

**THE LIFE CYCLE AND LARVAL PHOTOPERIODIC RESPONSES OF
COENAGRION HASTULATUM (CHARPENTIER) IN TWO
CLIMATICALLY DIFFERENT AREAS (ZYGOPTERA:
COENAGRIONIDAE)**

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The life-cycle of *C. hastulatum* was studied in southern (58° 42'N) and northern (67° 50'N) Sweden. In the southern area, the life cycle duration was 1-2 yr. The univoltine and semivoltine larvae separated as cohorts already before the first winter. During the second summer, the semivoltine cohort continued to develop slowly, while larvae that had overwintered in the last 3 instars, i.e. the univoltine cohort and older semivoltine larvae, rapidly proceeded to emergence, which mainly took place in June. Egg development was direct. In the northern area, the life-cycle duration was 3-4 yr, and almost all larvae overwintered in the final instar before emergence. A very low frequency of penultimate instars in the overwintering population seemed to be generated during the late part of the third summer by a cohort splitting suggestive of the one in the southern area. Experiments performed at different photoperiods partly explained the life-cycle pattern. Larvae overwintering below a certain critical size, associated with the frequency minimum produced by the cohort splitting, showed a slow development at subsequent long-day photoperiods, in particular in the penultimate instar. Larvae overwintering above the critical size rapidly proceeded to emergence under the same conditions. A 19.3 hr daylength produced a long-day response in the southern population only. The shorter days of September (southern population) produced a burst of moulting activity before a hibernation diapause, present in at least the 3 last instars, was induced. The winter critical size was concluded not to be a result of the previous cohort splitting, which was assumed to be under separate environmental control. The early cohort separation may serve to reduce intraspecific competition and to add further refinement to seasonal regulation.

INTRODUCTION

Despite the great interest in dragonfly seasonality created by the early papers of CORBET (e.g. 1956, 1962), which for the first time demonstrated a decisive influence of photoperiod on larval development in Odonata, few studies on seasonal regulation at higher latitudes have been carried out (e.g. NORLING, 1971, 1976). In particular, the knowledge of populations living in subarctic, or high boreal areas is poor. The present paper is part of a survey of intraspecific variation in the larval growth and photoperiodic responses of dragonflies at high latitudes, carried out to illuminate how different species adapt their life cycles to different conditions of development, both within a small area and between climatically different areas, i.e. southern and northern Sweden.

Coenagrion hastulatum is one of the most successful damselflies in the northern part of the boreal spruce forest area, and it is often the dominant coenagrionid species in small bodies of water throughout Sweden.

MATERIAL AND METHODS

Study sites

The principal study sites in southern Sweden were two barely separated, about 7 m wide pools (A and B) in the large open *Sphagnum* bog Fjällmossen, 58° 42'N, 16° 31'E, 84 m above mean sea level, about 20 km ENE Norrköping.

The vegetation, composed of *Sphagnum*, *Utricularia*, *Eriophorum* and low *Carex*, was mainly restricted to the edges. The bottom consisted of soft naked peat. Maximum depth in spring was approximately 1.5 m in pool A and 0.7 m in pool B. During the summer the level of both the water and the bog surface sank, and pool B could partially dry up. Pool A was studied during 1972 and 1973, pool B during 1972 only. Sampling was normally confined to the vegetation and, if possible, took place at 2-3 week intervals during the growth season. Temperature records were taken in pool A during 1973 (cf. NORLING, 1976, fig. 1).

In northern Sweden, three water bodies in the Sappisaasi area, 67° 50'N, 21° 40'E, 15-22 km north of Vittangi and some 140 km north of the Arctic Circle, were examined. Except at site 3, sampling was restricted to three occasions during 1972.

Site 1. — An approx. 25 m wide pool in the large mixed-bog area Ripakaisenvuoma; 335 m amsl. Sampling took place in floating *Carex-Menyanthes* mats and in other vegetation (mainly moss, *Carex*, *Comarum*, *Menyanthes* and *Sparganium*) down to 0.8 m depth. The invertebrate fauna appeared to be unusually rich for the area.

Site 2. An approx. 50 m wide, possibly deep lake between the low peaks of the Sappisaasivaara mountain; 400 m amsl. The lake is surrounded by boggy areas, and a coarse *Betula-Picea* forest grows on the adjacent slopes. The sampling was performed at the edge in *Menyanthes-Scorpidium* mats with *Carex* and *Eriophorum*, and at the open water in large *Carex* and *Potamogeton natans* down to about 1 m depth. The lake appeared to be partly spring-fed.

Site 3. An approx. 100 m wide, very shallow lake at one end of a bog, situated in a flat area with coarse *Betula-Picea* forest; 375 m amsl. Sampling took place in floating patches of low *Carex* and *Eriophorum*. The depth of the lake beside the patches was about 0.5 m.

Daylengths and mean air temperatures in the two study areas are shown in NORLING (1984a).

Collection methods and measurements

The bulk of the samples were obtained with a net (diameter about 0.3 m) with 1x1 mm mesh size. During 1972, the contents of this net were mostly placed on a plastic sheet for rapid examination, which was effective only for later instars (cf. NORLING, 1971, where this method was the only one used). To also obtain samples of the early, often extremely abundant instars, restricted and careful collection with a small net (diameter 120 mm, mesh size 0.2x0.7 mm) capable of retaining all instars, took place before the main collection with the large one. The contents of the small net were thoroughly washed out in wide plastic trays. Sometimes part of the contents from the larger net was also searched in this manner.

During the 1973 study of pool A, examination on a plastic sheet was largely replaced by washing in water through sieves with 20, 3 and 1 mm quadratic mesh sizes, which permitted an easier separation of larvae and plant debris, and which increased the yield of smaller larvae. However, the small net, with which too much plant debris and peat could be avoided, still was the principal source of the earliest instars. This year particular care was taken to find the smallest larvae in each sample taken with the small net.

The collecting and searching methods produced a strong bias towards the later instars, but could still provide a good record of the presence of or absence of early instars. However, the time available at the sites was often a limiting factor for the detection of small larvae.

Larvae that could be identified in the field, generally those with a head width above 1.7 mm, were usually measured in the field and subsequently returned to their habitat. Earlier instars were preserved in 80% alcohol for later examination. The head width of the last three instars were most easily and accurately measured in a wedge-shaped groove, as described by NORLING (1971). Smaller, live larvae, which could not be handled accurately enough for this method, were placed upon a soft, moist piece of cellulose sponge and measured with a magnifying-glass of 10x magnification supplied with a scale. Preserved material was measured using an ocular grid in a dissection microscope. Repeated cross-checking showed a good accordance between the different methods, including the effects of preservation. Even in larger specimens the differences rarely reached 0.05 mm.

In the later instars, the stage of development within the instar was estimated by means of observations of eye development (cf. ELLER, 1963; NORLING, 1971). The larva was observed from the front with 15-20x magnification, and the position of the pigmented anterior eye rim between the eye apex (0) and the base of the antenna (1.0) was estimated. The final (F) instar was subdivided into seven phases, the last of which were defined by wing and labium development: 1: eye rim position (ERP) ≤ 0.62 ; — 2: $0.62 < \text{ERP} \leq 0.7$; — 3: $0.7 < \text{ERP} \leq 0.8$, the space between the black eye rim and the antenna turns reddish; — 4: eye brownish or black up to the antenna, costal rib still straight; — 5: costal rib corrugated, labium without visible signs of histolysis (15-20x magnification); — 6: histolysis and retraction of the tissues of the prementum; — 7: prementum empty, costal rib with dark spots.

The F-1 (final minus one, penultimate) instar to the F-3 instar were subdivided into four phases: 1: $\text{ERP} < 0.58$; — 2: $0.58 \leq \text{ERP} < 0.66$; — 3: $0.66 \leq \text{ERP} \leq 0.7$; — 4: $\text{ERP} > 0.7$.

The lowest ERP value was usually 0.5, somewhat higher in the F instar, and the ERP value just before ecdysis (excepting the F instar) varied between little more than 0.7 up to 0.8.

Species separation

The shape of the prementum and the gill morphology were used for identification of the *C. hastulatum* larvae (cf. ANDER, 1926; PUUKKINEN, 1925). Very young larvae are difficult, or impossible, to distinguish from those of many other coenagrionid species. However, at the southern study sites (Fjällmossen) a most different species, *Lestes sponsa* (Hans.), was the only other

regularly breeding zygopteran, and during the study only two larvae could be identified as other coenagrionids. Therefore, all unidentified coenagrionid specimens, i.e. larvae with a head width below about 1 mm, could be treated as *C. hastulatum*.

In the northern area, other species were more troublesome. *C. armatum* (Charp.), not previously recorded from these latitudes, and *concinnum* (Johanss.) could not be distinguished from *hastulatum* up to a head width of about 1 mm. Larvae of *C. lunulatum* (Charp.), which are more similar to those of *hastulatum*, might even have been mistaken for the latter species up to a head width of 2 mm. However, the size distribution of the identified non-*hastulatum* *Coenagrion* specimens ($n = 50$) does not suggest that the results are seriously affected by regarding all young and unidentified larvae as *hastulatum*.

Experiments

Larvae were collected from the northern localities on 16-17 Sept. 1972, from the southern area on 12 Oct. 1972, 4, 17 and 31 Aug. 1973, 26 and 29 Oct. 1973, and 2 May 1974. The material from Fjällmossen was often supplemented with material from other sites within a distance of 30 km.

The 4 Aug. to 31 Aug. collections were kept at approximately natural temperatures and photoperiod (incl. Civil Twilight) until the start of the experiments; all other material was immediately stored at 0-4° C and occasionally interrupted darkness (cf. also NORLING, 1984a). Acclimation to experimental temperatures was allowed to take 5 hours or more. Experiments on both populations were performed at 20° C and LD 13:11 (13 hr light: 11 hr dark), LD 19.3:4.7 and LL (continuous light). The southern population was also examined at LD 16:8, 20° C and LD 13:11 and 19.3:4.7 at 15° C. The procedure and equipment are described elsewhere (NORLING, 1976, 1984a).

Diapause

Diapause is here used in a wide sense: a prolongation of development, or reduction in growth rate, that is not directly caused by adverse conditions (cf. BECK, 1980). Diapause usually serves to prepare the larvae for adverse conditions, e.g. winter, but also seems to regulate development for other purposes. In Odonata, there is often a continuous transition from a slight prolongation of development to a protracted, almost total arrest (e.g. NORLING, 1971). In this paper, an ecdysis interval approximately twice that at the maximum rate of development is arbitrarily chosen as a limit for the use of the term. At 20° C, this limit is about 30 days for the F instar and 15-20 days for earlier instars.

RESULTS

THE LIFE-CYCLE OF THE SOUTHERN POPULATION

Emergence started during the last days of May and probably continued to the end of June (Fig. 1, upper graphs). After oviposition, which mainly took place in June and early July, egg development was direct, and larvae of the next generation started to appear in the July samples. During 1973, the best documented year (Fig. 1c), hatching probably started during the very last days of June and continued at least throughout July. The hatching peak appeared to be situated in the early part of the period.

During subsequent development, the larvae gradually separated into two

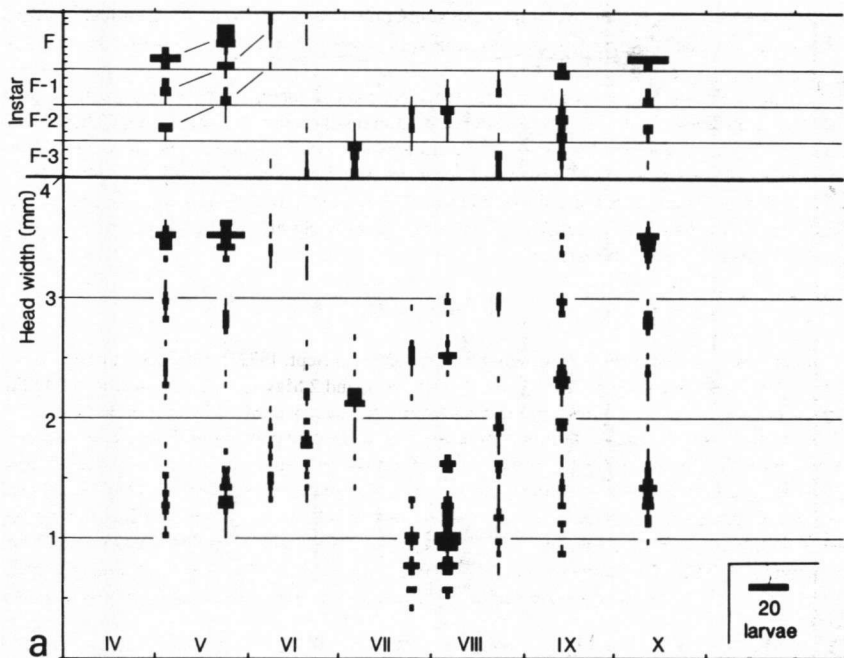


Fig. 1. Larval development of *C. hastulatum* at the study sites in southern Sweden, 58° 42'N. The months from April to October are shown with roman numerals. — (a) Pool A, 1972. — (b) Pool B, 1972. — (c) Pool A, 1973. — Lower part: Kite diagrams showing head width frequency distributions in different instars. The size-classes are 0.05 mm. In (c) the frequency peaks, which represent different instars, are assigned instar numbers, with the prolarval instar counted as the first. The incipient separation of the univoltine and semivoltine cohorts in the 1973 year-class is approximately indicated by a broken line. For reasons of space, this year-class is shown in a scale reduced to 50% in the 11 July to 4 August samples. — Upper part: Graphic presentation of the development in the last four instars, showing the distribution of larvae among the arbitrary instar subunits (phases) on the different dates. The ordinate shows the approximate relative duration of each instar, and each phase in the F instar, at 20° C and nondiapaused development. In the F-3 to F-1 instars the phases are shown equal, but their true relative duration is uncertain and varies between specimens. Each larva is represented by the same area as in the lower part, except when the scale is reduced there. A few larvae, where the instar assignment is most uncertain, have been omitted.

cohorts with different growth rates, as previously demonstrated for the related *C. puella* (L.) in Britain (PARR, 1970). In the present material the process of separation was revealed in greater detail than before. The fast-growing group appeared to hatch earlier than the slow-growing one, and the separation was produced by the combined effect of a difference in the size increase per ecdysis and a relatively slight difference in ecdysis intervals (Fig. 1c). When similar larval sizes are compared, the observed difference in growth rate between the cohorts is,

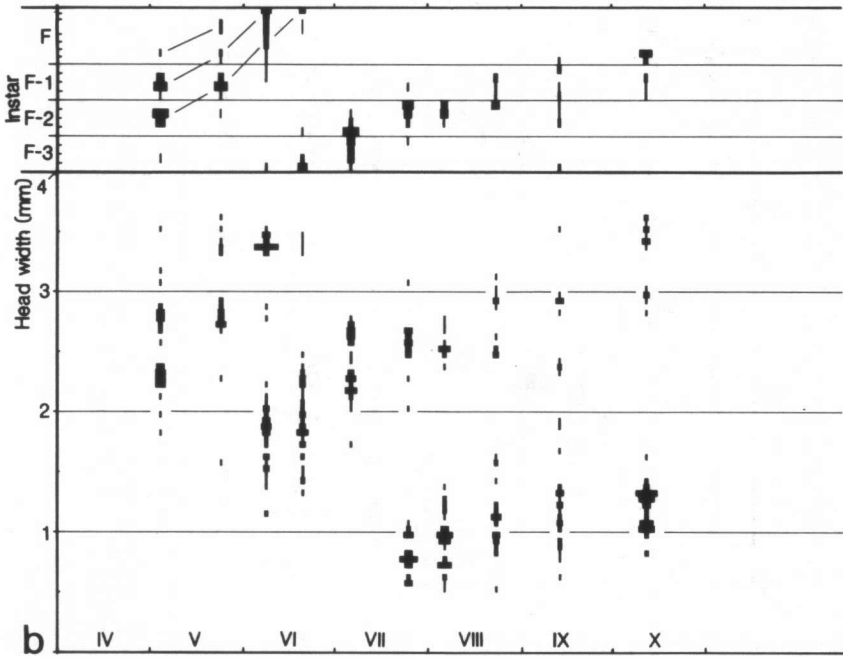


Fig. 1. (Continuation) — (b)

however, accentuated by temperature differences. As seen in NORLING (1976, fig. 1), the water temperature began to decrease about 1 July.

The last ecdyses for the season took place near the shift September-October. The fast-growing group spent the winter in the F-2 (9th) and F-1 (10th) instars, in 1973 probably also in the F (11th) instar. The larvae in the slow-growing group did not usually develop further than the 8th instar before winter. These 8th instars were as large as the 7th instar of the fast-growing group.

After overwintering, ecdyses started about mid-May. Larvae that had spent the winter in the last three, or possibly four instars, containing the whole fast-growing group, showed rapid development and emerged soon. The fast-growing group is thus univoltine, and it finishes larval development after 11 instars. The slow-growing group continued development at a reduced rate, which was particularly evident in the F-1 instar. The F instar was reached during September, and development halted in an early phase of the instar (diapause). After the second overwintering, the remaining development was rapid, and these semivoltine specimens were among the first to emerge during the season. The total number of instars required to complete development was probably predominantly 13, a figure corroborated during the experimental work.

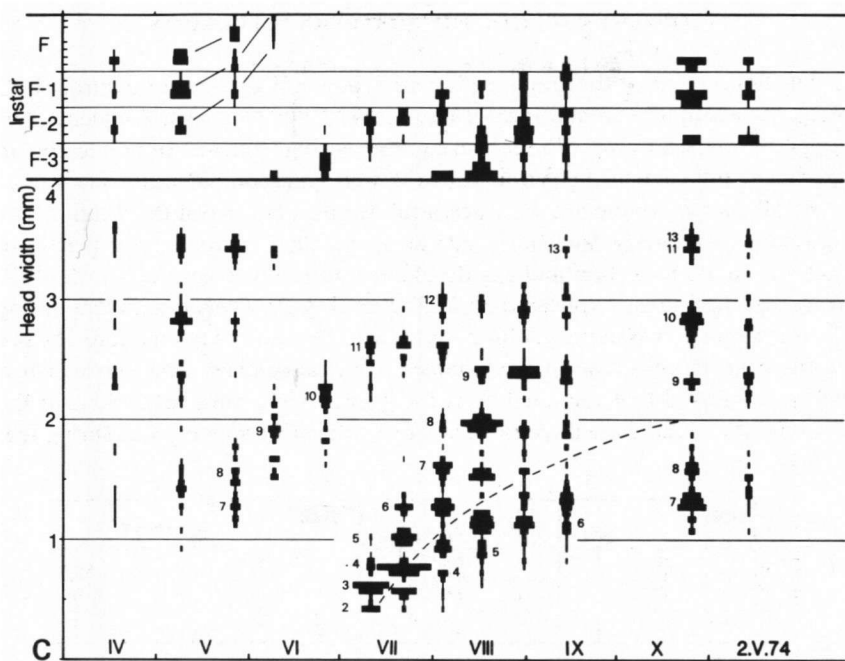


Fig. 1. (Continuation) — (c)

The development in pool A during 1972 was similar to that during 1973 (Fig. 1a). However, in pool B, the proportion of fast-growing, univoltine larvae, in the 1972 year-class was smaller than in pool A, and the slow-growing, semivoltine group grew even slower than in pool A. In this year most of the *Carex-Eriophorum* patches, the preferred habitat for *C. hastulatum* larvae, dried up in pool B during July. This forced many larvae out into *Sphagnum* moss and probably also into naked peat, both of which are assumed to be less suitable habitats. A decline in growth rate at this time was also noted among the semivoltine larvae from the preceding year.

Some material of *C. hastulatum* from other localities suggested that different mixtures of one and two year development, as described above, are a common feature in southern Sweden. In the southernmost part of the country, univoltinism seemed to dominate, and the winter was mostly spent in the F-I instar. A two year development appeared to dominate at a site some 30 km W Fjällmossen, and here a massive overwintering in the F instar took place.

THE LIFE-CYCLE OF THE NORTHERN POPULATION

The upper parts of the graphs in Figure 2 suggest that emergence during 1972 started at about the summer solstice at site 1 and 3, but later at site 2, which can be supposed to warm up slower during the spring. Furthermore, nearly all specimens must have emerged in an early, well synchronized peak.

At all sites reproduction was successful during 1972, when the weather was unusually hot during late June and the first half of July, i.e. the period of maturation, reproduction and egg development. The new generation, the 1972 year-class, had already appeared on 21-22 July (Fig. 2). The development during the first summer was more synchronous than in the southern population. The size of these northern larvae in the samples from September, when growth had halted, suggested that the conditions for growth were most favourable at the shallow site 3, and least favourable at site 2, which presumably was spring-fed.

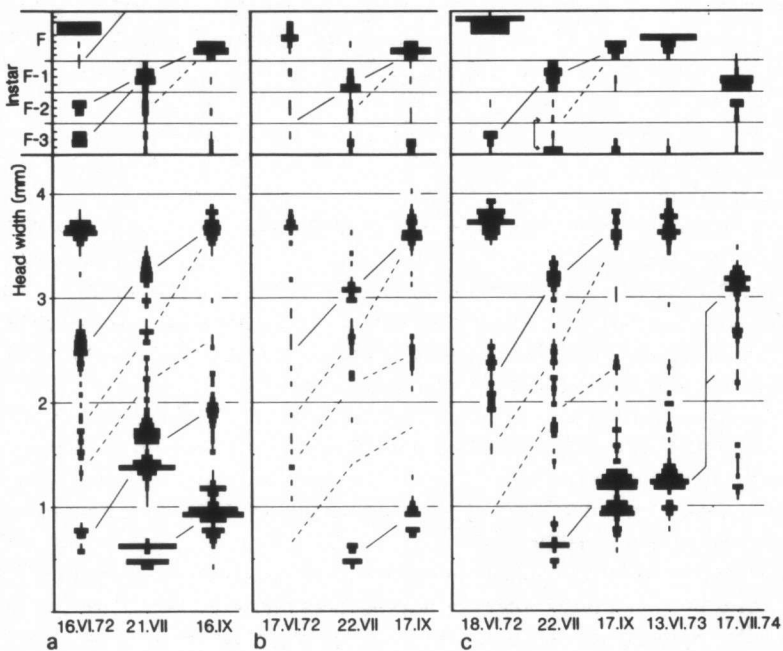


Fig. 2. Larval development of *C. hastulatum* at the northern study sites, 67° 50'N. The graphs are prepared as in Fig. 1. The different sampling dates are shown at the bottom. — (a) Site 1. (b) Site 2. — (c) Site 3. The main, reasonably certain development is indicated by solid lines, and the more uncertain interpretations are shown with broken lines. In (b), also the hypothetical development of the missing 1971 year-class is shown with a broken line. The instar assignment in the upper part is uncertain.

In the June samples, the preceding (1971) year-class was found only at site 1. The small size of these presumably overwintered larvae reflects the relatively cool summer of 1971. At site 3, the 1971 year-class showed up later, but it was less conspicuous than at site 1. At the cool site 2, reproduction appeared to have failed completely during 1971. During the summer of 1972, the 1971 year-class generally reached a head width somewhere around 2 mm. Precisely what happened with larvae that were at, or just below this size at the beginning of 1972 was not readily apparent, and it probably varied. It appeared that they either reached the F instar, or a stage with a head width about 2.5 mm (cf. below).

The winter before emergence was almost exclusively spent in the F instar, the size of which was distinctly greater than in the southern population. The data from site 1 suggest that also the few larvae that had overwintered in the F-1 instar emerged during the summer, but later than the main group. At the time of the June sample, two such larvae were in the process of entering the F instar. Larvae in the preceding instars were not yet ready for ecdysis at this time, and on 21 July they were accumulating in the F-1 instar. This pattern of accumulation was similar at all three sites, and the entry into the F instar probably started at the end of July, at least at site 1 and 3. On the average, the F instars spent the winter in a slightly more advanced state than in the southern population.

The remarkably low frequency of overwintering larvae in the F-1 instar appeared to arise from a cohort splitting during late summer, because no corresponding frequency minimum was present in the June or July samples.

These results suggest a dominating 3 year life-cycle at site 3, which appears to be corroborated by the July 1974 sample. At site 1 and 2, the life-cycle duration apparently was 3-4 or 4 years. The size distribution of the June sample from site 1 is distinctly quadrimodal, and the September sample from site 2 would probably have been, if the missing 1971 year-class had been present. The life-cycle duration certainly varies between different year-classes.

PHOTOPERIODIC RESPONSES OF THE SOUTHERN POPULATION

When larvae collected during the cold season and stored at low temperatures were subjected to LD 19.3:4.7 or LL at 20° C, the development was initially similar to that observed during spring and summer in the field (Figs 3, 4; Tabs I, II). With few exceptions, larvae overwintering in the last three instars proceeded rapidly to emergence, while larvae in the young semivoltine group showed a greatly delayed development. The prolongation of development appeared to occur in all instars present during the experiments, but was most distinct in the F-1 instar (Tab. I). In other words, the size difference between the semivoltine and univoltine cohorts during their first winter separated two different types of response to these long-day photoperiods.

Compared to the above photoperiods, LD 16:8 slightly, or moderately,

prolonged the development of the larvae in the last three instar groups (here: larvae that had overwintered in the last three instars). This effect was particularly noticeable in the overwintering F-1 instars (Fig. 4). On the other hand, the

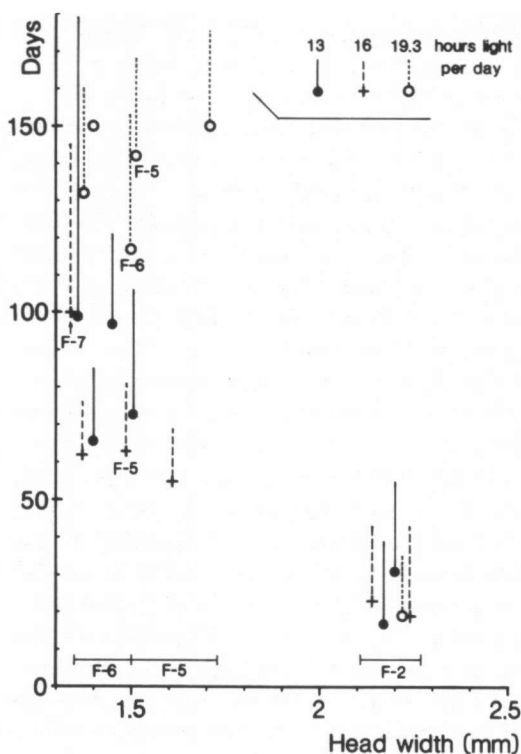


Fig. 3. Southern population. The effect of photoperiod and overwintering size on the time to entry into the F instar, and the duration of the latter, in an experiment started on 7 May 1974. The experimental temperature was 20°C. The symbols indicate the time of entry into the F instar at different photoperiods, the photophases of which are shown in the legend. The duration of the F instar, if recorded, is shown by the vertical line above each symbol. The head width of each larva at the beginning of the experiment is found on the abscissa. The instar in which the experiment was begun, reflecting the number of ecdyses that was required to reach the F instar during the experiment, is shown for size-groups and for some individual larvae.

development of the young semivoltine larvae was distinctly more rapid than at the long-day photoperiods, and it could even be close to the maximum rate observed in any larvae at any conditions at 20°C.

Also at LD 13:11, many specimens in the last three instar groups still showed an insignificant, or moderate, prolongation of development, but a stronger delay could occur, as in the 5 Dec. 1972 experiment (Fig. 4) and in overwintering F instars (Tab. II). The rate of development of young semivoltine larvae was not as greatly retarded as at long-day photoperiods, but it was usually slower than at LD 16:8 (Figs 3, 4). A distinct, short-day induced hibernation diapause of long duration was mostly absent in these experiments, but a weak diapause state appeared to occur in all examined instars at LD 13:11 (Tab. I). A comparison between the 5 Dec. 1972 and 7 May 1974 experiments reveals seasonal differences in the intensity of the responses.

Occasionally, larvae in the F-2 instar group could, like the semivoltine group, enter slow development at long-day photoperiods. On these occasions, a supplementary instar was added to the development. This

Table I

Southern population. Total duration in days of different instars in experiments on overwintering small (semivoltine) larvae at 20° C and different photoperiods. Durations of instars earlier than F-2 (head width always < 2.5 mm) are pooled. (N = number of measured durations, for the pooled data of earlier instars also the number of larvae. Divergent single values and some minimum values are shown separately in parentheses)

	Date of experiment	LD 13:11			N	LD 16:8			N	LD 19.3:4.7			N	LL			N
		Mean ± SD	Range			Mean ± SD	Range			Mean ± SD	Range			Mean ± SD	Range		
< F-2, < 2.5 mm	5 Dec. 72	16.2 ± 3.7	(9-25)	18/9	—	—	—	—	—	32.3 ± 8.8	(14-46)	15/6	—	25.1 ± 5.9	(17-41)	16/6	—
	30 Nov. 73	—	—	—	—	8.7 ± 0.6	(8-9)	3/2	—	—	—	—	—	—	—	—	—
	7 May 74	13.2 ± 3.6	(10-22)	10/4	—	12.0 ± 2.9	(8-18)	11/4	—	18.0 ± 2.9	(14-23)	13/5	—	—	—	—	—
F-2	5 Dec. 72	24.0 ± 5.5	(18-33)	7	—	—	—	—	—	25.3 ± 4.6	(20-30)	4	—	32.8 ± 3.7	(28-37)	4	—
	30 Nov. 73	—	—	—	—	8.0 ± 1.4	(7-9)	2	—	—	—	—	—	—	—	—	—
	7 May 74	16.0 ± 2.0	(14-18)	3	—	11.5 ± 3.5	(8-15)	4	—	20.3 ± 3.2	(18-25)	4	—	—	—	—	—
F-1	5 Dec. 72	42.5 ± 17.5	(25-64)	4	—	—	—	—	—	> 45-63	—	4)	—	43	—	—	1
	30 Nov. 73	(≥ 47-54)	—	2)	—	9.5 ± 0.7	(9-10)	2	—	—	—	—	—	> 42-59	—	—	2)
	7 May 74	23.7 ± 12.5	(11-36)	3	—	17.5 ± 9.1	(12-31)	4	—	60.3 ± 17.7	(39-80)	4	—	—	—	—	—
F	5 Dec. 72	32	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—
	30 Nov. 73	(≥ 22)	—	1)	—	15.0 ± 0.0	(15)	2	—	—	—	—	—	—	—	—	—
	7 May 74	25.3 ± 7.1	(19-33)	3	—	15.3 ± 2.3	(14-18)	3	—	29.0 ± 4.8	(26-36)	4	—	—	—	—	—
		(80)	—	1)	—	(45)	—	1)	—	—	—	—	—	—	—	—	—

situation did not occur at LD 19.3:4.7, 20° C (n = 9; only the F instar was prolonged in one larva), but at LL, 20° C (one of four) or at LD 19.3:4.7, 15° C (one of five). In an experiment at LD 19.5:4.5 and slowly rising temperatures (8° C on 27 March to 20° C on 12 June, 1971; cf. NORLING, 1984b) two "F-2" instar specimens of *C. hastulatum* from the vicinity of Lund (55° 40'N) were present. Both reacted with a strong diapause and two supplementary instars.

These experiments on "overwintering" larvae suggest that the different rates of development in the univoltine and semivoltine cohorts after the first winter are not predetermined. As in *Aeshna* (NORLING, 1971, 1984b), the critical size in the overwintering population, i.e., the "winter critical size", which separates individuals later showing two different rates of summer development, is not absolutely fixed: Extreme long-day photoperiods and low temperatures probably increase the winter critical size into the range of the univoltine cohort, and an intermediate photoperiod, such as LD 16:8, allows a relatively rapid development to emergence even in the "semivoltine" cohort.

The temperature-induced difference in the rates of nondiapause development observed between 15° and 20° C (Tab. II) was always near a factor of two, as in *Aeshna viridis* (Eversm.) and *Leucorrhinia dubia* (Vander L.) (NORLING, 1971, 1976, 1984a), which suggests a lower temperature threshold for development near 10° C in the examined instars. On the other hand, WARINGER (1982), who examined the influence of temperature on growth in *C. puella*, found little difference in the duration of the last 4 instars in the range 12-28° C; however the instar durations were long and his results probably apply to diapausing larvae.

The temporal separation of emergence between larvae overwintering in the last

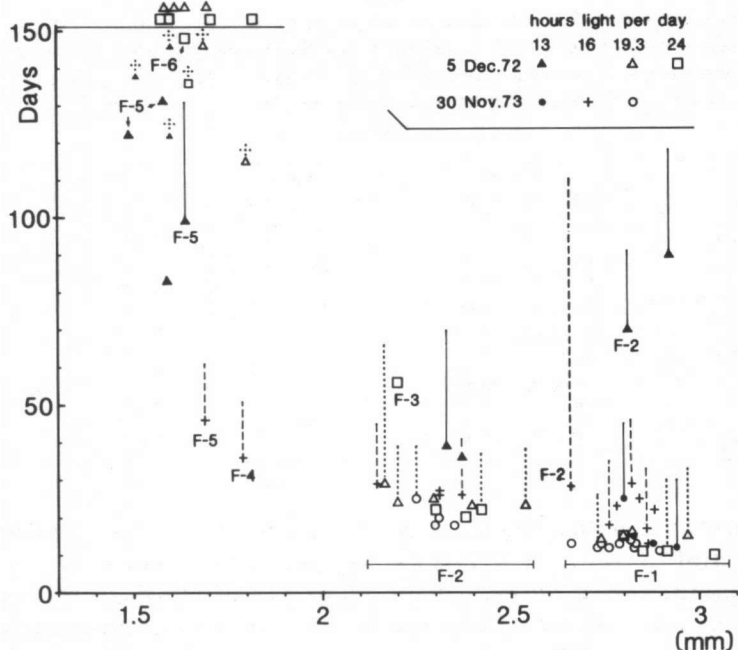


Fig. 4. Southern population. Same type of diagram as in Fig. 3, but based on different experiments (see legend). For reasons of space, the duration of the F instar is shown only for part of the material. Larvae that did not reach the F instar during the 5 Dec. 1972 experiment are shown with symbols above a horizontal line at 151 days, which represents the end of the experiment. The death of larvae in instars earlier than F during the later part of that experiment are shown with small symbols and five dots. After 25 days, the 30 Nov. 1973 experiment was disturbed by a fault in the automatic switches, causing continuous light for one or two days.

three instars seemed, at long-day photoperiods, to be simply related to the normal duration of the involved instars at rapid development. However, the small variation in ocular development in the F instar groups at the beginning of the experiments (cf. Fig. 1) was not found to be well correlated with the variation in the time to emergence.

Experiments initiated during August and early September (20° C only) demonstrated photoperiodic responses which were appropriate for this time of the year. Already in the first and largest of these experiments, begun on 6 Aug. 1973, the responses to LD 13:11 closely resembled what happened in the field later on. The larvae in the F-1 instar (old semivoltine) entered the F instar synchronously after 21-26 days ($n = 14$), whereafter a strong diapause was induced. The F instar duration was 102.3 ± 15.9 days ($\bar{X} \pm SD$; range 72-122; $n = 8$). If the larvae were transferred to LD 19.3:4.7 in close connection with the ecdysis, the diapause was soon terminated and the F instar duration was 23-27

Table II

Southern population. Effect of temperature and photoperiod on larvae overwintering in the last three instars: total duration of the F-I and F instars and time to emergence for larvae overwintering in the F instar (days). Experiment started on 30 Nov. 1973. (Values shown as in Table I). An asterisk (*) indicates values for diapausing larvae exhibiting supplementary moulting. Cf. also note in

Figure 4

		LD 13:11			LD 16:8			LD 19.3:4.7		
		Mean \pm SD	Range	N	Mean \pm SD	Range	N	Mean \pm SD	Range	N
20° C	F-I	—			12.0 \pm 0.8 (19*)	(11-13) 1)	4	8.5 \pm 1.3	(7-10)	4
	F	19.7 \pm 1.5	(18-21)	3	17.1 \pm 1.7 (82*)	(15-21) 1)	9	14.6 \pm 0.7	(13-15)	11
	To emergence from F	32.0 \pm 12.1	(21-45)	3	20.4 \pm 3.0	(17-25)	5	17.0 \pm 0.0	(17)	4
	F-I	—			—			16.8 \pm 1.0 (67*)	(16-18)	4 1)
15° C	F	32.7 \pm 1.5 (80)	(31-34)	3	—			27.8 \pm 1.8 (39; 50*)	(25-32)	11 2)
	To emergence from F	49.5 \pm 14.7	(35-68)	4	—			34.0 \pm 0.8	(33-35)	4
	F-I	—			—			—		

days ($n = 7$). If the transfer took place 5-12 days before the ecdysis, the diapause was averted and the F instar duration was only 17-20 days ($n = 5$).

Larvae that were in earlier instars on 6 August (two F-4 and two F-3 from the univoltine cohort, nine F-2 from the old semivoltine cohort) moulted within 16 days. Specimens moulting within 14 days also moulted a second time before entering diapause, which took place 21-29 days after 6 August ($n = 12$). The moulting interval was particularly short (12-16 days, $n = 6$) in larvae which performed their first ecdysis 7-14 days after the start of the experiment, when probably no lingering long-day influence was present. Including Civil Twilight, the daylength on 6 August is about 18 hours in the southern area (cf. NORLING, 1976). The exact instar duration of diapausing F-2 ($n = 2$) and F-1 ($n = 3$) larvae was not recorded, but it exceeded 50 days. For the smallest instars in this experiment it appeared that the premature short-day treatment produced a hibernation diapause in an earlier instar than would have occurred in the field.

Overall, the last ecdysis before diapause was remarkably well synchronized at LD 13:11, and took place after 21-26 days ($n = 25$), except in two larvae, which entered the diapause instar after 16 and 29 days, respectively. In a smaller experiment, started on 19 August, the results fitted entirely into the above pattern both at LD 13:11 (four F-2, three F-1) and LD 16:8 (two F-2, one F-1).

In the 6 August experiment, the responses to LD 19.3:4.7 were as follows. Of seven larvae in the F-I instar, two entered the F instar after 18 days, and emerged after a further 15 days. The other five postponed their entry into the F instar until after 31-41 days, and thus displayed a long-day diapause. The duration of the F instar in these larvae was almost the same as that for long-day diapausing larvae in the 7 May 1974 experiment (Tab. I): 31.3 ± 4.4 (27-37) days ($n = 4$). Evidently a

strong long-day diapause is absent in the F instar.

Larvae in earlier instars (one "F-4", one "F-3", two F-2) moulted after 7-16 days and thereafter displayed a long-day diapause. The two youngest larvae also made one supplementary moult each. The LD 19.3:4.7 group from 19 August (four F-2, three F-1) did not differ significantly from this pattern.

In the 2 September experiment all larvae at LD 13:11 (four F-2, seven F-1) moulted within 2-11 days and then entered the hibernation diapause. At LD 16:8 (three F-2, six F-1) the larvae moulted within 1-14 days, but two of the F-2 larvae moulted once more before entering diapause. At LD 19.3:4.7 (four F-2, six F-1) ecdysis took place within 1-10 days, but all larvae except one (F-2) now showed rapid development as in the winter experiments.

The August-September experiments show that LD 13:11 induces a distinct hibernation diapause in at least the last three instars at this time of the year. Before this occurs, any previously induced long-day diapause is terminated, and a delay in the onset of the hibernation diapause can permit some rapid development. The hibernation diapause can be terminated by long-day photoperiods. Also LD 16:8 shows these short-day effects at this time, but it probably permits a longer period of rapid development before the onset of the hibernation diapause. In late instar larvae, LD 19.3:4.7 generally produces an immediate diapause response in early August, but barely a month later the response has changed almost completely to a stimulation of development.

PHOTOPERIODIC RESPONSES OF THE NORTHERN POPULATION

Only two experiments were performed with material from this population, both of them during the winter of 1972-73 (Fig. 5; Tab. III). At LL, 20° C, which can be considered as roughly normal summer conditions in the northern study area (NORLING, 1984a), the winter critical size for later rapid development was similar to that observed in the field, i.e. just below the F-1 instar, or below a head width of 2.9 mm. Larvae that were below this size at the beginning of the experiments developed slowly at LL, and even the largest of them (head width about 2.6 mm) developed as F-3 instars despite their size. Also in this population the F-1 instar was responsible for a considerable part of the prolongation of development, but the intensity of the long-day diapauses was generally less than in the southern population, except perhaps in the F instar.

LD 19.3:4.7 did not act as a long-day photoperiod in these experiments. As far as can be seen in the two specimens tested, the development of the F-1 instar group was slowed down significantly. Larvae smaller than this developed more rapidly at LD 19.3:4.7, and to some extent at LD 13:11, than at LL. The effects of LD 19.3:4.7 on the northern population can be compared with that of LD 16:8 on the southern population.

Diapause-like phenomena were recorded in all examined pre-final instars at all

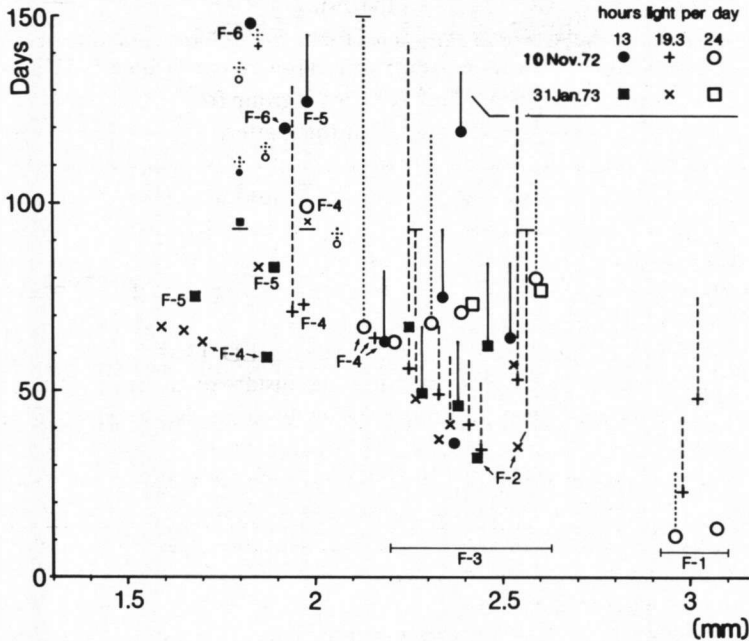


Fig. 5. Northern population. Same type of diagram as in Figs 3 and 4. A short horizontal bar below a small symbol, or at the end of an F instar line, indicates the end of an experiment.

tested photoperiods. However, the responses of the F instar were somewhat intriguing. No diapause ever occurred at LD 13:11 when the F instar was reached at this photoperiod. At LD 19.3:4.7 five out of the seven larvae in the F-3 instar group that reached the F instar within 50 days showed no diapause, whereas, as far as could be controlled, the remainder of these larvae showed a fairly intense diapause. At LL an F instar diapause appeared to be present in at least two larvae.

In overwintering F instars, as in the southern population, there was an increasing delay of the emergence with shorter daylengths and at LD 13:11 in the 10 Nov. 1972 experiment the response was in the range of diapause as defined here (p. 432). As in the southern population, the range in ocular development at the start of the experiments (cf. Fig. 2) showed only a slight correlation with the observed variation in that time to emergence.

In overwintering F instar larvae, and in the earliest of the examined instars, development was more rapid in the latest of the two experiments than in the earliest one (Tab. III), suggesting a promoting effect on subsequent development of long exposure to low temperatures. However, the total durations of the F-2 to F instars in the experiments did not appear to show these seasonal differences.

Table III

Northern population. Total duration of different instars in experiments on larvae overwintering in instars earlier than F-I, and time to emergence for larvae overwintering in the F instar (days). Temperature 20° C. (Values shown as in Table I)

	Date of experiment	LD 13:11				LD 19.3:4.7				LL		
		Mean \pm SD	Range	N		Mean \pm SD	Range	N		Mean \pm SD	Range	N
< F-2, < 2.5 mm	10 Nov. 72	28.8 \pm 7.2	(17-39)	9/6		16.5 \pm 3.8	(11-23)	8/6		22.3 \pm 7.6	(11-32)	8/5
	31 Jan. 73	16.3 \pm 5.9 (46)	(10-26)	6/4 1/1)		13.3 \pm 5.6	(9-24)	8/5		16		1
F-2	10 Nov. 72					14.6 \pm 4.0	(9-25)	18		17.8 \pm 4.0	(12-26)	9
	31 Jan. 73	13.0 \pm 2.4	(10-18)	15		(65)		1)				
F-I	10 Nov. 72					17.4 \pm 5.2	(8-31)	17		36.8 \pm 6.7	(28-48)	8
	31 Jan. 73	19.6 \pm 6.4	(9-30)	16								
F	10 Nov. 72					17.4 \pm 0.9	(16-18)	5*		38.0 \pm 17.0	(26-50)	2
	31 Jan. 73	18.7 \pm 2.2	(16-23)	12		73.0 \pm 13.0	(60-86)	3**		(> 112)		1)
To emer- gence from F	10 Nov. 72					19.4 \pm 3.6	(15-25)	5		17.2 \pm 0.8	(16-18)	5
	31 Jan. 73	35.2 \pm 16.8	(21-61)	6		(39)		1)				
		16.8 \pm 1.7	(14-19)	10		15.5 \pm 1.4	(14-18)	10		13.9 \pm 0.7	(13-15)	7
		(55)		1)		(28)		1)		(21)		1)

* The F instar was reached within 50 days from the start of the experiments in all of these larvae.

** Observations at the end of the experiments showed that an additional 8 larvae were in diapause.

DISCUSSION

The backbone of the seasonal regulation of *C. hastulatum* is provided by the same two-phase photoperiodic reaction as in many other odonate species: Autumn emergence is prevented by a long-day induced prolongation of development during late summer. After exposure to the short days and low temperatures of the autumn and winter, which suppress development, the long days of the spring promote development, and emergence can take place (CORBET, 1980; NORLING, 1984b)

A surprisingly good simulation of the natural summer development was achieved when "overwintering" larvae of *C. hastulatum* were suddenly subjected to constant artificial summer conditions, i.e. favourable temperatures and long-day photoperiods, as previously demonstrated also for *Aeshna viridis* and *Leucorrhinia dubia* (NORLING, 1971, 1976, 1984a). Individuals, that had overwintered above a certain genetically and, to some extent, environmentally determined critical size, were stimulated to rapid development by the long days, and they soon emerged. At this time, rapid development was further enhanced by the previous exposure to low temperatures, and even short-day photoperiods often did no more than slightly delay emergence. Larvae which had overwintered below the critical size showed the long-day induced prolongation of development, which culminated in the F-I instar.

The late-summer experiments with the southern population explained the

course of events during August-September. The long-day diapause in the F-I instar is terminated by the shortening days, the F instar is reached synchronously, and a short-day induced hibernation diapause virtually halts further development. Less advanced larvae, in particular those of the younger, univoltine cohort, show some relatively rapid development during the period of intermediate and short days immediately preceding the initiation of the hibernation diapause. The univoltine cohort seems to escape a noticeable long-day diapause because the most sensitive instars are not reached until very late in the summer, when the days are of intermediate length. The high sensitivity to short days and the great intensity of the short-day diapause in the late summer/early autumn experiments, as compared to the winter experiments, may be governed by the long previous exposure to long days and favourable temperatures.

The winter critical size is a key factor in the seasonal regulation of a population, because it ultimately determines the least advanced stage in which the winter before emergence can be spent (NORLING, 1984b). The exact emergence period is largely determined by the range of developmental stages above the critical size that is present during this winter, and the time it takes to reach emergence from each of these stages under natural conditions, i.e. the rate of development. When the last winter is spent in several instars, as in the southern population of *C. hastulatum*, an increased synchronization of emergence can be achieved by a system of rising lower temperature thresholds for development in the different instars (CORBET, 1957; LUTZ, 1968) or by different diapause characteristics of these instars (ELLER, 1963). However, no such mechanisms were detected in *C. hastulatum* (cf. Fig. 1). An early and relatively well synchronized emergence appeared to be achieved principally by means of a high rate of development.

The winter responses at LD 16:8, 20° C (Fig. 4) might suggest that some synchronization occurs between larvae that overwinter in the F-2 and F-1 instars. It is, however, uncertain whether these responses can contribute to synchronization in the field. LD 16:8 is the natural photoperiod at mid-April (Civil Twilight included in the photophase), a time when it is too cold for any development to occur (cf. NORLING, 1976, fig. 1). Moreover, larvae overwintering in the F instar are not included in these synchronization responses. SAWCHYN (1972) reported similar experimental results with larvae of *C. angulatum* Walker at LD 12:12, 16.5° C.

Most of the differences in development and photoperiodic responses found between the two populations of *C. hastulatum* can directly be related to climatic differences between the two study areas (cf. NORLING, 1984a, for more detailed data on climate). The difference in voltinism is a natural consequence of the much shorter and, on the average, cooler summers in the northern area, and the extremely long daylengths necessary for a long-day response in the northern population are related to the extreme photoperiodic regime which this

population is adapted to. In the northern area, there is continuous light during most of the summer, and LD 19.3:4.7, which does not act as a long-day photoperiod in the northern population, bears the same relationship to air temperature during late summer-autumn as LD 14:10 in the southern area.

The short, cool summers of the north also restrict the time available for maturation, reproduction, egg development and the very first part of larval development (the prolarva, possibly the second instar), which probably must be completed before winter conditions can be survived. The end of the emergence period in the southern population does not appear to be determined solely by climatical factors, but that in the northern population probably is. An extremely early emergence is needed in order to allow the above sequence to be completed before winter (cf. the more detailed discussion in NORLING, 1984a). Properties of the northern population related to this requirement are the large winter critical size and maybe the slightly more advanced state of development of the overwintering F instars.

The relatively weak long-day diapause in the northern population is also connected with the short summers. A lower diapause intensity is sufficient to prevent autumn emergence in the northern area, and the larvae must not be prevented to reach an advanced stage before winter, if the emergence is to take place early during the following summer. Similar population differences between these two areas have also been reported in *Leucorrhinia dubia* (NORLING, 1984a).

One of the most intriguing aspects of the life-cycle of *C. hastulatum* is the early splitting of the recently hatched larvae in the southern population into two cohorts with different growth rates. PARR (1970) showed this phenomenon in *C. puella*, and my own preliminary observations indicate that *C. pulchellum* (Vander L.) in southern Sweden is similar to *hastulatum* in this respect. While this distinct pattern is not universally recorded in the genus, it may still be widespread in appropriate climatic zones. A mixed 1-2 year development has also been reported by LAWTON (1972) for *C. puella* in Britain, BAKER & CLIFFORD (1981) for *C. resolutum* (Hag.) in Alberta, Canada (53° 31'N) and KHARITONOV (1977) for *C. hastulatum* in the Siberian taiga. In the first of these papers, the cohort splitting is present, but appears somewhat less distinct than previously reported for *C. puella*. In *C. resolutum* a real split even appears to be absent during the first year; however, the mode of presentation of the data, with instar assignments based on head width peaks derived from part of the material, might conceal a frequency minimum. KHARITONOV (1977) reports a pattern of development in *C. hastulatum* different from that reported here, with a small number of semivoltine larvae and the univoltine larvae mainly overwintering in the F instar. The winter critical size is below the F-I instar. No cohort splitting was mentioned, but might have been present as some larvae hibernated in "earlier instars (than F-I)".

In the southern population studied in Sweden, the difference in growth rate between the cohorts during the second summer can be entirely explained by the position of the winter critical size in the gap between the cohorts, and the experimental results indicate that it is not a predetermined continuation of the developmental rates from the previous year. The only experiment related to the development during the first summer shows that the univoltine cohort displays the same kind of responses to photoperiod during late summer as the older, semivoltine group of larvae. However, there are no experimental results related to the separation of the cohorts during the first summer.

The remarkably distinct difference between the cohorts concerning the size increase per ecdysis appears to be too large and abrupt to be explained entirely by a continuous variation like the one producing different numbers of instars in *Aeshna cyanea* (Müll.) (SCHALLER, 1960). Because the ratio between the number of specimens in each cohort appeared to be affected by environmental differences between adjacent pools (cf. Fig. 1a and b), the cohort splitting is probably not genetically predetermined. Although both genetical variation, and environmental factors acting during the egg stage (SCHALLER & MOUZE, 1970) certainly are important in this variation in development, the final split can tentatively be assumed to be the result of overriding environmental factors acting during the larval stage, producing responses which anticipate and refine the more definite separation which is tied to the winter critical size during the spring.

Late hatching specimens encounter a somewhat different environment as regards temperature and photoperiod than the earlier hatching ones. Specific responses to these factors may thus be involved in the cohort splitting; for example, different responses to shorter days in different sizes could affect the final stages of the process and the exact location of the winter frequency minimum (cf. NORLING, 1984b). During early larval life, the food situation, together with temperature, is probably of greater relative importance. Both with and without a specific response, food is likely to affect the early phase of the cohort splitting: LAWTON et al. (1980) showed that food shortage could both increase the ecdysis intervals and reduce the size increase per ecdysis in larvae of *Ischnura elegans* (Vander L.). At high population densities, late hatching specimens of *C. hastulatum* could be affected in this manner by competition for food or feeding sites with the high number of somewhat older larvae, which may have some initial competitive advantage. This would at least be expected to increase the variation in rate of growth and size increase per ecdysis, but unless a specific response is involved somewhere, a more continuous variation than that observed would be expected.

An early cohort separation may have the advantage of reducing later competition between the cohorts. The semivoltine cohort can perform more of its growth than otherwise during a time when univoltine larvae are either absent or very different in size, and the univoltine cohort can benefit from reduced

competition during the crucial time before winter when an overwintering stage above the critical size has to be reached, and during spring before emergence.

In the northern area there are indications of a somewhat similar cohort splitting during the third summer, which bears the same temporal relationship to emergence as does the first summer in the southern population. The peak of overwintering larvae with a head width around 2.5 mm (the F-3 and F-2 instars) may have been produced by a late accumulation in this size while most F-1 larvae entered the F instar. The frequency minimum thus produced is associated with the winter critical size, as the one in the southern population. The position of the winter frequency minimum in the northern population just above the critical size has an effect on phenology comparable to that of an increase in critical size. A higher true winter critical size (i.e. one above the F-1 instar) may be desirable in the population, but it may be impracticable in this species if the long-day diapause in the F instar is not intense enough to prevent attempts at autumn emergence when this instar is reached during the early part of the season (Tab. III).

A cohort splitting of a similar type, producing a low frequency of overwintering larvae near the critical size, and adding further refinement to seasonal regulation, seems to be present also in *Leucorrhinia dubia* (NORLING, 1984a). It is possible that this kind of cohort splitting is a widespread phenomenon in the seasonal regulation of phenologically early species with an average life-cycle duration exceeding one year (cf. also NORLING, 1984b).

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REFERENCES

- ANDER, K., 1926. Beiträge zur Kenntnis der schwedischen Odonaten. I. Beschreibung der Nympe von *Agrion hastulatum* Charp. 2. Über die Nymphen von *Agrion lunulatum* Charp. und *armatum* Charp. *Ent. Tidskr.* 47: 31-42.
- BAKER, R.L. & H.F. CLIFFORD, 1981. Life cycles and food of *Coenagrion resolutum* (Coenagrionidae: Odonata) and *Lestes disjunctus* (Lestidae: Odonata) populations from the boreal forest of Alberta, Canada. *Aquatic Insects* 3: 179-191.
- BECK, S.D., 1980. *Insect photoperiodism*. Second edition. Academic Press, New York.
- CORBET, P.S., 1956. Environmental factors influencing the induction and termination of diapause in the Emperor Dragonfly, *Anax imperator* Leach (Odonata: Aeshnidae). *J. exp. Biol.* 33: 1-14.
- CORBET, P.S., 1957. The life-histories of two summer species of dragonfly (Odonata: Coenagrionidae). *Proc. zool. Soc. Lond.* 128: 403-418.

- CORBET, P.S., 1962. *A biology of dragonflies*. Witherby, London.
- CORBET, P.S., 1980. Biology of Odonata. *A. Rev. Ent.* 25: 189-217.
- ELLER, J.G., 1963. *Seasonal regulation in Pachydiplax longipennis (Burmeister) (Odonata: Libellulinae)* PhD thesis, Univ. North Carolina, Chapel Hill, NC.
- KHARITONOV, A. Yu., 1977. Life cycles of some species of dragonflies in eastern Priuralje. *Izv. sib. Otdel. Akad. Nauk SSSR (Biol.)* 1977 (5): 55-60. (Russ.)
- LAWTON, J.H., 1972. Sex ratios in Odonata larvae, with particular reference to the Zygoptera. *Odonatologica* 1: 209-219.
- LAWTON, J.H., B.A. THOMPSON & D.J. THOMPSON, 1980. The effects of prey density on survival and growth of damselfly larvae. *Ecol. Ent.* 5: 39-51.
- LUTZ, P.E., 1968. Effects of temperature and photoperiod on larval development in *Lestes curinus* (Odonata: Lestidae). *Ecology* 49: 637-644.
- NORLING, U., 1971. The life history and seasonal regulation of *Aeshna viridis* Eversm. in southern Sweden (Odonata). *Odonatologica scand.* 2: 170-190.
- NORLING, U., 1976. Seasonal regulation in *Leucorrhinia dubia* (Vander Linden) (Anisoptera: Libellulidae). *Odonatologica* 5: 245-263.
- NORLING, U., 1984a. Photoperiodic control of larval development in *Leucorrhinia dubia* (Vander Linden) (Anisoptera: Libellulidae): A comparison between populations from northern and southern Sweden. *Odonatologica* 13(4). (In press).
- NORLING, U., 1984b. Life-history patterns in the northern expansion of dragonflies. *Adv. Odonatol* 2. (In press).
- PARR, M.J., 1970. The life histories of *Ischnura elegans* (Vander Linden) and *Coenagrion puella* (L.) (Odonata) in South Lancashire. *Proc. R. ent. Soc. Lond. (A)* 45: 172-181.
- PULKKINEN, A., 1925. Über die Larven einiger Odonaten. 1. *Agrion hastulatum* Charp. 2. *Agrion concinnum* Johanss. *Notul. entomol.* 5: 111-114.
- SAWCHYN, W.W., 1972. *Environmental controls in the seasonal succession and synchronization of development in some pond species of damselflies (Odonata: Zygoptera)*. PhD thesis, Univ. Saskatchewan, Saskatoon.
- SCHALLER, F., 1960. Etude du développement postembryonnaire d'*Aeschna cyanea* Müll. *Ann. Sci. nat. (Zool.)* 2: 751-868.
- SCHALLER, F. & M. MOUZE, 1970. Effet des conditions thermiques agissant durant l'embryogenèse sur le nombre et la durée des stades larvaires d'*Aeschna mixta* (Odon. Aeschnidae). *Annls Soc. ent. Fr. (NS)* 6: 339-346.
- WARINGER, J., 1982. *Der Einfluss der Wassertemperatur auf die Dauer der Embryonal- und Larvalentwicklung von Coenagrion puella L. aus einem Tümpel bei Herzogenburg (N.Ö.)*. Diss., Univ. Wien.