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THE STATUS OF RIVULUS MARMORATUS (PISCES: APLOCHEILIDAE) IN THE BAHAMAS

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ABSTRACT

Rivulus marmoratus, a small, cryptically colored killifish, has been seldom collected in the Bahamas. Two surveys for this species were completed in the Exumas (1997) and San Salvador (2001). Seventy-eight specimens were taken in the Exumas, and this included one phenotypic male fish among this otherwise normally hermaphroditic species. Salvador, 28 specimens were collected, and all were hermaphrodites. Rivulus marmoratus were taken from a variety of sub-habitats in the mangal, using either miniature funnel traps, standard minnow traps, or hook and line. Subhabitats included solution holes, Cardisoma guanhumi (great land crab) burrows, a flooded "swale" inundated by high lunar tides, and among roots and algae on the edges of saline ponds. The presence of the fish in some isolated hypersaline ponds with a depauperate fish fauna on San Salvador is most intriguing, as it suggests that initial colonization may have been subterranean. These particular populations of Rivulus marmoratus may offer some interesting insights on population genetics in this species. Our collections suggest that R. marmoratus is likely abundant in the Bahamas where appropriate sub-habitats are found, and further collections throughout the Bahamas are encouraged both to assess the presence of male fish, which potentially could lead to outcrossing populations, and to reveal additional subhabitats utilized by this species.

INTRODUCTION

Rivulus marmoratus (Pisces: Aplocheilidae) is a diminutive, cryptic killifish indigenous to mangrove marshes of the Atlantic coast in the new world tropics. Field accounts of this fish have been lacking until recently, primarily due to its secretive behavior and the difficulty of capturing the fish in the dense vegetation and leaf litter of mangrove marshes, where it occupies temporary pools and crab burrows (Taylor 1988; Davis et al. 1990; Taylor 2000). Rivulus marmoratus has been recorded from southern Brazil, north through the Atlantic and Caribbean coasts of South and Central America, the Caribbean islands, Bahamas and reaches the northern limit of its range along both coasts of central Florida (Davis et al. 1990; In the Bahamas, only a few Taylor 1993). (<15) specimens are known, from New Providence, Andros, and Bimini (Böhlke and Chaplin 1968; L. Brown, pers. comm.), San Salvador (M. Barton and D. Gerace, pers. comm) and Turks and Caicos (R. Heard, pers. comm.).

Most individuals of *R. marmoratus* are self-fertilizing hermaphrodites with functional "ovotestes", and internal fertilization produces groups of homozygous "clones". However, in some populations, the presence of fish that are completely male complicates this reproductive mode. Both high and low temperature are reported to occasionally produce males (Harrington 1967, 1971), but Davis *et al.* (1995) suggested that ecological or habitat disturbance may also produce males. In addition, Taylor et al. (1995) have suggested that other factors,

possibly "social" in nature, may also be involved in the induction of males. No "pure" female fish have been found.

In Florida, about 15 wild males have been reported among the 2,000 + specimens collected, and all but one have come from the southeast or southwest coasts and the Florida Keys (Davis et al. 1990; Taylor 2000; D.S.T unpub. data). A significant population of male fish also reported from Curação. was Netherlands Antilles (Kristensen 1970). although other recent collections from there have failed to find male fish (B. Turner, pers. comm.). Davis et al. (1990) found that 10-25% of R. marmoratus from some cays off the coast of Belize, Central America cays are male, and Lubinski et al. (1995) demonstrated a high incidence of heterozygosity in these populations, suggesting that males are functionally competent and that outcrossing likely occurs. significant population of male fish (2%) and limited heterozygosity has since been reported from one of the Bay Islands, Honduras (Taylor et al. 2001). An even lower proportion of male fish (1%) is reported from the Bahamas (see below), and these populations were uniformly homozygous (Taylor et al. 2001).

Theory suggests homozygous lineages may have "limited potential", yet *R. marmoratus* has colonized one of the most environmentally stressful and variable of marine systems, the mangrove marsh. The fish has been collected at salinities ranging from 0-68 ppt, at temperatures from 7-38° C, and at high levels of hydrogen sulfide (Davis *et al.* 1990; Taylor *et al.* 1995). However, homozygosity has been shown to not confer any additional developmental instability in this species (Taylor 2001).

Although widespread latitudinally, *R. marmoratus* collections have focused on Florida (>2,000 specimens), Central America (>700 specimens) and the Cayman Islands (>500 specimens). We report below on recent collections of *R. marmoratus* from two areas in the Bahamas, the Exumas and San Salvador.

MATERIALS AND METHODS Exumas

Collections for *R. marmoratus* were completed from October 15-22, 1997 on Norman's Pond Cay (NPC) and Lee Stocking

Island (LSI) (Figure 1). The survey focused on NPC, but the limited mangrove areas on LSI were briefly examined. On NPC, a small area of mangrove with sandy substrate was found just north of a site known as the "Waterfall" in the large interior pond. The area was vegetated with buttonwood (Conocarpus), black (Avicennia) and white (Laguncularia) mangroves. A central swale retained water at low tide (depth about 8 cm) and was flooded to a depth of 25 cm at high tide. Cardisoma burrows were on both edges of the swale, on a shoreline berm and at the upland edge. The burrow areas were vegetated with sandfly bush (Rachicallis americana), baycedar (Suriana maritima) and Conocarpus. Cardisoma guanhumi burrows were abundant here and were sampled with "cup" traps (inserted into the burrows) or with miniature fishing gear using oligochaetes for bait (Taylor 1990). Areas of permanent standing water in the swale were sampled with wire ("Gee"®) minnow traps. On LSI, solution holes in the pond just north of the Caribbean Marine Research Center were sampled with cup traps. Two "inland" mangrove ponds on LSI were also examined and trapped with cup traps and minnow traps.

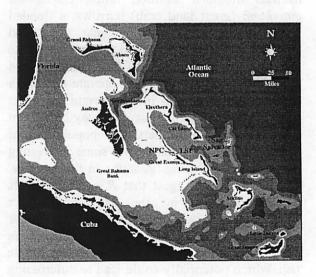


FIGURE 1. Map of the Bahamas, showing location of Lee Stocking island and Norman's pond Cay (Exumas) and San Salvador.

San Salvador

Collections on San Salvador (Figure 1) were completed from June 12-17, 2001 at five sites: Osprey Lake (OL), Little Lake (LL), Reckley Hill Pond (RHP) and Crescent Pond (CP). The physical and biological features of OL. RHP and CP have been previously described (Godfrey et al. 1994) At OL, cup traps were placed in shallow (5 cm) water along the shaded, rocky shoreline, among Avicennia pneumatophores and algal mats. Traps were left in place from varying periods of several hours to overnight. At RHP, cup traps were placed in a similar setting, although the shoreline here was vegetated with Rhizophora. At LL, cup traps were placed among depressions (5-6 cm deep) among a flooded Avicennia stand, and minnow traps were set on the edges of deeper (1 m) holes. At CP, cup traps were placed in solution holes flooded by a high tide. These traps were left overnight, and water level dropped nearly 25 cm during this period.

RESULTS Exumas

The number of specimens taken, salinity and water temperature for each collection site are summarized in Table 1. A total of 78 R. marmoratus was collected; 77 from NPC and one from LSI. The NPC site was the only area seen with substrate suitable to allow land crab recruitment and burrowing, and the sandy/mud substrates may be the result of alluvial deposits transported through the "Waterfall" tidal creek. Burrows were abundant in this area and some were exceedingly large (nearly 20-25 cm dia). At high water burrows were flooded 8-10 cm deep, probably an exceptional event as sampling was conducted during very high lunar tides. In all burrows where fish were collected, high tide inundated the burrows, and the water within the burrows was consistently clear and not tanninstained, as has been observed at many other R. marmoratus collection sites in the mangal. At low tide, these burrows were isolated and water

Table 1. Rivulusm marmoratus collections from the Bahamas: 1997 AND 2001

Site	Salinity (ppt)/ Temp(¡C)	Habitat code ¹	Catch ² (H/M)	GPS coordinates
Exumas (1997)		-		
Lee Stocking (LSI)	4/27	1	1 H	na
Norman@ Pond Cay (NPC)	34/29	2	6 H	na
Norman@ Pond Cay (NPC)	34/29	3	71 H, 1 M	na
San Salvador (2001)				
Osprey Lake (OL)	45/34	4	6 H	24; 06.7'
Little Lake (LL)	31/35	5	10 H	74 _i 27.9' 24 _i 03.0' 74 _i 31.2'
Reckley Hill Pond (RHP)	30/34	4	1 H	24 _i 06.9' 74 _i 27.5'
Crescent Pond (CP)	35/27	1	11H	24 _i 06.8' 74 _i 27.4'
Total			105 H,1 M	•

Note: Specimens (n=49) from the Exumas are deposited in the FLMNH, Gainesville, FL (UF #\tilde{G}\) 116516, 116524) and with CMRC, Lee Stocking Island, Exumas.

² H= hermaphrodite, M= phenotypic male

¹Habitat codes: 1= solution hole; 2= flooded mangrove &wale © 3= <u>Cardisoma</u> burrow; 4= edge water: <u>Avicennia</u>, rock, algae; 5= edge water: <u>Rhizophora</u>, rock, algae.

levels were well "inside" the mouth of the burrow.

A maximum of 11 fish was collected from a single burrow. Fewer than six specimens were taken with hook and line, and only six fish were taken in minnow traps set in the flooded central swale. Several *Gambusia* sp. were taken in the minnow traps. All other specimens of *R. marmoratus* were taken from burrows with cup traps. A single male fish was collected. It is noteworthy that not a single specimen was "drowned" in the traps, a phenomenon frequently observed in other collections where traps have been submerged by incoming tides, denying the fish access to the air/water interface (Davis *et al.* 1990).

On LSI, a single specimen was taken with a cup trap set in a solution hole. This solution hole, in which several R. marmoratus were observed, was located 2-3 m inland from the high tide mark and had no surface tidal connection (sub-surface connection to tidal water may have been present). Two other isolated mangrove pond areas on LSI were examined for R. marmoratus. One was a body of shallow, highly turbid water (salinity 5 ppt), several acres in size and with a heavy growth of No fish were Rhizophora on the edges. observed in the pond, and minnow and cup traps set for 24 h on the edges collected no fish. There is no surface tidal connection to oceanic water. The other pond was at least several acres in size, and has a dense overstory of Conocarpus, including some very large trees. The presence of cattails (Typha) and sedges (Carex) indicate that the site rarely, if ever, experiences high salinities. The area was heavily flooded by rainwater to a depth of 12-20 cm. The water was clear, tannin stained, and many tadpoles were present. Salinity was 2 ppt and temperature was 25 ° C. Several minnow and cup traps were set for 24 h, with no catch.

San Salvador

Physical and catch data for each site are summarized in Table 1. A total of 28 R. marmoratus was taken, and all were hermaphrodites. The OL collection site was in shallow, turbid water among decaying algal mats (Batophora sp.) and rocks on the shoreline of the lake. The site was shaded by overhanging,

stunted Avicennia. A maximum of three fish was taken in a single cup trap. Rivulus marmoratus from this site were noted to be pale in coloration, compared to most specimens taken from mangrove forests. Also taken in the traps were a few Gambusia sp. and Cyprinodon sp. The LL site was more "typical" of known R. marmoratus collection sites, with a dense large Avicennia growth of fairly topographically resembling a black mangrove basin forest. The site was located 25-50 m landward of a berm on the shoreline of the lake. Soils were very organic and soft, and the water (varying in depth from 3 cm-1 m) was deeply tannin-stained. Traps were set on the edges of depressions and deeper holes. Newly emerged mosquitoes (Ochlerotatus salt marsh taeniorhynchus) were very abundant at the site, so the site appears to be a breeding area for this species. A maximum of seven fish was taken in a single minnow trap, and only one fish was caught in the cup traps. All fish were hermaphrodites and exhibited the darker coloration typical of the species. Gambusia sp. and Cyprinodon sp. were also taken at the site. RHP was similar in some physical and biotic characteristics to OL, and the single fish was taken in traps set at the edge waters of the pond. However, the water in RHP was much less turbid than OL, and Rhizophora was the dominant shoreline plant at the collection site. The trap sites were less shaded, and the algal communities (predominately Batophora) were robust and green. Also taken in the cup traps were Cyprinodon sp. and Gambusia sp., Anisopteran larvae, Dysticid beetles molluscs (gastropods and bivalves). The single R. marmoratus taken was rather small (~30 mm SL) and normally colored. The collection sites at CP were all within several solution holes between 0.25 m and 1 m in diameter and varying in distance from 0.5 to 4 m from the permanent waters of the pond. When the eight cup traps were recovered after an overnight set, they were completely dry as a result of the outgoing tide, yet 11 fish (all hermaphrodites) were captured, and all but one was alive. Up to four fish were taken in a single trap.

DISCUSSION

This the first report presents comprehensive collections of R. marmoratus from the Bahamas. Previous, scattered collections have largely not been reported in the literature. The micro-habitat preferences of this fish have been well-defined in Florida and Central America, but the collection of the species from solution holes in the Bahamas adds a new element to its "fossorial" nature. The evidence from San Salvador could indicate that this fish may actually disperse via subterranean Don Gerace (Bahamian Field Station) has reported the collection of two specimens of R. marmoratus several meters within the San Salvador Lighthouse Cave system, where the only access was via underground tidal channels. Further, the fact that our collections were within isolated lake systems on San Salvador suggests arrival by "conduit", the underground channels connecting the lakes to the ocean, as many of these lakes are surrounded by high-relief terrain. depauperate fish fauna in these lakes strongly suggests limited oceanic access. An alternative hypothesis for the presence of R. marmoratus within isolated lakes may be that they have managed to persist here since the lakes were first formed some 3,000 ypb (Teeter 1995; J. Mylroie, pers. comm).

Our data from San Salvador suggest that R. marmoratus is likely found in many, if not all the lake systems there, as we examined only four systems and the fish was present in each one. Mike Barton (pers. comm.) has also taken R. marmoratus on San Salvador: three specimens from Moon Rock Pond (not examined by us) and one from Little Lake. A further intriguing question is why the fish was not found in isolated mangrove ponds on LSI, but it was not determined if subterranean pathways were present at/near that site.

The genetics of *R. marmoratus* has been the subject of intense scrutiny, and the potential for long-term isolation of some populations within San Salvador lakes could lead to some interesting analysis of the clonal structure within these populations. Our understanding of clonal structure within populations of this unusual animal is minimal, and we therefore encourage

further study and collection of this species throughout the Bahamas.

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