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LABORATORY REARING OF *TOXORHYNCHITES RUTILUS RUTILUS* (COQUILLETT) ON A NON-LIVING DIET[1]

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ABSTRACT. For the first time, *Toxorhynchites rutilus rutilus* (Coquillett), a predatory species of mosquito, was reared on a non-living diet of a commercially available food for tropical fish. Larvae reared on the non-living diet required an average development time of 107.5 days from egg to pupa compared with 15.6 days for larvae that were fed a diet of larvae of *Aedes aegypti* (L.). The daily survival rate of larvae reared on the non-living diet was 0.9901±0.0098, a value slightly less than the survival rate of 0.9973±0.0075 for larvae fed *Ae. aegypti.* Even though the daily survival rates for the 2 groups are about equal, the longer development time required for the non-living diet groups resulted in only ca. 24% pupation vs. ca. 98% for the group-fed larvae. Pupae reared on the non-living diet were much smaller (29.3±3.6 mg) and produced smaller adults than pupae in the control group (50.0±2.0 mg), but surprisingly, adult longevity and fecundity of the small adults did not differ significantly from the control. The significance of the above findings are discussed relative to the use of *Tx. r. rutilus* as a biological control agent for container-breeding mosquitoes.

INTRODUCTION

Toxorhynchites (Theobald) is a genus of large, non-biting mosquitoes that are predacious during the larval stages on certain mosquitoes that breed in discarded cans, bottles, tires, water cisterns, and treeholes. Brown (1973) considers species of the genus to be sufficiently promising as biological control agents of artificial-container breeding mosquitoes to warrant continued research toward this end. In this connection, inundative release of adult *Toxorhynchites* has been proposed as necessary to upset the normal predator-prey relationship, thus effecting control (Gerberg, unpublished talk at meeting of Eastern Branch of Entomol. Soc. Amer, 1974; Muspratt 1951). Inundative releases imply the mass rearing of large numbers of mosquitoes and in determining the practical utility of *Toxorhynchites* as a biological control agent, the cost of mass rearing could be as important a parameter as the biological aspects of the mosquito. In a separate report, Focks et al. (1977) wrote that one *Tx. rutilus rutilus* (Coquillett) larva requires ca. 100 *Aedes aegypti* (L.) as food; thus a rather large colony of *Ae. aegypti* would be required for the mass production of *Tx. r. rutilus.* In view of the expense involved in maintaining a large colony of mosquitoes as prey species, we are currently involved in a study directed toward the development of alternative food sources for *Tx. r. rutilus.* The present paper presents the results of investigations into the feasibility of using a non-living diet. The fecundity, daily adult survival, development time of immature stages and pupal weights are reported for *Tx. r. rutilus* reared solely on TetraMin®, a tropical fish food, and compared with observations on larvae fed solely on *Ae. aegypti* larvae.

[1] Mention of a proprietary product in this paper does not constitute an endorsement of this product by the USDA.

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MATERIALS AND METHODS

TetraMin Staple Food (manufactured in West Germany) is a dried, flaky material consisting of fish and shrimp meal, oat flour, fish liver, squid, fish roe, kelp, mosquito larvae, brine shrimp, aquatic plants, agar agar, chlorophyll and carotene[3]. By weight it is 45% crude protein, 5% fat, 6% fiber, and 44% water. It was selected for these experiments for the following reasons: (1) Considering the diversity of substances making up TetraMin, it was deemed reasonable that TetraMin contained the required nutrients for a predator, therefore, if *Tx. r. rutilus* failed to develop on TetraMin, the feasibility of rearing this predator on a nonliving diet would be dubious. (2) TetraMin has been used as the sole food source in rearing other mosquitoes, e.g., *Culex* spp., *Aedes* spp. and *Anopheles* spp. (Pappas 1973). (3) TetraMin does not promote as much scum formation on water as is found with other materials used as food for mosquitoes. (4) Since TetraMin is a commercially available, and only moderately expensive material, the cost of using it for rearing *Tx. r. rutilus* would not be prohibitive.

The *Tx. r. rutilus* used in these experiments were taken from the 5th and 6th generations of a colony maintained in the laboratory. The original material was collected as eggs from treeholes in Alachua County, Florida.

For the test, *Tx. r. rutilus* eggs were individually set in 8-dr glass vials that were held in a water bath at 28±1°C with a photoperiod of 14 hr light: 10 hr darkness. One group of 83 larvae was fed 5–10 mg of pulverized TetraMin every 3 days, and the control group of 48 larvae was fed larvae of *Ae. aegypti* as required. Every 10 days, the *Tx. r. rutilus* larvae were transferred to clean water.

Notes were kept on the development time required by each larval instar and on the comparative weights of pupae for the mosquitoes reared on the living and nonliving diets.

Adult fecundity (eggs/female/day), longevity and daily survival were recorded in an outdoor cage. The cage was a 1 × 1 × 2 m screened cage located within a larger 5.5 × 7.3 × 4.3 m screened building. These enclosures were situated under large oak trees that provided shade during the midday. The inner cage was protected from rainfall and contained water wicks, honey, apple slices and black 0.5-liter oviposition jars half-filled with water. Eggs were removed and counted on a daily routine to provide information on fecundity and percent hatch of the two groups of adults.

[3] Distributed in the U.S.A. by Tetra Sales of Hayward, CA.

RESULTS

The development time for *Tx. r. rutilus* larvae reared on TetraMin and *Ae. aegypti* are shown in Table 1. Larvae maintained on a diet of TetraMin required an average of 107.5±19.8 days compared to a relatively short 15.6±1.4 days for larvae reared on *Ae. aegypti*. Even this comparison is not complete, for after 160 days when the test was terminated, ten of the 83 larvae reared on TetraMin (12%) were in the 4th larval instar. These 10 remaining larvae were offered (on day 190) *Ae. aegypti* as prey and pupated within 10.4±3.0 days after consuming 87±34 1st- and 2nd-stage larvae. During the 160 days, only 24% of the larvae receiving TetraMin pupated, and 64% of the larvae died. In contrast, 95.8% of the larvae fed *Ae. aegypti* pupated. A daily survival rate (S_i) for the larval stages was calculated for

Table 1. Duration of larval instars of individually reared *Tx. r. rutilus* at 28±1°C when fed a diet of *Ae. aegypti* larvae or TetraMin.

	Mean development time (days)[a]		
Instar	*Ae. aegypti* fed (n=48)	TetraMin fed (n=83)	% Increase
I	1.2	8.6	720
II	2.6	5.2	200
III	3.8	14.4	380
IV	8.0	79.3	990
Totals±SD	15.6±1.4	107.5±19.8	690

[a] According to the arithmetic method of Southwood (1972); each entry is the time required for half the population to molt to the next instar.

the 2 groups of larvae by averaging the ratios obtained by dividing the number of larvae alive on a given day by the number of larvae alive on the previous day. The S_l values were 0.9901 ± 0.0098 and 0.9973 ± 0.0075 for larvae reared on TetraMin and *Ae. aegypti*, respectively.

A comparison of the average weight of the 2 groups revealed that pupae from the larvae reared on TetraMin were 40% lighter. Weights of the pupae were 29.3 ± 3.6 mg (n=21) and 50.0 ± 2.0 mg (n=8) for TetraMin and *Ae. aegypti*, respectively.

Daily survival (S_a) of the adults was calculated by the same method used in calculating S_l. Using this method, estimates of S_a were 0.988 ± 0.051 and 0.979 ± 0.020 for adults from larvae reared on TetraMin and *Ae. aegypti*, respectively. There was no significant difference in adult survival.

For convenience in comparing the fecundity (F) of the two groups of adult females, fecundity was expressed as eggs/female/day and was calculated by dividing the total egg production by the number of female days. Using this method, the fecundity averaged 0.98 ± 0.23 and 1.03 ± 0.28 eggs/female/day for adults reared on TetraMin and *Ae. aegypti*, respectively. Given $S_a = 0.979$, $F = 1.00$ egg/female/day, and total egg production $= nF \sum_{t=0}^{35} S_a^t$, a cage of n = 100 females would be expected to produce ca. 2500 eggs during a 5-week period. Percentage hatch for eggs from the two groups of females was identical, 98%.

DISCUSSION

Our results presented herein document for the first time that *Tx. r. rutilus* is not an obligate predator under laboratory conditions, albeit the mortality of 64% of the larvae fed the non-living diet would preclude the use of this technique for mass production of this species. In consideration of the biological parameters measured, the undesirable aspects of using TetraMin center on the extremely long development time and the reduced size and weight of the pupae. Obviously the larvae were not receiving an adequate diet, but whether the malnutrition was due to the lack of essential nutrients of ingestion is unknown. In our observations, we noted that the *Tx. r. rutilus* never consumed all the TetraMin offered and this fact indicated that the larvae simply do not feed vigorously on a non-moving diet. The other parameters we measured, which included larval survival (S_l), adult survival (S_a) and fecundity (F) were not different from those of the control group. However, in the context of mass rearing, the extremely long development time overshadows these bright points. For example, the larval mortality exceeded 60% and pupation was highly asynchronous due to the length of development. The cost of rearing larvae with a 3 to 4 month development time would be prohibitive.

One important fact concerning the results herein is the ability of *Tx. r. rutilus* to survive over long periods without prey. This aspect of the durability and flexibility of this predatory species could be of some importance during a biological control program. It may be possible to release adult females and obtain a fairly high population of larvae of *Tx. r. rutilus* in the environment preceding the expansion of the prey population (Trpis, unpublished WHO Document, 1972).

Further attempts to reduce the cost of mass production of *Toxorhynchites* could center on using a prey species which do not require an obligate blood meal in its life history. The elimination of maintaining animals would reduce the expenses incurred during culturing of the prey.

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CONTROLLED RELEASE ORGANOTINS AS MOSQUITO LARVICIDES

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ABSTRACT. A number of commercially available controlled release organotin molluscicides and their degradation products were evaluated against culicine larvae as a segment of a broad environmental impact and chemodynamics study. Test materials were observed in periodic bioassay, in some cases for over 400 days, and found effective against mosquito larvae at dosages considered to be practical for field application. The LT100 results were comparable to controlled release temephos and chlorpyrifos. Long term efficacy, low biological persistency (once released from the binding matrix), and the suspected proteolytic kill mechanism which may negate the development of tolerance would indicate that tributyltin fluoride and bis (tri-n-butyltin) oxide in sustained release compounds are suitable as mosquito larvicides.

INTRODUCTION

A number of controlled release organotin molluscicides were evaluated against culicine larvae and pupae as a portion of a broad environmental impact study. The bioassay results when coupled with other factors regarding the relative environmental safety indicate that these formulations would have significant merit as mosquito larvicides.

It has been recognized for a number of years that the trialkyltins and triaryltins are larvicidal. Bis (tri-n-butyltin) oxide and other organotins formulated in elastomers were examined in 1965–1966 against *Culex pipiens pipiens*, *Cx. pipiens quinquefasciatus*, and *Aedes taeniorhynchus* with good results (Smith and Roos 1966, Cardarelli 1976, Schultx and Webb 1969). Boike and Rathburn (1973), examined 23 organotin/elastomer compounds and found high larvicidal activity in clear water and generally poor activity in water laden with organic matter. Further work in this area was curtailed because organic content of field waters was believed too great a burden and the environmental effects were essentially unknown.

In the snail control area, however, a number of materials are commercially available, and the results of field tests have demonstrated not only efficacy but long term control (Cardarelli 1977, Castleton 1974, Shiff 1975, Gilbert, et al. 1973, Gilbert, et al. 1976, Upatham 1976, Arfaa 1976 and Santos 1977). Analysis of materials returned after 2 years field immersion indicated a biological half-life in excess of 4 years (Cardarelli, et al. 1977).

MATERIALS AND METHODS

The commercial organotin molluscicide formulations evaluated against mosquito larvae were BioMet® SRM, 6% bis (tri-n-butyltin) oxide in natural rubber manufactured by M&T Chemicals, Rahway, NJ; CBL–9B containing 20% tributyltin fluoride in natural rubber from the Creative Biology Laboratory, Inc., Barberton, OH; ECOPRO®–1330 and ECOPRO®–1230, both 30% tributyltin fluoride in ethylene propylene