

SOME BIOMETRICAL CONSTANTS FOR *ANOPHELES QUADRIMACULATUS* SAY LARVAE IN RELATION TO AGE WITHIN STADIA

JACK COLVARD JONES

Laboratory of Tropical Diseases, U. S. Department of Health, Education and Welfare,
Public Health Service, National Institutes of Health, National Microbiological
Institute, Bethesda, Maryland

The present writer has found only one paper giving size measurements on *Anopheles quadrimaculatus* larvae, that of Abdel-Malek and Goulding (1948) on head capsule widths.

In physiological and toxicological experiments on *A. quadrimaculatus*, some means of estimating the age of larvae within a given stadium was needed, for it had been observed that larvae from the same egg batch grew at different rates under uniform conditions. To determine larval age, measurements of total body lengths, thoracic widths, "collar" lengths, and head capsules were made. Measurements were also made on eggs.

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METHODS. All studies were made on material from a heterogeneous colony of *A. quadrimaculatus* Say (Diptera: Culicidae).

Eggs laid during the night were collected the next morning, measured in a film of distilled water on a slide at 75 diameters, and placed in 20 ml. of distilled water in numbered, filter-paper-lined petri dishes for hatching at 28 to 30° C. The larvae were fed daily on a 1:1 mixture of dog food and brewer's yeast until they pupated. Larvae took from 1-3 days to complete each of the first 3 stadia and from 2-4 days to complete the fourth stadium. Many larval measurements were made on living specimens to permit daily observations on single individuals of known history. At first, larvae were measured while floating in water in micro slides. Since this proved very inconvenient, larvae were placed in drops of distilled water on ordinary glass slides and all but a thin film of

water removed with filter paper strips prior to measurement at magnifications of 6.6 to 75 diameters. For studies on first instar collar lengths alcohol-fixed material was examined at 750 diameters.

DEFINITION OF TERMS. 1. Head capsule width: The widest portion of the head capsule, between the larval eyes, was measured.

2. Collar length: The posterior rims of the epicranial plates form a collar-like piece around the posterior end of the head. This structure was referred to by Smith (1914) in her beautiful study of *Anopheles punctipennis* as the collar. The collar grows progressively in length during each of the first 3 stadia. It is clearly a part of the head, for it is shed with the cuticle of the latter at each molt. The anterior to posterior dimension of the dorsal portion of the collar was measured.

3. Total head length: Includes both the growing collar region and the non-growing portion of the head anterior to it (pre-collar region).

4. Thoracic width: The widest portion of the thorax, the mesothorax, was measured.

5. Total body length: The distance from the anteriormost portion of the frontoclypeus to the anus was considered as the total body length. This measurement excluded mouth brushes and anal papillae. All measurements were made along the mid-dorsal line.

RESULTS. The various egg dimensions of *Anopheles quadrimaculatus* were found to be remarkably constant. The constancy of the figures (Table 1) suggests that there are no size differences between male and female eggs.

Mean head capsule widths and the ranges in total body lengths for the four instars of *A. quadrimaculatus* are given in Table 2. Variations in head capsule widths for each stadium and their frequencies are graphed in Fig. 1. The relation between the mean head capsule widths and total body lengths is shown in Fig. 2.

The values in Table 2 differ slightly but, nevertheless, significantly from those given by Abdel-Malek and Goulding (1948). While their data actually showed that the greatest proportionate differences (ratio of mean head capsule width of one instar to the next) in head capsule widths occurred between the second and third stadia, they concluded from other information that this was due to an experimental error in measurements, and that it should have occurred between the first and second stadia. The data in Table 2 confirm their conclusions and correct their experimental error.

The range in total body length measurements for a given stadium overlaps that of the preceding stadium (Table 2) as a result of different growth rates, but the

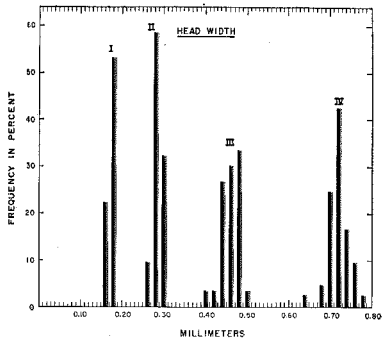


FIG. 1.—Frequencies in head capsule width variations for each of the instars of *A. quadrimaculatus*.

TABLE 1.—Mean dimensions of 25 eggs of *Anopheles quadrimaculatus* Say

Part measured	Mm.	S.E.	SD
Total egg length	0.56	0.004	0.019
Anterior (top) width	0.14	0.002	0.01
Center width (including floats)	0.21	0.002	0.01
Depth (dorso-ventral dimension at egg center)	0.14	0.001	0.01
Posterior (bottom) width	0.11	0.002	0.01
Float length	0.29	0.004	0.02
Float width	0.09	0.016	0.01

TABLE 2.—Mean head capsule widths and total body lengths for the four instars of *Anopheles quadrimaculatus*

Instar	Head capsule width*			Capsule ratios	Total body length			Magnif.
	No. measurements	Width (mm.)	SD		No. measurements	Length Min.	Length Max.	
I	75	0.174†	0.009	1.638	125	0.7	1.7	30 X
II	53	0.285†	0.012		98	1.4	3.0	30 X
III	60	0.459‡	0.019	1.611	104	2.5	5.6	13-30 X
IV	85	0.720	0.025	1.569	190	3.3	7.6	6.6-13 X

* All measurements at 75 x.

† Values differ from those of Abdel-Malek and Goulding, (1948) by ± 0.016 .

‡ Value is 0.009 less than Abdel-Malek and Goulding reported.

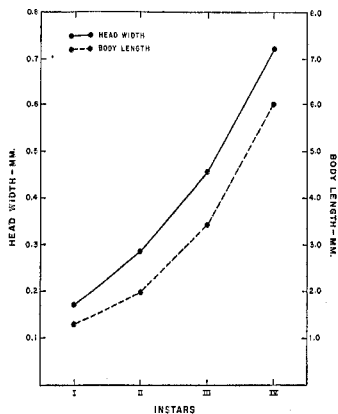


FIG. 2.—Relation between mean head capsule widths and mean total body lengths in the larval stage of *A. quadrimaculatus*.

mean total body lengths are different for each stadium. Increases in total body length occur at the beginning of each new larval stadium. For example, immediately after molting to the fourth stadium total body lengths increased 0.1 to 0.4 mm. (mean increase 0.23 mm.). A decrease in length is seen prior to pupation. Since body lengths overlap within a given stadium, they do not permit estimations of age within it; the same is true for thoracic widths.

Immediately after hatching the collar is either not present or only faintly indicated (Fig. 1, Plate I). Shortly thereafter it becomes clearly defined but does not completely encircle the head. Later as the collar lengthens it forms a continuous band around the base of the head (Fig. 6A, Plate I). The fully formed collar is made up of 3 distinct zones: (1) a dark anterior rim, (2) an intermediate tan zone, and (3) a terminal clear zone.

Since the collar undergoes changes in length within each of the first 3 stadia, while that portion of the head anterior to the collar (pre-collar region) undergoes no change in dimensions, it is possible not only to identify an instar by capsule width but to determine its age by collar length. Collar lengths in first instars grow from

approximately zero to 0.066 mm. (Table 4). Since almost every intermediate between these two extremes can be found, the growth is considered continuous. Measurements of collar lengths of larvae at different periods within the third stadium (Table 3) furnish a means of roughly estimating age to the nearest quarter of the stadium.

Observations on darkening of the head capsule and pigmentation of the palmate hairs also permit rough estimation of larval age. The head capsules of newly

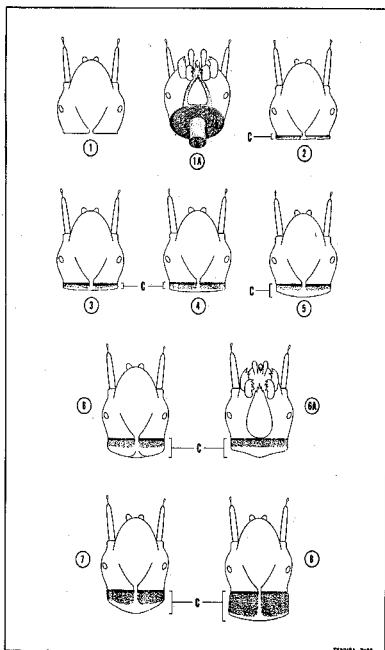


Plate I.—Development of the collar (c) in first instars.

- Fig. 1.—Dorsal view of head of 2 hour old larva.
 Fig. 1A.—Tipped ventral view of head of 2 hour old larva showing that the "collar" does not encircle the head.
 Fig. 2.—Dorsal view of head of 7 hour old larva.
 Fig. 3.—Dorsal view of head of 10 hour old larva.
 Fig. 4.—Dorsal view of head of 18 hour old larva.
 Fig. 5.—Dorsal view of head of 22 hour old larva.
 Fig. 6.—Dorsal view of head of 30 hour old larva.
 Fig. 6A.—Ventral view of head of 30 hour old larva.
 Fig. 7.—Dorsal view of head of 34 hour old larva.
 Fig. 8.—Dorsal view of head of 40 hour old larva.

TABLE 3.—Measurements of collar length, thoracic width, and body length in third and fourth instar *A. quadrimaculatus**

Part measured	Instar	Range in dimensions (mm.) at different periods within stadium			
		First quarter	Second quarter	Third quarter	Fourth quarter
Collar length	III	0	0-0.04	0.03-0.09	0.08-0.10
	IV	—	—	—	—
Thoracic width	III	0.6-0.7	0.7-0.9	0.7-1.1	0.9-1.1
	IV	1.05-1.30	1.2-1.45	1.3-1.55	1.4-1.55
Total body length	III	2.2-3.2	2.8-4.0	3.2-4.0	3.6-4.4
	IV	4.0-6.5	4.8-5.9	5.7-6.2	5.5-5.7

* Data based on measurements from 10 III instars and 10 IV instars.

hatched or molted larvae are always very light in color. In the third stadium the head capsule is very light during the first quarter, light tan to brown in the second quarter, and either tan or dark brown during the third and fourth quarters. Palmate hairs in the first stadium are single, thin, colorless, spindle-shaped structures; they remain clear in the second stadium and become pigmented first in the third stadium (usually only those on segments III-V are darkly pigmented). The palmate hairs are very light or just beginning to become pigmented in the first two quarters of the third stadium. As is well known, pigmented palmate hairs occur on segments II/III-VII in fourth instars. In the fourth stadium the palmate hairs are fully pigmented after the first quarter.

TABLE 4.—Changes in collar length during the first stadium of *A. quadrimaculatus* (10 larvae measured at each age)

Hours old	Range in collar length (mm.)	Mean collar length
2	0-0.006	0.003
7	0.002-0.008	0.005
10	0.004-0.010	0.006
14	0.006-0.014	0.009
18	0.011-0.024	0.015
22	0.014-0.030	0.024
26	0.020-0.030	0.026
30	0.020-0.048	0.030
34	0.026-0.060	0.039
40	0.034-0.066	0.052

Some additional observations not pertaining to age estimation are of interest. The so-called "notched organ" of Nuttall and Shipley (1901) is not at all notched in the first instar. There is no ventral brush on the anal segment in the first instar although the region from which it will form is clearly indicated. The imaginal compound eye disc first becomes apparent at the beginning of the third stadium. Green, pinkish, brown, and mixed pigmentation of larvae becomes evident only in the fourth stadium.

DISCUSSION. Measurements on eggs of *Anopheles quadrimaculatus* given in this paper are generally quite comparable to those reported for a number of other species of *Anopheles* (Smith, 1914; Sweet and Rao, 1939; Bellamy and Repass, 1950). There appear to be no measurements on egg depths in the literature on *Anopheles* mosquitoes.

The head capsule width of *A. quadrimaculatus* larvae in a given stadium is fixed as is the length of the head anterior to the collar, but the total head length progressively increases within each of the first 3 stadia due to development of the collar.

Immediately after a molt the new head capsule has no collar and the length of the new capsule is found to be almost the same as at the end of the preceding stadium. The new head capsule is continuously formed underneath the old one and extends underneath the collar. While it

is true that the change in head capsule widths from one stadium to the next appears discontinuous, the growth responsible for the change has been continuous. In other words, the formation of a new head capsule is not the result of explosive cellular divisions at the molt but rather is the result of a gradual growth throughout the given stadium and is made manifest at the molt by an expansion in width. There do not, then, appear to be periods when true growth is entirely absent in the development of the head capsules of the first 3 stadia of *A. quadrimaculatus*.

Mitrofanova (1929) made measurements on the collars and pre-collar head lengths of *Anopheles maculipennis* larvae and referred to the progressive growth of the collars, but she did not use this information for determination of age within a stadium. She found that the total head length between stadia always increased slightly at the molt.

SUMMARY. I. The following biometrical constants were obtained for *Anopheles quadrimaculatus* Say: Mean measurements of length, anterior width, center width, posterior width, depth, float length and width for the eggs; mean head capsule widths and ranges in total body lengths for the four stadia.

2. A region posterior to and continuous with the epicranial plates of the larval

head capsule was found to grow continuously within each of the first three stadia. This region of the head capsule is referred to as the collar.

3. Approximations of age within the first 3 stadia can be made by measurements of collar lengths alone.

4. Darkening of the head capsule and pigmentation of the palmate hairs are helpful in age approximation of larvae.

5. Thoracic widths and total body lengths overlap within stadia and therefore do not permit determination of approximate ages.

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