

Breeding behavior and early life history of the waccamaw killifish, *Fundulus waccamensis*

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Synopsis

The territorial, courtship and spawning behavior of *Fundulus waccamensis* was investigated in the field and laboratory. Spawning occurs from April to August. Sexual dimorphism is manifested through dichromatism, genitalia, morphometric differences and contact structures. In nature, nuptial males vigorously defend circular territories and court entering females by circling them, sidling, 'J' shaping and spawning in the sand substrate. In the aquarium, males perform eight advertisement motor patterns during courtship, of which dipping and flashing are most frequent. The female is relatively inactive, performing only two basic acts. The maximum number of mature eggs per ovary is 120. Both mature egg numbers and total egg numbers are highly correlated with standard length. Recently fertilized eggs are colorless and adhesive. The newly hatched young is well developed and relatively large in size.

Introduction

The waccamaw killifish, *Fundulus waccamensis*, is a lacustrine derivative of the banded killifish, *F. diaphanus* (Hubbs & Raney 1946, Miller 1955). The range of *F. waccamensis* is either restricted to Lake Waccamaw, a large (3618 ha), shallow bay lake located in Columbus County southeastern North Carolina, or shared with Lake Phelps (Bailey 1977) in northeastern North Carolina, pending taxonomic studies. The waccamaw killifish is common over sand or silty sand bottoms in the shallow waters of Lake Waccamaw from early spring to late

summer. During the fall and winter, however, it is uncommon in the lake, but individuals are found in manmade canals surrounding the lake, natural feeder streams, and the Waccamaw River headwaters. Because *F. waccamensis* has a localized distribution and because Lake Waccamaw has been threatened by increasing residential development, this killifish has been proposed for protection under various endangered species programs (Bailey 1977, Williams 1977) and lists (Miller 1972, Deacon et al. 1979). However, little published information exists on the biology of *F. waccamensis* on which to base sound recommendations on status. The threatened nature of the waccamaw killifish calls for future consideration for environmental mitigation. Additionally, the breeding behavior of both old world (e.g. Ewing & Evans 1973, Haas 1976, Kroll 1981) and new world cyprinodontids (e.g. Kodric-Brown 1981, Itzkowitz 1974) have been reported, thus,

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facilitating a comparison to the reproductive behaviors of *F. waccamensis* based on the breeding biology presented herein.

Methods and materials

Twenty-two stations in Lake Waccamaw were sampled monthly from February 1979 to September 1980 to provide specimens for gonad analysis. Station localities were chosen to represent a cross-section of habitats found in the Lake Waccamaw area including: vegetated and non-vegetated shoreline; sand and mud bottom deep waters; Waccamaw River headwaters; Big Creek (the principal feeder stream to the lake); and man-made canals surrounding the lake. Specimens were captured with various sizes of seines, ranging from 3 × 1.2 to 15.2 × 1.8 m (mesh size 3 mm), dip nets, and a small otter trawl (2.8 m × 1.3 m at the mouth and lined with 3 mm mesh netting). Each month a representative sample of *F. waccamensis* was preserved in 10% formalin.

A monthly gonad index was determined by removing ovaries from 169 females (22–83 mm SL) in 20 monthly collections, blotting them thoroughly, and weighing them to the nearest 0.01 mg on a Mettler single pan balance. The weight was expressed as a percentage of the total body weight of the female prior to removal of the gonad (Schenck & Whiteside 1977). After weighing, eggs were separated into size classes and counted. Male gonad indices were calculated by comparing the square root of the standard length of each of 203 individuals (21–85 mm SL) with the width of the left testis (Schenck & Whiteside 1977). An average gonad index was calculated for each sex each month.

On 29 July 1978 from 1220–1600 hours and on 10 August 1979 from 1030–1330 hours spawning observations were made from a private pier on the south shore of Lake Waccamaw approximately 1500 m northwest of the dam at the Waccamaw River headwaters. Maidencane (*Panicum*) provided a wavebreak allowing low turbidity and good visibility in a shallow (13–25 cm) area (3 × 3 m) of water. Other conspicuous vegetation present in the

study area included hatpin (*Eriocaulon*) and a benthic species of bladderwort (*Utricularia*). Unusually high water temperatures ranged from 35 to 39° C.

One male (80 mm SL) and one female (75 mm SL) *F. waccamensis*, collected on 10 April 1980, were placed the next day in a laboratory aquarium (30.5 cm deep × 61 cm high × 122 cm long) filled with 75 l of lake water to a depth of 30 cm. Maidencane and hatpin were established in the sand bottom. Water temperature in the aquarium was held at 26° C. The light:dark cycle was 14:10 hours, provided by two 122 cm long fluorescent lamps placed 35 cm above the water. Super 8 mm cine films were taken of courtship behavior on 23 April 1980 (1400–1600 hours). A Nikon R8 cine camera with macrolens was used in conjunction with two flood lamps placed above and to one side of the aquarium. Film was exposed at 18 frames per second. Films were then analyzed with a standard projector at regular speed (18 fps) and slow motion (5 fps). A standard movie editor was also used for a frame by frame analysis of motor patterns.

Hand recorded observations of aquarium spawning behavior were made on 24 April 1980 (1345–1400 hours) and 30 April 1980 (1145–1230 hours). Buried eggs were removed from the sand immediately after spawning and then incubated at 21–23° C in petri dishes suspended in a water bath.

Results

The spawning season of *F. waccamensis*, inferred from gonad indices, begins in April and extends through August (Fig. 1). The number of mature eggs per female ovary is significantly correlated with size (Fig. 2). Also, the total number of eggs is likewise correlated with size (SL). The largest total number of eggs contained in the ovary of one female was 996 in a 70 mm SL female captured in August 1980. The equation for the expression of the regression line is $Y = 6.7X - 54.1$, where Y = total number of eggs and X = SL. The value of $r = 0.43$ and $p < 0.01$ ($N = 55$).

Sexing of juvenile *F. waccamensis* is difficult from outward appearance, as both male and female have

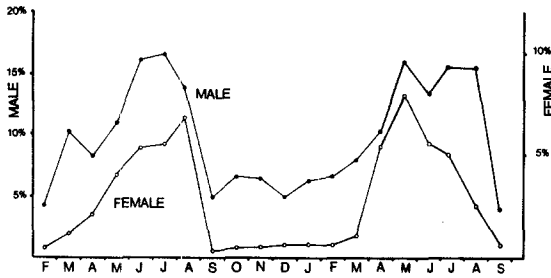


Fig. 1. Monthly changes in gonad indices for male (testes width per square root of standard length $\times 100$) and female ovary weight per body weight $\times 100$) *Fundulus waccamensis* during 1979 and 1980. Typical variability of mean for spawning season (Apr–Aug) is S.D. = 3.8% (males) and 3.0% (females); otherwise, S.D. = 2.6% (males) and 0.18% (females).

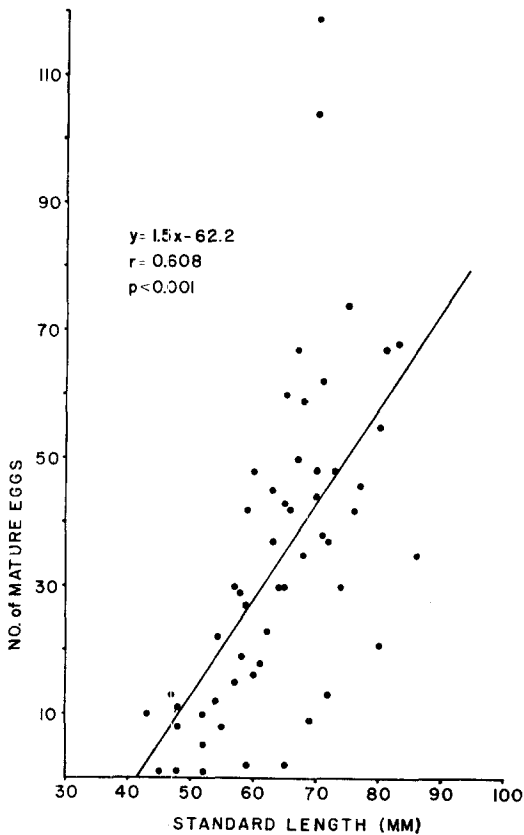


Fig. 2. Least squares regression analysis of numbers of mature eggs (1.75 – 2.0 mm diameter) and standard length of female *Fundulus waccamensis*.

similar patterns of coloration consisting of a series of dark, widely spaced, thin, short crossbars on an olivaceous background. At sexual maturity, marked chromatic differences occur, especially in the male. During the breeding season, the female retains the typical juvenile coloration and has an oviducal flap of tissue which lies back over the first few anal rays (Fig. 3). Males at sexual maturity lose the juvenile pattern, and develop longer, wider crossbars which are more closely spaced than the bars of the female. During the breeding season, these bars alternate with a metallic blue-green hue, especially ventrally. Yellow pectoral, pelvic and anal fins and throat membranes are also another manifestation of the sexual dichromatism of male *F. waccamensis*. But, more typically, the pelvic and anal fins appear as an iridescent blue-green color in active territorial and spawning males. Nuptial males have longer anal fins. The longest ray of the anal fin of males taken in April, May and July 1980 (N = 11) averaged 17.4% SL as compared to 13.7% SL for females (N = 11) from the same dates.

Another sexually dimorphic characteristic of *F. waccamensis* is the presence of 'temporary organs on the scales,' or 'fingerlike processes on the margins of all scales in certain regions' in spawning males (Newman 1907). These contact organs are largest and most numerous on the regions of the

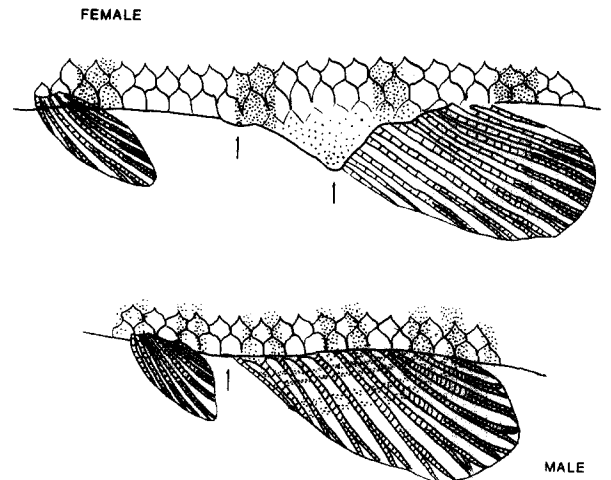


Fig. 3. Lateral aspect of genitalia of mature male and female *Fundulus waccamensis* during breeding season. Arrows indicate anus in both sexes and ovipositor at end of oviducal flap in the female. Not drawn to scale.

body where greatest pressure occurs during sexual contact (Newman 1909, Foster 1967). On *F. waccamensis*, contact organs occur laterally from the caudal peduncle almost to the snout, on the opercle, cheeks, and top of head. Specimens were captured with one to two contact organs per scale in April 1980, and one to three contact organs per scale in May through August 1980.

In nature, male *F. waccamensis* were observed on 29 July 1978 and 10 August 1979 to defend temporary (2–3 hours) territories by continually patrolling their boundaries (0.5–1.0 m diameter). The more vigorous males tended to be more brilliantly colored. Because of an ‘aggregation’ of these territories (Fig. 4A), there was a constant battle to defend a territory against rival males with nearby territories. Occasionally, a male would come into contact with a neighboring territorial male. If this was a head-on encounter (Fig. 4B), the result was a gular display by both males and spreading of all fins. Often they would nip at each other. This ‘facing’ behavior is similar to that described by Barlow (1961) for *Cyprinodon macularius*. ‘Arching’ and ‘tail-beating’ motor patterns (Barlow 1961) also occurred when males came into contact laterally. Each male would curve his body with the concave side toward the other fish in lateral display (Fig. 4C). The brightly iridescent blue-green coloration of the pelvic and anal fins was remarkably obvious to the observer. At the same time, each male would ‘beat’ his opponent with his tail (Fig. 4D). This resulted in the heads of the males gradually becoming farther apart from each other (Fig. 4E), and eventually they separated and continued patrolling their own territories. Non-territorial males did not display any aggression toward other males encountered. Indeed they schooled together, and when confronted by territorial males, they merely fled. On the second observation date, possibly due to a marked rise in temperature in the shallow waters of the study area, males defending territories gradually became less pugnacious. By 1330 hours all territorial males appeared to have deserted their territories, and were schooling and feeding.

During the above field observations, females swam slowly in the shallow water of the study area

in schools of 3–6 (probably feeding). A single female entered the territory of a male (Fig. 5–1) and positioned herself near the bottom. She then remained still, moving only her pectoral and caudal

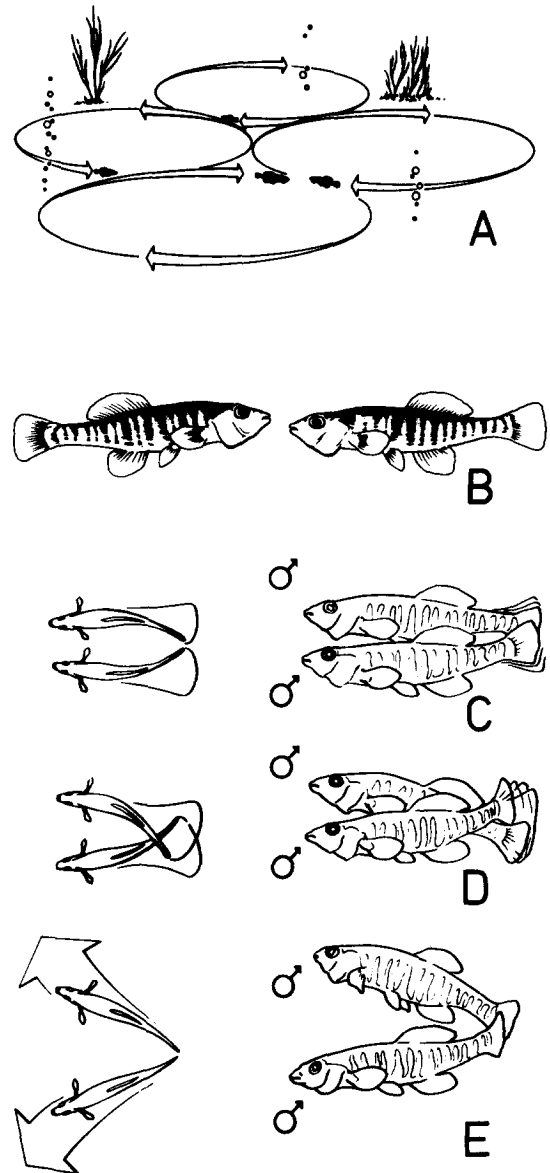


Fig. 4. Territorial males of *Fundulus waccamensis*. A. Males patrolling territorial boundaries within an aggregation of territories. B. Neighboring territorial males that have met head-on showing gular display and spread fins. C. Neighboring territorial males that have met in lateral display with bodies curved and concave sides toward opponents. D. The same males as in ‘C’ showing ‘tail beating.’ E. Eventually moving away from opponents.

fins (Fig. 5–2). The male then courted the female by rapidly circling her (see male, Fig. 5). The ‘dipping’ action (Carranza & Winn 1954) of the male (Fig. 5), although not discernible in the field due to the angle of observations, is inferred here from our aquarium observations. Again, the bright blue-green colors of the anal and pelvic fins of the male were obvious. The circles gradually decreased in size, bringing the male closer to the female. When the male was close to the waiting female, she was observed to tilt her head downward and nip at the substrate, often almost burying her entire head (Fig. 5–3). This ‘nipping’ behavior almost always resulted in the spawning act. The male positioned himself parallel to the female, and with his body forming a strong ‘J’, he pressed the female against the bottom. The dorsal fin of the male was folded around or ‘wrapped’ (Barlow 1961) around the dorsal region of the female. The female appeared to bury herself in the silty substrate, and only the anal regions of the pair were in contact (Fig. 5–4). This was followed by a short quivering of each partner. Presumably an egg was buried by the female at this time and sperm released by the male. Usually the female would retreat after one mating act and the male would continue to maintain his territorial boundaries and wait for another female to come near. Occasionally, however, one female was observed to engage in two or three mating acts with the same male, moving a short distance (a few cm) between the acts.

After spawning activities had ceased, the study area was searched for eggs. Large (2–3 mm diameter) demersal, adhesive eggs were found, often clinging to sand grains, algae or other plant material found near the bottom. No parental care of the eggs was observed.

Cinematographic analysis of 13 sequences of courtship behavior in our aquarium facilitated a convenient breakdown of the characteristic motor patterns of *F. waccamensis* as follows:

Dipping. – The male swims slowly forward while the head is pointed downward and then upward, thus appearing as an up and down bobbing movement. This movement is frequently followed or preceded by flashing.

Flashing. – The male spreads the pelvic, anal and dorsal fins showing the bright iridescent blue-green colors, particularly of the anal and pelvic fins. This display frequently occurs during the downward action of dipping when the caudal peduncle is upturned, thus allowing maximum spread of the anal fin.

Leading. – Male or female slowly swims in front of and preceding mate.

Following. – Male or female slowly swims behind mate.

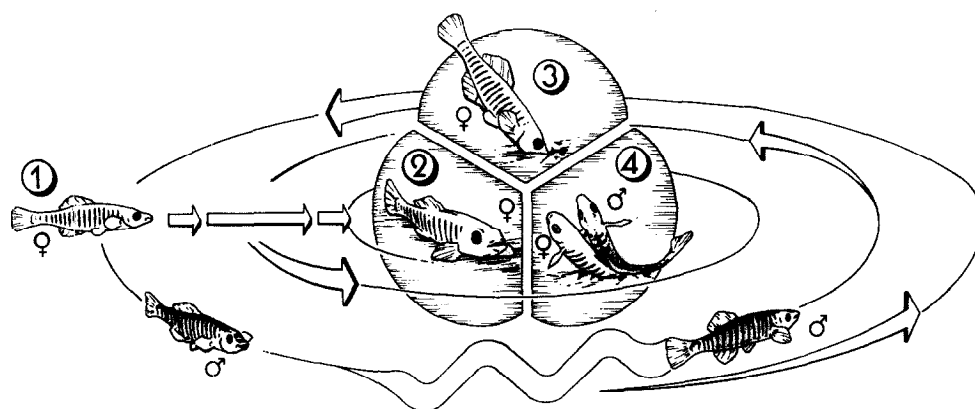


Fig. 5. Spawning of *Fundulus waccamensis*: 1 – female entering male's territory, 2 – remaining still near the bottom, 3 – tilting her head and nipping the substrate, 4 – the mating with the female partially buried and the male in a strong 'J' shape. Note gradually decreasing circles of male around female.

Circling. – Male slowly swims around the female while she is stationary.

Contacting. – Male gently touches females with snout (usually near vent), caudal peduncle or side of body.

Showing. – Male nears substrate in a slightly forward-tilted attitude and remains still.

Sidling. – Male nears female, 'J' shapes and presses body against female. This movement was never fully completed in the laboratory (compare with field observations above). Mating did not occur during filming.

Nipping. – Female tilts, takes a mouthful of sand and spits the grains in front of her.

The composite results of the 13 filmed sequences (Fig. 6) are based on a dyad analysis that records the frequency of pairs of motor patterns. Combinations of eight male and two female motor patterns occurred at least one percent of the total dyads (N = 268, Fig. 6). Clearly, the male is the more active participant, initiating 89% of the movement pairs. Most dyads (78%) were male motor pairs. Dipping was the most frequent motor pattern, occurring in 40% of the dyads.

Further hand recorded observations of actual aquarium spawnings indicated that nipping was invariably followed by sidling and egg deposition. Eggs were laid on sand, hatpin, or on vertical wood

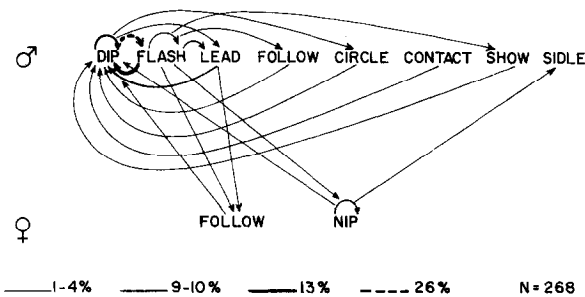


Fig. 6. Flow diagram of courtship motor patterns of *Fundulus waccamensis* based on cine analysis. Motor pattern pairs occurring less than 1% of the total are not shown.

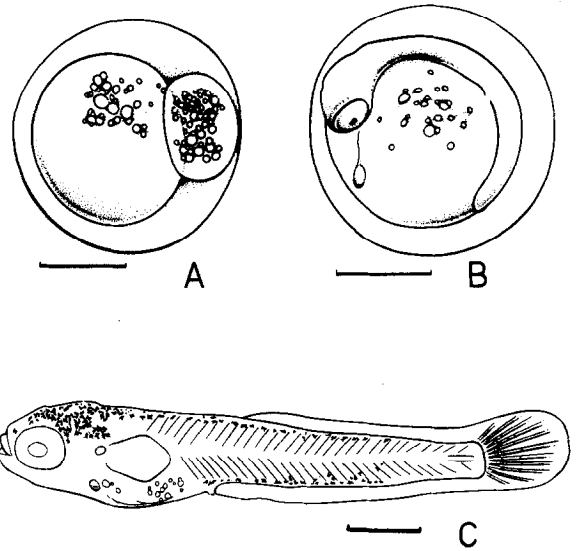


Fig. 7. Laboratory incubated young of *Fundulus waccamensis*: A – egg 24 hours after fertilization, B – embryo 5–6 days after fertilization, C – newly hatched young, 13 days after fertilization. Scales = 1 mm.

pieces. Eggs adhered to all but the latter object. No parental care was given the eggs.

Newly fertilized *F. waccamensis* eggs are colorless, spherical (2–3 mm diameter) and demersal with numerous oil globules (Fig. 7A). The late embryo (Fig. 7B) shows little development of pigmentation. Although *F. waccamensis* eggs are adhesive, no adhesive threads of chorionic filaments were observed, such as those described for eggs of *F. diaphanus* (Hardy 1978). Aquarium raised embryos hatched in 10–13 days at 21–23°C. A newly hatched *F. waccamensis* young (Fig. 7C) is larger (8.1 mm TL) than recently hatched *F. diaphanus* (5.3–6.4 mm TL, Hardy 1978). In our specimen, the dorsal finfold originates just posterior to the ninth myomere (total myomeres number 31, Fig. 7C). The large pectoral fin bud is evident and several caudal rays are developed. A concentration of melanophores is present on the head and dorsal and ventral aspects of the body.

Discussion

The males of many killifish species are known to maintain and defend territories during the repro-

ductive season (Newman 1907, Raney et al. 1953, Barlow 1961, Breder & Rosen 1966). *Fundulus diaphanus* has also been reported to defend territories (Greeley 1935, Richardson 1939, Foster 1967). A lek type breeding system, in some ways similar to our Figure 4A, has been observed for *Cyprinodon pecosensis* in a small lake with limited spawning habitat (Kodric-Brown 1977, 1978, 1981). Males on the lek competed for optimal spawning sites where large, brightly colored males were territorial and smaller 'satellite' males, in subdued nuptial coloration, were nonterritorial. Smaller females spawned with satellite males and larger females with the larger and more brightly colored males. Our observations compared favorably with those for *C. pecosensis* except that we did not observe any satellite males. Satellite males of *C. pecosensis* presumably developed in response to limited spawning sites (Kodric-Brown 1977). This suggests that the breeding habitats of *F. waccamensis* are not as severely limited as those reported for *C. pecosensis*.

Kroll (1981) reports the male-male agonistic behaviors of tail snapping, tail beat and tail flutter for the African killifish, *Aphyosemion gardneri*. Of these behaviors, tail beat is most like that observed for *F. waccamensis* (Fig. 4D). Tail beat clearly provides a severe mechanical stimulus which can forcefully displace the opponent killifish (Ewing 1975, Kroll 1981). While in lateral display (Fig. 4C), *F. waccamensis* males do 'flutter' the caudal fin. However, *A. gardneri* males are usually oriented head to tail and the median, pelvic and caudal fins are folded when performing the tail flutter (Kroll 1981). This behavior, as reported for another rivulin, *A. bivittatum*, presumably delivers a pheromone to the opponent (Ewing & Evans 1973). Tail flutter in *F. waccamensis* is least comparable to the rivulins and is likely to be a minor preparatory movement to tail beat.

The frontal and lateral displays in old and new world killifishes are similar in form and context to those observed for territorial *F. waccamensis*. The reproductive behaviors, contacting and sidling, are also comparable in form and context to those male-female behaviors (e.g. nuzzle, sidle, clasp, S-shaping) reported for cyprinodontids (Barlow 1961,

Haas 1976, Kroll 1981).

The 'nipping' behavior of female *F. waccamensis* has been widely reported for female killifishes during spawning (Newman 1907, Koster 1948, Barlow 1961, Haas 1976), including *F. diaphanus* (Foster 1967) and is possibly a mechanism for testing the suitability of the substrate for mating. The male 'dipping' act has been reported for *Fundulus notatus*, *F. olivaceus* and *F. chrysotus* (Foster 1967) but not for *F. diaphanus*, from which *F. waccamensis* was presumably derived. Dipping in *F. waccamensis* may be a derived or an abbreviated movement of the more complex looping behavior described for *F. diaphanus* (Foster 1967) and other cyprinodontids (Haas 1976). Dipping, flashing, leading, circling, contacting, and showing are interpreted by us as movements performed by the male that advertise the male's intentions of leading and maneuvering the female closer to a suitable mating site (Foster 1967).

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