

## GROWTH AND BEHAVIOR OF COLONIZED *TOXORHYNCHITES RUTILUS SEPTENTRIONALIS*<sup>1</sup>

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**ABSTRACT.** The predaceous mosquito *Toxorhynchites rutilus septentrionalis* (Dyar and Knab) was colonized by induced copulation and reared under controlled laboratory conditions to obtain data on each stage of the life cycle. Eggs hatched 3.0 days after oviposition and the following mean values were obtained for larvae which emerged as females when reared at 27°C: 1st instar 1.6 days, 2nd instar 2.0, 3rd instar 3.2, 4th instar 6.6, pupa 5.4. Total development from oviposition to adult emergence required 21.8 days (20.6–22.6) for females; males completed their development approximately 1 day sooner.

When fed with 1st and 3rd instar prey, female *Tx. r. septentrionalis* devoured 237.8 (222–245) *Ae. aegypti* larvae during their development. The males consumed fewer prey but killed a greater number without consuming them. Killing behavior was first evident in male larvae 4 days before pupation and increased daily until more than 50% of the available prey were destroyed on the day prior to pupation. Female larvae exhibited some killing behavior but did not hunt and kill prey as actively as the males. The biological advantage of sex-related compulsive killing is discussed.

### INTRODUCTION

The mosquito genus *Toxorhynchites* Theobald contains some of the largest mosquito species known. The adults are brightly colored and incapable of feeding on blood. The larvae are always predaceous, feeding primarily upon the immature stages of other mosquito species found in their habitat. The genus is mainly tropical in distribution but a single species is native to North America. Two subspecies are recognized on the basis of morphological characteristics, physiological responses and geographic distribution.

*Tx. rutilus rutilus* (Coquillett) is known to occur only in the extreme southeastern United States (Carpenter and LaCasse 1955) where the subspecies exhibits continuous development throughout the year. *Tx. r. septentrionalis* (Dyar and Knab) is more widespread. The subspecies has been reported as far west as the Great Plains (Carpenter and LaCasse 1955) and as far north as New England (Main et al. 1976). In the more northern parts of its range, *Tx. r. septentrionalis* overwinters in diapause as a 4th instar larva (Lake 1954, Jenner and McCrary 1965, Bradshaw and Holzapfel 1975).

Hemmerlein and Crans (1968) reviewed the biology of *Tx. r. septentrionalis* and assessed its potential as a biological control agent. They concluded that little was known since observations have been based upon small numbers of specimens collected from their natural habitat at various stages of growth. They also stressed the need for laboratory colonization which would allow more extensive study by providing numbers of individuals for experimentation.

*Tx. r. septentrionalis* has since been colonized. (Trimble and Corbet 1975, Slaff et al. 1975). This paper presents the results of studies designed to better understand the growth and behavior of this predaceous mosquito using induced copulation as a tool to acquire specimens of the same age.

### MATERIALS AND METHODS

A colony of *Tx. r. septentrionalis* was maintained as described by Slaff et al. (1975). Eggs for experimentation were removed from the container in the colony cage as soon as possible after oviposition and placed in separate 2 x 3 cm vials containing distilled water. All material was held at 27°C and monitored for eclosion. Growth rates and prey consumption were followed in all instars.

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Each freshly hatched *Tx. r. septentrionalis* larva was offered 50 1st instar *Ae. aegypti* as prey before the head capsule hardened. When the 1st molt occurred, the *Toxorhynchites* were transferred to clean vials and remaining prey were counted. The procedure was repeated supplying the newly molted 2nd instar *Toxorhynchites* with 100 1st instar *Ae. aegypti*. When the *Toxorhynchites* larvae molted to the 3rd instar, they were transferred to larger 5 x 7 cm jars and offered 30 third instar *Ae. aegypti* daily until pupation. All jars were cleaned at 24-hr intervals to minimize bacterial contamination.

During the 4th instar, *Toxorhynchites* were also monitored for killing behavior. Prey which were killed but not consumed were recorded separately. If the *Ae. aegypti* were killed immediately, fresh prey were not supplied until the next scheduled feeding period. When the *Toxorhynchites* larvae pupated, all prey were removed and the jar was covered with a screened hood. The specimens were maintained in distilled water until emergence when the sex of each individual was recorded.

## RESULTS AND DISCUSSION

**GROWTH RATES.** The growth rates of 12 male and 12 female *Tx. r. septentrionalis* are presented in Table 1. Embryonic development was relatively consistent in these studies and eggs hatched approximately 71 hr after oviposition. Most of the eggs were obtained in the late afternoon when the females were actively ovipositing, thus, eclosion regularly took place during the

afternoon of the 3rd day. The period of embryonic development appears considerably longer than that reported by Trpis (1972) for *Tx. brevipalpis* at comparable temperatures.

Growth in *Tx. r. septentrionalis* was relatively rapid during the 1st two instars, and no differences were observed between the sexes. Table 1 shows that sexual differences in developmental times became evident in the 3rd and 4th instars. No differences in the duration of the pupal period were found. Total development from oviposition to adult emergence at 27°C required a mean of 20.7 days for male *Tx. r. septentrionalis*. The developmental period for females was approximately 1 day longer.

Trpis (1972) showed that temperature greatly affected the developmental rate of the tropical species *Tx. brevipalpis*. In his studies, 29–30°C was optimal; temperatures above and below the optimal range prolonged the development of the embryos and the larvae. The temperature selected as a constant in these studies approximated conditions encountered by the larvae in nature during the breeding season in New Jersey and was very close to the ideal range for *Tx. brevipalpis*. Even so, developmental times for *Tx. r. septentrionalis* were approximately 1 wk longer than those reported for *Tx. brevipalpis*. No studies were conducted to determine the effects of higher or lower temperatures in these investigations.

**PREY CONSUMPTION.** Data on the number of prey consumed during devel-

Table 1. Developmental periods of 12 male and 12 female *Toxorhynchites rutilus septentrionalis* reared at 27° C.

Sex	Egg	Time in Days					Total Development
		1st Instar	2nd Instar	3rd Instar	4th Instar	Pupa	
<i>Male</i>							
Mean	2.9	1.6	2.0	2.6	6.1	5.5	20.7
Range	(2.9–3.1)	(1.5–2.0)	(2.0–2.1)	(2.0–3.0)	(5.5–6.6)	(5.0–6.0)	(20.2–21.6)
<i>Female</i>							
Mean	3.0	1.6	2.0	3.2	6.6	5.4	21.8
Range	(2.8–3.1)	(1.5–1.8)	(2.0–2.2)	(2.7–3.8)	(5.6–8.0)	(4.5–6.0)	(20.6–22.6)

opment are presented in Table 2. During their total larval development, *Tx. r. septentrionalis* males consumed an average of 227 *Ae. aegypti*. The females, with a slightly longer developmental period consumed 237.8.

A number of estimates have been given on the total numbers of prey larvae destroyed by *Toxorhynchites* spp. during their larval development (Basham et al. 1947, Newkirk 1947, Muspratt 1951, Corbet 1963 and Trpis 1972) but the figures are difficult to compare and can be misleading. Data from these studies show that size of the prey directly influences the numbers of prey consumed and studies by Trpis (1972) indicate that temperature also has an effect on predation. In view of these multiple variables, laboratory experiments on prey consumption can be used only to approximate roughly the amount of larval control obtained under natural conditions.

**PREDATORY BEHAVIOR.** *Tx. r. septentrionalis* larvae remained suspended at a 45° angle from the surface when resting but usually assumed an anopheline attitude while hunting for prey. The larvae remained parallel to the water's surface and crept along the meniscus with a snake-like motion using the air tube as a pivot point. Many prey were passed by before a single specimen was captured and devoured. After unsuccessful attempts, the predator often sounded and captured *Ae. aegypti* which were feeding on the bot-

tom sediment. Subsurface predatory behavior was most common when a disturbance caused predator and prey to sound simultaneously.

After an initial feeding period, 4th instar *Tx. r. septentrionalis* larvae often killed prey without eating them. Similar behavior, referred to as compulsive killing, has been described for *Tx. brevivalpis* (Muspratt 1951, Corbet 1963, Corbet and Griffiths 1963 and Trpis 1972) as well as other *Toxorhynchites* spp. (van Someren 1948, Lien 1965). When feeding, *Tx. r. septentrionalis* grasped the prey using the stiffened mouth brushes and quickly consumed the struggling larva. When killing behavior was exhibited, the prey was grasped and tightly held until all struggling ceased. The dead larva was then torn from the mouthparts with a deliberate sweep to the air tube. If the dead larva was not ripped from the mouthparts on the 1st attempt, a 2nd or 3rd motion was made. It was not uncommon for some *Toxorhynchites* larvae to have 1 or more dead *Ae. aegypti* impaled on the caudal hairs of the 9th abdominal segment. Dead *Ae. aegypti* were usually ripped in two; in no instance were *Tx. r. septentrionalis* observed to release dead prey by relaxing their mouth brushes.

Killing behavior was first noticed in 4th instar male larvae which began killing prey 4 days prior to pupation. As metamorphosis neared, the killing behavior increased and was most intense on the day

Table 2. Number of *Aedes aegypti* immatures consumed by 12 male and 12 female *Toxorhynchites rutilus septentrionalis* larvae reared at 27° C.\*

Sex	Number of Prey Consumed During:				
	1st Instar	2nd Instar	3rd Instar	4th Instar	Total Larval Period
<i>Male</i>					
Mean	32.8	78.6	30.3	85.6	227.3
Range	(30-39)	(77-80)	(23-33)	(69-91)	(205-237)
<i>Female</i>					
Mean	35.8	79.9	33.8	88.4	237.8
Range	(32-44)	(79-82)	(27-44)	(74-99)	(222-245)

\* 1st and 2nd instar *Tx. r. septentrionalis* fed 1st instar *Ae. aegypti*.  
3rd and 4th instar *Tx. r. septentrionalis* fed 3rd instar *Ae. aegypti*.

prior to pupation when the 12 *Tx. r. septentrionalis* males in the sample killed an average of 16.1 *Ae. aegypti* or more than 50% of the prey which were offered. In most samples, all prey which were not consumed were killed by the end of the 24 hr period. Killing behavior was less intense in larvae which transformed into females. The behavior did not occur until 3 days prior to pupation and only increased slightly as metamorphosis neared. As a result, the female larvae co-existed with many live *Ae. aegypti* at the end of each 24 hr feeding period.

During their larval development, the 12 male *Toxorhynchites* killed 412 *Ae. aegypti* without eating them; individuals in the sample killed 28-41 each. The 12 female larvae killed 88 *Ae. aegypti* over the same period ranging from 2-11 each.

The biological significance of these findings is unclear. Compulsive killing is probably most intense after the pre-pupa has formed and feeding is no longer possible but the males in these studies exhibited the phenomenon 4 days prior to pupation and other workers have shown that compulsive killing can occur much sooner (Corbet and Griffiths 1963, Trpis 1972). There is no reference in the literature to suggest that killing behavior in *Toxorhynchites* is sex-related, yet females did not hunt and kill prey as actively as males at any time during these studies. A number of variables may have been involved. The females may have required more food and compulsive killing could have been retarded in favor of nourishment. Trpis (1972) has also shown that temperature can greatly influence killing behavior in *Tx. brevipalpis*, thus, the constant temperature utilized in these studies may have been a factor.

There is also the possibility that sex-related compulsive killing is beneficial to the species. Corbet and Griffiths (1963) have interpreted the phenomenon as a protective mechanism on the part of *Toxorhynchites* to avoid predation after pupation when an individual is most vulnerable to its siblings. Data from the present study show that males have a shorter larval period, thus, males would pupate first and

kill a large percentage of the sibling females in nature if killing behavior occurred within the species. This might be a mechanism for the predator to keep population levels below those of the prey. The overlap between the developmental periods of male and female in the present study suggests that some females pupate early enough to escape sibling predation and ensure continuation of the species.

No *Toxorhynchites* were kept together during these studies thus no conclusions can be drawn concerning survival between opposite-sexed larvae in direct competition. Compulsive killing appears to offer a fruitful area for future behavioral research.

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## HUMAN URINARY METABOLITES OF ORGANOPHOSPHATE INSECTICIDES FOLLOWING MOSQUITO ADULTICIDING

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**ABSTRACT.** Adult mosquito control practices generally employ chemical toxicants, and thus, unavoidably expose inhabitants of treated areas to insecticides. Human exposure to most organophosphate insecticides results in the excretion of specific urinary metabolites. In Dover, Delaware, urine specimens were collected from people residing in and adjacent to an area treated with naled for adult mosquito control. Chemical analysis of the spray solution revealed that it was contaminated with traces of temephos, another organophosphorous insecticide used in mosquito control. Urine samples from the same individuals were

taken prior to and just after aerial spraying and analyzed for organophosphate insecticide metabolites. Levels of these metabolites varied; increases in dimethyl phosphate (from exposure to both temephos and naled) and dimethyl phosphorothionate (solely from temephos exposure) in post-treatment samples could be attributed to this spraying. Metabolite levels observed in this study did not approach concentrations normally associated with cholinesterase inhibition or other clinical repercussions. Other aspects of human exposure to insecticides are discussed.

Intensive mosquito suppression activities are practiced in many communities throughout the United States. The actual methods and procedures employed vary greatly from one place to another and, in some cases, take advantage of behavioral or ecological phenomena indigenous to specific locales. One characteristic which most adult mosquito abatement programs share is their reliance on chemical pesticides. These insecticides are applied by numerous aerial and ground means. Treated areas are usually in or near population centers. Therefore, these practices unavoidably expose the general population of the treated, and perhaps adjacent, areas directly to certain amounts of pesticides.

Human exposures to organophosphate insecticides result in the excretion of urinary metabolites which are generally

characteristic of the type of pesticide used. The identity and rate of excretion as well as the amount excreted vary and are functions of the specific pesticide, the exposure, and the intrinsic and extrinsic factors influencing individual metabolism.

Changes in cholinesterase levels have also been noted as a sequela of mosquito control treatments. In several published reports from other countries, the use of fenthion, an organophosphate insecticide used for mosquito control, has produced moderate depression of whole blood and plasma cholinesterase in spraymen and in inhabitants of sprayed dwellings (Taylor 1963, Elliott and Barnes 1963). During studies of the exposure of mosquito control workers to fenthion in the United States, Wolfe et al. (1974) found that there was no important change in erythrocyte cholinesterase activity. However, there