ACKNOWLEDGMENTS. The authors thank Dr. R. E. Cline, Chemistry Section, for his valuable suggestions and cooperation in the chemical aspects of this investigation and Mrs. Betsy Noonan, Biological Laboratory Technician, Biology Section, for making most of the egg and larval counts.

Literature Cited

CLINE, R. E., WILTON, D. P., and FAY, R. W.

Aliphatic amines ovicidal for the mosquito, Aedes aegypti (L.). J. Econ. Entomol., in press.

Mulla, M. S. 1967. Biocidal and biostatic activity of aliphatic amines against southern house mosquito larvae and pupae. J. Econ. Entomol. 60(2):515-522.

Mulla, M. S. and Chaudhury, M. F. B. 1968. Ovicidal activity of aliphatic amines and petroleum oil against two species of mosquitoes. J. Econ. Entomol. 61(2):510-515.

WILTON, D. P., CLINE, R. E., and FAY, R. W. 1968. Two formulations effective in the laboratory as ovicides for *Aedes aegypti* (L.). Mosq. News 28(4):602–606.

THREE ESTUARINE KILLIFISH AS FRESH WATER MOSQUITO LARVIVORES

J. M. DOLL AND T. F. BAST

Bur. of Epidemiology, New York State Dept. of Health, Albany, N.Y.

Inland fresh waters of New York State lack indigenous fish that are efficient mosquito larvivores. Recently, specimens of three killifish, Fundulus confluentus, Goode and Bean, Fundulus heteroclitus (L), and Cyprinodon variegatus Lacepede partially acclimatized for fresh water, have been obtained for colonization and field observation.

The value of these fish as mosquito larvivores has been studied by several authors. Fundulus heteroclitus and Cyprinodon variegatus were listed as suitable salt water or brackish water fish by the Bureau of Fisheries as early as 1915 (Radcliff, 1915). About this same time the New Jersey Agricultural Experiment Station studied these two species and named F. heteroclitus as by far the most important salt marsh larvivore of those studied, (Childester, 1917). In Florida a study indicated that where abundant, mosquitoes contributed to total food volumes up to 85.5 percent for F. confluentus, while in a similar situation mosquitoes made up about 78 percent of the food volumes of Gambusia, (Harrington and Harrington

All three species are euryhaline and native to the Atlantic Coast, *C. variegatus* and *F. confluentus* ranging generally farther south than *F. heteroclitus* (Miller, 1955).

Since these killifish all range into fresh water under certain situations, introduction into most of New York would constitute little more than a slight extension of their natural range and they should, therefore, not be considered as "exotic."

Fundulus confluentus has been observed to lay stranded eggs which are resistant to desiccation for several months, an accomplishment previously only associated with Grunion in California and certain South American and African Cyprinodontidae, (Harrington, 1959).

The more northern ranging F. heteroclitus has been reported living in pools where the water temperature dropped to the 40° Fahrenheit range. Burrowing into soft muck on the bottom of the pool enabled them to withstand the low water temperatures. Childester (1917) noted that where given an opportunity, these fish migrated to brackish water as cold weather approached.

Colonization of these various killifish is being attempted to acquire data on their fresh water habits as well as to study possible mass production of desiccation resistant eggs for use in intermittent aquatic habitats.

The authors are greatly indebted to Dr. Frank Murphey and Mr. Robert Lake of the University of Delaware and Mr. Fred Lesser of the Lee County Mosquito Control District, Ft. Myers, Florida, for their cooperation in securing specimens of the fish.

Procedures and Results. In mid-November 1968, a stock of *F. heteroclitus* and *C. variegatus* was obtained from experimental pools at Newark, Delaware, and flown to Syracuse, New York.

Some 300 Fundulus and 200 Cyprinodons were equally divided between two containers with about 10 gallons of habitat water. Temperature during transit was brought down to about 55° F. by the addition of crushed ice between the outer, rigid container and inner liner of heavy gauge plastic.

Oxygen, in a portable "D" tank, was used to saturate the air space of the sealed plastic liners before and once during the 1½ hour flight.

On arrival the fish were transferred to glass aquaria with habitat water brought from Delaware.

A sample of each species was maintained as a reserve at the aquatic biology laboratory, Forestry College, State University of New York, Syracuse.

Fresh water Fundulus confluentus were shipped January 20, 1969, via air express from Ft. Myers, Florida. Approximately 50 specimens were shipped in a commercial styrofoam shipping carton with plastic bag liners.

Fish were divided equally between two large plastic bags, in about a half-gallon of water. The bags were then inflated with oxygen, tied off and laid side by side in the container and the styrofoam box

was sealed. The flight, including a 3-hour layover in Tampa, Florida, took 8½ hours and shuffling of the specimens to and from the airports another 30 minutes.

These specimens, arriving at about 70°F, were placed immediately into waiting tanks of aerated dechlorinated tap water. Both forms of transportation resulted in zero mortality, except for a couple of cannibalized younger fish, and one suspected of injury during handling.

The conversion to fresh water of *F. heteroclitus* and *C. variegatus* was made slowly, by adding 1/10 volume dechlorinated tap water every 3 days to replace a similar volume of habitat water removed. This took approximately three weeks.

This slow conversion was prompted by an observation of the straight transfer of specimens from the habitat water, approximately 4100 ppm salinity pH 6.5, to tap water. C. variegatus exhibited extreme shock and impaired movement when introduced suddenly, but F. heteroclitus could withstand this change with apparent ease.

During stabilization and acclimitization the two Delaware species sustained close to 10 percent mortality, mostly attributed to overcrowding, overfeeding or cannibalism.

During the conversion to tap water, extreme overcrowding, 150 to 175 per 10-gallon tank, placed a heavy load on the aeration and filtering system and the tanks became grossly polluted. The ability of these fish to withstand pollution and low oxygen is obvious and has been noted by other writers (Childester, 1917). This adaptability could be of great value in controlling *C. pipiens*.

Soon after conversion to tap water an infestation of the parasitic protozoan *Ichthyophthirius* and accompanying *Saprolegnia* fungus became apparent. It is not known whether this common aquarium fish disease was initiated by the acclimitization or whether it just became noticeable in the clearer water. The infestation is sometimes triggered by water temperature change, such as that occurring during transport.

The infestation was cleared up in the *F. heteroclitus* with one 24-hour course of treatment using 1 drop per gallon of 0.75 percent Malachite green solution (Perma Life "Ich-out") but remained in the *C. variegatus* for over a month despite treatment.

Due to the reinfection of "Ich" by *C. variegatus* and their excessive aggressive tendencies, all *F. heteroclitus* were removed to separate tanks and both species further subdivided according to size to prevent cannibalism.

The F. confluentus fresh water strain, acquired 2 months after the others, adapted immediately to fresh, chlorinated tap water and has shown no indication of Ichthyophthirius infection. On the night of their arrival, four mature F. confluentus vaulted out of the aquarium and were out of water for an estimated 5 to 10 minutes before being noticed. Three of the fish were noted in a condition of extreme dryness and were nearly motionless but recovered completely when reintroduced. A single fish later died, approximately 24 hours after the accident. Screen wire lids are kept on the F. confluentus aquarium, but have not been necessary with the other species.

Tanks containing mature F. heteroclitus and F. confluentus have been elevated in temperature from the 70° F. ambient to 80° F. and are receiving 14 hours supplemental incandescent light in addition to approximately 9 hours of natural daylight.

Float-supported spawning mops have been introduced to the mature tanks. Spawning behavior and appearance such as enlargement of urogenital pouch, searching for spawning sites and pairing with the male, as described by Foster (1967) has not been observed as yet in *Fundulus*, but territorial behavior and some color change (Fanara, 1964) has occurred in the *C. variegatus* colony.

SUMMARY. Specimens of F. confluentus, F. heteroclitus, and C. variegatus are being colonized to study egg laying behavior and viability, as well as for eventual introduction into fresh waters of New York State.

Colonization is presently aimed at fresh water acclimitization and obtaining an abundance of eggs for testing, especially in regard to over-wintering and desiccation under Northeastern fresh water conditions. Fresh water acclimitization has been accomplished.

Ultimate objectives of the project are to develop the resistant eggs for transportation and mechanical dispersion (perhaps aerially) into extensive semi-permanent mosquito breeding habitats.

References

BIGELOW, H. B., and SCHROEDER, W. C. 1953. Fishes of the Gulf of Maine. Fish Bull. 74 vs. Fish and Wildlife Serv. vol. 53; pp. viii, 1–577.

CHILDESTER, F. F. 1916. A biological study of the more important of the fish enemies of salt marsh mosquitoes. Bull. 300, N.J. ag Exp. Sta. pp. 1–16.

FANARA, DEAN M. 1964. Notes on the biology of a salt marsh minnow Cyprinodon variegatus. N.J. Mosq. Ext. Assn. Proceedings 51:152-159.

FOSTER, NEAL R. 1967. Comparative studies on the biology of killifish (Pisces, Cyprinodontidae) Thesis, Cornell Univ.

HARRINGTON, R. W., Jr. 1959. A delayed hatching on stranded eggs of marsh killifish, Fundulus confluentes. Ecology 40(3):430-437.

HARRINGTON, R. W., Jr., and HARRINGTON, E. S. 1961. Food selection among fishes invading a high sub-hospital salt marsh: from onset of flooding through the progress of a mosquito brood. Ecology 42(4):646–666.

RADOLIFF, L. 1915. Fishes destructive to the eggs and larvae of mosquitoes. Dept. Commerce,

Bur. Fish., Economic Circular 17.