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SEASONAL REGULATION IN *PYRRHOSOMA NYMPHULA* (SULZER) (ZYGOPTERA: COENAGRIONIDAE) 2. EFFECT OF PHOTOPERIOD ON LARVAL DEVELOPMENT IN SPRING AND SUMMER

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In the laboratory, larvae of the Western Palaearctic *P. nymphula* were exposed to conditions of temperature and food conducive to uninterrupted development, and to one of three, constant photoperiods: 13.5 h (equivalent to either equinox), 18 h (14 May and 30 July) and 19.5 h (5 June and 8 July), and their responses were interpreted in the light of seasonal development in the field. Synchronised entry to F-0 (the final instar) in late summer is caused by the summer arrest in F-1 (the penultimate instar) that is induced by exposure in F-1, but not in F-2 or F-3, to 19.5 h, but not to 13.5 h. The refractoriness of F-1 declines during summer so that the response to 19.5 h changes abruptly during late August (16-16.5 h; maximum daily increment of change of 5 min/day), when F-0 is entered promptly. Termination of emergence in summer is caused by the long-day arrest that prevents F-1 larvae from entering F-0 during the second half of May (18 h or more). Photoperiod does not evidently affect the mean duration of F-2 or F-3, or the onset and rate of metamorphosis in spring. Persistent exposure to 19.5 h during their summer arrest causes about 25% of F-1 larvae to undergo a supernumerary moult, still within F-1; such individuals can be distinguished from normal F-1 (after the supernumerary moult) by their larger wing-sheaths. Supernumerary moulting occurs also in the field. Remaining unknown are: the responses of F-0 which prevent emergence (i.e. the onset of metamorphosis) in autumn; and the role, if any, of changing photoperiods in regulating rate of development or sensitivity to absolute photoperiod.

INTRODUCTION

In an earlier account (CORBET & HARVEY, 1989) the seasonal development of the coenagrionid dragonfly, *Pyrrhosoma nymphula*, in a northerly site is described, particular attention being given to stages at which development is

arrested or accelerated to achieve seasonal placement and restriction of emergence. Three events play a key role in the seasonal regulation of *P. nymphula* (CORBET & HARVEY, 1989). In this paper we report experiments designed to identify responses that bring about two of these events: synchronised entry to the final instar in early autumn (which usually results in > 90% of the senior age-cohort overwintering as F-0); and temporal restriction of emergence during spring and early summer. Responses bearing on the third key event (absence of emergence in autumn) will be addressed in a later paper.

The correspondence between these key events and the annual march of photoperiod at the study site, Dykehead Pond (Fig. 1), suggested that, as in many other insects (DANKS, 1987), and especially Odonata (see CORBET, 1980), response to daylength might help to control their seasonal placement. In designing and interpreting experiments to explore this possibility, we were fortunate to have access to the elegant and illuminating studies by NORLING (1984a, 1985b, 1984c) on certain Palaearctic Odonata, especially *Coenagrion hastulatum* (Charpentier) (NORLING, 1984b). In southern Sweden (at 58°42′ N.) 19.3-h photoperiods prolong development in each of the last four instars in the young semivoltine group of *C. hastulatum*, but particularly in F-1 (the penultimate larval instar), and so prevent entry to F-0 until late summer (September).

Larvae for experiments were collected before and after the summer solstice (21 or 22 June) in 1983. Those collected before the solstice (during May and early June) comprised the 1983 emergence cohort. Those collected after the solstice (during June through September) were assumed to belong to the 1984 emergence cohort, entering F-0 in early autumn, overwintering as F-0, and emerging in May or June 1984.

Two kinds of response were investigated: (1) the effect of photoperiod on development of larvae which had overwintered as F-1 or perhaps as F-2 or F-3: here we wished to test the hypothesis that such larvae, by virtue of having recently overwintered, might, when entering F-0 in spring, forgo the developmental arrest that is an invariable feature of larvae entering F-0 in autumn; and (2) the effect of photoperiod on entry by F-1 to F-0.

The first effect is difficult to investigate. Usually, very few larvae overwinter in F-1 and even fewer in F-2; so it is hard to obtain sufficient experimental material. Fortunately, the 1983 emergence cohort in Dykehead Pond included an unusually high proportion of larvae that, in spring 1983, even after having entered F-0, could be recognised (on the basis of interecdysial eye stage) as having overwintered in F-1. Our first hypothesis was that photoperiods in mid-May (ca. 18 h between Civil Twilights) would avert (i.e. override) the developmental arrest that in autumn prevents newly moulted F-0 from proceeding without delay to metamorphosis (thus obliging them to overwinter as F-0) and that longer photoperiods, closer to those at the summer solstice (perhaps 19-20 h) would induce arrest and thus postpone emergence for one year. Responses of this kind would

have produced the observed seasonal restriction of emergence, but the hypothesis was not supported by our preliminary experimental results. However our findings of the effect of photoperiod on F-1, F-2 and F-3 during the rest of the year enabled us to formulate another hypothesis to describe the responses of larvae to photoperiod in spring. Although we lack sufficient data for this hypothesis to be tested rigorously, it is supported qualitatively by our data and merits inclusion here and adoption as a working hypothesis.

The second response is relatively straightforward to investigate. We exposed larvae collected as F-1 or F-2 at different times during June through September to "short" (13.5-h, equinoctial) photoperiods or "long" (19.5-h, close to solstitial) photoperiods equivalent to those on 5 June and 8 July).

MATERIAL AND METHODS

General

Photoperiods at Dykehead Pond, Angus (NO 375606; 56°44'N., 3° 1'W.) (Fig. 1) were calculated from an algorithm provided by YALLOP (1978). For purposes of determining the correspondence between calendar date and "perceived" photoperiod, the latter was regarded as the interval between morning and evening Civil Twilights (see NORLING, 1984b, p. 432). Although the lowest light intensity able to elicit a photoperiodic response has seldom been determined for dragonflies (but see LUTZ & JENNER, 1964) it is likely to be far less than that obtaining at sunrise and sunset. At 56°44'N., between either equinox and the summer solstice, Civil Twilight occurs 38-65 min before sunrise or after sunset.

Larvae were collected from Dykehead Pond in 1983 on 10, 18, 25 May; 1, 8, 15, 22 June; 15, 26 July; 9, 17, 24 August; and 1, 8, 14, 22 September. A single collection was made on 25 June 1983 from the Dubh Lochan outflow stream, Argyllshire (NS 375965; 56°8' N., 4°36'W.). Methods for handling, measuring and categorizing larvae are described by CORBET & HARVEY (1989).

During experimental treatment each larva was kept in an individually numbered glass cylinder (height 75 mm and diameter 25 mm) the lower end of which was covered with nylon gauze (mesh diameter 0.1x0.03 mm) secured by a rubber ring halfway up the tube. Thirty such cylinders were held in a rack in a bowl of water, placed so that the top 20 mm of each cylinder was above the water level. The water was kept at 20 ± 1° C by a small thermostatically controlled immersion heater and constantly aerated by compressed air, thus achieving water circulation through the gauze into each cylinder; so each larva experienced approximately the same conditions of aeration and temperature, regardless of its position in a bowl. Two such bowls were placed in a cabinet (a modified domestic refrigerator) in which photoperiod was controlled, light being provided by four 12-V, 2.2-W bulbs placed above each bowl. A 4-cm length of paper drinking straw in each cylinder provided a support for the larva to cling to. Larvae of the mosquito, *Aedes aegypti* (L.), of instars II-IV (usually III) were provided ad libitum as food. Initially it proved difficult to ensure that living prey were always available: if many mosquito larvae were provided, the dragonfly often killed them all. However during mid-July it was realised that if no more than eight mosquito larvae were present in a cylinder, the dragonfly would not display such "wasteful killing" (see JOHNSON et al., 1975) and so exhaust its supply of living food. Cylinders were checked daily and the food replenished; therefore it can be assumed that, even before mid-July, the dragonflies never experienced a shortage of food. The head width and length of any larva that had moulted were measured as described by CORBET & HARVEY (1989) and the (larva's) exuvia preserved. The interecdysial stages in the compound eyes

(E.1-E.5) and wing-sheaths (W.1-W.4) (CORBET & PROSSER, 1986) were recorded daily until 7 December and every four days thereafter; but any larva which had reached stage W.2 (indicating the onset of metamorphosis) was examined daily thereafter. When a larva reached stage W.4 it was transferred to a separate container in which it could emerge.

To compare the sizes of larvae in instars F-1, supernumerary F-1 and F-0, the greatest length (base to tip) of the fore wing-sheath was measured to within 0.1 mm by the method used for measuring head width. The head width of all larvae had been recorded routinely immediately after ecdysis but wing-sheath length was measured later, using exuviae which had been preserved for three or four years in 70% alcohol. For this purpose 30 F-1 and 26 F-0 exuviae were chosen at random from material which had been used in these experiments and their dimensions compared with those of eight of the nine exuviae of supernumerary F-1 (one specimen had dried out).

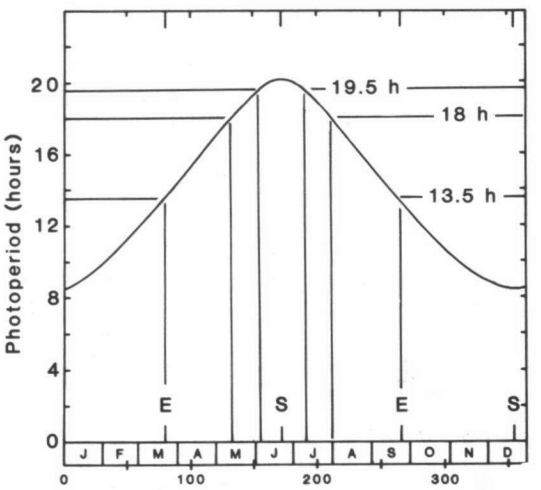

Fig. 1. Dykehead pond. The annual march of photoperiod (expressed as the interval between morning and evening Civil Twilight). The positions are shown of equinoxes (E) and solstices (S) and of the three photoperiods used in experiments.

Experimental design

Larvae were exposed to one of three photoperiods: 13.5 h ("short" (S), corresponding to either equinox); 18 h ("middle" (M), corresponding to 14 May and 30 July); and 19.5 h ("long" (L), corresponding to 5 June and 8 July) (see Fig. 1). Larvae collected up to and including 25 May were exposed to M or S, those collected from 1 to 22 June to all three photoperiods, those collected from 27 June to 14 September to S or L and those collected on 22 September to S. For each collection an attempt was made to place equal numbers of each instar into each experimental treatment. The position of larvae in bowls was randomised.

RESULTS

SERIES 1

Experiments testing the effects of S, M and L photoperiods on larvae collected as F-0, F-1 and F-2 up to the summer solstice (10 May to 22 June).

The three experiments in this series were distinguished according to the instar in which larvae were collected: experiment 1.1 (collected as F-0); 1.2 (F-1); and 1.3 (F-2). Table I summarises the results. In interpreting these results it must be noted that an exceptionally large proportion of the 1983 emergence cohort overwintered during 1982/83 as F-1 or earlier instars (for evidence see CORBET &

Table I

P. nymphula: effects of short (13.5-h), medium (18-h) and long (19.5-h) photoperiods on development of larvae in the last three instars collected up to the summer solstice

Experiment No.	Instar at collection	Collection date	N	Photoperiod treatment S M L	Synopsis of result
1.1	F-0	10.5-22.6	60	+++	All proceeded without delay to, or continued with, metamorphosis. No effect of photoperiod on the rate of development was detected (Fig. 2).
1.2	F-1	10.5-8.6	19	+++	All entered F-0 without delay (within 13 days) except for 2 larvae (exposed to M and L photoperiods) which postponed entry to F-0 for 50 days* and then did not proceed to metamorphosis in 5 and 112 days of life in F-0 (Fig. 3).
1.3	F-2	18.5(2) and 22.6(1)	3	++	All entered F-1 promptly and then spent a very long time (64-109 days)* in F-1.

* Including a supernumarary moult in F-1.

HARVEY (1989)). The F-0 used in experiment 1.1 were obtained only from those that had moulted a few days before collection and that therefore were known to have overwintered as F-1 or earlier instars, namely type (b) (CORBET & HARVEY, 1989). Numbers of larvae available for treatment were small in experiment 1.2 and especially in experiment 1.3. Moreover, because of the unusually high proportion of larvae overwintering in F-1 and possibly F-2 in 1982/83, the numbers available for experiments of this kind will normally be far fewer than were available to us.

EXPERIMENT 1.1 (Fig. 2). — Photoperiod had little, if any, effect on the onset or progress of metamorphosis. The samples of 18 and 25 May and 1 June are large enough to be tested statistically. No significant differences exist between photoperiods in mean interval from collection to attainment of stage W.3 (for 18 May, $t = 1.58$, $df = 8$ and $0.2 > P > 0.1$; for 25 May, $t = 0.24$, $df = 15$ and $0.9 > P > 0.5$; for June, $F = 3.49$, $df = 2, 12$ and $0.1 > P > 0.05$). The decline in mean duration of the interval between collection and the attainment of stage W.3 is due to the fact that larvae collected later were at a more advanced stage of interecdysial development. Stage W.3 is reached about three days after stage W.2 which marks the first visible signs of metamorphosis. This means that, on average, the larvae collected after 8 June (see Fig. 2) had already begun metamorphosis before collection, an

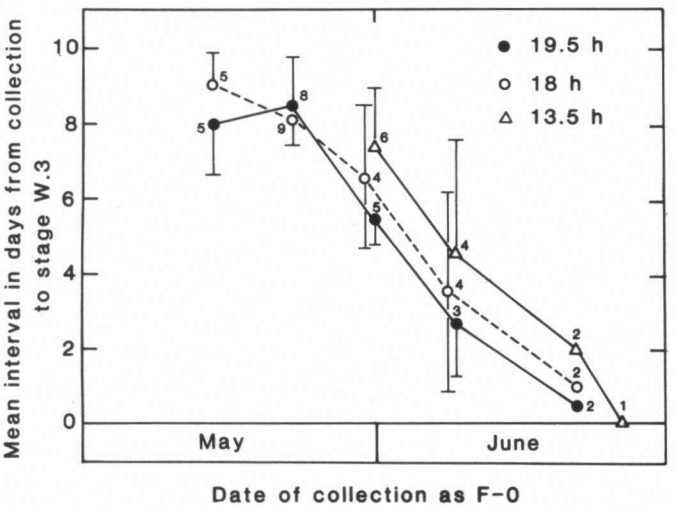

Fig. 2. *P. nymphula.* Effect of photoperiod on the incidence of metamorphosis in larvae collected as F-0 during May and June 1983 (experiment 1.1). Stage W.3 occurs ca three days after the first visible signs of metamorphosis at 20° C; so some or all of the larvae collected after 1 June had already begun metamorphosis when collected. A vertical bar shows 95% confidence limits for the estimate of the mean; a number shows the sample size. — Photoperiods: closed circles — 19.5 h; open circles — 18 h; triangles — 13.5 h.

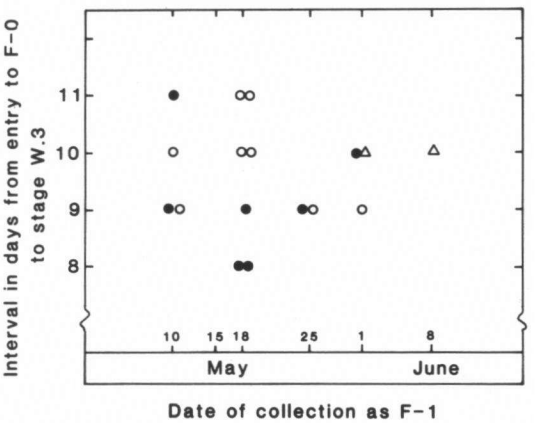

Fig. 3. *P. nymphula.* Effect of photoperiod on the incidence of metamorphosis in larvae collected as F-1 during May and June 1983 (experiment 1.2). All these larvae had overwintered as F-1 and entered F-0 after collection. Stage W.3 occurs ca three days after the first visible signs of metamorphosis at 20° C. Data points represent individual larvae. The only two larvae which showed developmental arrest in F-1 are omitted. — Photoperiods: closed circles — 19.5 h; open circles — 18 h; triangles —13.5 h.

inference which conforms precisely with the finding that by 15 June some of the larvae known to have entered F-0 in spring had begun metamorphosis in Dykehead Pond (CORBET & HARVEY, 1989).

EXPERIMENT 1.2 (Fig. 3). — All but two larvae entered F-0 soon (maximum 13 days) after collection. The two exceptions, collected on 18 and 25 May, one exposed to M and one exposed to L photoperiods, each took 50 days to enter F-0, the larva exposed to M undergoing a supernumerary moult on 15 June to produce an interpolated, extra F-1 instar (see experiment 2.1 below). Neither of these larvae proceeded to metamorphosis during the period (15 and 112 days) for which it subsequently survived in F-0.

EXPERIMENT 1.3 — The three larvae were exposed to M and L photoperiods. All entered F-1 promptly: the two collected on 18 May did so 10 and 20 days later and the one collected on 22 June did so 24 days later. Of the two larvae collected on 18 May, one spent 88 days as F-1, undergoing a supernumerary moult during this time, on 7 June; and the other larva underwent a supernumerary moult on 1 July and then died, still as F-1, on 3 September. The larva collected (as F-2) on 22 June spent 109 days as F-1. So M and L photoperiods prolonged F-1 in larvae collected as F-2.

SERIES 2

Experiments testing the effects of S and L photoperiods on larvae collected as F-1, F-2, F-3 and F-4 after the summer solstice.

The three experiments in this series were designed to measure the following periods: experiment 2.1 — duration of F-1 in larvae collected as F-2, F-3 or F-4; 2.2 — residual duration of F-1 in larvae collected as F-1; and 2.3 — duration of F-2 and F-3 in larvae collected as F-3 and F-4 respectively. Table II summarises the results.

EXPERIMENT 2.1 (Fig. 4). — The duration of F-1 for each collection at each photoperiod is shown in Figure 4, as means up to and including 9 August and as records for individual larvae thereafter. During August, field collections comprised 90-100% F-1 and so very few F-2 and F-3 larvae were available. The results show clearly that L photoperiods greatly prolonged F-1 among larvae collected as F-2, F-3 or F-4, regardless of collection date. The mean durations of F-1 under S and L photoperiods differ significantly for 25 June ($t = 8.95$, $df = 19$, $P < 0.001$), 15 July ($t = 6.94$, $df = 7$, $P < 0.001$), 26 July ($t = 7.35$, $df = 19$, $P < 0.001$) and the remaining dates combined ($t = 3.27$, $df = 12$, $0.01 > P > 0.001$).

Mortality in F-1 was significantly greater under L photoperiods: all of 41 larvae under S photoperiods reached F-0 but 10/34 under L photoperiods died before doing so. Of these, one died two days after collection, one died as F-2 and the remaining eight died after spending a long time as F-1 (Tab. III). Thus the mean duration of F-1 for larvae exposed to L photoperiods as given in Figure 4 is an

Table II

P. nymphula: effects of short (13.5-h) and long (19.5-h) photoperiods on development of larvae collected as instars F-1, F-2 and F-3 after the summer solstice

Experiment No.	Instar at collection	Collection date	N*	Synopsis of result
2.1	F-2 or F-3	25.6-22.9	41(S) 34(L)	Long photoperiods greatly prolong F-1 (Fig. 4) and cause higher mortality in F-1 (Table 3) and cause supernumerary moulting in F-1.
2.2	F-1	15.7-22.9	75(S) 55(L)	Residual duration of F-1 (Fig. 5); - at short photoperiods declines steadily with collection date; - at long photoperiods is much longer than at short photoperiods until late August after which it is the same as at short photoperiods (Table 5); and - at long photoperiods declines steadily with collection date except for an abrupt decrease in late August.
2.3	F-3 or F-4	25.6	F-2: 15(S) 14(L) F-3: 10(S) 10(L)	Photoperiod did not evidently affect the mean duration of F-2 (15.7-17.8 days) or of F-3 (10.8-13.1 days).

* Numbers in short (S) and long (L) photoperiods are listed separately.

underestimate in that it is based only upon larvae that survived to attain F-0.

Some larvae under L photoperiods underwent a supernumerary moult as F-1 (see also experiment 1.3) producing an extra F-1 instar (the duration of which we have included in the total recorded for F-1). Details of the supernumerary moults interpolated in F-1 by the nine individuals that did so are given in Table IV. Such supernumerary F-1 instars can be distinguished unequivocally from normal F-1 or from F-0: although there is a slight overlap of head width (large F-1 overlap with small supernumerary F-1) the wing-sheath lengths lie discretely between those of normal F-1 and those of F-0 (Fig. 5). The range of head widths of normal F-1 is 2.7-3.2 mm and that of supernumerary F-1 is 3.0-3.5 mm. The mean head width of those F-1 larvae which later underwent a supernumerary moult was 2.88 mm (LCL = 2.78, UCL = 2.98, N = 7) and did not differ significantly from that of F-1 larvae (exposed to L photoperiods) which did not undergo such a moult (mean 2.97 mm, LCL = 2.91, UCL = 3.03, N = 17) ($t = 1.80$, $df = 22$, $0.1 > P > 0.05$). A more realistic comparison of mean head width may be made by considering F-1 exposed to S photoperiods and which did not undergo such a moult

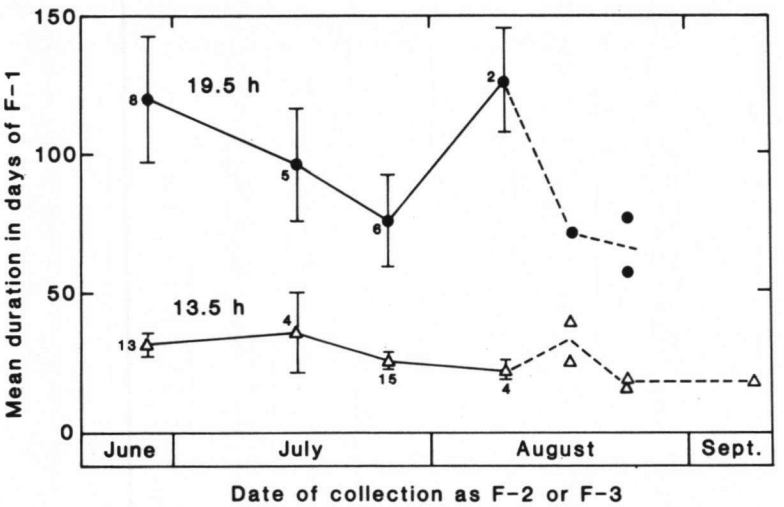

Fig. 4. *P. nymphula.* Effect of photoperiod on the duration of F-1 in larvae collected as F-2 or F-3 during June to September 1983 (experiment 2.1). For each collection up to and including August 9 a vertical bar shows 95% confidence limits for the estimate of the mean and a number the sample size; for collections thereafter data points represent individual larvae. — Photoperiods: closed circles — 19.5 h; triangles — 13.5 h. — Durations recorded under 19.5 h include supernumerary instars.

(mean 2.95 mm, LCL = 2.92, UCL = 2.97, N = 41). Again the mean head width of F-1 larvae which underwent a supernumerary moult is less, but not significantly so, than the mean head width of F-1 larvae which did not undergo such a moult ($t = 1.65$, $df = 46$, $0.1 > P > 0.05$).

In Figure 4 the error bars for L photoperiods are much longer than those for S photoperiods. This represents an increased variance in F-1 duration under L photoperiods which is due in part to the occurrence of supernumerary moults.

EXPERIMENT 2.2 (Fig. 6). — The residual duration of F-1 for each col-

Table III

P. nymphula: mortality among F-1 larvae exposed to a 19.5-h photoperiod (experiment 2.1)

	Date (1983)		
Collection	Entry to F-1	Death	Interval between entry to F-1 and death (days)
	23 July	10 Nov.	110
	29 July	13 Dec.	137
25 June	31 July	10 Sept.	41
	4 August	26 Dec.	144
	13 August	19 Dec.	128
15 July	29 July	18 Dec.	142
26 July	25 August	19 Dec.	116
17 August	25 August	25 Nov.	92

23.5% (8/34) larvae exposed continuously to 19.5 h as F-1 died in that instar. In contrast none of 41 larvae exposed to 13.5 h as F-1 did so.

Table IV

P. nymphula: details of supernumerary moults undertaken by larvae in F-1

Collection		Experiment		Details of moults to:					
				F-1	Supernumerary F-1		F-0	
Date	Instar	Series	Photoperiod	Date	Head width	Date	Head width	Date	Head width
18.5	F-2	1.3	18.0	7.6	3.1	1.7 (died 2.9 as F-1)	3.5	—	—
25.5	F-1	1.2	18.0	—	2.8	15.6	3.0	4.12	3.8
25.6	F-4	2.1	19.5	29.7	2.8	31.8	3.2	28.11	3.8
25.6	F-4	2.1	19.5	31.7	2.9	6.9 (died 10.9 as F-1)	3.2	—	—
15.7	F-2	2.1	19.5	25.7	2.9	13.8	3.2	13.10	3.9
15.7	F-2	2.1	19.5	17.7	3.0	1.9	3.4	23.10	3.8
15.7	F-2	2.1	19.5	17.7	3.0	1.8	3.3	15.11	3.6
9.8	F-2	2.1	19.5	15.8	2.9	5.9	3.3	16.12	3.8
9.8	F-3	2.1	19.5	17.9	2.7	11.10	3.2	23.1*	3.9

All head widths are mm; — all dates are in 1983 except the one marked * which is in 1984.

lection at each of two photoperiods is shown in Figure 6. The steady decline with successive collection date shown by larvae exposed to S photoperiods is consistent with the assumption that for larvae in long-day arrest F-1 has a certain finite duration, progressively more of which has been completed as the season progresses. The results for L photoperiod also show a de-

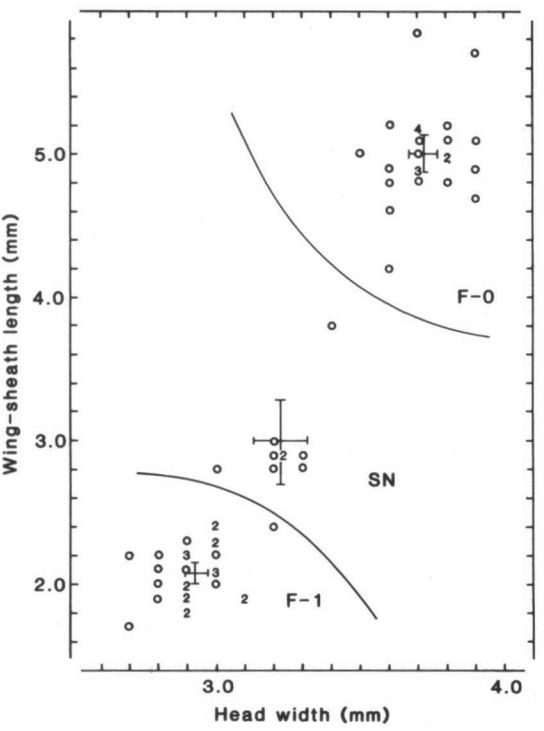

Fig. 5. *P. nymphula.* Comparison between dimensions of three larval instars (sample sizes in brackets): F-1 (N=30), supernumerary F-1, designated SN, (8) and F-1 (26). Vertical and horizontal bars shown 95% confidence limits for estimates of the means. Head widths overlap but wing-sheath lengths do not.

cline in duration but an abrupt discontinuity takes place during late August: before this, the duration under L photoperiods is significantly longer (Tab. V); for larvae collected on or after 24 August the duration is the same under both photoperiods. During the period 17-24 August (natural photoperiod 16.5-15.9 h) an abrupchange occurs in the response of F-1 to L photoperiods.

Table V

P. nymphula: the effect of photoperiod on residual duration of F-1 (experiment 2.2): the significance of differences between mean duration at photoperiods of 19.5-h and 13.5-h according to collection date

Date of collection (1983)	t	df	P
26 July*	7.48	9	$P<0.001$
9 August*	3.13	9	$0.02>P>0.01$
17 August*	3.54	14	$0.01>P>0.001$
24 August	1.03	13	$0.4>P>0.2$
1 September	1.07	18	$0.4>P>0.2$
8 September	1.09	23	$0.4>P>0.2$
14 September	2.01	12	$0.1>P>0.05$

* Dates when differences are significant.

EXPERIMENT 2.3. — Only one collection (25 June) contained sufficient numbers of F-3 or F-4 to provide material for this experiment, a situation likely to be

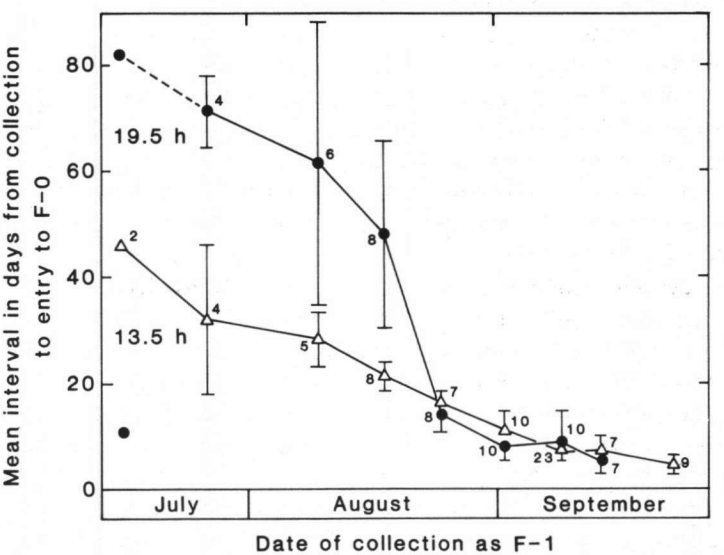

Fig. 6. *P. nymphula.* Effect of photoperiod on the residual duration of F-1 in larvae collected as F-1 during July to September 1983 (experiment 2.2). A vertical bar shows 95% confidence limits for the estimate of the mean and a number the sample size. Two larvae collected on 15 July and exposed to 19.5 h entered F-0 in 11 and 82 days and are shown as separate data points. Photoperiods: closed circles — 19.5 h; triangles 13.5 h. No larva underwent a supernumerary moult as F-1.

habitual because of the seasonal pattern of development of *P. nymphula*. Another difficulty encountered arose from the fact that the exuviae of F-3 and F-4 are small enough to be eaten sometimes by the larvae of *Aedes aegypti* provided as prey. If this occurred the moult would not have been recorded by us. Four larvae (two in each photoperiod) appeared to spend an exceptionally long time in F-3 and examination of their records revealed an unrealistically large increase in head width at the moult to F-2. On the assumption that we had overlooked a moult, we have excluded these four larvae from the analysis. Photoperiods did not evidently affect development rate in F-2 or F-3. The mean duration of F-2 under S or L photoperiods respectively was 15.66 days (LCL = 13.29, UCL = 18.03. N = 15) and 17.79 days (LCL = 15.86, UCL = 19.71, N = 14) for which $t = 1.50$, $df = 27$ and $0.2 > P > 0.1$. The mean duration of F-3 under S and L photoperiods respectively was 13.10 days (LCL = 10.80, UCL = 15.40, N = 10) and 10.80 days (LCL = 8.78, UCL = 12.82, N = 10) for which $t = 1.70$, $df = 18$ and $0.2 > P > 0.1$.

DISCUSSION

Based on the results reported here, we propose that the responses shown by *P. nymphula* contribute to its seasonal regulation in the following way (relevant experiments are cited by numbers in parentheses).

Larvae that enter F-1 in summer before early August are prevented from entering F-0 by long photoperiods (1.2, 2.1, 2.2). Between 17 and 24 August a permissive photoperiod regime (PRG) exists that can allow, or induce, such larvae to enter F-0, even though they may (as F-1) be subsequently exposed in the laboratory to long photoperiods formerly able to prevent entry to F-0 (2.2). This response alone could account for the accumulation of F-1 larvae up to mid--August and the synchronous entry to F-0 in September. Larvae prevented from entering F-0 during summer can be regarded as featuring a "long-day diapause" such as exists in certain other Palaearctic Odonata (NORLING, 1984a).

A brief exposure to the PRG during August must be sufficient to commit larvae in F-1 to enter F-0 because some larvae collected as F-1 on or after 24 August would presumably have spent only a few days in F-1 and yet they then entered F-0 promptly even though exposed to long photoperiods thereafter (2.2). A brief exposure to the PRG in F-2 does not, however, appear to allow entry to F-0 without delay when photoperiods are long; all larvae collected as F-2 and then exposed to 19.5-h photoperiods experienced a prolonged F-1 (2.1).

The PRG shows little variance: only one larva collected on 17 August and exposed to 19.5 h developed rapidly, and no larva developed slowly among those collected on 24 August and then exposed to 19.5 h (2.2; Fig. 6).

The responses of F-1 larvae in spring may be the same as in late summer. The period in spring when the photoperiodic regime corresponds most closely to that between 17 and 24 August is 19-26 April. Larvae that overwinter as F-1 will

experience photoperiods shorter than this while in F-1 and so will be able, or already committed, to enter F-0 in spring when temperature permits, regardless of photoperiod. Long photoperiods in spring will not then be able to induce a long-day diapause. Such larvae accordingly metamorphose and emerge without delay. The conduct of larvae that overwinter as F-2 will depend on temperature in spring: if such larvae develop rapidly enough to enter F-1 before 19-26 April, as F-1 they will experience a "short" photoperiod and so acquire a commitment to enter F-0 without delay. Although we did not test this directly, we conclude that neither the incidence nor rate of metamorphosis in F-0 is influenced significantly by long or increasing photoperiods in spring: in the laboratory larvae began metamorphosis about a week after entry to F-0 (this being close to the minimum possible period) at all three experimental photoperiods (1.2). So we find no evidence that in *P. nymphula* long days induce rapid development, as obtains for several species of Odonata in Sweden (NORLING, 1984a). If however, a larva enters F-1 after the PRG in spring, it will enter long-day diapause and postpone entry to F-0 until the PRG occurs again in August. Thus larvae collected on 18 May as F-2 and then exposed to photoperiods of 18 or 19.5 h entered F-1 promptly and then showed long-day diapause (1.3). Also, whereas most larvae collected as F-1 between 10 and 25 May did not show a diapause in F-1, two larvae did so (1.2): these were collected on 18 and 25 May and so may have entered F-1 in the field after the PRG (19-26 April) in which case they would not have experienced a "short" photoperiod as F-1.

Persistent exposure in F-1 to 18 or 19.5 h causes about 25% of larvae to undergo a supernumerary moult within F-1 (1.2, 1.3, 2.1). Extra moults, often associated with abnormal wing-sheath formation, have been reported in F-1 larvae of two species of *Coenagrion* (SAWCHYN, 1971) and two species of *Enallagma* (INGRAM, 1976; INGRAM & JENNER, 1976) in North America. In these examples supernumerary moults were induced by exposing larvae to a combination of conflicting stimuli: a temperature that induced rapid development and a photoperiod that arrested development. This supports the conclusion that supernumerary moulting is the consequence, sometimes at least, of experimental conditions in which the seasonal march of temperature and photoperiod are uncoupled (INGRAM, 1976). However such moulting has been detected in field populations of *Enallagma aspersum* (Hagen) by INGRAM & JENNER (1976) and of *P. nymphula* by us at Dykehead Pond: on 10 March 1982 one F-1 was collected with wing-sheaths of intermediate length and a head width of 3.3 mm. Until more is known about the incidence of supernumerary moulting in nature it would be premature to infer its adaptive significance. We note, however, that undergoing an extra moult would incur a cost if mortality is increased at ecdysis and that the production of new cuticle would incur metabolic costs. We note also that a benefit would accrue from the production of a larger F-0 larva in as much as larval and adult sizes are strongly correlated; and larger adult males

enjoy enhanced short-term mating success (HARVEY & CORBET, 1985) and larger females probably enjoy enhanced fecundity. So it is possible that a supernumerary moult serves to increase fitness by increasing adult size. There is a suggestion in our records that only small F-1 larvae undergo supernumerary moults (2.1).

So far we have referred to the permissive photoperiod regime (PRG), obtaining between 17 and 24 August (and perhaps also between 19 and 26 April) without inferring the nature of the cues it presents. Responses by insects to absolute photoperiod are well known (see BECK, 1980; DANKS, 1987) but in long-lived insects there are a-priori reasons to expect that changing photoperiod may provide a clue (CORBET, 1956). The PRG at Dykehead Pond features an absolute photoperiod of 15.93-16.52 h; it also features a daily rate of change in photoperiod which, at ca 5 min/day, is close to the annual maximum (Fig. 7). Although it is not necessary to invoke a response to change in photoperiod to account for the seasonal development of *P. nymphula*, we cannot exclude the possibility that at least part of the pattern observed in nature constitutes a response to rate of change of photoperiod as well as to absolute value — a view in accord with that of NORLING (1984a, p. 135) based on his more extensive studies of Swedish Odonata. Here two points should be made. First, the PRG is largely confined to the period 17-24 August whereas the daily rate of change remains high until early November (Fig. 7); so, on this count, the temporal restriction of the PRG is more likely to reflect a response to absolute photoperiod than to either daily increment or rate of daily change. Second, for several reasons, the effects of changing photoperiod are unlikely ever to be rigorously explored: the pattern of seasonal development is such that it can be difficult or impossible to obtain from the field sufficient material in a stage, or with a recent history, appropriate to the needs of an experiment; the design of experiments simulating

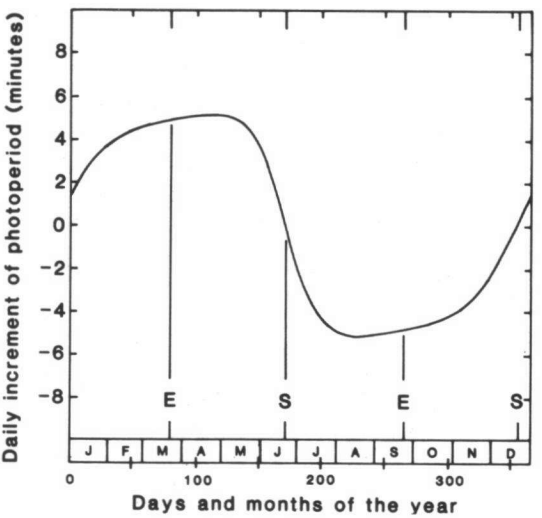

Fig. 7. Dykehead pond. The annual march of daily change in photoperiod (expressed as the interval between morning and evening Civil Twilight) based on data in Fig. 1. The greatest rate of daily change occurs in April and August. The positions are shown of equinoxes (E) and solstices (S).

natural rates of change presents formidable problems (see CORBET, 1956) and to test possibilities thoroughly large numbers of larvae are needed. For the moment, however, we can state with confidence that the responses we have detected could account for the two key phenomena we address in this paper: the synchronised entry to F-0 in early autumn; and the temporal restriction of emergence during spring and early summer. In a future paper we plan to report on the responses of the F-0 larvae which prevent emergence in autumn, and those which bring about the termination of this developmental arrest before the next spring.

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