GROWTH OF THREE MOSQUITOES ON TWO LARVAL DIETS MEASURED BY PROTEIN ACCUMULATION[1]

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ABSTRACT. Larval growth recorded as accumulation of protein was measured in *Aedes aegypti, Culex nigripalpus* and *Culex quinquefasciatus,* raised on liver powder or dry brewer's yeast. In the early stages all 3 species grew faster on liver powder, but at pupation there was no significant difference in protein content between diets and among species. *Aedes aegypti* pupated one day ahead of *Culex.*

INTRODUCTION

Larval development of mosquitoes is divided into 4 discrete instars. Assignment of instar depends on estimated larval size. More stringent criteria depend on measurement of head capsules (Nemjo and Slaff 1984) but this is tedious and time-consuming. Since cuticle is incapable of growth, it was assumed that growth takes place exclusively during molting (Wigglesworth 1939). When one defines growth as accumulation of body mass, it can be shown that much activity takes place between molts, and the "dogma" of growth by molting alone was therefore eloquently rejected by Williams (1980). Bradshaw (1983) proposed biomass determined by dry weight as a criterion for growth of mosquito larvae. This method is time consuming and since most mosquitoes vary between 0.002 (first instar) and 1 mg (fourth instar and pupae), requires rather large pools for accurate results.

In the present work I have used protein accumulation as a criterion for growth. This method appeared to be sensitive enough to identify small differences in growth rates between larval populations. Numerous diets are in use to raise mosquitoes (Gerberg 1970, Nayar and Sauerman 1970, Friend and Dadd 1982). Often different diets are used for different species. I have compared the difference in protein accumulation between 2 popular diets, brewer's yeast and liver powder, in 3 species developing simultaneously under the same conditions.

MATERIAL AND METHODS

Aedes aegypti (Linn.), *Culex nigripalpus* Theobald and *Culex quinquefasciatus* Say, all from established colonies, were raised under 12 hr light—12 hr dark at 26°C in 22 × 32 cm enamelware pans containing 500 ml water and 150–160 larvae. Each treatment consisted of at least 2 pans. On the day of hatching, and 3, 4 and 5 days later, 100 mg food was added. The following modification of the protein assay of Lowry et al. (1951) was used to evaluate growth. Pools of larvae or pupae from each pan (25 on day 3 and 4, 10 on day 5, 6 and 7) were homogenized in culture tubes in 5 ml of an alkaline solution (20 g water-free or 54 g crystalline sodium carbonate and 4 g NaOH per liter) in the presence of a sliver of cover glass to facilitate grinding. After several hours or standing overnight, duplicate aliquots containing between 0.1 and 0.2 mg protein and standards containing 0.1 and 0.2 mg protein, were transferred to 16 × 100 mm culture tubes marked at the 5 ml level, and filled to that level with the same alkaline solution. Then 0.2 ml of copper reagent (0.5 g copper sulfate and 2 g K-Na tartrate per 100 ml) and 0.2 ml Folin-Ciocalteau reagent were added and mixed immediately. The blue color was read at 650 nm after 30 min. As a standard, we used 100 mg bovine serum albumen preserved with 100 mg sodium azide per 100 ml. The protein assay was repeated until the difference among the four replicates was less than 5%. Pupae were analyzed when most larvae had pupated, to minimize sex bias. In preliminary experiments the differences between diets appeared to be rather small, with occasional overlap. The entire experiment was therefore repeated 8 times. The average protein level of 2 pools of insects was treated as a single data point, and the average of the 8 trials was used in the calculation of standard error. To prevent overlap the data were plotted slightly to the right (yeast) and left (liver) of the time points. Analyzed with the same method, liver powder had 60% and yeast powder 55% protein.

RESULTS AND DISCUSSION

Aedes aegypti started to pupate after 5 days on both diets. *Culex nigripalpus* and *Cx. quinquefasciatus* developed almost synchronously and started to pupate after 6 days. In some trials the first

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pupae appeared on the liver diets several hours before the yeast diet. This occurred in all three species but not necessarily in the same trial. Judged by size, *Aedes aegypti* reached fourth instar after 4 days and pupated on the sixth day. *Culex nigripalpus* and *Cx. quinquefasciatus* reached fourth instar after 5 days and pupated on the seventh day. The data (Figs. 1, 2, 3) strongly suggest that growth as measured by protein accumulation continues in fourth instar larvae. Wigglesworth (1942) had already shown with histological techniques that *Aedes aegypti* in their fourth instar continue to synthesize several body constituents including protein.

In all species, accumulation of protein was slightly faster on liver than on yeast diet. However, at the time of pupation the difference between yeast and liver was no longer statistically significant. In *Cx. quinquefasciatus*, where the difference was largest and persisted to the day before pupation, it had almost disappeared in the pupae, mainly because there was a drop in protein from fourth instar to pupation on the liver diet (Fig 3). The other 5 curves showed little difference between the late fourth instar and pupae. In all three species, reared on liver or yeast, pupal protein varied from 400 to 450 µg. *Aedes aegypti* larvae had already grown on the third day to almost half the final protein content (Fig. 1). At this time, *Culex* had barely accumulated one-tenth of the final amount (Figs 2, 3). On the liver diet, almost linear growth took place between day 3 and 5 in *Culex*, whereas in *Aedes aegypti* it slowed down after day 4. On the yeast diet, maximum growth rates occurred between day 4 and 5 for all three species. Judged by early growth rate, liver seems to be superior to yeast, but in the pupal stage, a difference between these two diets could not be demonstrated.

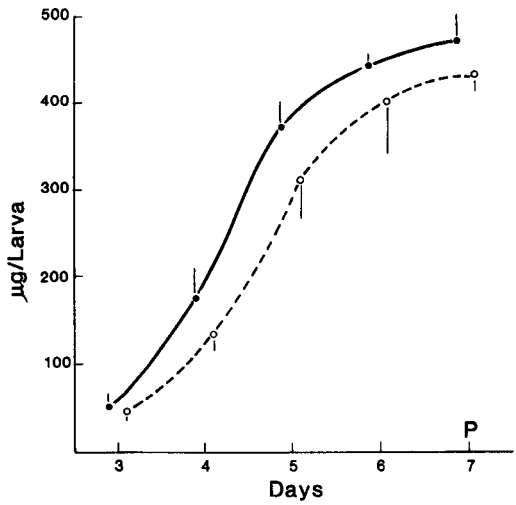

Fig. 2. Protein content of *Culex nigripalpus* raised on liver (solid line) or on yeast (broken line).

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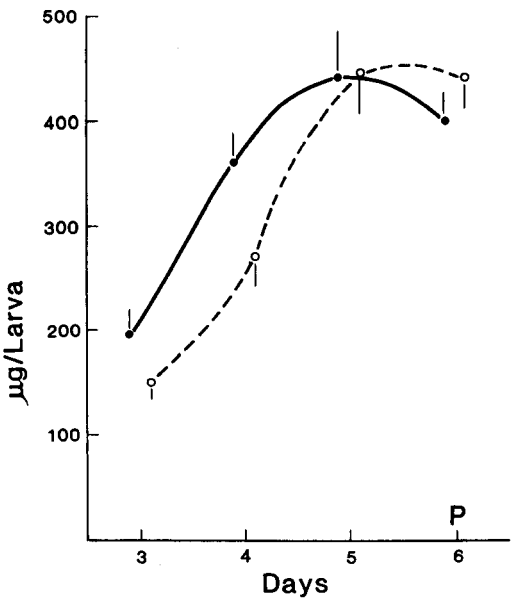

Fig. 1. Protein content of *Aedes aegypti* raised on liver (solid line) or on yeast (broken line). The vertical line is one standard error.

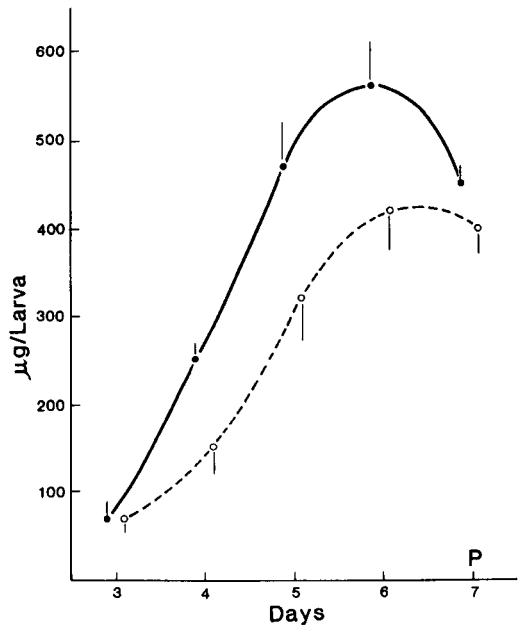

Fig. 3. Protein content of *Culex quinquefasciatus* raised on liver (solid line) or on yeast (broken line).

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