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DIAGNOSIS OF INTERECDYSIAL DEVELOPMENT IN FINAL-INSTAR LARVAE OF *PYRRHOSOMA NYMPHULA* (SULZER) (ZYGOPTERA: COENAGRIONIDAE)

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Changes are described in the appearance of final-instar larvae of *P. nymphula* which indicate, respectively: recent entry to the instar; developmental stages that precede the onset of metamorphosis; and apolysis before emergence. Approximate durations of some stages are given.

INTRODUCTION

Since the detailed observations by GRIEVE (1937) on *Ischnura verticalis* (Say) and by STRAUB (1943) on *Aeshna cyanea* (Müll.), it has been recognized that externally visible changes, mainly in the compound eyes, wing-sheaths and labium, can be used to monitor interecdysial development in the final larval instar (F-0) of Odonata. Such changes, especially those involving the compound eyes, have been described for several species of Anisoptera, in which they have been used to monitor the onset and progress of metamorphosis, and thus to predict the time of emergence (CORBET, 1957; SCHALLER, 1960; MIYAKAWA, 1969; BULIMAR, 1971; HASSAN, 1977) and to characterize pre-metamorphosis stages of development, especially in regard to the onset of diapause (ELLER, 1963) and to the morphological stage in which F-0 larvae overwinter (NORLING, 1971, 1976, 1984a). Although these changes have recently been described for *Coenagrion hastulatum* (Charp.) by NORLING (1984b) they are less readily discerned and measured in Zygoptera, partly because the facetted area of the compound eye does not extend so far mesially during metamorphosis as in Anisoptera (cf. SHERK, 1978). Our aim in this study was to identify in the coenagrionid, *Pyrrhosoma nymphula,* characters which could be used quickly

and incisively to monitor interecdysial development during studies of its seasonal development.

MATERIAL AND METHODS

Larvae of *P. nymphula* were collected in the penultimate (F-1) or final (F-0) instar from Dykehead Pond, Angus (56°44'N, 3°1'W) and placed at 20 ± 1° or 23 ± 1°C under photoperiods of 6, 13.5, 19.5 or 20 h in the laboratory. They were fed daily *ad libitum* on instar-3 larvae of the mosquito, *Aedes aegypti* (L.). Each dragonfly larva was segregated throughout and inspected regularly, usually every four days until stage W.2 and daily thereafter. During inspection (under a Nikon SM-2 stereoscopic microscope at x 10 and sometimes at x 20) the living larva was beneath the water surface in a petri dish and provided with a short piece of drinking straw which it clasped and so remained immobile — even under bright illumination. Larvae were collected at several times of year. Those collected in October (when F-0 larvae are usually in diapause) were induced to proceed with normal morphogenesis by exposing them to a photoperiod of 20 h (at 23° C). Where appropriate, a value (TTE) is recorded for the interval in days between the onset of a stage and emergence. Only minimum intervals are recorded for stages E.1-E.4, in which development can be suspended during winter or during diapause; these values were derived from larvae in which diapause had been artificially averted in the manner described and so would apply also to larvae metamorphosing without delay in spring. For two stages (E.5, E.6) data were available for very few individuals; so the interval cannot be expressed quantitatively.

RESULTS

Changes were distinguished in the external appearance of the head (compound eyes, ocelli, prementum of labium, frons and vertex) and wing-sheaths. These changes are described in the order in which they occur after entry to F-0.

EYES

Stage E.1 (Fig. 1). — An ill-defined dark band lies obliquely and anteroposteriorly across the facetted area of the compound eye about ⅘ of the distance between its lateral and mesial boundaries when viewed dorsally. At the mesial boundary of the facetted area there is no brownish-black band with distinct lateral and mesial margins. In five larvae examined daily after the moult to F-0, this stage lasted until 2-8 days (average 4.0) after ecdysis at 20° C and a photoperiod of 13.5 h. In 100 larvae examined every four days under the same conditions, the cumulative percentages that had completed this stage (i.e. entered E.2) by 4, 8, 12 and 18 days after ecdysis were 33, 72, 98 and 100 respectively. In our experience this stage is always transitory and so can be used to recognize in field collections larvae that have recently moulted to F-0.

Stage E.2 (Fig. 2). — At the mesial boundary of the facetted area there is a narrow brownish-black band with distinct margins, laterally and mesially. The ill-defined dark band that characterized E.1 is no longer visible. E.2 can last

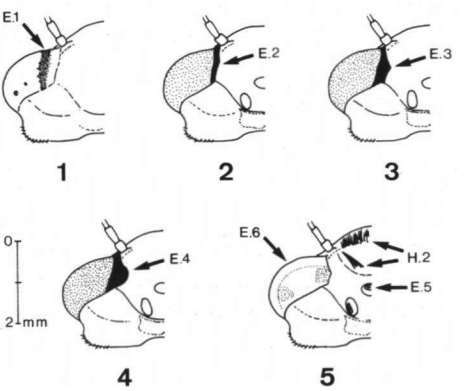
throughout the winter in the field. TTE at 23°C: 17 (N=1).

Stage E.3 (Fig. 3). — The brownish-black band that marks the onset of E.2 has begun to extend mesially but has not yet achieved a smoothly curved mesial boundary. Larvae in E.3 are found in the field at the beginning and end of winter; so it is likely, though not certain, that some larvae may overwinter in this stage. TTE at 23°C: 13(3), 14(1).

Stage E.4 (Fig. 4). — The brownish-black extension that characterized E. 3 has reached its full extent, its mesial boundary now being evenly rounded. Some larvae spend the winter in E.4. In larvae developing without delay from E.2, E.4 is first reached 4-5 days after the first signs of E.3. E.4 is always reached before the onset of metamorphosis. TTE at 23°C: 9(3), 10(2).

Stage E.5 (Fig. 5). — The ocelli, which hitherto (except in E.1) have appeared as pale, oval patches on the dorsum of the head, contract to become crescent--shaped. This contraction takes at most 2 days and occurs between stages W.3 and W.4 (*q.v.*).

Stage E.6 (Fig. 5). — The mesial part of each compound eye becomes opalescent and pale grey, and the mesial boundary no longer appears smoothly curved, probably because the cuticle of the pharate adult has become separated from that of the larva. It occurs at about the same time as stage H.2 (*q.v.*).



Figs 1-5. *Pyrrhosoma nymphula*: left side of head in dorsal view to show stages in the development of the eyes (E.1-E.6) and frons and vertex (H.2). For details see text.

WING-SHEATHS

Stage W.1 (Fig. 6). — The thorax and both pairs of wing--sheaths (WS) are unswollen. The fore (mesothoracic) WS are overlain and hidden distally in dorsal view by the hind (metathoracic) WS. Stage W.1 lasts from ecdysis to F-0 until the onset of externally visible metamorphosis (W.2).

Stage W.2 (Fig. 7). — The thorax and both pairs of WS have started to swell so that the distal ends of the fore WS are now visible when viewed dorsally. The onset of W.2 provides the first external sign that metamorphosis has begun. TTE at 20°C: median 13.6, range 12-17 (N=17).

Stage W.3 (Fig. 8). — The costal vein of the adult wing first becomes visible, folded concertina-like within the WS. Because the onset of W.3 is unequivocal, this stage is the most useful indicator for comparing the times at which metamorphosis begins in laboratory experiments. In spring at 20°C larvae developing

without delay reach this stage 8-11 days after entry to F-0 (CORBET et al., unpublished) thus completing the instar in about 18-21 days. TTE at 20°C: median 10.4 range 9-12 (N=17).

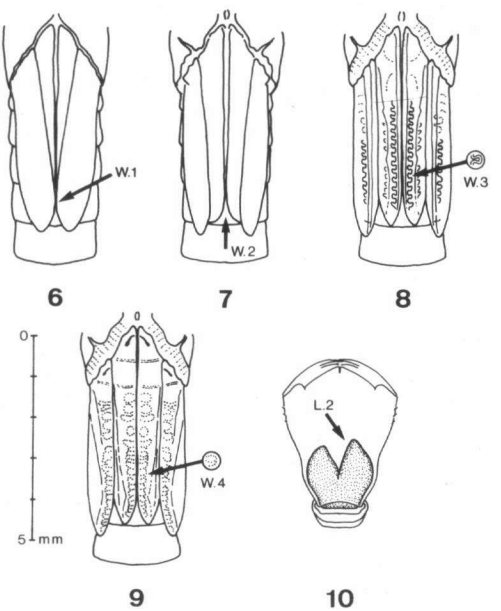
Stage W.4 (Fig. 9). — Under x 20 (and usually x 10) magnification the microtrichia on the adult wing are visible as distinct black spots within the WS along the costal border. The onset of W.4 also is unequivocal. TTE at 20°C: median 3.7 range 2-5 (N=17).

LABIUM

Stage L.1. — There is no sign of shrinkage of tissues within the prementum. The labium remains functional.

Stage L.2 (Fig. 10). — Tissues of the developing adult labium are visible within the prementum of the larva and are separated from the distal margin of the prementum. L.2 is associated with cessation of feeding, which in captive larvae can provide a guide to its onset. TTE at 20°C: median 3.8, range 2-8 (N=24).

Stage L.3. — Passages of the tissues through the prementum has been completed. TTE at 20°C: median 3.3, range 1-3 (N=13).



Figs 6-10. *Pyrrhosoma nymphula:* part of thorax and abdomen viewed dorsally (Figs 6-9) to show stages in the development of the wing-sheaths (W.1-W.4) and labium viewed ventrally (Fig. 10) to show shrinkage of the developing adult tissues within the prementum (L.2). For details see text.

HEAD

Stage H.1. — Dark setae on the frons and vertex of the pharate adult are not yet visible.

Stage H.2 (Fig. 5). — Such setae are now visible. TTE at 20°C: median 2.7, range 1-4 (N=14).

ABDOMEN

In most, though not all, individuals the abdominal dorsum became reddish

shortly before emergence — as noted by GARDNER & MacNEILL (1950). Surprisingly, this change occurred in some individuals up to 31 days before entering W.2; in some it occurred a day or so before emergence; and in others it was not detectable at all. So it is clearly not useful as an indication that emergence is imminent.

DISCUSSION

Emergence in captivity occurs over a range of several days among individuals which, to judge from their conspicuously pharate condition and by the performance of those that emerge promptly, are morphologically ready to emerge. The reasons for this variability (i.e. postponement of emergence) under conditions which appear to be identical are unknown. Partly because of the uncertainty associated with finding suitable emergence sites, one may expect this variability to be even greater in nature (cf. CORBET, 1957, tab. 9); so the durations of stages listed here must be regarded as conservative when applied to the field.

At present the interecdysial stages described here are of value to investigators of Zygoptera in several respects. For studies in nature, the stages can be used: (1) to characterize and thus compare the developmental stages in which populations of F-0 larvae overwinter; (2) to detect the onset of metamorphosis (stage W.2); and (3) to recognize larvae that have recently moulted to F-0 (stage E.1). For studies in the laboratory, the stages can be used: (1) to trace the progress and rate of metamorphosis; and (2) to provide an incisive measure of the onset of metamorphosis and therefore, under permissive conditions, of the termination or absence of diapause (stages W.2 and, especially W.3 — a measure of great value when the critical photoperiod for averting diapause is being inferred from populations in the field in early summer).

Not all Zygoptera are likely to feature eye stages as easily categorized as are those of *P. nymphula*. For example, in *Ischnura verticalis* (cf. GRIEVE, 1937) and *I. elegans* (Vander L.) (our observations), the facetted area of the compound eye begins to extend mesially about 10 days before emergence at 23°C, but the mesial margin of this area is indistinct and only becomes clearly defined about three days before emergence. In *I. elegans*, to judge from our few laboratory observations at 23°C, stage W.3 occurs only three days before emergence and is followed one day later by stages W.4 and L.2, and about two days later by stages E.6 and H.2.

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