THE KILLIFISH *RIVULUS MARMORATUS*: A POTENTIAL BIOCONTROL AGENT FOR *AEDES TAENIORHYNCHUS* AND BRACKISH WATER *CULEX*

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ABSTRACT. In the laboratory, newly hatched fry of *Rivulus marmoratus* were effective predators on 1st-instar *Aedes taeniorhynchus*. Adult fish consumed 4th-instar *Ae. taeniorhynchus* and egg rafts of *Culex quinquefasciatus* at a rate increasing with standard length of the fish. Predation of *Rivulus marmoratus* on *Ae. taeniorhynchus* larvae in the field was documented by observing the passage of larval/pupal remains through the gut. Laboratory reared fish released at known *Ae. taeniorhynchus* breeding sites survived over 1.5 years, but did not reproduce.

Larvivorous fish have been used as mosquito control agents for nearly 100 years, and during this period a number of fish species have been investigated for use in diverse applications ranging from cisterns to rice fields. *Gambusia affinis* (Baird and Girard) has proved to be hardy and adaptable to a variety of environmental parameters, but the widespread introduction of *Gambusia* has resulted in negative impacts on indigenous small fishes and, in some cases, predation on the young or eggs of food fishes (Laird 1977). Since the 1960s, interest has increased in alternative biocontrol fish species, primarily in Africa and Asia. Among the species investigated have been the fresh-water "annual" killifishes, the adults of which produce drought resistant (or drought dependent) eggs that survive the drying of transitional pools in tropical through mild-temperate regions. These transitional aquatic habitats are often too fragmented (separate pools of water) to allow uniform distribution of fish. In contrast, nearly all such habitats are likely to breed mosquitoes, with the result that larval predation by annual fishes may have a negligible result (Bay 1985).

Mangrove swamps in the tropical-subtropical regions of the eastern Atlantic present similar characteristics to the habitat of the annual killifishes described above: irregular topography, with isolated depressions often producing large numbers of the black salt-marsh mosquito *Aedes taeniorhynchus* (Wied.) upon flooding by rain or tidal inundation. While *Gambusia* and other larvivorous species are often abundant in mangrove swamps, these fishes are sometimes unable to cope with the large broods of *Ae. taeniorhynchus* produced or may be unable to access isolated depressions in high-marsh areas due to changes in topography or heavy vegetation (Todd and Giglioli 1983, Carlson and Vigliano 1985).

The killifish *Rivulus marmoratus* (Poey) is found in mangrove swamps from Brazil north to central Florida (Huehner et al. 1985). *Rivulus marmoratus* is unique among vertebrates in being a simultaneous, "selfing" (synchronous, internal self-fertilization) hermaphrodite. Eggs produced from an adult hatch into exact clones of the parent (Harrington 1961). In addition, *R. marmoratus* can withstand conditions of hypoxia and high hydrogen sulfide concentrations (both common features of mangrove swamps), often leaving the water for damp terrestrial locations (Abel et al. 1987). This ability allows the fish to withstand water quality conditions which could be fatal to other fishes (Abel et al. 1987, Taylor 1990) and would thus allow the fish to survive in transitional, isolated mosquito-producing habitats.

Rivulus marmoratus resembles the annual killifishes in that its eggs are capable of estivation under drought (no standing water) conditions and, like the eggs of *Ae. taeniorhynchus*, will hatch spontaneously once conditioning thresholds are met (Ritchie and Davis 1986, Taylor 1990).

Rivulus marmoratus is a predator, with insects (including mosquito larvae) forming a principal part of the diet of the few wild-caught specimens examined (Huehner et al. 1985; Taylor, in press). Webber (1982) suggested that *R. marmoratus* might be an effective larvivore in saltmarshes and recommended further study of the species.

Incidental aquarium observations of intensive predation on mosquito larvae by *R. marmoratus* led to the following laboratory study on the predatory capabilities of fry and adult *R. marmoratus* on *Ae. taeniorhynchus* larvae and *Culex quinquefasciatus* Say egg rafts. Supplemental field observations of both introduced and locally occurring *R. marmoratus* populations in known breeding habitats of *Ae. taeniorhynchus* were also made.

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Rivulus marmoratus fry (age 1, 3, 7 and 10 days) were placed in 90 × 12 mm Petri dishes with 35 ml of 15% seawater. Newly hatched (1st-instar) larvae of *Ae. taeniorhynchus* were counted into the Petri dish. One-day-old fish (*n* = 38, x̄ SL (standard length) 4.0 mm) were fed 10 to 50 larvae, 3-day-old fish (*n* = 26, x̄ SL 5.0 mm) were fed 20 to 100 larvae, 7-day-old fish (*n* = 14, x̄ SL 5.5 mm) were fed 50 to 100 larvae, and 10-day-old fish (*n* = 6, x̄ SL 6.0 mm) were fed 100 larvae. The same group of fish was used in all tests. The 3-day-old fish were starved for 24 h prior to trials, and 7 and 10-day-old fish were starved for 48 hours. Surviving (unconsumed) larvae were counted after 24 hours. Eighty-seven adult *R. marmoratus* (19–55 mm SL) were collected from mangrove swamps near Naples, FL. Fish were isolated in separate glass laboratory bowls containing 125 ml of diluted seawater (salinity 18%). Each bowl contained a single mangrove leaf to provide cover for the fish. Fish were starved in the bowl for 48 h prior to the introduction of 4th-instar *Ae. taeniorhynchus* (reared from field-collected eggs). Fish were fed from 3 to 150 larvae (total no. larvae fed = 3,919), and the number consumed was calculated after 24 hours.

Forty-four trials with *R. marmoratus* (18–55 mm SL) were run using the same technique as described for 4th-instar *Ae. taeniorhynchus*, except that fish were fed *Cx. quinquifasciatus* egg rafts. Fish were fed 10–150 rafts for a total of 1,825 rafts. Mean number of eggs/raft was estimated at 165 (James Thomas, unpublished data). Unconsumed rafts were counted after 24 hours.

Natural populations of *R. marmoratus* in mangrove swamps near Naples, FL were observed and sampled concurrent with 4 broods of *Ae. taeniorhynchus*. Fish were collected with a modified Gee® trap (Ritchie and Johnson 1986) and transferred to individual bowls. Fecal material was collected at 24 h intervals (up to 72 h) and examined for evidence of mosquito larvae/pupae predation.

Twenty-six laboratory reared *R. marmoratus* (15–25 mm SL) were released into 5 known habitats (50–1,000 m^2) of *Ae. taeniorhynchus* in Brevard County, FL. These habitats contained no other fish and were isolated from sources of water containing fish. The survival/reproduction of these *R. marmoratus* was documented over a 1.5 year period by using modified Gee and plastic cup traps for recapture (Taylor 1990).

One-day-old *R. marmoratus* consumed a maximum of 21 larvae/fish. However, 18 of 38 fish in this age group consumed no larvae. Three-day old fish consumed considerably more larvae (max. 79/fish), and 7 and 10-day-old fish continued this trend (max. 87/fish and 100/fish). All fish in the 3, 7 and 10-day age group consumed larvae. These data are summarized in Table 1. Supplemental trials with the 7 and 10-day age group of fish revealed that once fish had fed to repletion they frequently continued to kill (but not consume) 4th-instar *Ae. taeniorhynchus* larvae.

The number of 4th-instar larvae and egg rafts consumed by adult fish increased dramatically with standard length (Fig. 1). Of 3,919 larvae offered, 3,273 (83%) were consumed, and of 1,825 rafts fed, 1,688 (92%) were consumed.

Eighteen (42%) of the 43 fish collected concurrent with 4 mosquito broods defecated remains of larvae and pupae within 48 hours. In the first 2 trials, 34 fish were collected in traps set for 18 hours. Only 9 (26%) were positive for larvae. In the latter 2 trials, traps set for only 3 h collected 9 fish, all positive for larvae or pupae. These data suggest that the fish rapidly digested and evacuated the larval remains. Of the 26 fish released at the 5 mosquito producing sites, 3 fish were recaptured from 2 sites over the 1.5 year period (at 6, 10 and 18 months). These fish were apparently from the original release stock, and no evidence of reproduction or juvenile fish was found. These original release fish did not exhibit an ocellus on the base of the tail, which is characteristic of hermaphrodites (Harrington 1967). All recaptured fish had the phenotypic coloration of male fish, probably indicating that the released specimens matured into males, a phenomenon sometimes found in laboratory-raised fish (Davis et al. 1990). The 5 release sites dried up for up to 2 months during the winter-spring dry season.

The high rate of feeding among 1-day-old *R. marmoratus*, in conjunction with rapidly expanding larvivorous capabilities as the fish age through 10 days, suggests that synchronous

Table 1. Feeding of *Rivulus marmoratus* fry on newly hatched *Aedes taeniorhynchus* larvae.

Fish age (days)	No. fish, no. eating larvae	No. of larvae fed	Larvae ingested (24-h) Mean ± SE*
1	(2, 0)	10	—
1	(29, 12)	20	7.0 ± 1.4
1	(7, 6)	50	11.2 ± 2.6
3	(3, 3)	20	14.0 ± 5.5
3	(10, 10)	30	16.8 ± 3.2
3	(6, 5)	50	48.6 ± 0.5
3	(7, 6)	100	67.0 ± 4.9
7	(3, 3)	50	43.3 ± 5.7
7	(11, 11)	100	59.2 ± 5.4
10	(6, 6)	100	71.8 ± 7.7

* Mean and standard error of the mean, based on tests where fish ate larvae.

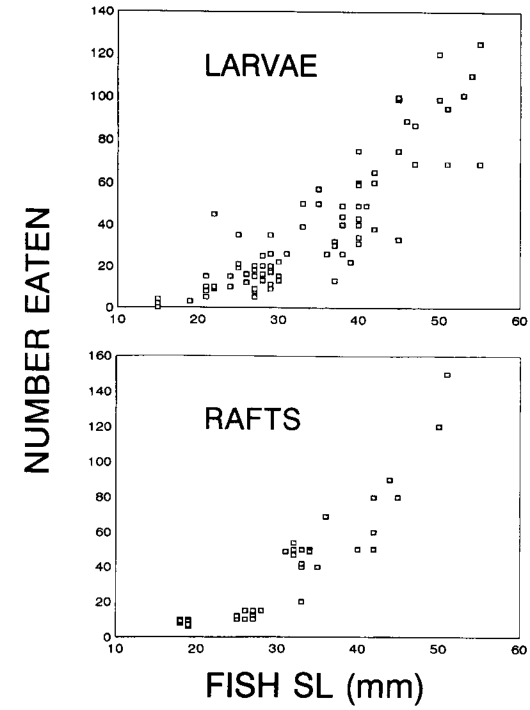

Fig. 1. Relationship of *Rivulus marmoratus* standard length (SL) to the number of 4th-instar *Aedes taeniorhynchus* larvae and *Culex quinquifasciatus* egg rafts ingested in 24 hours.

hatching of *R. marmoratus* and *Ae. taeniorhynchus* eggs may control some of the early instars.

The data on feeding in both fry and adult *R. marmoratus* must be considered preliminary, because of the small size of containers and the fact that older/larger fish were offered more prey. However, increased feeding capability appears to be concurrent with increase in size in both fry and adult *R. marmoratus*. Although *Cx. quinquefasciatus* is not a mangrove swamp species, other *Culex* (e.g., *Cx. nigripalpus* Theobald and *Cx. salinarius* Coq.) are known to live in these habitats (Carlson and Vigliano 1985).

The field releases confirm natural history observations that *R. marmoratus* can survive in transitional habitats producing *Ae. taeniorhynchus*. A single hermaphrodite of *R. marmoratus* may lay up to 8 eggs/day in the laboratory, but very little is known about overall reproductive capability in the wild (Taylor 1990). Obviously, to be a viable candidate for biocontrol, the "fecundity" criteria must be met. Natural populations of *R. marmoratus* never seem to reach the density of some poecilids nor do they seem numerous enough to control large broods of *Ae. taeniorhynchus* (Ritchie, unpublished data). Temperature limitations may restrict application of *R. marmoratus*. While hardier than many tropical species. *R. marmoratus* becomes moribund following prolonged exposure to water temperatures below 9°C.

As a clonal organism, *R. marmoratus* can be tracked through various genetic tagging techniques (e.g., DNA fingerprinting), and current research on the fish focuses on this. Release of the fish in the wild should thus be coordinated with specialists in this field to avoid possible genetic "contamination" of resident populations. *Rivulus marmoratus* seems to exhibit some of the desirable qualities of both the topminnows and the annual killifishes. Since the fish is easily reared in the laboratory, perhaps some effort should go into mass culture and stocking of appropriate clones of this interesting species in suitable habitats for a closer examination of its true potential as a larvivore.

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