LABORATORY COLONIZATION AND BIOLOGICAL OBSERVATIONS OF *TOXORHYNCHITES RUTILUS RUTILUS*[1]

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ABSTRACT. *Toxorhynchites rutilus rutilus* (Coquillett) was successfully colonized and studied in the laboratory to determine the potential usefulness of this predatory species of mosquito as a biological control agent for container-breeding mosquitoes. When *Tx. r. rutilus* larvae were reared at 28±1°C in individual containers with a surplus of larvae of *Aedes aegypti* (L.) as prey, the duration of the immature stages averaged 1.6, 15.6, and 6.0 days for eggs, larvae, and pupae, respectively. Contrarily, with mass rearing conditions, the duration of the larval stage was significantly reduced to 11.1 days and pupation was more uniform than in individual containers. Adult females survive for 7 wk in laboratory cages and oviposit an average of 1 egg/day. Fourth-stage larvae of *Tx. r. rutilus* can survive for about 2 months without food. Adult females preferred to lay their eggs in water previously used to rear *Ae. aegypti.*

Extensive use of synthetic pesticides has resulted in the phenomenon of resistance to them. The ramifications of this problem are providing increased impetus to reconsider mosquito control strategies involving biological control agents. Several of the more important mosquito vectors of human disease breed in discarded cans, bottles, tires, water cisterns, and tree holes. Control of larvae of these species is difficult because the larval habitats are small, dispersed and often inaccessible. Mosquitoes of the genus *Toxorhynchites* are larval predators of container-breeding mosquitoes, and possibly could be highly effective control agents because the female *Toxorhynchites* might more efficiently find these breeding sites than the mosquito-control worker (Brown 1973). However, the literature is replete with examples where *Toxorhynchites* spp. failed to control prey species (Newkirk 1947, Paine 1934, Swezey 1930 and 1931, Williams 1931). This has been attributed to intrinsic factors such as long life cycles, low fecundity, and survival rate (Nakagawa 1963). Gerberg (1974) and Muspratt (1951) contend these shortcomings can be overcome with inundative releases of *Toxorhynchites,* a situation that upsets the normal predator-prey relationship.

Currently we are conducting research on the feasibility of using *Tx. rutilus rutilus* (Coquillett) as a biological control agent against container breeding mosquitoes. The present paper summarized our progress in laboratory studies and describes the colonization, mass rearing, and other aspects of the biology of this species.

The genus, *Toxorhynchites,* in North America north of Mexico is represented by two subspecies (Jenkins 1949) and perhaps a third, the status of which is uncertain (Zavortink 1969). *Tx. rutilus septentrionalis* (Coquilllett) is known to occur in the eastern United States north to New Jersey and Pennsylvania and west to the great plains of Kansas, Oklahoma, and Texas. *Tx. r. rutilus* is known only from the extreme southeastern United States in peninsular Florida, southern and coastal Georgia and coastal South Carolina north to Myrtle Beach (Carpenter and LaCasse 1955). Intergrades occur in the zone of overlap of the ranges of these two subspecies (Jenkins 1949). The egg, larva, pupa,

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adult stages (Carpenter and LaCasse 1955; Dodge 1964), oviposition habits (Olinger 1957) and habitats (Seabrook et al. 1946; Basham et al. 1947) of *Tx. r. rutilus* have been described.

COLONIZATION AND BIOLOGICAL OBSERVATIONS

We collected *Tx. r. rutilus* eggs from cavities in various trees in Alachua County, Florida, during September and October 1975. Larvae were reared to the pupal stage individually in 8-dr glass vials. Each egg was individually set with approximately 250 first stage larvae or eggs of *Aedes aegypti* (L.); additional prey were added as required. The prey larvae fed on 5–10 mg of TetraMin® (a commercially available tropical fish food) supplied every other day. Subterranean well water was used in all rearing. The photoperiod was 14 hr of subdued light:10 hr dark. Reared at 28±1°C, the development time from egg to pupation for 156 individuals was 17.2±3.3 days, and the total mortality was 8% (Table 1).

Table 1. Duration of eggs, larval and pupal stages of *Tx. r. rutilus* and total numbers of *Ae. aegypti* larvae[a] eaten.

Stage	No. ($\bar{X}$ ± SD) prey devoured	Development time (days)
Larvae		
Egg		1.6 ± 0.55
1st	6.4 ± 4.2	1.2 ± 0.45
2nd	6.6 ± 2.8	2.6 ± 0.42
3rd	8.0 ± 2.0	3.8 ± 0.76
4th	73 ± 14	8.0 ± 1.58
Pupae		6.0 ± 0.82
Total	93 ± 15	23.2 ± 1.36

[a] 1st stage larval predators offered 1st and 2nd instar prey; 2nd, 3rd and 4th stages offered 3rd and 4th instar prey.

The first cohort of 150 pupae reared in the above fashion were placed in a 1 m x 1 m x 2 m high screened aluminum cage covered with a clear plastic film to maintain humidity. Water on sponge wicks, honey, apple slices, and black jars (0.5-liter size) half filled with water for oviposition sites were provided. The cage was within an environmentally controlled room lacking windows. Light was provided by 8 fluorescent tubes (40-watt). The conditions were: photoperiod 14 hr light: 10 hr dark, temperature 24±1.5°C, relative humidity (RH) 85±10%. No attempt was made to simulate twilight. No mating was observed and no eggs were produced. No mortality was observed for the first 6 wk, but 75% died within the next 2 wk and no individual lived longer than 10 wk.

A second cohort replaced the 1st under similar temperature and humidity conditions and a number of things were tried in an attempt to induce mating. J. S. Haeger (personal communication) has had success in colonizing difficult species by using either singly or in combination the following: (1) plants within the cage to act as swarm markers; (2) twilight simulation using incandescent lights on a rheostat; (3) the addition of a second species (*Ae. aegypti*); and (4) casting shadows across the interior of the cage. Using the above techniques, Haeger has been able to stimulate mating and oviposition in *Tx. r. rutilus*. However, we tried his techniques with no success. Varying the numbers of *Tx. r. rutilus* within the cage (n = 25, 50, 100, or 150) was also unsuccessful.

A third cohort of about 30 adults was placed in a 0.5 m x 0.5 m x 0.5 m Plexiglass® (acrylic plastic) cage. The conditions were as for the first cohort. Mating was observed on the 2nd day after emergence, and oviposition was observed 6 days postemergence. Females fly in a vertical circle several inches above the oviposition jar and eject the eggs singly onto the surface of the water (Olinger 1957). Sixteen females observed for a period of 3 wk after oviposition began produced an average of 1.23 eggs/female/day. All the eggs were fertile but no ovarian cycle was immediately apparent. Using the techniques described above, *Tx. r. ritulus* has been maintained in the laboratory for 9 generations.

Rearing in outdoor cages was attempted during July and August 1976 in an at-

tempt to increase egg production. The same 1 m x 1 m x 2 m high cage described previously, but without the plastic covering, was used. This cage was located within a longer 5.5 m x 7.3 m x 4.3 m screened building. The enclosures were situated under large oak trees which provided shade during the middle of the day. An average daily temperature of 29°C and RH of 85% were recorded inside the smaller cage. Various numbers of *Tx. r. rutilus* (n = 18, 30, 150, 250, or 450) were placed in the inner cage with water wicks, honey, apple slices, and oviposition jars (half filled with well water). At each density of adults, 1st matings and oviposition were observed at 2 and 6 days, respectively.

During the outdoor cage work, 15 females were removed after 12 days of being with 15 males and confined in individual containers supplied with honey, water wicks, and oviposition jars. The containers, held at 28°C, RH of 85±10% and a photoperiod of 14 hr light:10 hr dark, were checked daily for oviposition over a period of 25 days. Thirteen (or 87%) of the females laid eggs. Fecundity was 0.83±0.66 eggs/female/day, a value not significantly different ($p = 0.05$) from 1.23 eggs/female/day reported above for the indoor cage. The oviposition cycle can best be described as highly irregular. Three of the females laid 1–3 eggs daily, and 5 of them oviposited 5–18 eggs on successive days with 5–15 day intervals of no oviposition. A 3rd group of 5 females was intermediate in behavior. As evidenced by the standard deviation, differences in total egg production between females were great. The egg fertility was virtually 100% suggesting that females do not oviposit unless they have mated.

Before colonization procedures were established for *Tx. r. rutilus*, induced mating was used to maintain laboratory stocks. Techniques described by Gerberg (1970) and Trimble and Corbet (1975) were successfully employed with the exception of ethyl ether being used as the anesthetic. Fertile eggs were laid 2 days after forced mating. Typically, however, oviposition stopped after only a few days.

The amount of time larvae of *Tx. r. rutilus* can withstand fasting may be an important parameter in biological control considerations. To investigate this, variously aged larvae and eggs were placed in individual vials containing clean well water and observed until death by starvation occurred. Larvae were fed the usual diet of *Ae. aegypti* before the test began. From the results in Table 2, one can readily see that the ability of *Tx. r. rutilus* larvae to survive fasting will indeed be of value in the use of this predator for control. The first 2 larval stages survived for about a week, and 3rd stage larvae lived for 18 days without food. The fourth larval stage was exceptional in its ability to withstand fasting, and this noteworthy fact is all the more important because this stage also is capable of eating more prey than the first 3 instars.

The existence of an oviposition stimulant, e.g., the presence or past presence of a prey species could be important in biological control by *Tx. r. rutilus*. A simple 2-choice test between well water and water

Table 2. Length of larval life of *Tx. r. rutilus* when deprived of food.

Larval stage in which starvation began	Age at beginning of test (days)	Days before death	No. individuals observed
1st	0	6.9 ± 0.3	9
2nd[a]	4 & 5	8.4 ± 0.5	10
3rd[b]	9 & 10	18.0 ± 6.5	10
4th[b]	13	59.0 ± 22.4[c]	8

[a] Temperature = 27°C.
[b] Temperature 27°C during first 11 days then raised to 30°C.
[c] One individual fasted 88 days.

which had been used in rearing *Ae. aegypti* from egg to pupa (hereafter referred to as colony water) was conducted to determine whether *Tx. r. rutilus* preferred one or the other as an oviposition media. Four pairs of black oviposition jars (0.5-liter size) were placed in the 1 m x 1 m x 2 m high cage under the previously described outdoor conditions. Each pair consisted of 1 jar half full of well water and 1 jar half full of colony water. During the test, the colony water did not contain any *Ae. aegypti.* Each pair was placed in a corner of the cage floor. Daily, the positions of the jars within each pair were alternated to nullify any positional effects. At 3-day intervals, the water in each of the 8 jars was replaced with new well or colony water as appropriate. The cage contained approximately 750 adults (ca. 375 females). The number of eggs in each type of water was recorded daily for 6 days. The mean daily oviposition in the jars containing well water and colony water was 74±47 eggs/day and 247±87 eggs/day, respectively. The difference between the 2 means is highly significant (t-test).

The preference of *Tx. r. rutilus* females to oviposit in the polluted water can be quite important in regard to using this predator for control. Although the need for more work is indicated, it appears that the females prefer to oviposit in water similar to that found in natural settings (treeholes, discarded tires, etc.) rather than in containers (water bottles, rain barrels, and cisterns) commonly employed to hold water for household uses.

MASS REARING

Since the rearing of *Tx. r. rutilus* in individual vials is a laborious, impractical process, experiments were conducted to determine the feasibility of mass production. Cannibalism was the principal problem encountered in mass rearing *Tx. r. rutilus* in the same container; therefore, all of these trials were conducted in complete darkness. Fourteen plastic trays (38 cm x 51 cm x 10 cm) were filled with well water to a depth of 4 cm, and the water temperature was maintained at 28±0.3°C. To these trays were added 110 *Tx. r. rutilus* eggs (less than 24 hr old), ca. 10,000 *Ae. aegypti* eggs and 1.7 g TetraMin. The same day the *Tx. r. rutilus* eggs were set, a 2nd identical tray was set with ca. 10,000 *Ae. aegypti* eggs and 1.7 g TetraMin. Each tray received an additional 1.7 g TetraMin on alternate days. The 2nd tray containing *Ae. aegypti* was added to the 1st tray containing *Ae. aegypti* and *Tx. r. rutilus* 8 days after the trays were set. Overcrowding and underfeeding the *Ae. aegypti* result in a mixture of 3rd and 4th instars being added to the *T. r. rutilus.* Seventy±6% of the *Tx. r. rutilus* survived to the pupal stage with cannibalism being the most likely cause of mortality. The initial larval density of 18 cm^2/larva changed to 25 cm^2/larva at pupation. Reared in this manner, the average development time from egg to pupation was 12.66±1.22 days for 547 larvae. The difference between length of development (egg to pupa) when 156 larvae were reared individually (17.2±3.3 days) and when reared in mass was highly significant (t-test), but the reason for this observation was not readily apparent.

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