8 and 17.7:6.3, at 20°C, the response was dependent on the season. In the winter W-X larvae, though not always at LD 16:8, reacted similarly to the long-day groups. In mid-August at LD 16:8 there was a marked developmental arrest, however in LD 17.7:6.3 only partly so. As the rapid development in the long-day controls seemed to start somewhat later

in some larvae, a diapause-like state (arrest) may have been induced prior to the start of the experiment, i.e. in the early half of August.

The penultimate instar had a slightly longer duration in the long-day (LD 19.3:4.7 and LL) conditions, i.e. 24.6 ± 0.4 (n = 68) days at 20°C, than at LD 13:11 and 16:8 when the short-day arrest failed: 20.1 ± 0.8 (n = 15) days at 20°C. This long-day retardation is conspicuous in larvae that had spent the winter in this instar, thus corroborating the results from the field-study.

DEVELOPMENT AND PHOTOPERIODIC RESPONSE IN THE FINAL INSTAR

In the studies of intra-instar development it was found that the early part of the instar was best defined by median expansion of the compound eyes (A in Fig. 2), the middle part by caudal expansion (B, C, D). However, the relation between values of A on one hand, and B, C and D on the other, was dependent on the magnitude of developmental retardation. The subdivision of the instar, presented in Figure 2, has the disadvantage that phases 2 and 3 overlap when retardation is weak, and are separated by an appreciable interval when development is strongly retarded. To show the relations between median and caudal expansion of the eyes, the duration of a phase called 2a (end of phase 1 = A 1 to start of phase 3 = B, C 1) is compared with phase 2 (A 1 to A 2, Fig. 2, cf. Tabs. I, II, Figs. 3, 4).

When the final instar was entered without a recent and substantial increase in day-length, development always became more or less retarded, irrespective of photoperiod. The observed effects of temperature and photoperiod on this retarded development are summarized in Tables I and II, Figures 3 and 4. Unfortunately, identical experiments performed on different occasions could sometimes result in slightly, and even significantly, different values. Differences between different experimental groups were, however, more important in all but a few cases (cf. Tab. II).

In phases 1 - 3 at 20°C the degree of retardation continuously increased with day-length up to LD 19.3:4.7 (Tab. I, Fig. 3). Except in phase 1, the responses to LL seemed to reverse this trend. The sensitivity to photoperiod was greatest near LD 17.7:6.3. In phase 4 at 20°C, a strong developmental delay was produced in all photoperiods between LD 13:11 and 19.3:4.7.

Groups previously subjected to longer days showed a faster development in the first three phases than similar groups kept in constant conditions from the outset. In the LD 17.7:6.3 groups, which, however, were not really simultaneous (Tab. I), the difference was considerable. In the groups at LD 17.7:6.3 and 19.3:4.7 there was a tendency for the effect of the previous treatment to decline in phase 3, at the entry of which these larvae had spent a rather long time in constant photoperiod. The responses in phase 1 of the larvae in the LL groups

Table I

Duration (days) of the first four developmental phases of the final instar in five different photoperiodic regimes at 20°C

		LD 13:11			LD 16:8			LD 17.7:6.3			LD 19.3:4.7			LL	
	decr.	W 73, S 74			W 73, S 74			S 74			S 74		incr.	S 74	
	const.	W 73			W 73			W 73, S 74 (late)			W 73, S 74			S 74	
		$\bar{X}$ SE	n		$\bar{X}$ SE	n		$\bar{X}$ SE	n		$\bar{X}$ SE	n		$\bar{X}$ SE	n
Ph. 1	decr.	6.21 ± 0.15	6 + 2	-	6.49 ± 0.31	7 + 3	+	8.38 ± 0.52	9	-	10.7 ± 1.35	5	incr.	19.3 ± 2.49	4
		-			-			+			-			-*	
	const.	5.80 ± 0.29	4	+	7.62 ± 0.40	15	++	11.0 ± 0.56	10 + 4	+/-	13.5 ± 0.40	9 + 13	-	13.9 ± 0.95	6
Ph. 2/2a	decr.	7.63 ± 0.35	6 + 2	-	8.79 ± 0.61	7 + 3	+	11.7 ± 0.70	9	+	26.9 ± 6.59	5	incr.	52.0 ± 6.14	4
		6.60 ± 0.42		-	7.94 ± 0.54		+	11.1 ± 0.80		+	28.3 ± 8.00			61.5 ± 10.9	
		+/-			++/+			++/++			++/+			-/-	
	const.	9.87 ± 0.51	4	+	13.9 ± 0.92	15	++	33.6 ± 1.90	10 + 4	++/+	49.8 ± 2.05	9 + 12	-	43.4 ± 3.24	6
		7.30 ± 0.65		++	13.8 ± 1.42		++	35.5 ± 2.07		++/+	57.7 ± 3.39		-	48.4 ± 5.58	
Ph. 3	decr.	6.31 ± 0.51	6 + 2	-	7.90 ± 1.36	7 + 3	+	18.3 ± 2.62	9	+	40.8 ± 4.35	4	incr.	21.2 ± 2.52	4
		-			++			+			-			-	
	const.	9.07 ± 1.41	4	-	17.1 ± 2.17	13	++	29.2 ± 1.97	10 + 4	++/-	44.3 ± 4.22	9 + 8	-	25.1 ± 5.38	6
Ph. 4	decr.	67.7 ± 6.03	6 + 1	-	78.3 ± 8.35	7 + 3	-	62.1 + 11.8	4	–			incr.	13.4 ± 2.32	4
		-			-			NC						-	
	const.	92.2 ± 25.8	4	-	82.3 ± 12.3	13	-	102.0 ± 14.7	9 + 0	-	72.3 ± 5.13	7 + 2	-*	18.4 ± 6.63	6

In LD 13:11 to 19.3:4.7, a comparison is made between groups where a substantial decrease in day-length, usually from LD 19.3:4.7, had preceded the entry into the final instar with less than 20 days ("decr.") and groups where no decrease had occurred, or did occur more than 60 days before entry into the final instar ("const."). The LD 19.3:4.7 "decrease" group was previously kept in LL. In LL the effect of an increase from LD 19.3:4.7 is shown ("incr."). The experiments were usually begun with larvae in the W and X instars and the changes in photoperiod occurred after 35-40 days or more. The head shows the composition of each group with respect to timing of the start of experiments: W = winter, S = summer (August). The year of collection is added. Means, standard errors and number of larvae are given. The results of non-parametrical significance tests on differences between adjacent values are shown: + = $P < 0.05$, ++ = $P < 0.01$, - = non significant. The tests are made on values from approximately simultaneous experiments. When no comparisons could be made on these terms, it is shown with NC. An asterisk denotes a level of significance of 95% obtained with an ordinary t-test.

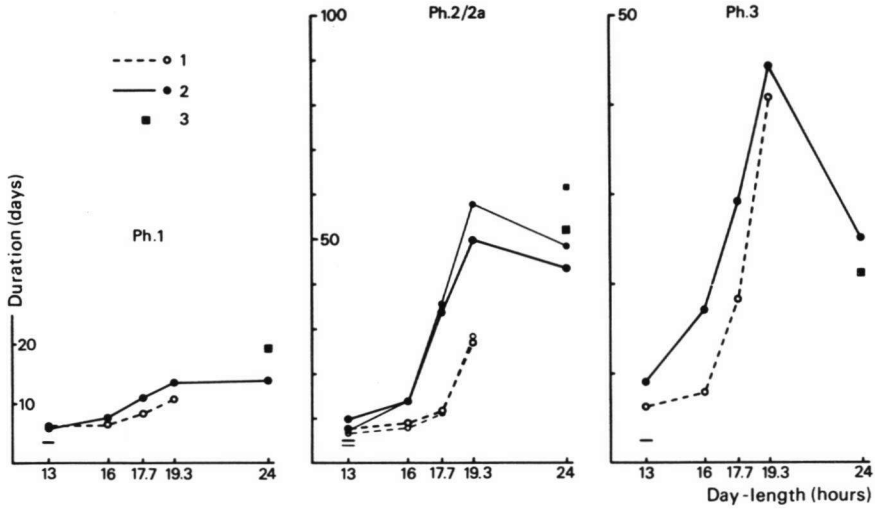


Fig. 3. Effects of different photoperiods on developmental time in the first three phases of the final instar, based on data in Table I. The time scales (ordinates) are so adjusted that the duration of each developmental phase at stimulated development at 20°C is at approximately the same level except phase 2a (indicated by the symbol –): 1, with a previous decrease in day length; – 2, constant photoperiods; – 3, with a previous increase in day length. Phase 2a is shown with smaller symbols.

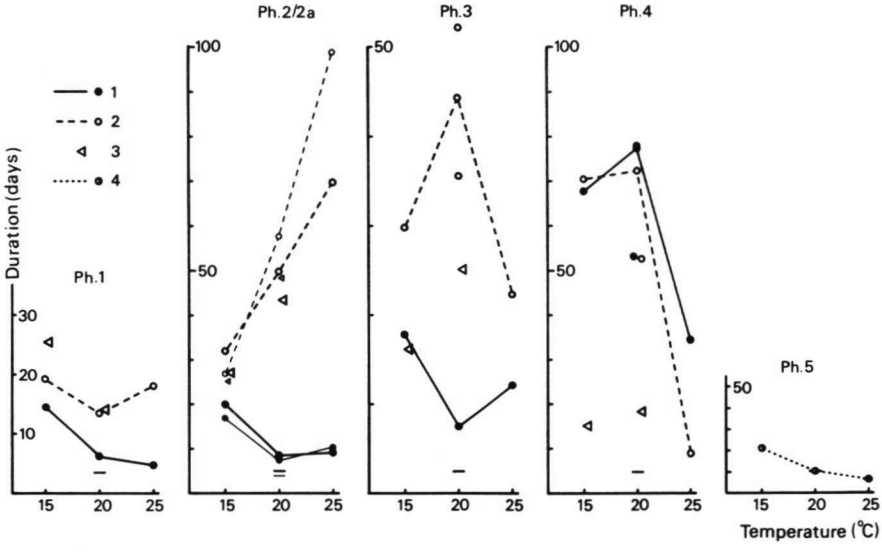


Fig. 4. Combined effects of temperature and photoperiod on development in the final instar, based on the data in Table II. The ordinates are adjusted as in Figure 3. The mean values of the two LD 19.3:4.7, 20°C groups are used for drawing the lines. 1: short-day groups; – 2: LD 19.3:4.7; – 3: LL; – 4: all groups.

indicate that an increase in day-length may have an effect opposite to that of a decrease.

The combined effects of temperature and certain photoperiods are shown in Table II and Figure 4. In the first three phases, the influence of photoperiod on development generally increased with temperature. However, in phase 3 the long-day group (LD 19.3:4.7) at 25°C began to show a similar trend as the LL groups. When the speed of development was governed mainly by retardation, the temperature coefficient for development was often negative. A positive temperature coefficient occurred when the retardation was weak, especially in the interval 15° - 20°C. In phase 4 at 15° and 20°C the developmental rate was extremely low, except in LL, and variation within the groups and between non-simultaneous experiments could be considerable. At 20°C development in phase 4 was more rapid, in particular in the long-day group. Phase 5 was governed by temperature alone, and photoperiod never affected the rate of development.

During the course of final instar development, the degree of retardation varied according to a pattern determined by photoperiod and temperature. Under short-day conditions retardation was weak in the two first phases. In phase 3 an increase in intensity was noted and a maximum was reached in phase 4 at all temperatures. In LD 19.3:4.7 the retardation intensity increased earlier, in particular at higher temperatures. At 15° and 20°C the approximate level of phase 4 is already reached in phase 3, while at 25°C a maximum occurs in phase 2 (2a). The LL groups initially show a response similar to that in LD 19.3:4.7, but deviate later. Unfortunately, the LL and LD 19.3:4.7 groups were to a very minor extent simultaneous concerning the material in the later phases, but the differences are great and probably reflect different photoperiodic responses.

Responses of final instar larvae to different photoperiods changed during late summer and autumn (Fig. 5). A reversal in response to LD 19.3:4.7 from strongly retarded development (Fig. 5a) to stimulated development (Fig. 5c, d) occurred during the latter part of August. The transition seemed to appear first in more advanced larvae (Fig. 5b). After 2 September the response remained rather constant. Continuous illumination (LL) could stimulate development at a time when LD 19.3:4.7 was ineffective in this respect, even at 25°C (Fig. 5k, l).

Both LD 13:11 and 16:8 produced typical short-day responses (cf. Tab. I) during the period 6 Aug. to 16 Sept. (Fig. 5e, f, h, only part of the results are presented). The winter experiments indicated a late change in response to these photoperiods. The effect of LD 16:8 (Fig. 5i) was then rather close to that of LD 19.3:4.7, especially in the latest experiment. In LD 13:11 (Fig. 5g), the responses obtained for the less advanced larvae were essentially as in September, while the more advanced ones showed a much faster development than before.

Similar experiments conducted at LD 13:11 and 19.3:4.7 at 15°C (on 30 November 1973) produced similar results as the corresponding groups at 20°C,

Table II
Duration of the developmental phases of the final instar at different combinations of photoperiod and temperature

	Photoperiod	15°C		20°C		25°C	
	LD						
sd	13:11 decr.	W 73		W 73, S 74, (S 73)		S 74	
sd	13:11 const.	W 73		W 73		–	
sd	16:8 decr.	–		W 73, S 74, (S 73)		S 74	
	19.3:4.7	W 73		W 73, S 74		S 74	
	LL	W 74		S 74		–	
		$\bar{X}$ SE	n	$\bar{X}$ SE	n	$\bar{X}$ SE	n
Ph. 1	sd	14.5 ± 0.54	13	6.26 ± 0.16	22	4.75 ± 0.27	22
Ph. 1	19.3:4.7	19.2 ± 0.53	16	13.5 ± 0.40	22	18.0 ± 1.18	15
Ph. 1	LL	25.5 ± 2.10		13.9 ± 0.95	6	–	
Ph. 2/2a	sd	20.0 ± 1.51 16.8 ± 1.60	13	8.56 ± 0.35 7.33 ± 0.32	22	9.18 ± 0.62 10.3 ± 0.82	22
Ph. 2/2a	19.3:4.07	31.9 ± 2.30 26.8 ± 2.24	16	49.8 ± 2.05 57.7 ± 3.39	21	69.8 ± 3.06 98.8 ± 4.72	15
Ph. 2/2a	LL	27.0 ± 2.10 25.1 ± 2.19	6	43.4 ± 3.24 48.4 ± 5.58	6	–	
Ph. 3	sd	17.8 ± 2.98	12	7.53 ± 0.70	22	12.1 ± 0.64	22
Ph. 3	19.3:4.7 W 73	29.8 ± 3.36	16	52.1 ± 5.71	9	–	
Ph. 3	19.3:4.7 S 74	–		35.6 ± 4.90	8	22.3 ± 3.65	14
Ph. 3	LL	16.2 ± 3.12	6	25.1 ± 5.38	6	–	
Ph. 4	sd W 73, S 74	67.6 ± 11.7	11	77.4 ± 6.42	21	34.5 ± 2.60	22
Ph. 4	sd S 73	–		53.1 ± 2.60	20	–	
Ph. 4	19.3:4.7 W 73	70.4 ± 8.69	10	77.9 ± 4.44	7	–	
Ph. 4	19.3:4.7 S 74	–		52.7 ± 6.15	2	8.90 ± 1.20	13
Ph. 4	LL	15.1 ± 1.67	6	18.4 ± 6.63	6	–	
Ph. 5	All photoperiods	21.5		10.5		6.5	

The short-day groups (sd) are composite and consist of all groups in LD 13:11 and those LD 16:8 groups where a decrease in day-length preceded the entry into the final instar with less than 20 days (cf. Tab I). Statistical treatment of the phase 5 values has not been made, since the differences were small (usually ± 1 day) and mainly due to observational errors. The explanation to the head corresponds with that of Table I.

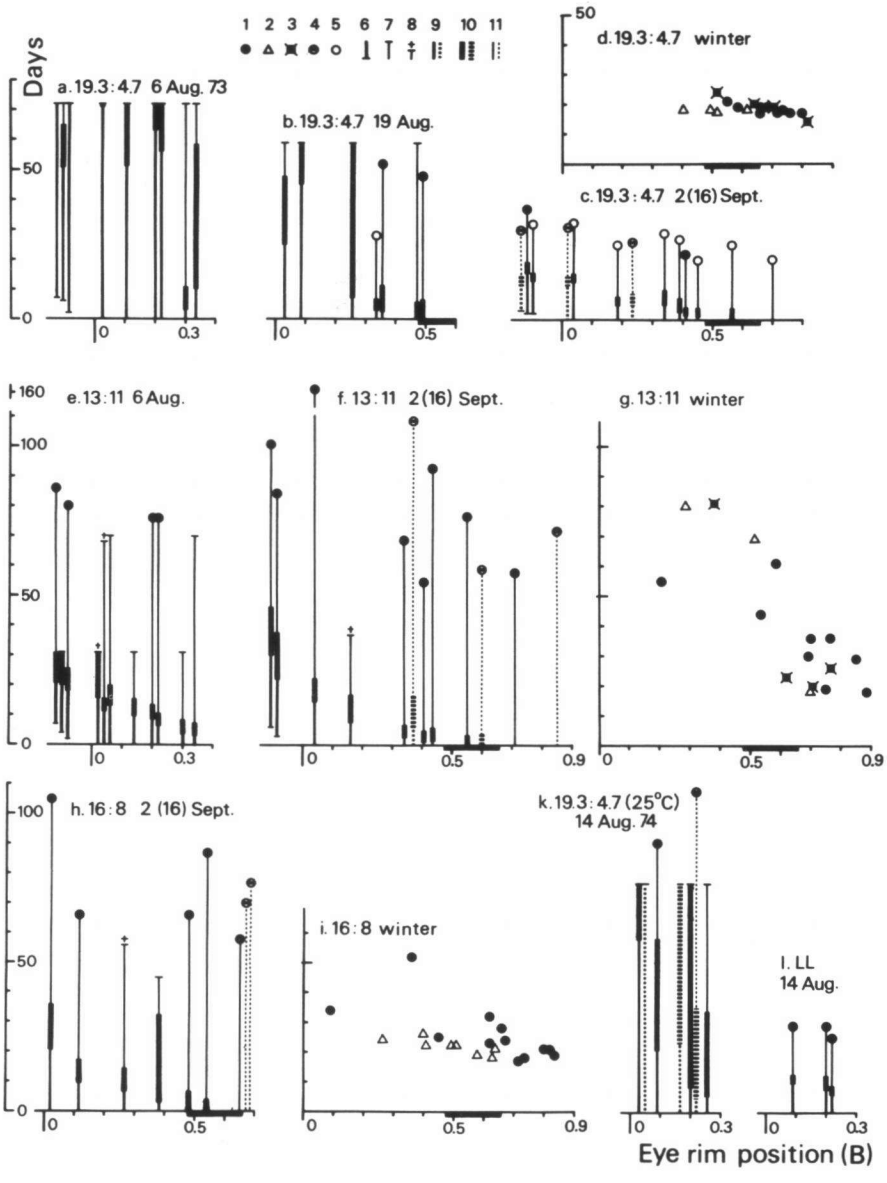


Fig. 5. Individual photoperiodic responses (time to emergence) of final instar larvae on different occasions during the period August to January. Above each diagram the experimental photoperiod and time for start of the experiments are shown. Data within parenthesis refer to the broken lines. Temperature 20°C, except partly in k. The abscissa corresponds to B in Figure 2, and is a measure of the stage of intra-instar development for

but the developmental rate was about half of that at 20°C.

Certain experiments performed at 20°C support the view that the change in response to long-day conditions was the result of changes in photoperiod (two stage reaction). Larvae kept for at least 5-6 weeks in constant photoperiods were subjected to longer days shortly before, at, or after, the entry into the final instar (Fig. 6). The material is, however, small and temporally heterogenous, but shows that even rather small increases in day-length may stimulate development, at least at intermediate photoperiods (LD 16:8 to 17.7:6.3). In the upper photoperiodic range sensitivity to the increase appears to be higher in the final instar than before or at the final moult (cf. also Fig. 5b).

The final-instar duration of larvae kept at LD 19.3:4.7 at 20°C was shortened when they were subjected to LD 13:11 at 15° or 20°C for the first 3-6 weeks after the final moult. They required 56-62 days (n = 7) to pass the instar in comparison with 171-230 days (n = 7) for long-day (LD 19.3:4.7) controls and 86-125 days (n = 8) for short-day (LD 13:11) controls. The development after short-day treatment appeared to be most rapid when the short day occurred at 15°C.

DISCUSSION

To a great extent larvae of *L. dubia* exhibited a continuously variable response to photoperiod, which is evidently a common feature in Odonata (Jenner in CORBET, 1962, p. 97; cf. SAWCHYN, 1972, figs. 15, 25). A gradual response was most clearly seen in the different degrees of developmental retardation at certain stages of the final instar (Fig. 3). Nevertheless, a LD 17.7:6.3 photoperiod appeared to be near-critical. It could act as both long- and short-day (Figs. 3, 6), which is thought to demonstrate the importance of changes in photoperiod (cf. *Anax imperator*, CORBET, 1956; *Chrysopa carnea*, TAUBER & TAUBER, 1970; *Gerris odontogaster*, VEPSÄLÄINEN, 1971). However, a

each larva at the start of experiment. The B values of phase 3 are indicated by a thickening of the axis. The least advanced larvae are ranked in the space $0 < B < 0.3$ from their values of A. A = 0.3, which separates phases 1 and 2, here corresponds to B = 0.16. The ordinates show the number of days from the start of each experiment. In a, c, e, and f, the development of larvae, reaching the final instar soon after the start of experiment, are shown to the left of the B-scale. – S y m b o l s: 1-4: Recorded emergence (see below); – 5: estimated time for emergence, either when the larvae died during phase 5 or, in c only, when the emergence could not be recorded properly; – 6: entry into the final instar; – 7: larva removed from experiment; – 8: larva dead; – 9: period before phase 3; – 10: phase 3; – 11: period after phase 3. – In d, g, and i, the symbols for emergence indicate different experiments: 1: start 30 November 1973. Through a fault in the automatic switches the larvae were exposed to continuous light for probably 1-2 days about 25 later. Emergence dates later than 10 days after this may have been affected; – 2: placed in LD 13:11 at 15°C on 24 January, start 26 January, 1974; – 3: start 5 December, 1972.

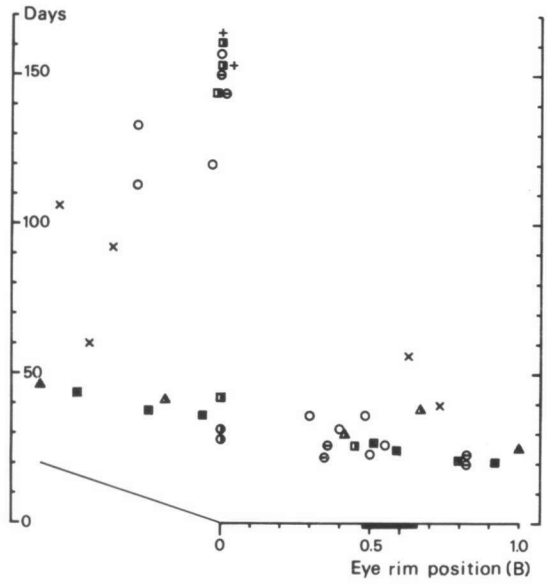


Fig. 6. Effects on development of sudden artificial increases in day-length during the late penultimate and early ultimate instar at 20°C. The time between the increase and emergence is shown (ordinates). The abscissa (0-1.0) refers to the stage of final instar development at the increase (cf. Fig. 5). The oblique line to the left indicates the time of entry into the final instar for larvae in the penultimate instar. The larvae were kept at least 5-6 weeks in constant photoperiods at 20°C before the experiments. Results from several years are combined. S y m b o l s: X: change from LD 13:11 to 16:8; – Triangles: change to LD 17.7:6.3; – Squares: to LD 19.3:4.7; – Circles: to LL; – Symbols all black: change from LD 13:11; – Half black: from 16:8; – With horizontal line: from LD 17.7:6.3; – Open: from LD 19.3:4.7. – A + indicates that the adjacent symbol refers to the date of removal of the larva from experiment instead of emergence.

complicating effect due to an endogenous seasonal rhythm (cf. GEISPITZ et al., 1974) cannot be excluded as the experiments were not strictly simultaneous. The sensitivity to changes in photoperiod appears to be proved (Tab. I, Figs. 5, 6), but the material is insufficient to demonstrate conclusively the existence or non-existence of the seasonal rhythm.

The critical photoperiods are estimated to be encountered in early May, when temperatures just become high enough to permit development, and in early August, when temperatures are still high (Fig. 1). Through the sensitivity to changes in photoperiod a long-day response (usually stimulation) is ascertained in the spring at the same time as short-day responses (different degrees of retardation) are permitted to start early in late summer.

The dramatic reversal in long-day response of final instar larvae in August is a

conspicuous result of the two-stage photoperiodic reaction. During late summer and autumn, "short-day sensitization" (ZASLAVSKY, 1972) takes place after which the developmental stages showing the two-stage reaction, i.e. the one or two last instars, can be stimulated to a rapid completion of larval development by the action of longer days (Fig. 5). Thus, the development of final instar larvae was greatly stimulated in the spring and they soon became adults. Small larvae appeared always to be stimulated by long days, but retardation became evident in the two last instars. Even hibernating Y larvae were not advanced enough to be finally stimulated, at least not under the environmental conditions prevailing in the locality studied.

In *Aeshna viridis*, a summer species, several of the last instars can be stimulated by long days after hibernation. The number of stimulated instars is influenced by temperature and photoperiod during the spring, and four was found to be a common figure at a locality in southernmost Sweden (NORLING, 1971). The capacity to be stimulated by long days (LD 19.5:4.5) seemed to appear first in the final instar near mid-August (NORLING, 1971, p. 184 and Fig. 6D, also supported by later experiments). The data in Figures 5b and 6 suggest that in *L. dubia* the most advanced larvae are also the first ones to become sensitive to long-day stimulation, but in contrast to summer species, the capacity of hibernating larvae to be finally stimulated by long days is mainly confined to the final instar. It appears likely that environmental influences on the number of instars susceptible to the final long-day stimulation are, as in *A. viridis*, present in *L. dubia*, determining the subsequent development of the hibernating Y larvae (cf. the larva on which Fig. 2 is based). The sensitivity to increasing day-length may be of special importance in the spring (cf. CORBET, 1956).

However, the interpretation of Figures 5b and 6 is uncertain. Some of the results shown in Figure 6 may be partly explained by the special properties of continuous illumination, a photoperiod not normal for the population. The rather rapid development in phases 3 and 4 shown in Table I and Figure 4 may alone be sufficient to explain, in a few cases, the responses obtained by transferring more advanced larvae to LL. Furthermore, the more advanced larvae in Figure 6 had, on the average, spent a longer time in pre-transfer conditions than the less advanced ones, which may have affected the results.

In August, the temperature is still fairly high (Fig. 1), and the conspicuous change in response to long days (Fig. 5a-c) can be attributed solely or mainly to photoperiod. It is, however, likely that exposure to low temperature played a most important role in the changes in responses to LD 16:8 and 13:11 (Fig. 5) that occurred later. In the more complete reversal occurring in the response to LD 16:8, photoperiod might have been active as stimulator, but it may also have been more passive, neither retarding nor stimulating larvae which were reactivated by low temperature. The varying response to LD 13:11 might then be

explained in the following way:

Development started immediately after exposure to a favourable temperature. Through reactivating effects of previous low temperature, photoperiod probably needed some 5-10 days (dependent on the degree of reactivation), to exert its full influence on development. The most advanced larvae could, to a great extent, pass the stage where retardation is possible within this time, in contrast to the less advanced larvae, which were strongly retarded in phase 4.

Thus, through an increasing sensitivity to long-day stimulation produced by the successively shorter days (short-day sensitization), and later a stimulating effect of low temperatures on subsequent development, the shortest day-length able to produce a rapid development continuously decreases during late summer and autumn (cf. the shift in critical photoperiod during winter reported by INGRAM, 1972 for *Coenagrion angulatum*). A contribution from an endogenous seasonal rhythm is, however, also possible. Similar shifts in the response to different day-lengths are also well documented for *Tetragoneuria cynosura* (LUTZ, 1974a) and *Enallagma aspersum* (INGRAM, 1971). The effect of previous low temperature ensures a most rapid final instar development in the spring.

The pronounced short-day induced and very diapause-like developmental delay in smaller larvae may be an adaptation for surviving the long Scandinavian winter in the shallow water-bodies that *L. dubia* often inhabit and was not recorded for *T. cynosura*. It seems therefore reasonable that still smaller larvae than examined here show a similar reaction. The sensitivity to short days appears to be very great in late summer (decreasing day-length and/or activity of a seasonal rhythm), and the arrest apparently starts early, while the temperature is still high, except perhaps in the penultimate instar. The possibly higher tendency for Y larvae to escape the short-day arrest and then to cease moulting later in the season indicates the beginning of the transition to the short-day responses of the final instar. It is probably more advantageous to hibernate in early phases of the final instar and emerge somewhat later in the season than to postpone the emergence by one year, which certainly would be the case for some larvae if they were prevented from reaching the final instar in the autumn.

In his original definition of spring species, CORBET (1954) stressed the synchronizing effect of a final instar diapause, successively accumulating larvae in the final instar before winter. As is already evident from the classical studies on *Anax imperator* (CORBET, 1956, 1957) a certain degree of synchronization can be achieved in the penultimate instar by means of a long-day developmental arrest. In another spring species, *Tetragoneuria cynosura*, this arrest in the penultimate instar has been found to be a major synchronizing factor (LUTZ & JENNER, 1964; LUTZ, 1974 b). The larvae accumulated in this instar during late summer, and entered the final instar synchronously in October, when shorter days brought the arrest to an end.

In *L. dubia*, as in *A. imperator*, the synchronization of the entry into the final

instar, though still present, was less evident. Instead the larvae accumulated in the final instar, but in contrast to *A. imperator* the development did not cease when this instar was reached. Intra-instar development seemed to proceed in such a manner that the degree of synchronization slowly increased, finally resulting in an accumulation of larvae at a fairly advanced intermoult stage. The material obtained in the field was not large enough to provide details of this process, but the results of the experimental work suggest that the following mechanism is operating.

The long-day retardation found in the penultimate instar continues with successively increasing intensity in the final instar (Fig. 4). The high intensity of the long-day retardation in the beginning of this instar prevents the larvae from reaching more advanced intermoult stages during long-day conditions and allows larvae in earlier instars to catch up with those in the final instar. The increase of the long-day retardation intensity during final instar development may allow a slow intra-instar synchronization. However, in the early part of the final instar, the strong short-day-induced arrest of the earlier instars has been replaced by a weak retardation permitting a relatively rapid development. Decreasing day-lengths thus tend to speed up development in the earlier phases (1-3) and displace the higher retardation intensities towards more advanced stages (Figs. 3, 4). Thus, larvae reaching this instar early are more retarded in their early phases than larvae reaching the instar at a later time. In August, the combination of photoperiod causing a short-day response and fairly high temperature releases a rather rapid development in the first two or three phases (cf. Figs. 1, 3). Under these conditions the degree of retardation does not increase until phase 3 (Figs 3, 4), which was reached by the most advanced larvae in mid-August. The retardation reaches its maximum in phase 4, which in the field was never observed until late August. Thus, in late summer, the most advanced larvae still develop more slowly than the less advanced ones, at least those in the final instar. The latter larvae now rather rapidly catch up with the former ones. This is the final phase of the synchronization of larval development, which will, however, never be complete since the process is interrupted by the onset of winter. Larvae reaching the final instar late are forced to spend the winter in earlier phases.

Many responses shown in Figures 3 and 4 are of doubtful ecological significance. Phase 1 is the only stage which is likely to experience extreme and fairly constant long-day conditions, often combined with high temperature. Here the responses to long days, even to LL, appear quite functional (Fig. 4). In phases 3 and 4, long day-lengths are unnatural when development is retarded, and the responses to such photoperiods were varied. A strong retardation in an early phase might also have affected the development in the later phases. For phase 4, the responses of larvae at LD 13:11 at 15°C are likely to be the most relevant ones (cf. Figs. 1, 4).

The best synchronization should occur in a warm summer. High temperatures are expected to produce a strong long-day retardation in the first larvae to reach the final instar, but also to allow late larvae to pass the first two phases more rapidly than otherwise. On the other hand, when low temperatures prevail, the synchronization can be assumed to be less effective. The relatively weak long-day retardation at low temperatures should, however, make it easier for early larvae to reach a fairly advanced stage before winter.

The concentration of a great number of the hibernating final instar larvae to phase 4 certainly has phenological consequences. At summer temperatures and stimulated development it may result in emergence occurring seven to ten days earlier than that would be the case if all larvae suspended development as soon as they reached the final instar. At high latitudes, where the summer is very short, the earlier emergence may be a factor of great importance for the survival of the local populations. Phase 4 is also the most advanced stage where a diapause-like state can occur, and thus the last of the phases in which the winter can be spent.

The strong direct relationship between the degree of retardation in the final instar and photoperiod, here suggested to be important in the seasonal regulation, is known also from *T. cynosura* (LUTZ, 1974 b), but appears to be absent from summer species such as *A. viridis* (NORLING, 1971) and *Pachydiplax longipennis* (ELLER, 1963). Field observations suggest that at least *Leucorrhinia rubicunda* and *Cordulia aenea*, both very early species, use essentially the same mechanisms for seasonal regulation as *L. dubia* (NORLING, 1971, 1975).

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