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GROVELING IN THE MANGROVES: 16 YEARS IN PURSUIT OF THE CYPRINODONT FISH *RIVULUS MARMORATUS* ON THE BELIZE CAYS

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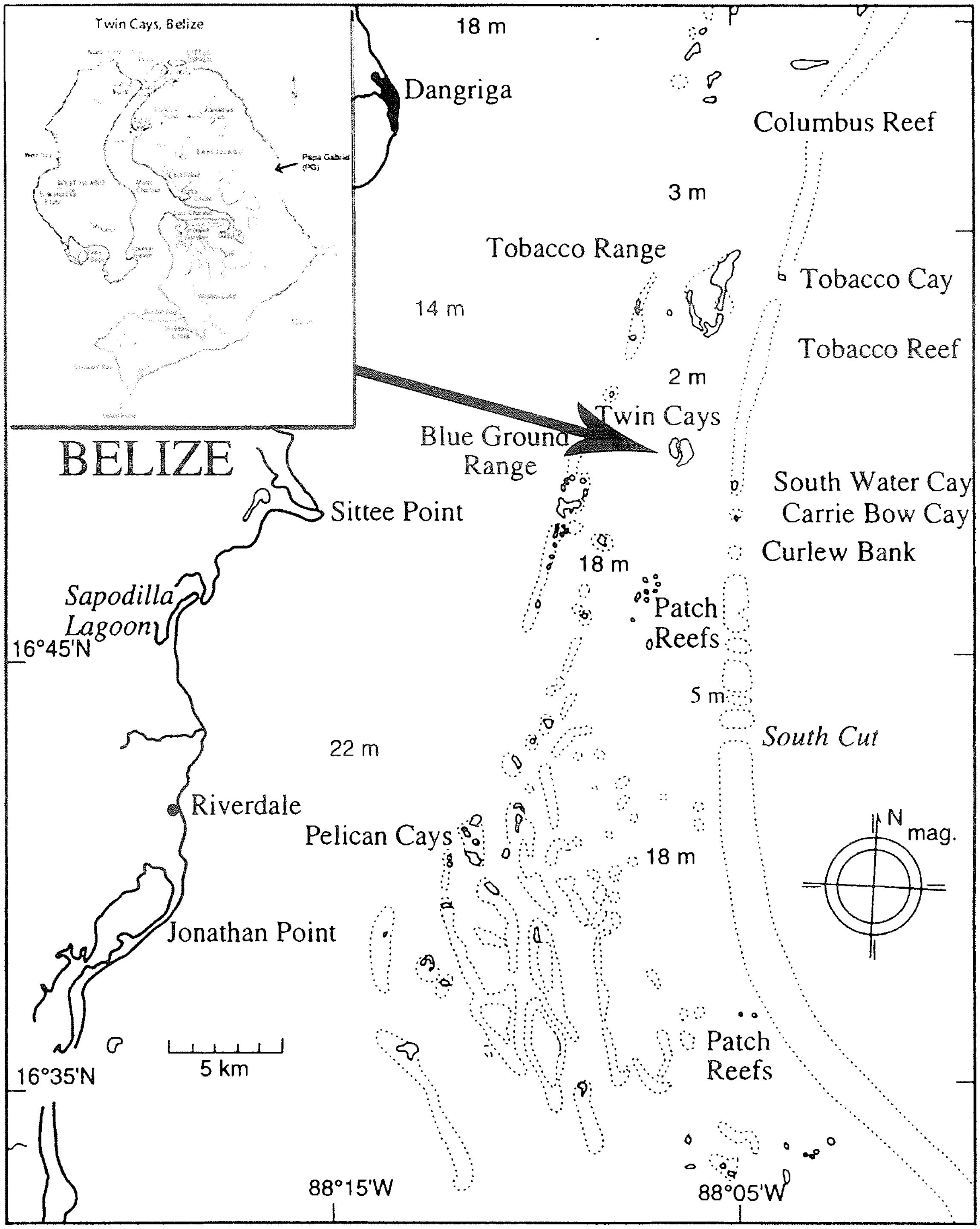

Figure 1. Map of the Belize Cays showing the range of collections for Rivulus marmoratus. Glovers Reef is not shown.

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ABSTRACT

The cyprinodont fish *Rivulus marmoratus* is strongly affiliated with the red mangrove *Rhizophora mangle* and distributed throughout the tropical western Atlantic including the Belize Cays. We have studied this animal for 16 years on the cays during six investigations since 1988. We summarize our findings in the field and explore the significance of the continued presence of high numbers of male fish on certain cays, a phenomenon unknown elsewhere. We also present data on mainland (Dangriga) collections where male fish were not found, an as-yet unexplained contrast to the Belize Cays.

INTRODUCTION

The cyprinodont fish (Aplocheilidae) *Rivulus marmoratus* (Poey) is widely distributed from southern Brazil through the Caribbean islands and Central America to the Yucatan and along both coasts of Florida north to central Florida (Lat 26° N) (Taylor, 2000). The fish is strongly affiliated with the red mangrove (*Rhizophora mangle*) and tracks the distribution of this plant in the western Atlantic, thus giving rise to the common name "mangrove rivulus" (Davis et al., 1995). *Rivulus marmoratus* was first described from Cuba by Poey (1880). However, until well into the next century, *R. marmoratus* was thought to be very rare (Harrington and Harrington, 1961; Harrington, 1971) and, until 1988, fewer than 50 specimens were captured in the state of Florida, an area intensely collected by ichthyologists for several decades. At least in some locations, the apparent scarcity of the species appears to be a factor of inappropriate sampling methodology that failed to target specific microhabitats within the mangal.

It is now clear from our combined work in several western Atlantic locales that *R. marmoratus* has been largely overlooked using standard ichthyological methods because of the unique and sometimes "fossorial" nature of its habitats, including crab burrows, transitional pools and even terrestrial refuges, under leaf litter and logs (Davis et al., 1990; Taylor, 2000). Our collection efforts over the last two decades have focused in Florida, the Bahamas, Honduras, and the cays of the Belize barrier reef (Fig. 1). We have developed a search strategy for appropriate *R. marmoratus* habitat as well as specialized

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collection techniques. Our studies demonstrate that *R. marmoratus* can be abundant in appropriate habitat. However, we have also observed that habitat alteration, such as mosquito impoundment construction and destruction of mangroves and saltmarsh, can negatively impact populations (e.g. east coast of Florida) (Davis et al., 1990; Taylor, 2000; Taylor et al., 2003

Rivulus marmoratus has behavioral and physiological adaptations that are novel among fishes in general although perhaps widespread among the approximately 100 species of the genus. *Rivulus marmoratus* survives in water-quality conditions that are adverse for other fishes (high temperature, widely varying salinities, high levels of H_2S and ammonia) with a remarkable suite of physiological and behavioral adaptations. Salinity variation is apparently compensated for with highly efficient chloride cells which allow acclimation in the laboratory to 114 ppt. (King et al., 1989). With this mechanism, *R. marmoratus* has been found in the wild at salinities ranging from 0-68 ppt (Taylor, 2000). Ammonia concentrations can also be quite high in crab burrows (up to 4.7 umol l^{-1}) and *R. marmoratus* tolerates these levels (Frick and Wright, 2002). Hydrogen sulfide is acutely toxic to fishes at even very low concentrations and *R. marmoratus* avoids this hazard by leaving the water, a behavior known as "emersion" (Abel et al., 1987). Upon leaving the water, the fish respires in moist environments via an expanded capillary network, absorbing O_2 through the skin and fins (Grizzle and Thiyagarajah, 1987). As long as a damp refuge is available (e.g. mud, rotting logs, leaf litter), *R. marmoratus* can survive emersed in a laboratory setting for at least 66 days. Although considerable weight-loss occurs during a lengthy emersion, fish quickly resume feeding and normal activity when reflooded (Taylor, 1990). Once emersed in the wild, we presume that *R. marmoratus* reenters the water when conditions improve. These remarkable adaptations perhaps explain a common observation of ours: few other fish species are found in most *R. marmoratus* habitats.

Apart from these attributes reminiscent of a salamander, most scientific interest in this species is generated by its unique reproductive system and genetics. *Rivulus marmoratus* is the only vertebrate animal known to reproduce as a self-fertilizing hermaphrodite (Harrington, 1961). The gonad is an integrated ovotestis organ that simultaneously produces eggs and sperm with fertilization in the gonoduct at ovulation (Harrington, 1961). This reproductive strategy results in the fish cloning itself, producing exact genetic duplicates (at the level of multilocus DNA fingerprinting) that are stable for at least 20 generations (Laughlin et al., 1995). This reproductive mode produces homozygous lines in wild populations and most *R. marmoratus* populations are now identified as groups of homozygous clones (Kallman and Harrington, 1964; Harrington and Kallman, 1968; Turner et al., 1992b). However, unlike conventional invertebrate/plant clonal systems, clonal diversity is very high with essentially a 1:1 ratio of fish:clone. This high diversity is enigmatic (Turner et al., 1992b). Further, a more basic question remains about how a homozygous clonal organism, with presumed genetic "limitations", can successfully occupy such a vast geographical range.

This presumably infallible reproductive system is confused by the presence of male fish. Male fish, distinguished by having only testicular gonadal tissue, were first noted in laboratory cultures reared at low temperatures (20° C) (Harrington, 1967). Males occur in specific wild populations (Taylor, 2000) and show evidence of loss of ovarian tissue. A significant number of male fish were described in the wild from Curacao (Kristensen, 1970) and a few have been found in Florida, Honduras and the Bahamas (Taylor et al., 2001).

The greatest number of males, however, have been observed in the Belize Cay populations and our observations here and subsequent genetic studies have exploded previous concepts of the reproductive/genetic system of *R. marmoratus*. Our first collection in Twin Cays (1988) revealed a large number of males clearly distinct from hermaphrodites by their unique coloration and confirmed by histology (Davis et al., 1990; Turner et al., 1992a) (Fig. 2, 3). These males contain functional testes, often with little or no trace of ovarian tissue. Subsequent genetic investigation revealed almost uniform heterozygosity on some Belize Cays (Lubinski et al., 1995) and this trait has continued for at least several years (Taylor et al., 2001). The most parsimonious way for heterozygosity to occur in this clonal system is via outcrossing. However, the mechanism by which this occurs has not been identified. We hypothesize that, in the presence of males, hermaphrodites suppress internal fertilization and lay unfertilized eggs, that are fertilized externally by males. This is an intriguing mechanism and neither the histology nor physiology have been elucidated nor have functional "females" been identified in the wild.

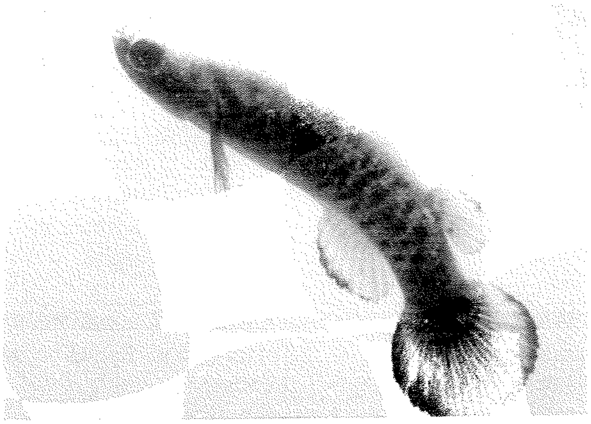

Figure 3. *Rivulus marmoratus*, male.

Figure 2. *Rivulus marmoratus, hermaphrodite*

A lower percentage of male fish (2%) has been described in populations from Utila, Bay Islands, Honduras and a limited outcrossed, heterozygous population (~2%) has resulted here (Taylor et al., 2001). It is further intriguing to note that the Utila population (135 km distant from Twin Cays) appears to operate under a completely different genetic system with minimal outcrossing.

Thus, the male "issue" continues to be one of the great unanswered questions in *R. marmoratus* research. It may be that although outcrossing is intermittent and infrequent in all populations it appears to have persisted in the Belize Cays for over a decade. The broader question, of course, is the significance of the phenomenon in an apparently highly successful clonal organism that has managed to colonize a huge area of a highly inhospitable environment, the mangal of the western Atlantic.

With this larger question looming, we have persisted in our field investigation of the Belezian mangal and below we summarize our collections of this intriguing animal during six investigations since 1988.

MATERIALS AND METHODS

We sampled *R. marmoratus* populations on Twin Cays and adjacent cays and mainland Belize (Dangriga) during 1988, 1989, 1990, 1992, 2000 and 2003 (Fig. 1). We utilized a number of collection techniques within temporary pools and burrows of the mangrove land crab, *Ucides cordatus*, which have been described elsewhere and are summarized in Table 1 (Taylor, 1990; Davis et al., 1990; Davis et al., 2003). We recorded basic water-quality data including salinity via optical refractometer, temperature, and tidal stages at different times of the year and attempted to correlate these data with behavior of the fish. Each collection period encompassed approximately two weeks. Our collection on Twin Cays focused on the site "Papa Gabriel" (PG) (Fig. 1; Davis et al., 1990). We also have documented extensive clearing of mangroves at this site over the 16-year period.

Table 1. Gear types used for collection of *Rivulus marmoratus* on the Belize Cays.

Gear	Description	Technique
Cup trap/'pipe' trap	8-10 oz. plastic cup fitted w/ plastic funnel or misc. plastic tubular material fitted w/ fiberglass screen funnel	Inserted funnel-down into crab burrows or set in shallow, flooded pools and covered w/ debris to form a 'refuge'
Wire minnow trap (Gee®)	Cylindrical, commercial wire trap w/ funnel on both ends	Set in shallow pools of depth sufficient to flood funnel openings. Covered w/ debris to form 'refuge'
Hook and line	Small 'stick' w/ 25 cm. of light monofilament line and small hook w/ marine polychaete worm bait	Dangle bait in crab burrow. Fish seizes worm end and is lifted out for capture
Dip net	Custom-made wire-frame net (~70 cm dia) w/ 3 mm mesh netting	Thrust rapidly into crab burrows to capture fish

All specimens collected were released except for those retained for ongoing genetic research or for maintenance of a diverse set of clonal lineages in our respective laboratories. Those retained alive were stored after collection and transported in small Whirl-Pac® bags (Davis et al., 1990).

RESULTS

We collected a grand total of 1,271 *R. marmoratus* during our six investigations. We have summarized the collections from PG (n= 986) in Table 2. The following narrative summarizes each collection from PG, surrounding cays and the mainland site.

Table 2: *Rivulus marmoratus* collection data from Twin Cays, Belize Cays for six different years.

Year	Total collected (#herms./#male	% male
1988	121 (85/36)	29.7
1989	183 (161/22)	12.0
1991	252 (240/12)	4.7
1992	265 (246/19)	7.1
2000	134 (111/23)	17.1
2003	31(23/8)	25.8

July 28 to August 10, 1998

Some data on collections for 1988 and 1989, as well as a description of the PG site, were reported by Davis et al. (1990). During this visit, our first, we encountered very wet conditions with heavy rainfall and high tidal stages. A tidal "pool" formed behind the dwelling at PG from rainfall and receding high tides, reaching a maximum depth of 16 cm. Salinity within crab burrows and pools ranged from 13 ppt to 36 ppt and temperature varied from 25° C in burrows to 37° C within the pool in full sun. Many specimens (n=63) were taken from this pool including rarely collected juveniles (5-9 mm TL). We took 33 fish from crab burrows. The first male fish taken in Belize was collected at this PG site. We also took two fish from the Boa Flats area and two from Man 'O War Cay confirming that the species was not confined to Twin Cays. We noted that some hermaphrodites were so gravid that many viable embryos were released into the collection bags.

July 7 to July 16, 1989

By contrast, conditions this year on the Cays were much drier than during 1988 with lower tides and less rainfall. Salinities were as high as 60 ppt in crab burrows and drying pools during this period but had dropped to 17 ppt due to heavy rainfall by the end of the period. Temperature ranged from 24-28° C within burrows and 34-36° C in exposed pools. Extensive clearing of mangroves at PG had occurred during the past year and a swath about 15m x 50m had been clear-cut. In addition to collections on Twin Cays, we expanded our survey to include Tobacco Range (18 hermaphrodites, 1 male) and Blue Ground Range (2 hermaphrodites, 1 male). "Dry" conditions, however, apparently hampered collection efforts as the catch-per-unit-effort (CPUE=1 trap-night) was less during 1989 than 1988 (1988= 0.35 CPUE; 1989= 0.097 CPUE) (Davis et al., 1990). In addition, fish appeared "thin" and no embryos were released in the bags. This year also included our first observation in Belize of what we have termed "sulfured"

R. marmoratus, in which individual fish have patches of white on the epidermis often covering much of the body and fins. We have subsequently observed this phenomenon repeatedly during other drought conditions and believe that it represents growth of sulfur-oxidizing bacteria, possibly *Beggiatoa* sp.

The year 1989 also provided an opportunity to compare collections in the newly cleared site vs. the mangrove areas of PG intact from the previous year. We compared the catch results from 51 traps set in the newly cleared area over a period of 11 days vs. the results of 28 traps set in the uncleared area during the same period. The CPUE for the cleared area was 0.86 vs. 0.61 for the uncleared area.
We are not certain whether this represents a correlative observation, beginning of a trend or the range of variation of field observations.

January 10 to January 23, 1991

This was another "dry" event and resulted in some interesting observations. In addition, at PG further clearing of the site had taken place extending the cleared swath 50 m north of the dwelling and west all the way to Hummingbird Pond. Large piles of cut mangrove were formed for burning and all the crab burrows in the cut shoreline area were gone. Crab burrows that were still present in the newly cleared areas in 1989 were reduced by an estimated 90%. A considerable amount of sand and rock had been placed in the area adjacent to the dwelling and the formerly productive pool area was filled in. Although tides were very low, salinities were not high (ranging from 26 ppt at the inception of the sampling period and dropping to 10 ppt on 1/14/91 following 2.5 cm rainfall). Water temperature fell within a narrow range of 26°-29° C.

The CPUE with cup traps was relatively low (0.27 for a total of 163 trap-nights). Many of the *R. marmoratus* observed within burrows exhibited the "sulfured" condition once again, perhaps more commonly than in 1989. Two new observations were presented to us during this collection. We inadvertently discovered a large number of *R. marmoratus* emersed under some plastic sheeting debris near the shoreline at PG. Further investigation found this to be a common phenomenon and nearly 80 individuals were collected in this fashion from under debris and logs. Apparently, *R. marmoratus* had left the confines of the crab burrows and emersed in these habitats. Emersion under logs and leaf litter was described in Florida (Huehner et al., 1985; Davis et al., 1990), but ours represents the first observation of this behavior for Belize. Another startling example of emersion was found on Peter Douglas Cay. Here we discovered a drying pool (8 m x 3 m: salinity 22 ppt) about 50 m from the shoreline (Fig. 4). It contained several species of dead fish (poeciliids, gerreids) at the water's edge. When we moved a small log (1.5 m long, 9 cm dia) at the edge of the water, a number of *R. marmoratus* fell out. Upon breaking the log open, we found it riddled with termite galleries all full of *R. marmoratus* (Fig. 5). We estimate that over 100 individuals were contained therein and many of these were returned to our stateside laboratory still within a portion of the log. Presumably, this represents yet a further adaptation to poor water quality conditions.

We also found that shallow depressions, usually around the bases of stumps and associated with one or more crab burrows along the intertidal sections of the

Figure 4. Temporary mangrove pool at Peter Douglas Cay. Over 100 Rivulus marmoratus were found emersed inside a galleried log at this site.

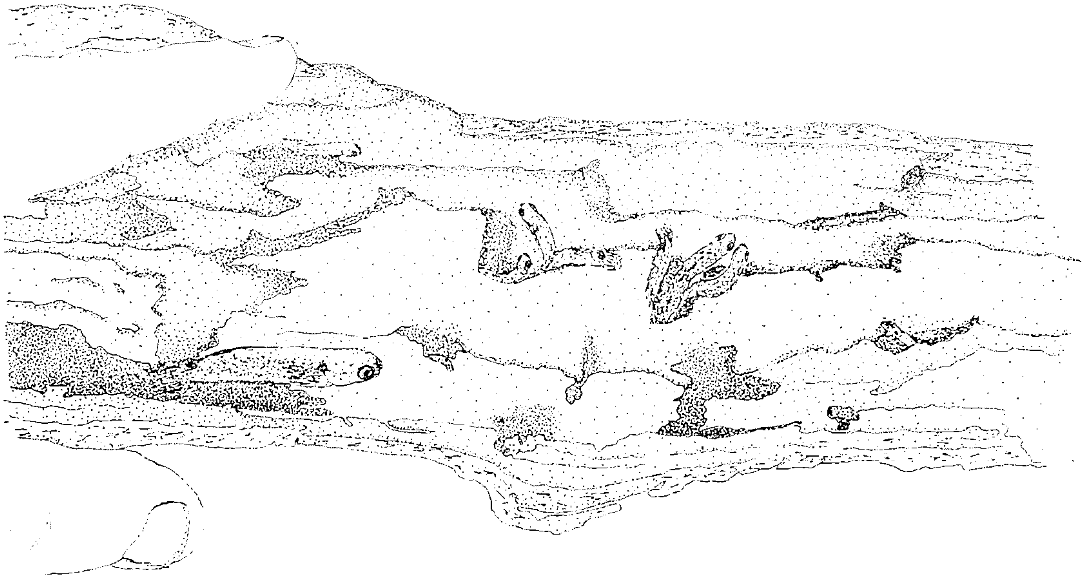

Figure 5. A sketch of the log from Peter Douglas Cay, with emersed Rivulus marmoratus visible.

shoreline, contained large numbers of very large *R. marmoratus*. We collected from 8-21 individuals from three of these sites at PG and most were very large hermaphrodites. Once again, we speculate that these were fish that had moved down the marsh profile due to inhospitable conditions at higher elevations.

We sampled other locales this year also. We collected 11 hermaphrodites on Coco Plum Cay, 2 on Elbow and 3 on Pelican Cays and 21 hermaphrodites and 1 male on Ragged Cay. A limited sampling effort at the minimal mangrove habitat on Middle Cay, Glovers Reef produced no *R. marmoratus*.

March 19 to April 1, 1992

This collection period represented yet another dry period, with low tides and little rainfall. At PG we collected *R. marmoratus* at salinities ranging from 34 ppt within burrows to a maximum of 65 ppt in drying pools. This latter represents the highest salinity at which we have collected the fish in any locale. Temperature ranged from 27° in burrows to 37° C in pools. We observed again many "sulfured" fish and considerable emersion inside crab burrows and also noted that the incidence of emersion apparently decreased with flooding tides (we observed tides rise as much as 5 cm in 1.5 h during this period). We completed additional collections at Man 'O War (35 hermaphrodites, 7 males) and Peter Douglas Cays (70 hermaphrodites, 4 males). Another trip to the same mangrove site on Middle Cay, Glovers Reef was again unproductive for *R. marmoratus*.

January 24 to February 6, 2000

We spent two days collecting in the mangroves on the mainland south of Dangriga, Belize. Our goal was to determine the status of mainland populations with a particular focus on a search for male fish not previously reported from mainland sites. We had previously (1991) taken a single hermaphrodite from a site north of Dangriga where we also had found *Rivulus tenuis*, a typically freshwater form, in low-salinity habitats (10 ppt) in the mangroves in shallow pools and crab burrows. The 2000 Dangriga site was located east of the main coastal road, about 12 km south of the center of the city. The site was a heavily shaded, black mangrove forest with numerous burrows and flooded swales and depressions. Salinity was 10 ppt and the water temperature was 24°C and was heavily tannin- stained. These conditions and numerous late-stage salt marsh mosquito larvae present in the pools indicated recent heavy rainfall. We observed frogs throughout the area. Both *R. marmoratus* and *R. tenuis* were collected from crab burrows and pools. We frequently collected both species within the same burrow. A total of 46 *R. marmoratus* was collected and all were hermaphrodites along with 13 *R. tenuis*. We noted that the Dangriga *R. marmoratus* seemed larger than those typically found on the Cays so we compared the SL of this population with those collected at PG during the subsequent week. In fact, the Dangriga population was significantly larger (mean SL= 27.9 mm) than PG (mean SL= 22.9 mm) (*T* test: $p < 0.0001$). The Dangriga fish were also very robust and several oviposited viable embryos in collection bags.

Conditions on the Cays during this collection period were markedly different than on the mainland with low tides and no recent rainfall apparent. Salinities ranged from 33-41 ppt and water temperature in pools and burrows ranged from 23-26°C during the sampling event. A high incidence of "sulfured" *R. marmoratus* was once again observed and emersion was common. We also found a number of *Ucides* dead within their burrows, a possible result of hydrogen-sulfide toxicity. As in previous years with low water, we found large numbers of very large *R. marmoratus* (both hermaphrodites and males) along the shoreline areas concentrated in crab burrows associated with stumps. At one such location, we collected 37 hermaphrodites and eight males. The largest hermaphrodite we collected in these habitats was 51 mm SL, one of the largest fish we have collected in the wild. In spite of the preponderance of large hermaphrodites taken, none of these oviposited in collection bags as did Dangriga fish. The concentration of *R. marmoratus* in these intertidal areas led to one of the higher CPUE we have experienced: for 88 trap-nights we collected 87 fish with a CPUE= 0.99.

In 2000, we also collected on Peter Douglas Cay, Cat Cay and Little Lagoon Cay. On Douglas, we relocated the pool where the remarkable occurrence of *R. marmoratus* within a log was found in 1991. The pool was completely dry in 2000 but 13 fish were taken from inside a 5 cm dia log. *Rivulus marmoratus* were also taken on Cat and Little Lagoon Cays (n=4 and 8, respectively).

October 23 to November 5, 2003

Rivulus marmoratus was an incidental part of the focus of this particular trip which focused on a broad investigation of fishes utilizing the mangroves of the Cays. At PG we found further extensive mangrove clearing both north and south, extending several hundred meters south and nearly 100 m north. Crab burrows were completely absent from much of the cleared area and extensive erosion of surface sediments had occurred. Our visit this year was unique in that we encountered the highest water ever in the Cays due to spring tides. We made only a limited trapping effort not specifically targeting *R. marmoratus* in the course of our collections, setting cup traps on the marsh surface only, not in crab burrows. Nevertheless, we did collect 31 *R. marmoratus*. Due to the high tidal inundation, salinities and temperatures were both moderate with salinity very close to 35 ppt and temperatures from 28-30° C. We were able to visit the Peter Douglas site again where the pool site was flooded 12-15 cm deep. Although we did not attempt collection of *R. marmoratus* we observed the following fishes within the pool: *Lutjanus apodus, Sphyraena barracuda*, *Poecilia orri* and *Mugil* sp.

DISCUSSION

We have documented that *R. marmoratus* is abundant within the mangal of the Belize Cays and can be collected in appropriate habitats during different seasons and tidal inundation stages. Probably in no other western Atlantic location where we have collected were we able to collect the species in such great numbers and with such consistency. The only exception appears to be at Glovers Reef where we were unable to find the fish. The limited mangrove habitat there may have prevented establishment of the species. There is no question that Glovers should be included within the range of *R. marmoratus* as it has successfully colonized islands, albeit larger islands, more remote that Glovers.

During our trap sampling efforts in Belize we have set hundreds of traps of various types (Table 1) and have experimented with traps constructed of various available materials, including a variety of flotsam (plastic, bamboo) found in the mangroves. None of our traps have been "baited", and we conclude that *R. marmoratus* enters traps set upon the marsh surface (pools, flooded habitats) because they offer "cover". Since we attempt to shield our traps with leaves, branches or sometimes sheets of black plastic, they clearly offer a refuge for the fish. We have observed that fish in flooded habitats dart quickly from one sheltered location (logs, debris) to another and they are rarely exposed for more than an instant. Our practice of setting traps that provide cover, mimic natural shelters, or are set adjacent to existing shelters has proven productive. We also suspect that any single fish entering a trap becomes an enticement for other fish to enter, although in aquaria the fish are normally aggressive toward each other.

Although we did collect many specimens in flooded habitats, the focus of *R. marmoratus* habitat, especially during low-water conditions, is the burrow of the mangrove land crab, *Ucides*. As we have previously described, *R. marmoratus* are orced periodically to the air-water interface in order to respire effectively in the low dissolved-oxygen environment of burrow water (Taylor, 1990). Therefore, when cup traps are properly set bisecting this interface, fish are easily collected within a short period. Other collection techniques, such as dip net and hook and line (Table 1), were of limited importance in our overall collection efforts and were more useful as quick survey tools when assessing crab burrows at a new site. We have observed that Belizean *R. marmoratus* are less likely to be taken consistently by hook-and-line than they are in Florida where fish are more inclined to aggressively attack the worm bait. It would be interesting to determine if this behavior is the result of more limited food resources in the Florida burrow habitat.

Interpretation of water-quality data did not reveal any significant trends. Unsurprisingly, we note that salinities were consistently higher during low-water/ drought conditions, for example, when we encountered a salinity of 65 ppt and were still collecting viable *R. marmoratus*. Salinities this high easily become reduced to nearly 0 ppt after heavy downpours which are common on the Cays.

We frequently observed emersion of *R. marmoratus* in the Belize Cays, especially common during drought or low-water conditions. We observed two patterns of emersion in Belize: 1) when fish were under debris, logs, etc. or inside galleried logs, and 2) emersion onto the sides or near the mouths of crab burrows. We suspect that in case 1, emersion is longer-term, that is, when temporary pools have dried or water-quality conditions within crab burrows are unacceptable. *Rivulus marmoratus* would then often be concentrated in large numbers and frequently be clumped together. We speculate that this may be a moisture-conserving behavior. This aggregating behavior is in strong contrast to behavior in aquatic habitats where the fish are normally quite aggressive to one another. Some of the largest collections were made during 1991 when many fish were emersed under shoreline debris. When emersed fish were exposed by removal of debris they typically would 'flip' away so collectors had to move quickly but if successful, literally handfuls of fish could be collected. Emersion in case 2 is shorter-term, with the fish often flipping in and out of the water several times within an hour. We have noted that incidence of emersion inside burrows seemed to increase at night (we made several night-time observations), but is reduced with the onset of a flooding tide, day or night. The influence of H_2S on emersion has been well-documented (Abel et al., 1987) but the dynamics of H_2S within crab burrow water or within temporary pools is unknown and an interesting avenue for future research. In addition, there may well be other physiochemical factors that elicit emersion in *R. marmoratus* in the wild (e.g. ammonia).

We have noted consistent clearing of mangroves originating from PG over the years. While our field sampling frequency does not allow specific tracking of the effect of clearing on *R. marmoratus* populations, we suspect that, in the long-term, removal of mangroves is detrimental to the fish. One piece of evidence may be the comparative set of trapping observed in 1989 indicating higher catches in the recently cleared area and perhaps making the fish more vulnerable to capture. We have noted significant erosion of peat substrate (up to several cm) has occurred within a few years after clear-cutting with little or no regrowth of vegetation of any type. Significant reduction from a few to no *Ucides* burrows was found in clear-cut areas after one or two years. Loss of burrows and substrate, together with the lack of any other vegetative cover probably renders these areas unsuitable for sustaining *R. marmoratus* populations.

We have documented carefully the number of phenotypic males collected on Twin Cays during each visit. Although sexing hermaphrodites is relatively easy (the presence of a dark ocellus on the caudal fin representing the distinguishing feature), it is sometimes difficult to determine if smaller presumptive males are in fact male or immature hermaphrodites. In most cases, however, the distinctive reddish-orange tint of males is clearly visible along with the lack of an ocellus, and larger males are spectacularly colored with the distinctive addition of black edges to the caudal and anal fins. The percentage of males varied from 4.7%-29.7% (Table 2) and these ratios are significantly different across years (Chi-square= 62.64; df=5; $P<0.001$). There appears to be a decreasing trend in number of males from 1988 to 1991, but by 2003 the ratio had increased again (although a small sample size limits assessment). It is unfortunate that the eight-year gap in collections from 1992 to 2000 prevents accurate interpretation of trends. The presence of relatively large numbers of males remains the most interesting aspect of our collections in Belize and, hopefully, monitoring of this aspect will continue along with continued genetic work assessing heterozygosity of Belize Cays populations.

Our collection onshore in Dangriga was motivated by a search for male fish but none were found. While we have documented that males are found along a wide span of the central barrier reef (30 km-from the Pelican Cays to Tobacco Range, Fig. 1), it is curious that they were not observed on the mainland at Dangriga (a distance of about 18 km from the Cays). Other factors contrast these two collection sites: lower salinities in this coastal area (subject to more frequent heavy rainfall and allochthonous run-off) than those encountered on the Cays, at least based on our single collection, and the presence of the sympatric congener *R. tenuis*. Our collection of a few specimens of this species in this oligohaline habitat within crab burrows was previously reported (Davis et al., 1990) but the 2000 collection effort was much more extensive. Other than *Rivulus caudomarginatus* (a sexually dioecious species, as is *R. tenuis*) in coastal areas of Brazil (Huber 1992), we know of no other *Rivulus* species that is sympatric with *R. marmoratus.*

There may be as yet undescribed interesting behavioral interactions between these two species at the Dangriga site. Finally, the contrast in size between collections of *R. marmoratus* in Dangriga and Twin Cays is interesting and a long-term study of the Dangriga site would be needed to determine if this is a consistent feature and to identify the ecological factors influencing it.

The continued presence and high percentage of male *R. marmoratus* found on the Belize Cays remains a subject of intense interest. Turner et al. (1992a) and Lubinski et al. (1995) had initially hypothesized that there was probably an ecophenotypic factor inducing male production on the Belize Cays. However, we have yet to identify any environmental factor here which differs from those in other sites where males are not found. In addition, there is no reason to think that Belizean *R. marmoratus* are intrinsically different from other populations. Selfing has been defined as a derived character within the genus *Rivulus* (Huber, 1992) but Weibel et al. (1999) have determined through mtDNA analysis that Belize *R. marmoratus* do not differ from populations in Florida or other Caribbean locations that is, the Belize population is not ancestral to the others.

However, one of us (BJT) has recently demonstrated that "maleness" may in fact have a genetic basis on Twin Cays. In a "common garden" experiment he has demonstrated that fish from Twin Cays continue to produce a high percentage of males over multiple generations, as compared with lines from Dangriga which produced only the typical low percentage of males commonly seen in laboratory colonies (Turner et al., in prep.). These data add a new element to investigations of this interesting phenomenon.

The past two decades have seen a great body of information added to our knowledge of *R. marmoratus,* and a 16-year span perhaps seems like a long period of time to devote to field research on a single organism in a single locale. But, as the results indicate, observations based upon long-term sample sets lead to more profound understandings. We continue to encounter new phenomena that raise challenging questions. We find the Belize Cays have provided an ideal setting to continue this pursuit.

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