

PRIVATE LIBRARY
OF WILLIAM L. PETERS

TRANSACTIONS
OF THE
ROYAL SOCIETY OF NEW ZEALAND

ZOOLOGY

VOL. 1

No. 19

DECEMBER 14, 1961

[Continued from *Transactions of the Royal Society of N.Z.*, Volume 88, Part 4.]

Studies on Ephemeroptera
I.—*Coloburiscus humeralis* (Walker); Early Life History
and Nymph

By BAUGHAN WISELY,

Zoology Department, Canterbury University,
Christchurch, N.Z.

[Received by the Editor, April 12, 1961.]

Abstract

THE egg mass measures 2.0 x 1.0–2.0 x 1.5 mm and contains 3,000–6,000 eggs. It appears to be dropped into water during flight and does not disintegrate immediately. Each egg is ellipsoidal, measuring 0.20–0.25 mm in length and 0.10–0.15 in breadth, and has 3–4 adhesive organs at each pole. During the early instars the number of antennal segments and abdominal gills increases; the latter appear to be single at first and later become bifid. When the full number of gills (14) appears a rudimentary maxillary gill develops from the base of the maxillary palp. Over the last four stadia male nymphs can be distinguished from females by differences in sternite nine, and in the eyes. The external features of the nymph are described. A description is given of the method used by the nymph to sieve potential food from the swift currents which it inhabits.

INTRODUCTION

THE order Ephemeroptera or mayflies are freshwater insects which spend almost their entire life history in the larval stage underwater as nymphs; the latter differ from other aquatic insect larvae in possessing paired and usually vibratile gills on the abdominal segments, and in having three caudal setae. The nymphs of many species are clingers, some are swimmers, and a few burrowers. Usually after six to twelve months the nymph is fully developed and metamorphoses into a winged subimaginal stage, which is not found in other orders. After one or two days the subimago transforms into the imago which it superficially resembles. The subimago differs, however, in colouration and possesses a fringe of setae or macrotrichia along the posterior edge of the wings. It is not sexually mature. Both the subimago and imago have vestigial mouth-parts and are unable to feed; the imagines usually live several days and congregate in swarms above lakes, rivers, and streams during

Published by the Royal Society of New Zealand, c/o Victoria University of Wellington, P.O. Box 196, Wellington.

their mating flights. In many countries the nymphs and imagines form a substantial proportion of the food of freshwater fishes.

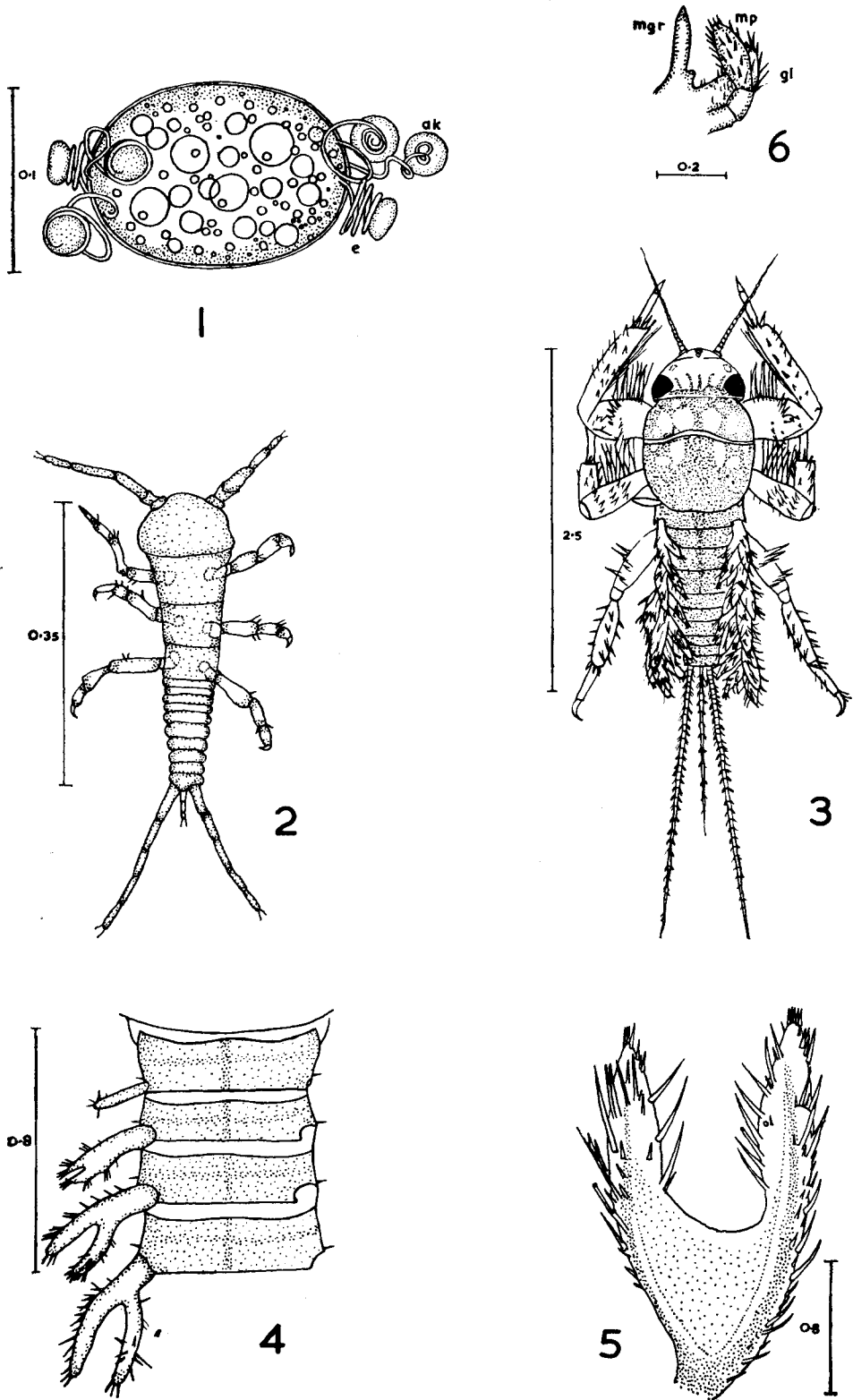
The family Siphonuridae which includes *Coloburiscus* consists of relatively large and archaic mayflies; Phillips (1930) has given a systematic account of the New Zealand genera and Riek (1955) a comparable one for the Australian genera. The genus *Coloburiscus* is endemic to New Zealand and is represented by one species, *C. humeralis*, which is widely distributed throughout the country. The Australian genus *Coloburiscoides*, which closely resembles it, has three species, hitherto recorded only from south-eastern Australia.

EGG MASS

The egg mass of *C. humeralis* is a bright orange colour and usually measures about 2.0 mm in length, 1.0–2.0 in breadth and 1.5 in depth. It is circular in cross section but slightly flattened on the face apposed to the abdominal sternites. The eggs/volume ratio is approximately 1,000 eggs/cu mm, hence egg masses of the above dimensions could be expected to contain 3,000–6,000 eggs. Females producing egg masses were located at Cass, Canterbury, during the breeding season (late November, December) by searching the vegetation bordering Grasmere Stream at dawn and dusk when the air was still. Such conditions were conducive to mating flights and consequently copulation and egg mass production. Detection of suitable females was facilitated by the conspicuous colour of the egg mass. The eggs were forced out of the twin oviducal openings between abdominal sternites seven and eight by a slow dorso-ventral flexing of the abdomen accompanied, at the termination of each downstroke, by a slight contraction. The abdomen was held humped, and as the egg mass increased in volume it spanned the arch so formed from the anterior end of sternite six to the posterior end of sternite nine. As it became larger it was held by the further curling of the abdominal segments ventrally. Of these, segment nine, being almost terminal, moved through the greatest arc so that the two sharp latero-posterior projections of its tergite wedged into the egg mass. The corresponding projections of the sternite were rotated forwards still further through about 120°.

OVIPOSITION

European and North American mayflies are known to deposit their eggs in three ways—viz., (1) By crawling underwater to the undersides of stones, where the eggs are placed singly or in rows, as recorded by Eastham through Percival and Whitehead (1928) for *Baetis* sp. (2) By periodically alighting on the water so that a few of the extruded eggs are washed off each time—e.g., *Heptagenia hebe* and *Stenonema ithaca* (Smith 1935). (3) By flying low over the current so that the egg mass is swept off. The egg mass may remain as an entity in *Ephemerella rotunda*, *E. dorothea*, and *E. needhami* (Smith 1935), or disintegrate into individual eggs as in *Ecdyonurus forcipula* (Gros 1923) and *Ephemerella danica* (Percival and Whitehead 1926). Since *C. humeralis* forms an egg mass, it seems likely it has egg laying habits similar to those of (3). However, as noted by Tillyard (1933a) and by Smith (1935) the onset of darkness often prevents observations on oviposition; this was also experienced during the present study. Oviposition was only observed once. A female was seen flying very low upstream, and as she passed over some ripples in a rapid her abdomen touched the water surface and the egg mass disappeared from sight into the water. A number of egg masses was detached from females subsequently and dropped into beakers of water. These did not disintegrate immediately, suggesting that the egg masses resemble those of *Ephemerella* in this respect.



Figs. 1-6.—Fig. 1—Lateral view of egg: c = chorionic extension, ak = adhesive knob. Fig. 2—Larvule (after Phillips 1930). Fig. 3—Young nymph. Fig. 4—Anterior abdominal tergites of a young nymph showing transition in gill structure. Fig. 5—Gill of last instar nymph. Fig. 6—Maxillary gill rudiment (mgr) attached to base of maxillary palp (mp) and galea-lacinia (gl) in a young nymph. All measurements in mm. Camera lucida.

EGG

In discussing North American species Smith (1935) pointed out that the form of the egg was frequently inconsistent at the subgeneric and generic levels, and not at all constant for the families. Also, there was surprisingly little relation between the form of the eggs and the places where they were found. Eggs both with and without special anchoring devices were laid in rapid streams and quiet ponds. Some of the New Zealand mayfly eggs, including that of *C. humeralis*, have already been described by Phillips (1930). His figure (p. 284) is useful for recognition purposes but somewhat misleading; the large terminal knobs of the chorionic appendages are not shown, and the egg is still partly enclosed in overlying tissue. The outline is more irregular than is typical. Accordingly the egg has been refigured (Fig. 1) and described more fully. The egg is ellipsoidal and measures 0.20–0.25 mm in length and 0.10–0.15 mm in breadth. Its chorion is minutely pitted and has three or four adhesive organs at each pole, each organ consisting of a rope-like extension coiled like a conical spring with a terminal knob at the apex. There are no polar caps. Once the egg is wetted each terminal knob becomes adhesive in a few seconds, grips on contact, and becomes flattened and circular in outline. The spring-like extensions give the anchored egg elasticity: if the egg is pulled these extensions stretch up to five or six times their original length and snap the egg back into its original position when the tension is released. According to Smith (1935) all known species of the genus *Heptagenia* have eggs with this type of adhesive organ; it also occurs in some species of *Caenis*, and in some other genera. Smith's (1935) figure of *Caenis perpusilla* from India shows a close resemblance to the egg of *C. humeralis*; *C. perpusilla* has four adhesive organs at each pole, but the chorionic extensions of these are many times the length of those in *C. humeralis*.

LARVULE

Phillips (1930) has already described the newly hatched larvule of *C. humeralis* (Fig. 2). Development took 18 days, the larvule at hatching measuring 0.35 mm in length, the outer caudal setae adding another 0.20 mm. In 10 days the larvule was 0.50 mm long, excluding the now five-segmented outer caudal setae which were about 0.30 mm long. The antennae were five-segmented, about 0.15 mm long, and ended in a pair of bristles; there were no gills but the alimentary canal was complete.

EARLY INSTARS

A number of early instars were obtained in April, 1952, by washing the undersides of stones in Purau Stream, Banks Peninsula, and passing these washings through a series of bolting silk sieves. The smallest nymph obtained was not much larger than the larvule; the head width (hw.) was 0.15 mm, the thoracic length (tl.) 0.25 mm, and the total body length (tbl.) 0.72 mm (excluding the caudal setae). Ocelli and compound eyes were visible, the latter being smaller than the former. Each antenna consisted of a basal scape, a pedicel and two other segments; the interior of the pedicel was subdivided into three lobes, suggesting that this was the proliferating segment as in many other insects. A long spike was based on the distal segment and equal in length to the rest of the antenna (0.17 mm), and four smaller bristles radiated out intersegmentally. None of the 10 abdominal segments bore gills.

In nymphs of hw. 0.17 mm, tl. 0.25–0.32 mm, and tbl. 0.85–1.15 mm, the antennae were six-segmented and there were six pairs of peg-like gills present on the third to eighth abdominal segments. In a nymph of h.w. 0.20 mm, tl. 0.40 mm, and tbl. 1.25 mm the gills were still peg-like, but

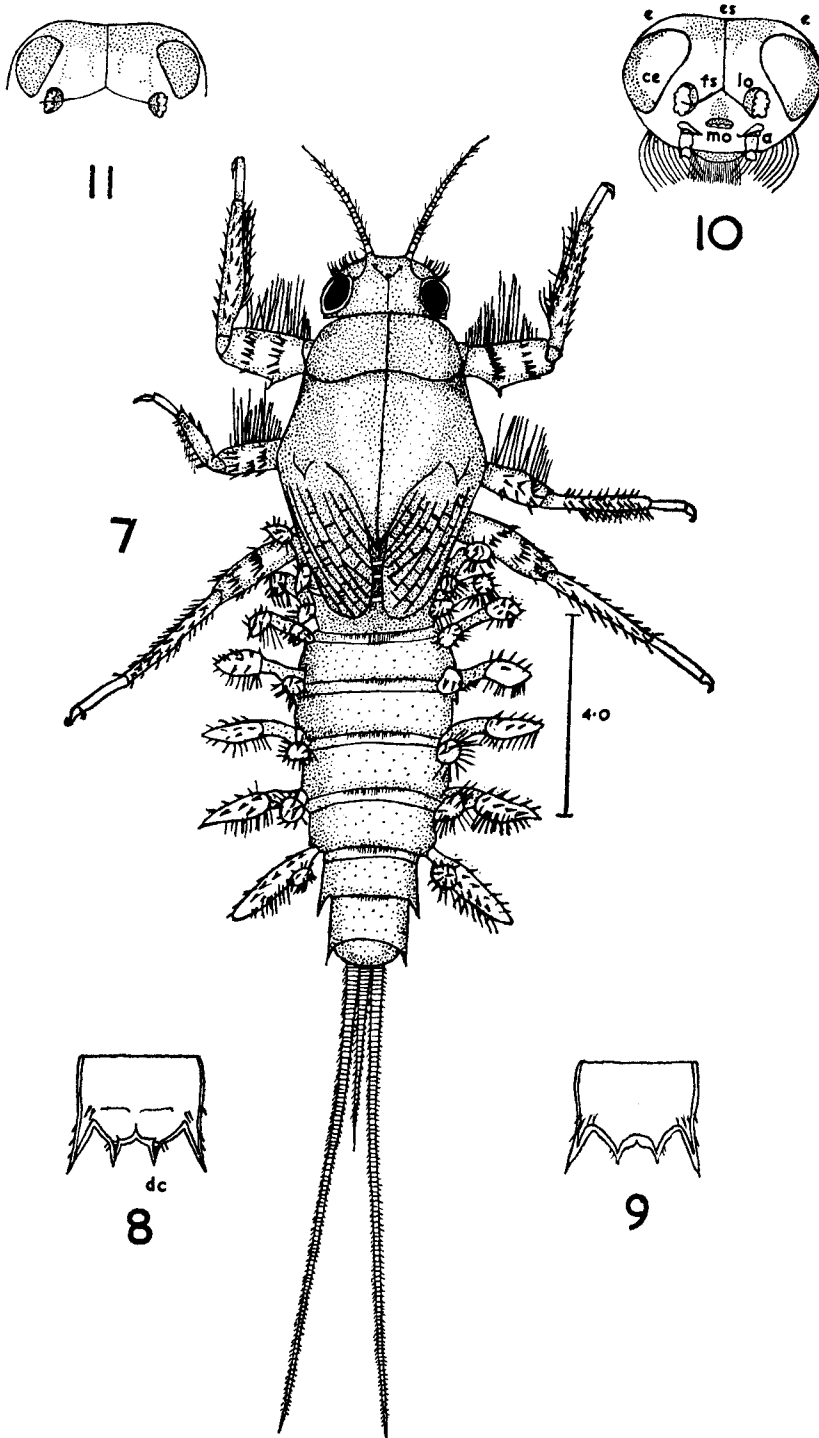
the compound eyes were larger than the ocelli. The antennae had nine segments. In a larger nymph (hw. 0.35 mm, tl. 0.47 mm, and tbl. 1.30 mm) the antennae had 12 segments and there were seven pairs of gills. The latest pair appeared on the second abdominal segment. In this nymph and one other (hw. 0.47 mm, tl. 0.52 mm, tbl. 1.80 mm) the gills showed evidence of bifurcation and in the latter specimen the antennal segments numbered 17.

A nymph of hw. 0.55 mm, tl. 0.62 mm, and tbl. 2.5 mm (Fig. 3) bore 14 gills arranged in pairs on the first seven of the nine abdominal segments. The gills on the anterior part of the abdomen of a slightly younger nymph than this are shown in Figure 4; the gills of the first segment were peg-like, those of the second slightly bifid, and those of the third distinctly forked, bearing spines, and differing little from those of segments four, five, six and seven. Apparently as each gill increased in size it became increasingly bifid and spined more like the characteristic *C. humeralis* gill (Fig. 5). Nymphs of this size were partially transparent and pale in colour; the dark brown colour of heavily chitinized structures was limited to the grinding plates, prothecae, and canines of the mandibles, the gill and leg spines, and the posterior abdominal sternites. The buccal cavity, oesophagus, and rectum were not clearly delimited in the tubular gut. A slight increase of diameter was apparent in the midgut region and there were usually five Malpighian tubules present at the midgut-ileum junction. The ganglia of the nervous system resembled those of older nymphs but were relatively less pigmented and closer together in the abdomen.

At this stage the galea-lacinia and the maxillary palp originate from a common base (Fig. 6) together with a rudimentary axillary gill which already shows evidence of bifurcation. As growth proceeds the maxillary gill of each side bifurcates to form two long white filaments which project into the water below the head; each contains tracheae continuous with the longitudinal head and thoracic region trunks. Maxillary gills have not been recorded previously for this genus but additional gill surfaces have been noted in a number of other genera. In the European genus *Oligoneura* and the North American *Isonychia* there are, in addition to the full series of seven pairs of abdominal gills, a cluster of gill filaments at the base of each maxilla and at the base of each leg (Needham, 1935). The Australian *Coloburiscoides giganteus* has a cluster of filaments on the posterior face of the abdominal gill (Tillyard 1933b, Fig. 43) and it seems likely, from Tillyard's descriptions, that a second species in the genus, *Coloburiscoides munionga*, has similar structures. The peculiar Chilean genus *Murphyella* lacks abdominal gills but has a single long gill attached midventrally on each thoracic sternite. There is an added pair beside the single gill on the prothorax, as well as two pairs on the maxillae and one pair on the labium (Needham and Murphy 1924).

NYPH

The forked spiny gills (Fig. 5) of a *C. humeralis* nymph (Fig. 7) distinguish it from all other described mayfly nymphs, with the exception of the Australian genus *Coloburiscoides* which has similar structures. Seven pairs of gills are present on the first seven abdominal segments, those on segments three and four being the largest. Each gill has an upright lamella studded with fine bristles and containing numerous tracheoles; it is borne between a stout inner and outer arm. The latter bears stout spines. Although these structures and the maxillary gills are probably used in respiration, it is not uncommon to find apparently healthy nymphs with up to seven out of the 14 abdominal gills absent or in the process of regeneration. When the nymph is in its normal clinging position among the stones of rapids it is exposed to swift currents, and it seems likely that the gills, which are held vertically, may sometimes be broken off by current-borne objects.



FIGS. 7-11.—Fig. 7—Dorsal view, last instar female nymph. Fig. 8—Sternite nine of male nymph showing developing claspers (dc). Fig. 9—Comparable sternite of female. Fig. 10—Anterior view of head of male nymph; a = antenna, ce = compound eye, e = epicranium, es = epicranial suture, fs = frontal suture, lo, mo = lateral and median ocellus. Fig. 11—Anterior view of part of head of female nymph. All measurements in mm. Camera lucida (except Fig. 7).

Over the last four stadia two pointed lobes appear on the posterior border of sternite nine (Figs. 8 and 9) which contain the developing claspers in the male and the egg valve in the female. Males can be distinguished at this stage by the presence of two segments in each lobe, in contrast to the unsegmented lobes of the females. Segment ten is rounded and consists, according to Needham (1935), of the epiproct and paraprocts of segment 11 in addition to its own elements. As in many Siphonuridae the median caudal seta is short. The lateral setae consist of tapering segments each bearing a circle of large spikes at the posterior ends and smaller spikes and bristles intersegmentally. The caudal setae are used in swimming, particularly when the nymph is rising to the surface before emergence as a subimago, and are also probably sensory; nymphs crawling backwards swing segment ten and the setae from side to side and tap the substratum with the latter.

The thorax is heavily chitinized and humped dorsally. The pronotum is narrow longitudinally and wide transversely; its posterior limit with the metathorax is marked by an emarginate suture. The mesothorax accommodates, in the later instars, the large developing wing muscles and wing buds, and is fused to the metathorax which bears the smaller hind wing buds, the two forming a synthorax. The prothoracic legs consist of basal coxa, trochanter, a large flattened femur, tibia and tarsus, the latter with a single claw. The meso- and metathoracic legs are similar. Both the pro- and mesothoracic legs have two parallel lines of bristles on the anterior edge of the femora which are used, as discussed later, in feeding.

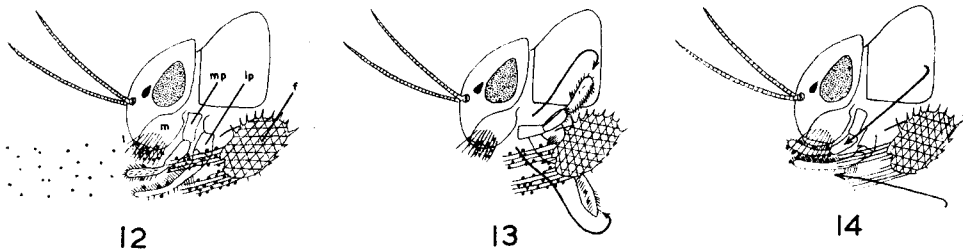
The larger part of the head capsule consists of the epicranium (Fig. 10) which is considered to be composed of fused vertex, occiput and genae. It is bisected medianly by the epicranial (or coronal) suture which bifurcates anteriorly into two frontal sutures. The fronto-clypeus occupies the area between the latter and the antero-lateral margin of the head and contains the median ocellus and antennae. The clypeus is distinct, rectangular, and overlies the labrum. The basal scape of the antenna is short and thick, the pedicel is twice the former's length, and the antennal segments number up to approximately 40. The compound eyes of the male nymph are relatively larger than those of the female and are reddish in their inner halves (cf. Figs. 10 and 11). In contrast the female eyes are relatively wider apart, uniformly dark brown, and the distance between the lateral ocelli is less. The mouth-parts of the nymph, consisting of labrum, mandibles, maxillae, hypopharynx, and labium have been described and figured by Phillips (1930).

FEEDING

Most mayfly nymphs are herbivorous, feeding on diatoms, algae, and detritus (Lubbock (1886), Eaton (1888), Murphy (1922)). *Chiloporter*, *Ameletopsis*, and *Prosopistoma* are considered carnivorous and *Chirotenetes* is said to feed on insect larvae and vegetative detritus (Needham 1935) thereby utilizing two sources of food. In describing the mouth-parts of *Coloburiscus* Phillips (1930) suggested "the fringes of hair on the forelegs and mouth-parts . . . would seem to form a plankton straining apparatus." Observations on this point were not made in the present study on nymphs in their natural habitat since they inhabit the undersides of stones in comparative darkness; however, laboratory observations tend to substantiate Phillips' suggestion.

To make these observations, each nymph was placed on its back under a dissecting microscope, being almost sandwiched by two sheets of glass. The weight of the upper sheet of glass was supported by two strips of cardboard arranged in a large V with the head of the nymph facing a small opening in the apex. The lower sheet lay on a tray fitted with a water outlet. A large tank containing

water and stirred up washings from stones taken from Purau Stream was placed above this so that a steady jet of water could be siphoned from it. When this jet was directed through the small apical opening in the V, starved nymphs quickly moved into position facing the current and commenced feeding.



FIGS. 12-14.—Feeding of nymph. Potential food is represented by black dots, current borne from the left; l = labrum; m = mandible base, mp = maxillary palp, lp = labial palp, f = femur of prothoracic leg. The latter extends some distance laterally to the former (see Fig. 7). Fig. 12—Potential food is accumulating. Fig. 13—The labial and maxillary palps at the beginning of the return stroke. Fig. 14—The palps have brought the potential food in towards the mandibles and other mouth-parts. Diagrammatic.

As feeding commences the glossae move dorsally and the maxillary palps move towards each other so that the galea-laciniae (which are attached to the inner side of the maxillary palps) come together in a groove which is bounded anteriorly by the labrum and posteriorly by the glossae and paraglossae. As the maxillary palps are moving outwards the labial palps are moving inwards, and *vice versa*; since the apposing faces of these structures are studded with bristles which point towards the midline, this action shuffles food material towards the mandibles and mouth. Meantime (Fig. 12) the long bristles fringing the anterior borders of the femur and tibia of each prothoracic leg have been accumulating food material from the current. These bristles lie in two parallel lines, one slightly more dorsal than the other. Similar bristles on the anterior edge of the labrum and the mandibular bases act in the same way. Potential food material on these structures is brought within the range of the galea-laciniae and mandibles by synchronous sweeps of the maxillary and labial palps (Fig. 13). These sweep backwards and outwards and as they return towards the mouth the maxillary palp combs the upper line of femoral bristles and the labial the lower. The movement is performed extremely quickly and is accompanied by a short inward flick of the prothoracic leg. The completion of the movement is shown in Fig. 14. There are supplementary movements. Sometimes a quick circular flick of the maxillary palp is used to collect from the labrum and mandible bristles, but more commonly the whole leg is used. In this case the tibia and tarsus are brought over the eyes and towards the labrum. When this occurs the bristles on the inner side of the leg comb those of the mandible and labrum and sweep the collection to the maxillary and labial palps. At times the prothoracic legs are moved back towards the mouth and the tibial and tarsal surfaces cleared. Although the mesothoracic legs resemble the prothoracic and also collect food material these do not appear to be greatly utilized; only the very distal parts of the bristles are combed by the palps. These actions result in a longitudinal rope of food material being formed which passes steadily posteriorly between the labial palps. Much of it disappears from view between the mandibles which are constantly triturating. Sometimes a peculiar movement takes place; the head is withdrawn posteriorly against the prothorax and then pushed anteriorly again, the complete cycle lasting about a second. Concomitantly, a similar movement occurs with the mandibular bristles, but the remainder of the mouth-parts remain stationary.

Possibly this movement is a swallowing and compressing movement which may aid food passing posteriorly through the alimentary canal. When starved nymphs were placed in an aerated tank containing washed diatom and algal-covered stones from their habitat they commenced feeding as just described, but at no time were they observed to scrape food material from the stones with their mouth-parts. This was in marked contrast to *Deleatidium lillii* nymphs which, as soon as they were added, began immediately "grazing" the diatoms and algae.

The gut contents of freshly caught nymphs from Purau Stream consisted mainly of diatoms, algae, and detritus (i.e., vegetative matter too macerated to permit identification). In addition to this, nymphs from Grasmere Stream, Cass. contain material indistinguishable from the faeces of a mollusc *Potamopyrgus* sp. which is extremely abundant there. Animal fragments were not observed in any gut contents. In general these observations suggest that *C. humeralis* nymphs collect current-borne and mainly vegetative material from the rapids in which they live, and that they are not "grazing" or predatory feeders.

REFERENCES

- EATON, A. E., 1833-87. A revisional monograph of recent Ephemeridae or mayflies. *Trans. Linn. Soc. London*. Sec. Ser. Zool. 3: 1-352.
- GROS, A. J., 1923. Etudes sur les Primeres Stades des Ephemerides du Jura Francais. *Ann. de Biol. lac.* 12: 49.
- Lubbock, J., 1866. On the development of *Chloeon (Ephemera) dimidiatum*. *Trans. Linn. Soc. London*. 24: 61-78.
- MURPHY, H., 1922. Notes on the biology of some of our North American species of mayflies. *Bull. Lloyd Library* 22, Ent. Ser. 2: 1-46.
- NEEDHAM, J. G., 1935. *The Biology of Mayflies*. Comstock Pub. Coy. N.Y.
- NEEDHAM, J. G. and MURPHY, H. E., 1924. Neotropical mayflies. *Bull. Lloyd Library* 24, Ent. Ser. 4: 1-79.
- PERCIVAL, E., and WHITEHEAD, H., 1926. Observations on the biology of the mayfly, *Ephemera danica*, Mull. *Proc. Leeds Phil. Soc. Sci. Sect. 1*: 136-148.
- 1928. Observations on the ova and oviposition of Ephemeroptera and Plectoptera, *Proc. Leeds Phil. Soc. Sci. Sect. 1*: 271-288.
- PHILLIPS, J. S., 1930. A revision of New Zealand Ephemeroptera. *Trans. N.Z. Inst.* 61: 271-390.
- RIEK, E. F., 1955. Revision of the Australian Mayflies (Ephemeroptera). I. Subfamily Siphonurinae. *Aust. J. Zool.* 3: 266-80.
- SMITH, O. R., 1935. The eggs and egg-laying habits of North American mayflies. In Needham (1935) pp. 67-89.
- TILLYARD, R. J., 1933a. The trout-food insects of Tasmania, Part I. A study of the genotype of the mayfly genus *Atalophlebia* and its life-history. *Papers and Proc. Roy. Soc. Tas.* 1933: 1-16.
- 1933b. Mayflies of the Mt. Kosciusko region, N.S.W. Introduction and Siphonuridae. *Proc. Linn. Soc. N.S.W.* 58: 1-32.

B. WISELY, M.Sc.,
C.S.I.R.O. Division of Fisheries and Oceanography,
Cronulla, New South Wales, Australia.