# LABORATORY AND FIELD STUDIES WITH $^{32}P$ LABELED *TOXORHYNCHITES RUTILUS RUTILUS*

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ABSTRACT. Females and eggs of *Toxorhynchites r. rutilus* were labeled with $^{32}P$ by feeding fourth-stage larvae $^{32}P$ labeled *Aedes aegypti* larvae. Eggs from females up to 3 weeks in age had detectable levels of radioactivity and individual eggs contained ca. 0.3% of the mother's total radioactivity. Comparisons of labeled and unlabeled females in indoor and outdoor cage tests indicated that survival and fecundity of the 2 groups were approximately equal. No differences were noted for dispersal and fecundity of labeled and control females released in field tests. The $^{32}P$-labeled *Tx. r. rutilus* females behave similarly to unlabeled females, and this method of radiolabeling provides a sound tool for tracking laboratory-reared females released into an area with an indigenous population.

## INTRODUCTION

In recent years there has been considerable interest in using species of the genus *Toxorhynchites* as predators in the biological control of species of mosquitoes that breed in artificial and natural containers. Focks et al. (1982) obtained ca. 74% control of *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* Say by the addition of one or 2 *Toxorhynchites rutilus rutilus* (Coquillett) larvae to containers in a substandard housing area. Bailey et al. (1983) observed that a dense natural population of *Tx. r. rutilus* significantly reduced a natural population of *Ae. aegypti* in a tire dump when compared with 2 other tire dumps with low levels of *Tx. r. rutilus*.

Ovitraps have been used extensively to sample natural populations of *Tx. r. rutilus*, but the results of releasing small numbers of laboratory-reared females are often difficult to evaluate because of the presence of indigenous populations. Since Smittle and Seawright (1983) were successful in labeling *Ae. aegypti* eggs with radioactive phosphorus ($^{32}P$), the use of this technique was evaluated to assess its usefulness for the identification of eggs from released *Tx. r. rutilus* females. This paper presents the results of laboratory labeling of immature *Tx. r. rutilus* with $^{32}P$ to produce females that would deposit radioactive eggs and the field release of radiolabeled females with subsequent recovery of radiolabeled eggs.

## METHODS AND MATERIALS

The *Tx. r. rutilus* used in these studies were reared in the laboratory on a diet of *Ae. aegypti* by methods previously described by Focks et al. (1979). Briefly, the *Tx. r. rutilus* larvae were reared on a diet of *Ae. aegypti* larvae in 50 × 40 × 10 cm trays of well water at 27.2°C; the photoperiod was 14 hr light:10 hr dark. On day 0, 0.05 ml of 0 to 24-hr-old *Tx. r. rutilus* eggs (ca. 330) were added to a rearing tray containing 0.15 ml of *Ae. aegypti* eggs and a slurry of 3 g of hydrolyzed yeast and liver powder (1:1). Other trays containing only *Ae. aegypti* eggs and food were set on day 0, 3, 5 and 7. These *Ae. aegypti* larvae were placed in the *Tx. r. rutilus* trays as required for adequate feeding.

In the preliminary tests, late third- and early fourth-stage *Ae. aegypti* larvae were placed in tap water containing either 0.0325 (low) or 0.0650 (high) μCi/ml of $^{32}P$. After 3 hr the larvae were fed a slurry of yeast and liver powder. After 48 hr the larvae were removed from the $^{32}P$ treatment solution, rinsed to remove most external radioactivity and offered to late third- and early fourth-stage *Tx. r. rutilus* larvae. The *Tx. r. rutilus* larvae were fed the radioactive *Ae. aegypti* larvae until pupation. For emergence, the *Tx. r. rutilus* pupae were placed in water-filled cups in 0.5 × 0.5 × 0.5 m, clear acrylic cages which were maintained at 80% RH, 27°C, and with the same photoperiod used in larval rearing. The adults were provided with cotton rolls soaked with a 50% honey-water solution and a black 0.5-liter oviposition jar half filled with water. *Toxorhynchites r. rutilus* begin ovipositing 5 or 6 days after emergence.

The radioactivity of the various stages was measured using a liquid scintillation counter having an efficiency for $^{32}P$ of greater than 95%. Only those eggs with counts greater than 10 counts/min above background were considered labeled. The effects of the $^{32}P$-labeling were monitored by comparing the daily survival and oviposition of the labeled and control adults. The radioactivity of the females and their eggs during the 2-week period following the first oviposition was recorded.

For each of the 3 field releases conducted, similar rearing methods were used but only the 0.0325 μCi/ml of $^{32}P$ treatment was used for radiolabeling the *Ae. aegypti* larvae. All labeled

*Tx. r. rutilus* pupae in the second and third releases were individually assayed in a G-M or proportional counter to assure that adequate levels of $^{32}P$ would be present in all females. For the first 2 releases, pupae were placed in cups within 0.5 × 0.5 × 0.5 m screened cages and, after emergence, the adults were held in the laboratory for about 1 week before release to allow for mating and start of oviposition. For the third release, the adults were held in screen cylinders 48 cm in diameter and 58 cm high in an outdoor screened building under ambient conditions.

The approximately square experimental release area covering about 12.6 ha on the University of Florida campus, has been described previously (Focks et al. 1979). Releases were made from the center of a student housing complex that occupied an area of 5.3 ha. This inner area was separated from the outer area by roadways. The plot was interspersed with various hardwood and pine trees, shrubs, and open expanses of lawn. It was bordered on 3 sides by a more densely wooded area and on the fourth side by a lake.

Oviposition by *Tx. r. rutilus* was monitored with 64 oviposition traps (0.5 liter black jars half filled with water) placed on tree boles (ca. 2 m above the ground) with a uniform spacing of ca. 60 m intervals throughout the study site. Thirty ovitraps were within the housing area (inner area), and the rest were in the outer, surrounding wooded areas. The jars were checked daily for *Tx. r. rutilus* eggs, which were removed and returned to the laboratory for assay of radioactivity. Prior to the releases, the ovitraps were monitored for 3 to 5 days to detect the presence of indigenous *Tx. r. rutilus*.

The *Tx. r. rutilus* adults were released at sundown in the center of the inner area. Both $^{32}P$ and control females were released to allow comparison of oviposition and dispersal following field release. The number of females released was 102 $^{32}P$ and 102 control, 175 $^{32}P$ and 67 control, and 159 $^{32}P$ and 157 control in the three releases, respectively. For each release, $^{32}P$ and control groups were maintained at the laboratory for oviposition and survival data.

## RESULTS AND DISCUSSION

PRELIMINARY TESTS. The mean radioactivity levels for the *Ae. aegypti* larvae and the various stages of *Tx. r. rutilus* are presented in Table 1. These data indicate that it is possible to use this technique for obtaining uniformly labeled *Tx. r. rutilus* adults. Except for the pupae of *Tx. r. rutilus*, all of the stages from the tray treated with 0.0325 μCi/ml were about one-half as radioactive as the stages from the 0.065 μCi/ml treatment. The eggs laid 13 days after oviposition commenced had 56% (low dose) and 49% (high dose) of the radioactivity present in eggs laid on the first day of oviposition. Radioactive decay alone would result in eggs retaining 56% of the amount present on the first day of oviposition, thus substantial amounts of $^{32}P$ were not lost by the female through routes other than oviposition. Eggs from the 0.0325 μCi/ml treatment could be identified for at least 2 wk after oviposition began.

Table 1. Mean radioactivity[a] of *Aedes aegypti* larvae and various stages of *Toxorhynchites r. rutilus* labeled with $^{32}P$.

| Species : Stage | Radioactivity of specimens exposed to: : 0.0325 μCi/ml | : 0.065 μCi/ml |
|---|---|---|
| *Ae. aegypti* | | |
| IV-stage larvae | 33,166 ± 3,996 | 77,845 ± 5,848 |
| *Tx. r. rutilus* | | |
| IV-stage larvae | 114,033 ± 24,842 | 239,411 ± 33,328 |
| pupae | 25,116 ± 2,027 | 70,433 ± 14,450 |
| newly-emerged adults | 22,549 ± 1,323 | 53,659 ± 4,918 |
| eggs | | |
| day 1 | 32.7 ± 2.8 | 85.1 ± 5.2 |
| day 13 | 18.4 ± 3.4 | 41.9 ± 3.2 |

[a] Counts/min less background and ± standard errors.

Pupal production and mean daily survival for caged adults did not indicate a treatment-related reduction due to the $^{32}P$ labeling. Total oviposition by the radiolabeled females was reduced over 14 days by 35 and 61% for the low and high doses, respectively, but the observed reductions were not significantly different from the control (ANOV, $P = 0.05$) due to high day-to-day variability. For the field release studies, the lower dose of 0.0325 μ/Ci/ml was used, because it provided adequate levels of radioactivity for identification of eggs.

FIELD RELEASES. A summary of the radioactivity of various stages from the 3 field releases is presented in Table 2. The *Ae. aegypti* larvae fed to the *Tx. r. rutilus* in the first and third releases had similar amounts of radioactivity and were lower than the amount in larvae used for the second release. However, the level of radioactivity in *Tx. r. rutilus* females varied considerably, with a range of approximately 7,000–40,000 counts/minute. Apparently, slight differences in the physiological age of the larvae of *Tx. r. rutilus* can have a marked effect on the amount of $^{32}P$ label incorporated into the adult females and their eggs. Age is an important consideration, because consumption

Table 2. Mean radioactivity[a] of *Aedes aegypti* larvae and adult females and eggs of *Toxorhynchites r. rutilus* labeled with $^{32}P$ from the 3 field releases.

| Species : Stage | Radioactivity of specimens from indicated release. 1 | 2 | 3 |
|---|---|---|---|
| *Ae. aegypti* | | | |
| IV-stage larvae | 9,333 ± 1,327 | 16,236 ± 2,922 | 9,069 ± 946 |
| *Tx. r. rutilus* | | | |
| adult females | 6,923 ± 709 | 17,420 ± 969 | 39,494 ± 1,504 |
| eggs | 21.4 ± 1.2 | 60.9 ± 2.2 | 97 ± 6.7 |

[a] Counts/min less background ± standard errors.

of the radiolabeled prey depends on the number of days in fourth stage. Radioactivity taken in initially may be eliminated through metabolism prior to pupation, and radioactivity taken in late may not be absorbed but merely passed out of the gut prior to pupation.

On the first day of oviposition, the amount of radioactivity in an individual egg averaged about 0.30 ± 0.05% of the radioactivity of the female on the first day of oviposition. However, 10% of the eggs from females that averaged 7,000 counts/min had less than 10 counts/min above background on the first day of oviposition. Therefore, for field releases, it is advisable to use females which have an average radioactivity of above 10,000 disintegrations/min on the first day of oviposition.

Figure 1 shows the average survival and fecundity of caged females from the three groups (pooled) that were released in the field. Daily fecundity was calculated as the ratio of the number of eggs laid on any particular day to the number of females alive on that day. The daily survivals for labeled and control females were virtually identical with the control females having slightly higher survival for the first 5 days. With the exception of 2 days out of 14, the $^{32}P$ females, had higher fecundity. In fact, females from the third release which were the highest in radioactivity also had the highest survival and oviposition rates. This is in contrast with the preliminary studies and indicates that differences in fecundity may be due to factors other than radiolabeling procedures.

Oviposition in the 64 ovitraps in the release study area for the 3 releases (pooled) is shown in Figure 2. Prerelease oviposition by indige-

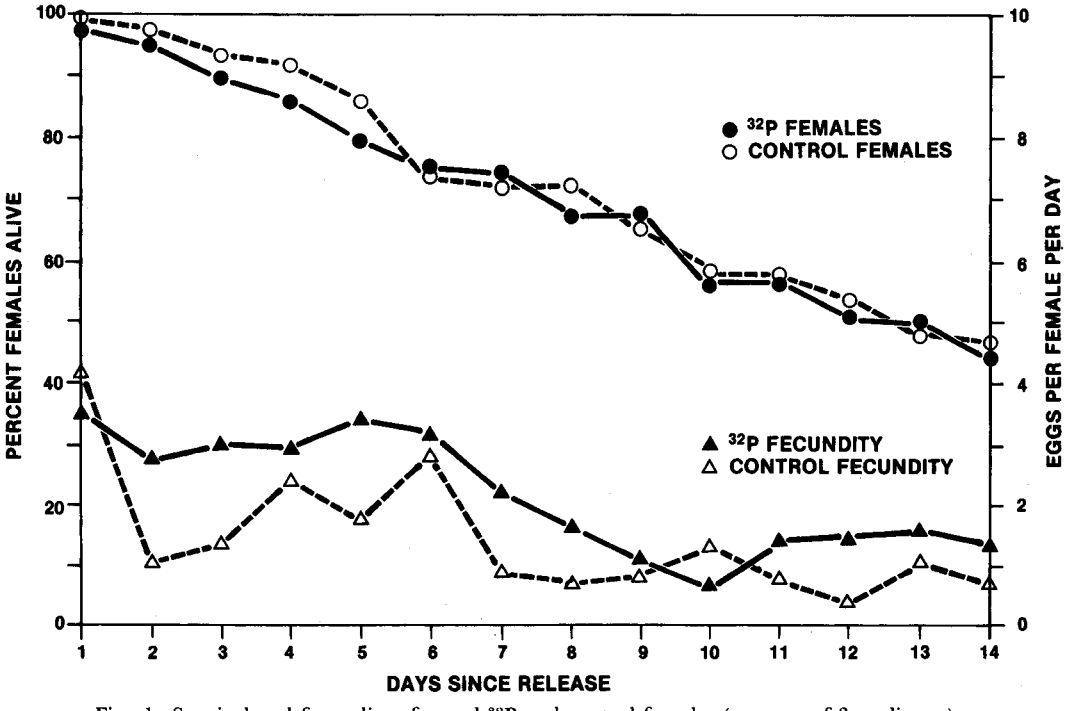


Fig. 1. Survival and fecundity of caged $^{32}P$ and control females (average of 3 replicates).

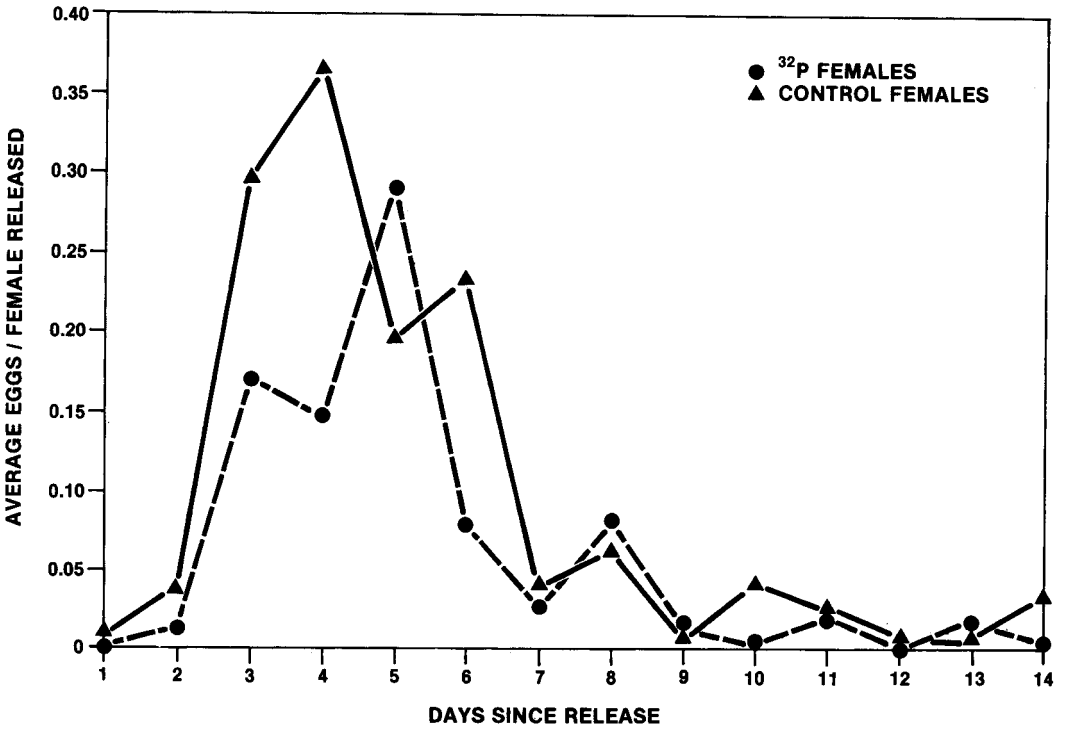


Fig. 2. Oviposition of $^{32}P$ and control females released in housing area (average of 3 releases).

nous females ranged from 2.3 to 4.0 eggs/day; the average daily indigenous oviposition was subtracted from the nonradioactive eggs collected each day to achieve the average eggs/control female released. The control females had a higher average oviposition rate initially with little difference between the 2 groups on the other days. Overall, 1.0 and 0.9 eggs/female released were recovered from the ovitrap monitoring system for the control and $^{32}P$ females, respectively. Since cage tests indicated that at least 10% of the eggs from radioactive females in the first release were classified as nonradioactive, the average fecundity of the $^{32}P$ group would have been slightly higher and thus there would be little difference in fecundity of field released $^{32}P$ and control females.

Radioactive eggs were first found in the most distant ovitraps 3 to 6 days after release indicating movement away from the release location at a rate comparable to that of unlabeled *Tx. r. rutilus* observed previously by Focks et al. (1979). Some of the $^{32}P$ females stayed near the release site as radioactive eggs were collected nearby throughout the 14-day collection period.

The methods and results presented here indicate that it is possible to radiolabel *Tx. r. rutilus* females in the laboratory. These tests indicate that $^{32}P$ labeled *Tx. r. rutilus* females, ranging between 10,000 and 40,000 disintegrations/min, had survival, dispersal and oviposition rates that were comparable to unlabeled females. Since the $^{32}P$ label provides positive identification of laboratory-reared material, the use of $^{32}P$ labeled females should be a useful tool in field studies with this and perhaps other species of *Toxorhynchites*.

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