A METHOD OF COUNTING EPHEMEROPTERAN EGGS

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In order to determine the number of eggs laid by an insect it is essential to know that no eggs have already been laid. This initial difficulty can be overcome conveniently in the Ephemeroptera by examining ovaries from the subimago when the eggs are fully developed, none being laid until the insect becomes an imago. The present note describes a method of counting the eggs of *Cloëon simile* Eaton, using a standard Sedgwick-Rafter plankton-counting cell (Whipple, 1899). The method was tried out by the writer whilst working at the Scottish Home Department Brown Trout Research Laboratory, Pitlochry, during the autumn of 1955.

Subimagines of *C. simile* previously preserved in formalin alcohol were used. A subimago was placed in the Sedgwick-Rafter cell. The head, prothorax, mesothorax and the two terminal segments of the abdomen were removed, and the metathorax and the abdomen were then opened dorsally. The ovaries were removed as intact as possible; the rest of the specimen was then discarded. A drop of methylene blue was added and left for about a minute to stain the eggs. The eggs tend to adhere in clumps (20–50). The ovaries were therefore transferred to a sieve made of a piece of glass tubing (internal diameter 10 mm. and length 33 mm.) over the end of which was stretched a piece of bolting silk, 20 meshes to 1 cm., held in place by a strong elastic band. The eggs were brushed through the bolting silk, using a fine camel-hair brush, into the counting cell, to which had been added about 0.5 ml. of boiled water plus a drop of detergent. Boiled water was used to prevent bubbles forming when the cover-glass was added to the cell, and the detergent prevented the eggs from floating by reducing surface tension. The brush which had been used in sieving the eggs was brushed over the underside of the cell cover-glass to transfer eggs adhering to it. The cover-glass was then placed transversely across the counting cell and twisted into position, boiled water being added at the corners as necessary. Any eggs which were washed out of the cell were counted. A thick coating of glycerine was then brushed round the junction of the cell wall and the cover-glass to prevent evaporation of the enclosed water.

The eggs in the cell were counted by counting longitudinal rows alternately left to right and right to left. The differential staining of the eggs (the outer ones of the ovary taking up the dye more heavily) aided in counting when there were a fair number in the square of the counting cell. As a check on any eggs still remaining the brush used for sieving was wiped clean on a slide and any eggs which came off counted and the sieve itself examined for attached eggs. All counting was done under a binocular dissecting microscope.

A series of four subimagines of *C. simile* counted by this method contained the following number of eggs—

2269, 2533, 2071, 2259.

The advantage of sieving was evident in the difference in number of eggs obtained (e.g. 1518) before this step was introduced. All specimens were taken in late August and early September. Allowing for some individual variation, the numbers obtained are sufficiently consistent to suggest that this is a useful method of counting eggs in the Ephemeroptera and possibly other suitable insects.

REFERENCE

Whipple, G. C., 1899. *The microscopy of drinking water*, London.

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