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Baughan Wisely.*

STUDIES ON EPHEMEROPTERA

III. *COLOBURISCUS HUMERALIS* (WALKER); MORPHOLOGY AND ANATOMY OF THE WINGED STAGES

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*See footnote next page

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THE WINGED STAGES

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Summary

Females dominated over males in all collections of late stage nymphs made prior to and during the breeding season. The latter takes place mainly in spring and summer (October–February) but probably extends into the autumn (March, April). Emergence of the subimago from the nymph is described; it can take place rapidly (in 10–12 seconds) and sometimes the subimago uses the nymphal exuviae as a raft which it flies off from in mid stream. The external anatomy of the nymph, subimago, and imago are compared briefly. Ephemerid wing venation theories are reviewed; observations made on the wing bud tracheation tend to confirm an earlier suggestion made by the late Dr R. J. Tillyard that the radial sector does not cross the anterior median in recent mayflies. The subimaginal and imaginal venation is described and a general account given of the anatomy of the reproductive, nervous, and excretory systems, and the tracheation and the alimentary canal. There is some evidence that a ventral sympathetic nervous system is present, as in some other orders of insects. The frontal ganglion and recurrent nerve of the visceral sympathetic nervous system are present. Copulatory behaviour is described.

INTRODUCTION

Earlier papers (Wisely 1961, 1962) described the early life history of *Coloburiscus humeralis* and the general ecology and distribution of the nymphs. The present paper describes the winged stages, mainly in terms of comparative anatomy, and gives a broad outline of the remainder of the life cycle.

SEX RATIOS OF NYMPHS

Female nymphs predominated over males in all collections made between August 1951 and May 1952 (Table 1). These collections were made by overturning loose stones in rapids in the mouth of a net so that the dislodged nymphs were swept into it. The nymphs were not visible during collecting so it is unlikely that a visual bias could account for the ratio difference; the male nymphs were smaller than the females, however, and it is possible that they were not as easily dislodged into the net by the collecting method.

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TABLE 1—Sex Ratios of Late Stage *C. humeralis* Nymphs

Locality	Date	Males	Females	Totals
Grasmere Stream	20/ 8/51	11	66	77
"	21/ 8/51	172	201	373
Purau Stream	21/ 9/51	93	148	241
"	25/10/51	201	634	835
"	22/11/51	362	474	836
"	23/ 1/52	84	141	225
"	22/ 2/52	88	112	200
"	25/ 3/52	35	47	82
"	25/ 4/52	53	61	114
Totals		1099	1884	2983

EMERGENCE PERIODS

Hudson (1904) stated that the winged stages of *C. humeralis* first appeared about the end of October, were extremely abundant throughout November, and could be observed as late as the middle or end of January. Phillips (1930) considered that the emergence period was from about mid October to the beginning of February. These remarks probably refer to the Wellington district where both authors were based. During the present study winged stages were found most abundantly in late October and early November at Purau and late November and early December at Cass. Brief descriptions of these habitats appeared in Wisely (1962, p. 210). Records of the winged stages in the collections are given in Table 2 and substantiate the view that *C. humeralis* emerges in spring and summer, predominantly between October and February.

Nymphal samples taken from Purau Stream during 1952 suggest, however, that emergence was still taking place in autumn (March, April). These samples were taken between September 1951 and April 1952 from a rapid in Purau Stream where the nymphs were particularly abundant (see Wisely, 1962, Plate 1, fig. 2). The nymphs were netted as described previously, preserved in 70% alcohol, and their headwidth measurements taken using a micrometer eyepieces reading to 0.025 mm (± 2 of these units error). As shown in Fig. 1, final instar nymphs of 2 mm headwidth were common from September through to February. In March they began to decline and concurrently a peak of younger nymphs of 1 mm headwidth appeared. In April nearly all the 2 mm headwidth nymphs had disappeared and the 1 mm headwidth group had become dominant. This evidence is not sufficient to suggest that there are distinct summer and autumn emergences,

TABLE 2—Museum Records of Winged Stages of *C. humeralis* in 1952

Locality	Date	Collector	Museum
Raurimu	6/ 1/1919	C. E. Clark	Auckland
Wellington	—/10/1886	G. N. Hudson	Dominion (Wellington)
„	25/ 9/1893	„	„
„	29/10/1898	„	„
„	5/11/1898	„	„
„	16/11/1898	„	„
„	9/12/1920	„	„
„	24/ 9/1944	„	„
Maruia Springs	4/ 1/1940	S. Lindsay	Canterbury (Christchurch)
Purau Stream	20/12/1919	G. Howes	„
Akaroa	—/10/1920	R. J. Tillyard	„
Cass	—/11/1924	A. Tonnoir	„
„	—/ 2/1925	„	„
White Rock	—/11/1919	S. Lindsay	„
Glentui	27/12/1921	„	„
Dunedin	3/12/1916	C. E. Clark	Auckland
„	—/12/1919	„	„
„	3/12/1920	„	„

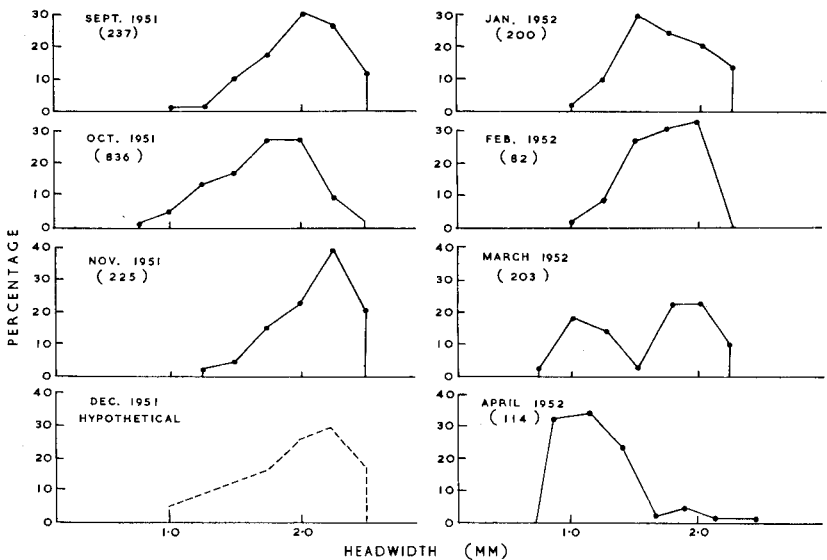


FIG. 1—Headwidth frequencies for monthly samples of nymphs from Purau Stream. Banks Peninsula. (See text.)

as has been reported for *Baetis vagans* (Needham, 1935). It does, however, indicate that the emergence period of *C. humeralis* extends further into the autumn than has been reported previously.

EMERGENCE OF THE SUBIMAGO

During the last stadium the nymph shows distinctive colour changes. The dorsal half of the body and the wing buds become blackish and the bright yellow wing bases and mauve sternites of the developing subimago become visible through the nymphal cuticle. Just before emergence the nymph leaves the substratum and swims feebly towards the surface as it is carried down stream by the current. On several occasions the emerged subimago was seen using the nymphal exuviae as a raft before flying off, but more usually the nymph was washed against stones or vegetation and crawled partly out of the water before emergence took place.

Emergence is rapid and spectacular. A narrow split appears in the mid dorsal line of the thoracic tergites and regular peristaltic movements undulate up the body from behind forwards. As each expanding bulge enters the thorax it pushes the split open more and more until the subimaginal thorax protrudes. The split then extends forwards between the compound eyes and backwards to the first or second abdominal segments. Rapid vibrations of the meso and metathoracic wing muscles aid at this stage, particularly in unravelling the wings from the wing buds. The subimago slides out of the exuvia jerkily with the legs assisting in the final stages. The hindwings vibrate first and the forewings follow with flight resulting, in some cases, in the incredibly short period of 10-12 seconds after the initial opening of the split. As in other species, the subimago (Fig. 2) is readily distinguished from the imago by the presence of a fringe of macrotrichia, along the posterior edge of the wings. It is distinguished from other New Zealand subimagines by the presence of a bright yellow spot at the base of each forewing. Laboratory kept specimens usually moulted within several days into the imago or sexual phase and during this interval they usually remained quiescent. Subimagines seen in the field were also quiescent; the only active flight noted was just after emergence and this was characteristically an upward linear flight taking the insect high into the air.

COPULATION OF THE IMAGINES

Field observations made at Cass, Canterbury, showed that hovering groups of males tended to concentrate above Grasmere Stream, particularly in the early morning or evening or at other times when there was a lull in the wind. On several occasions females, distinguishable by their larger size and bulky abdomens, were seen to fly into these groups and rise and fall several times in hovering flight before they were observed by a male. The male approached the female from below and seized her by the thorax with his prothoracic legs. At the same time he arched the tip of his abdomen so that his claspers encircled her abdomen at segments seven and eight. This enabled the penes to engage in the oviducal openings during flight and usually the couple flew off from the group before separating.

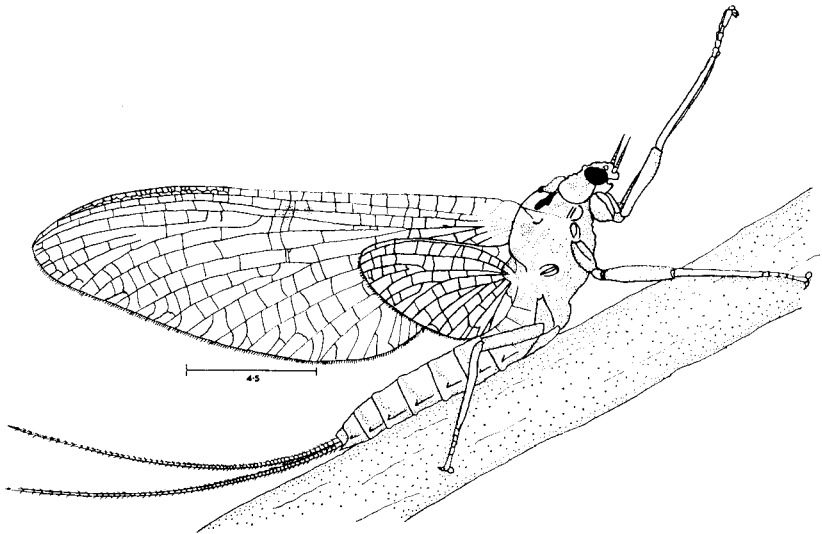


FIG. 2—Female subimago. Traced from a photograph. Scale in mm.

GENERAL EXTERNAL FEATURES

The nymphal head with its eye differences in male and female has already been described (Wisely, 1961, p. 255). In the subimago and imago the eyes become greatly developed. In the male they occupy most of the dorsal surface of the head and are divided into a dorsal brown section and a ventral olive green section. In the female they are dark brown and relatively smaller. The male ocelli are markedly elevated, cone-shaped and bunched together; the female ocelli are only slightly elevated and further apart. The antennal stape and pedicel persist in both sexes but the filament becomes greatly shortened and loses its segmental appearance. The mouth parts become vestigial and are apparently non-functional. The pronotum becomes markedly concave in the midline and extends down further laterally; the mesonotum becomes oval in outline and much more markedly concave and the mesopleuron becomes broader posteriorly. Two pairs of spiracles appear as oblique elongated slits: the mesostigmata are situated antero-dorsally to the bases of the middle legs and the metastigmata are situated lower and close to the base of the hind legs. The wings are discussed later. General and specific characters of the legs have been described by Eaton (1868) and Phillips (1930). Corresponding legs of both sexes are similar, corresponding legs of the subimago and imago of the same sex are also similar, but there are marked sexual differences in the prothoracic legs of the winged stages. In the female subimago or imago the prothoracic leg is nearly smooth and has a 5-segmented tarsus (Fig. 2). Two tarsal claws are present; one blunt, the other hooked. In the male subimago or imago the tarsal segments of the prothoracic legs are greatly elongated and facilitate gripping the female during the mating flight (Fig. 3). Genitalia differences are discussed later.

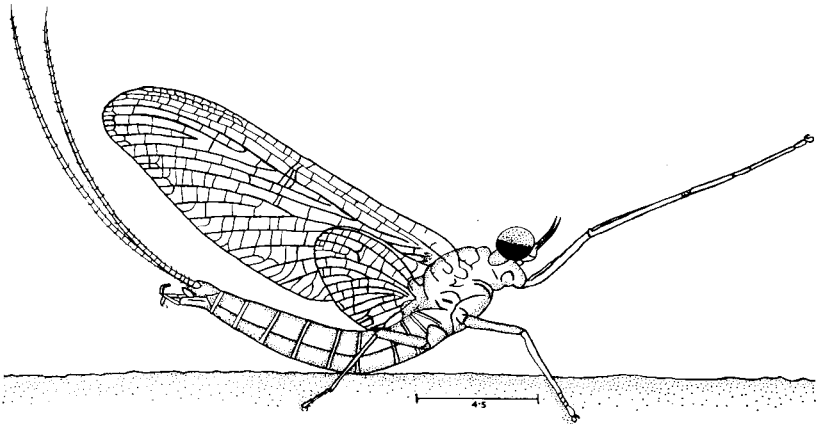


FIG. 3.—Male imago. Traced from a photograph. Scale in mm.

INTERPRETATION OF WING VENATION THEORIES

There has been considerable disagreement over the homologies of ephemerid wing venation, chiefly arising from varying interpretations of (1) the venation of fossil ephemerid and other insect wings; (2) the relationships of nymphal wing bud tracheation and adult venation in recent species; and (3) the alternation of high and low (convex and concave) veins over most of the forewing. Table 3 attempts to summarise major treatments of the subject and is derived from Eaton (1883), Comstock and Needham (1898), Morgan (1913), Comstock (1918), Lameere (1922), Martynov (as quoted by Tillyard, 1923), Tillyard (1926, 1932), and Needham (1935). The major changes seem to have been:

1. Tillyard's reorientation (1923) of the anal region of the wing was accepted by Needham (1935). Thus 1A of Comstock and Needham (1899), and Morgan (1913) became CU in Tillyard (1923, 1926), and in Needham (1935).

2. Tillyard's reorientation of the middle region of the wing was mainly accepted by Needham (1935). The former showed that in four archaic New Zealand mayflies, including *C. humeralis*, the radial sector, R_s , did not cross the first branch of the median, M , as Morgan (1912) had intimated; her M_1 was considered to be R_s , or some branch of it, and M_1 was placed posterior to the radial sector.

3. Tillyard's views changed later in 1923 after he read Lameere's (1922) paper. The latter contended that in most of the Paleozoic insects, and in the recent Ephemeroptera, the median consisted of a convex anterior branch and a concave posterior branch. Tillyard's acceptance was in two stages; in 1926 he wrote of MA_1 and MA_2 , still retaining Comstock and Needham's M_1 and M_2 for the latter. Although Martynov agreed with him (see

TABLE 3—Interpretations of Ephemeropter Wing Venation (See text)

Sign	Eaton (1883)	Comstock-Needham (1899)	Morgan (1913)	Martynov (as quoted by Tillyard (1923))	Tillyard (1923)	Tillyard (1926)	Needham (1935)	Tillyard (1932)	Name
+	1	C	C	C	C	C	C	C	Costa
-	2	Sc	Sc	Sc	Sc	Sc	Sc	Sc	Subcosta
+	3	R ₁	R ₁	R ₁	R ₁	R ₁	R ₁	R ₁	Radius
-	4	R _s	M	R _s	R _s (part)	R _s	R _s	R _s	Radial
-	..	R ₂	M ₁	R _{s1}	IR _{2a}	R ₂	R ₂	R ₂	Sector
+*	..	R _{s2}	2R _{2a}	IR ₂	..	IR ₂	Sector
-	R _{s3}	3R _{2a}	R _{3a}	..	R _{3a}	Sector
+	R _{s3}	4R _{2a}	IR _{2a}	..	IR _{3a}	Sector
-	..	R ₃ †	R _s	R _{s3}	5R _{2a}	R _{3b}	..	R _{3b}	Sector
+	4	R ₄	INT. V. 1	R _{s4}	R _{2b}	IR _{3b}	..	IR _{3b}	Sector
-	5	R ₅	M ₂	R _{s5}	R ₃	R ₄₊₅	R ₃	R ₄₊₅	Sector
+	6	M ₁	M ₃	MP	R _{4a}	MA ₁	R ₄	MA ₁	Ant. Median
-	..	M ₂	..	MP	R _{4b}	IMA	..	IMA	
+	6	M ₃	M ₄	MP	R ₅	MA ₂	R ₅	MA ₂	
-	7	CU ₁	CU ₁	CU ₁	M ₁	MP ₁	M ₁	MP ₁	Post. Median
+	INT. V. 2	..	M ₂	IMP	..	IMP	
-	7	CU ₂	CU ₂	CU ₂	M ₃₊₄	MP ₂	M ₂	MP ₂	
+	8	1A	1A	A ₁	CU _{1a}	CU _{1a}	CU ₁	CUA ₁	
-	..	1A	1A	A ₁	CU _{1b}	ICU ₁	CU ₂	ICUA	
+	8	1A	1A	A ₁	CU _{1c}	CU _{1b}	1A	CUA ₂	Ant. Cubitus
-	9 ₁	2A	2A	A ₂	CU ₂	CU ₂	..	CUP	Post. Cubitus
+	9 ₂	3A	3A	A ₃	1A	1A	..	A ₁	First Anal.
-	9 ₂	3A	3A	A ₃	2A	2A	..	IA	Int. Anal.
+	9 ₂	3A	3A	A ₃	3A	3A	..	A ₂	Second Anal.
+	A ₃ A ₄	Axillaries

+ = convex. - = concave vein. INT. V. = interpolated vein *Needham (1935) has R₃ here but Tillyard (1932) has not. †Omitted by Needham (1935), but not by Tillyard (1932).

Tillyard, 1932, p. 271), Needham (1935) did not accept the suggestion that an anterior median was present in recent mayflies. Consequently his M₁ and M₂ correspond to Tillyard's (1932) MP₁ and MP₂; his R₄₊₅ corresponds to Tillyard's (1932) MA₁ and MA₂.

4. Tillyard (1932) also introduced the concept of anterior and posterior cubitals (CU A and CU P) to replace CU₁ and CU₂ but this also was not accepted by Needham (1935).

THE WING BUD ORIGINS OF THE RADIAL SECTOR AND ANTERIOR MEDIAN

It was possible to discern the tracheation in the wing buds of newly ecdysed young nymphs; older nymphs were wax embedded and dissected. The final instar nymph is referred to here as n ; hence the penultimate nymph is $n-1$ and so on. Diagrams of the tracheation of the last four nymphal stages are shown in Fig. 4.

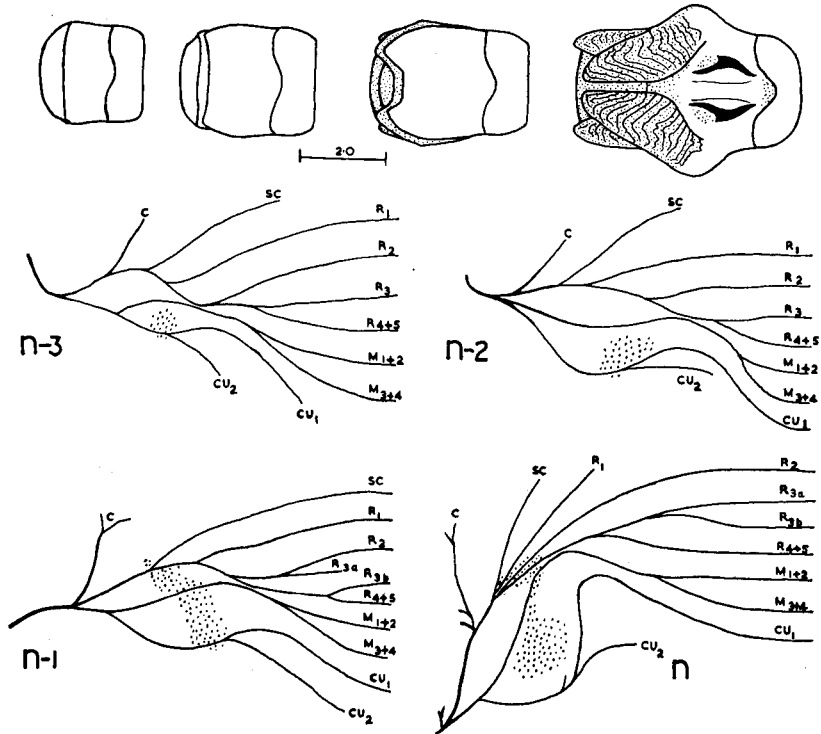


FIG. 4—Wing bud tracheation. The external appearance of the wing buds in the series are shown above. Diagrams of the relationships of the main trachea in the series are shown below.

In the $n-3$ nymph the costal-radial trunk consists of six trunks, interpreted here as C , Sc , R_1 , R_2 , R_3 , and R_{4+5} . The median trunk consists of M_{1+2} and M_{3+4} , the cubito-anal trunk of CU_1 and CU_2 . There is little change in the $n-2$ nymph but the pigmented area already present alongside the cubito-anal trunk increases in size. In the $n-1$ nymph this pigmented area increases still further and extends across the median and costo-radial trunk. Two new branches, apparently R_{3a} and R_{3b} , appear in the radial sector. At the beginning of the final stadium (n) the arrangement is still similar but the pigmented area (which ultimately forms the callus in the

winged stages) now lies across the bases of the main trunks and obscures them. During the latter part of this stadium the developing wings become greatly convoluted and the secondary and cross veins appear. The evidence from this series lends support to Tillyard's (1923) contention that the radial sector does not cross the anterior median in recent mayflies.

THE IMAGINAL VENATION

Tillyard's (1926) terminology is used in the following description of the imaginal venation, which is illustrated in Fig. 5.

The costal (C) is short, curved and contiguous with the humeral veinlet (HM), which runs through the subcosta (Sc) to the main vein of the radius (R_1). Both Sc and R_1 continue distally as unbranched veins, each bearing a pigmented bulla halfway along its length. Between Sc and the anterior edge of the wing there is a reticulum of cross veins forming the pterostigmatic region. Next in order is the radial sector comprising at least seven prominent veins— R_2 , IR_2 , R_{3a} , IR_{3a} , R_{3b} , IR_{3b} , and R_{4+5} . The median anterior vein follows and splits into MA_1 , IMA , and MA_2 ; it is followed by the three branches of the median, namely M_{1+2} , IM , and M_{3+4} . The first cubital (CU_1) is almost straight and bears pectinate branches posteriorly; it is followed by the prominently curved second cubital (CU_2). Between the latter and the thorax lie the anals and between the first of these (IA) and the second (2A) there is a concave and presumably interpolated vein. The two veins following are both slightly convex and may be 3A and 4A, or 3A and possibly another interpolated vein. It is usual to consider the anal region of the insect wing as consisting of convex veins; however, mayflies are characterised by a regular alternation of concave and convex veins across their wings but these become difficult to differentiate in the proximal part of the anal system.

The prominent humeral angle of the hindwing overlaps the cubito-anal area of the forewing and provides an example of the "amplexiform" arrangement of Tillyard (1926). The humeral angle is formed by C, which curves anteriorly and then turns sharply through about 90° . At this point it becomes parallel to, but external to, CU_1 of the forewing. The specific name "*humeralis*" refers to this characteristic. The remainder of the veins are similar to those of the forewing but only IA can be placed with certainty in the anal region. The remainder of the anal veins vary from specimen to specimen.

REPRODUCTIVE SYSTEM

In the freshly moulted female nymph entering the last stadium the ovaries are paired, slender, subcylindrical bodies, extending from the middle of the sixth abdominal segment to the metathoracic segment and lying dorsolaterally to the gut (Fig. 6A). The oviducts curve ventrally to open separately on the posterior margin of the seventh abdominal segment. In cleared specimens a small chamber appears near the external openings of

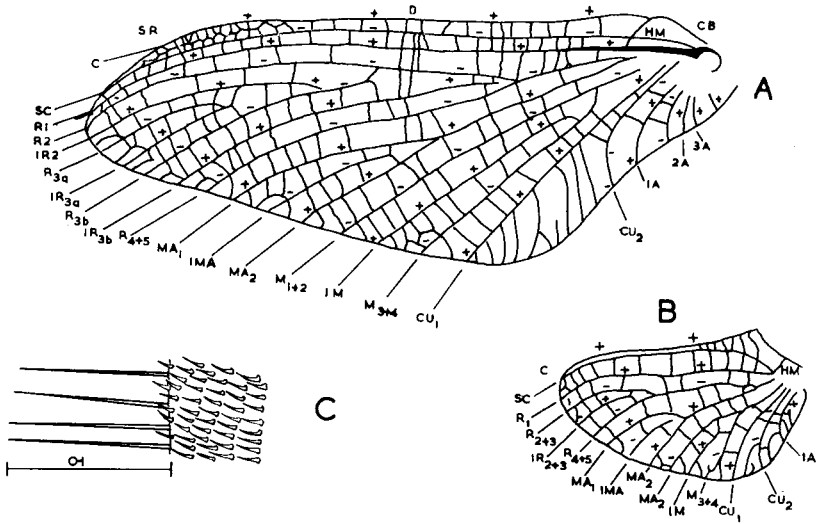


FIG. 5—Venation. A the imaginal forewing; B the imaginal hindwing; C part of the posterior edge of a subimaginal wing showing the fringe of macrotrichia. Traced from photographs. Scale in mm.

the oviducts which might be a receptaculum seminalis (Fig. 6B). Throughout this stadium the ovaries enlarge and mature until eventually they cram the abdomen and extend anteriorly into the prothoracic segment.

The ovarioles or egg tubes (Fig. 6C) consist of a peritoneal sheath, vitellarium, germarium, and a terminal filament. The germarium contains about 20 large nucleated cells, and since only about five of these mature into eggs some of the others may be nurse cells. In the next proximal dilatation there is one egg nucleus with granular inclusions in the cytoplasm. In the next dilatation the egg is larger, contains more vacuoles and globules, and has follicle cells present around its periphery. The remaining eggs increase in size, globule and vacuole contents the nearer they are to the proximal end of the ovariole. The follicle cells of the proximal egg are usually broken or sloughed off, the chorion is formed and the adhesive knobs are recognisable on the poles.

Several changes occur in the transition to the imago. There is shortening of the oviducts, increase in the number of mature eggs and differentiation of the egg value (Figs 7A and B). The latter is a posterior growth of segment seven which becomes external in the imago. The sternite of segment nine, which is used later to partially hold the egg mass, is now capable of a much greater forward rotation.

In the last instar male nymph the testes are nodular structures lying along each side of the alimentary canal. They are enclosed in a thin peritoneal sheath and lie between the anterior end of the fourth abdominal segment and the posterior end of the sixth (Fig. 6E). The vasa deferentia lead away on each side and constrict before dilating into the vesicula

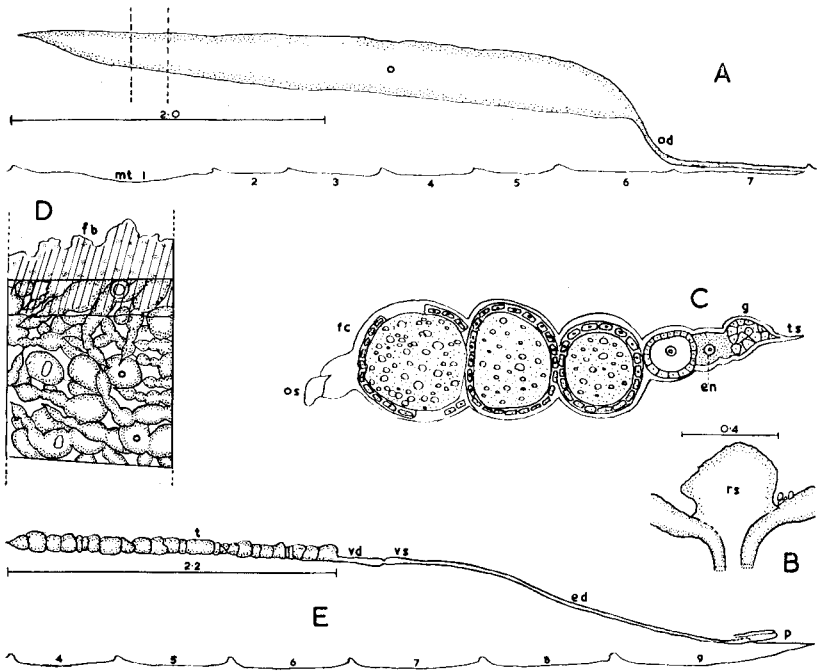


FIG. 6—Reproductive system. A lateral view of last instar female nymph. B optical section of female imaginal oviducal openings. C single ovariole from a last instar female nymph about to moult into a subimago. D section through part of the developing ovary shown in A. E lateral view of last instar male nymph. ed ejaculatory duct, en egg nucleus, fb fat body, fc follicular cells, g germarium, o ovary or ovarioles, os ovariole stalk, p penes, rs receptaculum seminalis t testis, ts terminal spike, vd vas deferans, vs vesicula seminalis, mt 1 metathoracic and first abdominal segment, 2-9 abdominal segments. Drawn from dissections. Scale in mm.

seminalis. A slimmer ejaculatory duct leads posteriorly from each of these traversing segments 7, 8, and 9 before ending in the respective penis.

Important changes occur in the transition to the winged stages. The testes shrivel, the vesiculae swell enormously and the anterior ends of the ejaculatory ducts thicken in the imago (Fig. 7c). The external genitalia are not fully protuberant until the imaginal stage is reached. The claspers have three segments which curve inwardly and bear minute mating spines (Fig. 7d). The penes (Fig. 7e) are paired with only the tips protruding.

CENTRAL AND PERIPHERAL NERVOUS SYSTEM

The supraoesophageal ganglion is dorsal to the gut in the last instar nymph, as in other insects, and the suboesophageal, pro, meso, and meta-thoracic and the seven abdominals are all ventral. These ganglia are linked

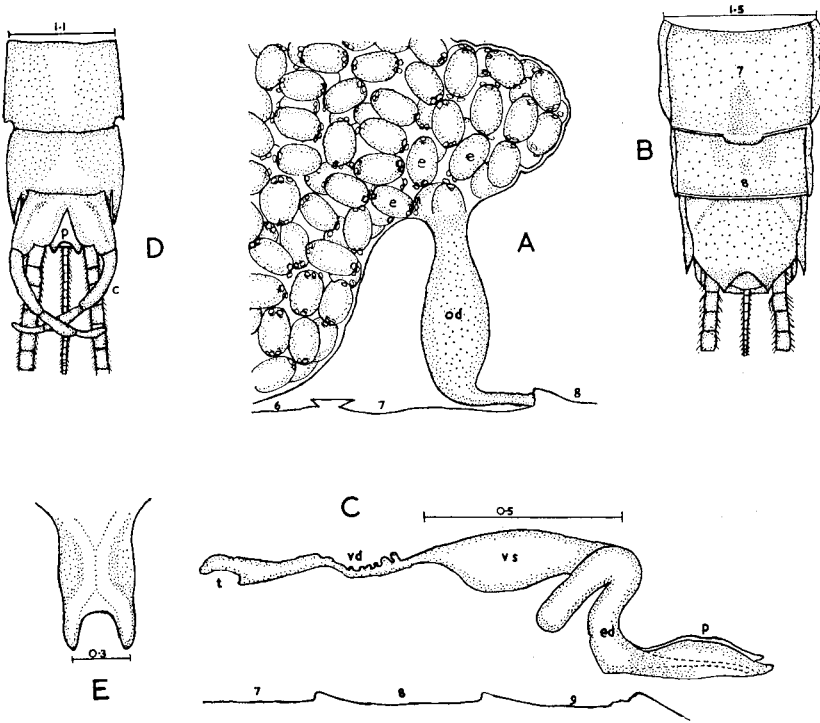


FIG. 7.—Reproductive system. A lateral view of female imago. B ventral view of the posterior abdominal segments of a female imago. C lateral view of male imago. D ventral view of the posterior abdominal segments of a male imago. E penis. c clasper, ed ejaculatory duct, od oviduct, p penes t testis, vd vas deferens, vs vesicula seminalis, 7-9 abdominal segments. A and C drawn from dissections. Scale in mm.

by paired connectives but there is asymmetry in the origins of some of the peripheral nerves.

The supraoesophageal ganglion (Fig. 8A), which is usually considered to represent the fused ganglia of the first three embryonic segments, consists of three regions. The protocerebrum is bilobed, large, and gives off three ocellar nerves before it becomes contiguous with the optic lobes of each side. There is a large antennal nerve on each side of the deutocerebrum. The bilobed tritocerebrum is immediately posterior to the deutocerebrum, the labrofrontal nerves leaving its anterolateral surfaces. Ventrally it gives rise to two stout crura cerbri which almost encircle the oesophagus, join through the postoesophageal commissure and enter the suboesophageal ganglion separately. Three pairs of nerves originate from the latter; viz., the mandibular nerves which leave the anterolateral borders, the maxillary nerves which emerge along its lateral margins, and the labial nerves which extend out from the posteroventral surface.

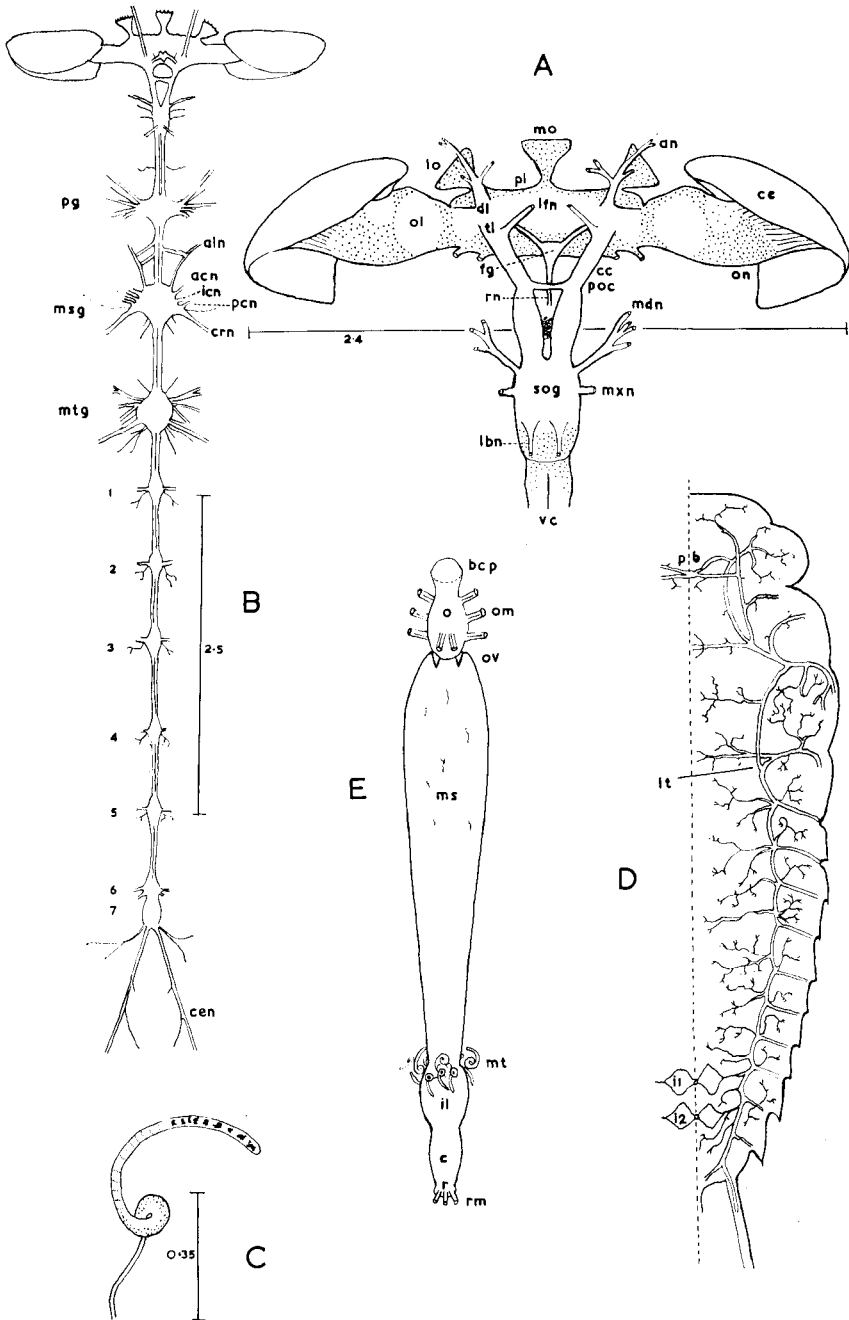
The prothoracic ganglion is depressed dorsoventrally and bears at least four pairs of nerves supplying the surrounding tissues (Fig. 8B). The first, the "alar" and the last the "crural" are the main nerves of the prothoracic leg muscles. The mesothoracic ganglion is large and has nine pairs of nerves leaving it. The first, the alar, supplies the developing wing muscles and the sixth, the crural, enters the mesothoracic leg. Between these two large nerves there are four smaller ones supplying the leg muscles, i.e., the anterior, the inferior, and posterior coxals and the trochanter extensor. The metathoracic ganglion also possesses nine pairs of legs but in this case the alar is the second nerve; the first nerve runs forwardly ventrally into muscles.

Five of the seven abdominal ganglia are similar in size and shape and each of the former bears a pair of nerves. Before they run dorsally to other tissues these nerves innervate the ventral longitudinal muscles. The sixth ganglion lies in the seventh abdominal segment, supplying it with an extra pair of nerves and the seventh ganglion follows closely, lying between segments seven and eight. It supplies a pair of nerves to the eighth segment but its most prominent feature is a pair of thick nerves that continue posteriorly towards the caudal setae. These give off nerves to the ninth segment and more median nerves to the tenth segment before they enter the setae as caudal nerves.

SYMPATHETIC NERVOUS SYSTEM

Certain nerves correspond in position with the ventral sympathetic nerves in other orders of insects. These include nerves midway along the ventral connectives joining the pro-, meso-, and metathoracic ganglia, the latero-posterior nerves of the first five abdominal segments and the smaller nerves to the hind gut and reproductive organs. The last-mentioned nerves leave the cercal nerves of the seventh abdominal segment. Visceral sympathetic nerves can be placed with more certainty (Fig. 8A). The frontal ganglion lies anterior to the brain and dorsal to the buccal cavity; it is joined to the tritocerebral lobes by two curved connectives which also give rise to the

FIG. 8—A ventral view of the brain of a last instar female nymph. B central and peripheral nerves of a last instar female nymph. C malpighian tubule. D half the tracheal system of a last instar female nymph. E diagram of the gut of a nymph recently moulted into the last instar. acn anterior coxal nerve, aln alar nerve, an antennal nerve, bcp buccal cavity and pharynx, c colon, cc crura cerebri, ce compound eye, cen caudal nerve, crn crural nerve, dl deutocerebral lobes, fg frontal ganglion, i1 induvia, i2 induvia, icn inferior coxal nerve, il ileum, lbn labial nerves, lfn labrofrontal nerve, lo lateral ocellus, lt longitudinal trunk, mdn mandibular nerve, mo median ocellus, ms mesenteron, msg mesothoracic ganglion, mt malpighian tubule, mtg metathoracic ganglion, mxn maxillary nerve, o oesophagus, ol optic lobe, om oesophageal muscles, on optic nerve, ov oesophageal valve, pb Palmen's body, pcn posterior coxal nerve, pg prothoracic ganglion, pl protocerebral lobes, poc postoesophageal commissure, r rectum, rm rectal muscles, rn recurrent nerve, sog suboesophageal ganglion, tl tritocerebral lobes, vc ventral connectives, 1-7 abdominal ganglia.



labrofrontal nerves. The recurrent nerve leaves the frontal ganglion posteriorly and passes dorsally along the oesophagus before it disappears into its tissue, about midway along its length.

This basic arrangement does not appear to alter greatly in the subimago and imago. Despite the atrophy of the mouthparts the mandibular, maxillary, and labial nerves are still present. The optic nerves of the males are relatively large (associated with the well developed compound eyes) and in both winged sexes a branch of the nerve supplying the tenth abdominal segment clearly innervates the genitalia.

EXCRETORY SYSTEM

The nymph of Malpighian tubules increases through the nymphal life to reach about 150 in the last instar. Comparable figures for other species are over 100 in *Siphonurus lacustris* (Drenkelfort, 1910) and around 150 in *Stenonema vicarium* (Needham, 1935). In *C. humeralis* the number of tubules in the winged stage is usually 140–154; these are attached in a ring around the gut at the mesenteron and proctodaeum junction forming a tangled mass in the body cavity. Each tubule consists of three parts (Fig. 8C). The distal glandular portion is curved, ends bluntly and contains dark crystalline deposits in its distal half. The central part of the tubule is thickened and looped and the proximal part is attached to the gut.

FAT BODY

The fat body is attached dorsolaterally to the ovaries or testes (Fig. 6D). It is most prominent in the last instar nymph and at this stage oval adipose cells are studded throughout the matrix. Its extent is reduced in the subimago and more so in the imago; in the latter traces are present around the internal genitalia.

TRACHEAL SYSTEM

C. humeralis nymphs have 14 gills arranged in pairs on the first seven abdominal segments; there are also filamentous maxillary gills on the head (Wisely, 1961). The tracheoles of the gills converge at their bases to form 14 spiracular trunks which run inwards a short distance before joining the great longitudinal trunks; the latter lie on each side of the nymph throughout most of its body length (Fig. 8D).

In segment ten the longitudinal trunks bifurcate and each supplies the caudal setae on its side and contributes one of the two trachea in the small median seta. In both segments eight and nine one of the tracheal branches runs towards the midline, bifurcates, rejoins and meets its partner from the other side. There are minute white bodies present at these junctions and these correspond to the smaller Palmen bodies or induvia of Hsu (1933).

Throughout the abdominal segments there are numerous trachea leaving the longitudinal trunks near their junctions with the spiracular trunks. In each segment these trachea supply the adjacent muscles, gut, gonads, fat body, and segmental ganglion. In the metathorax a large trunk leaves the main longitudinal trunk of each side and bifurcates. One branch goes to the metathoracic ganglion and the other supplies the flight muscles, leg, and the hind wing bud. Between the meso and metathoracic segments each longitudinal trunk curves sharply and laterally to give off the metathoracic leg trachea, the flight muscle trachea and the wing bud branch. Each longitudinal trunk then angles towards the midline to give off the prothoracic leg trachea and the ganglion trachea before it divides into two parallel trunks leading towards the head; one of these is dorsal and the other ventral. The dorsal branch gives a trachea to the prothoracic leg before it enters the occipital foramen.

Palmen's body, a spherical, concentrically layered structure, lies suspended between these trunks on the anterior side of the occipital foramen. Four fine trachea radiate from it, one joining each longitudinal trunk. Wodsedalek (1912) found that Palmen's body could be used as an index to the number of instars. Needham (1935), describing this structure in *Stenonema vicarium*, pointed out that the entire structure is surrounded by a continuous hypodermal layer. This encloses concentric layers of tracheal lining which are retained at each successive moult. The longitudinal trunks continue into the nymphal head to supply the mouthparts, brain, and head muscles; the ventral member of each side opens into the lumen of its maxillary gill.

In the winged stages the gills are replaced by eight pairs of spiracles along the abdomen. The eighth pair develop posteriorly to the last pair of nymphal gills. The two pairs of thoracic spiracles are oblique elongated slits with lateral flaps but no special closing structures. Additional trachea appear supplying the flight muscles, particularly in the males.

ALIMENTARY CANAL

In the freshly moulted nymph entering its last stadium the gut is a nearly straight tube extending from mouth to anus and consisting of stomodaeum, mesenteron, and proctodaeum (Fig. 8E).

The stomodaeum consists of buccal cavity, pharynx, and oesophagus, the posterior limit of the latter being at the occipital foramen. The mouth is a wide undivided cavity which is bounded above by the labrum, laterally by the mandibles and maxillae, and ventrally by the labium and hypopharynx. The frontal ganglion shows through the mid-dorsal surface of the walls converging into the pharynx. The latter constricts into the narrow oesophagus, which passes dorsally and posteriorly between the brain and the suboesophageal ganglion, dilates slightly, and then runs through the rest of the head to project back into the mesenteron. At this point there is a single oesophageal valve and the aortic vessel leaves the dorsal surface of the alimentary canal and ascends posteriorly through the indirect flight muscles of the thorax.

The mesenteron extends from the occipital foramen to the sixth and seventh abdominal segment and attains its greatest diameter in the mesothorax. A peritrophic membrane is present. The proctodaeum extends from the Malpighian tubules to the anus and consists of a well defined ileum, colon, and rectum. Radial muscles attach the rectum to the exoskeleton of segment ten.

In *C. humeralis* nymphs approaching the subimaginal stage longitudinal cracks appear between the superficial muscle cells of the mesenteron; these cells become granular and disappear, leaving behind a thin transparent region which is slightly elastic. During the present study neither the subimago nor the imago has been observed feeding and they are considered to live on stored reserves, as do other mayflies. The mouthparts atrophy, the gut no longer contains food, and the thin-walled mesenteron becomes distended with gas. The details of these processes in other mayflies have already been discussed by a number of workers, including Palmen (1877), Drenkelfort (1910), Pickles (1931), and Needham (1935).

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