

*Rivulus is an oddball fish, living in a most unusual abode with habits like a salamander and a kinky sex life that still has scientists puzzled!*

## **Mangroves, Mud and Miracles**

Counting mosquitoes is never easy, especially when they are biting you, but that's what I get paid for. Nice work, if you can get it, and it's just part of a summer day in the life of a mosquito biologist in east central Florida. So, there I was, knee deep in the mud of a mangrove swamp- the heat, the thick, tangled vegetation, the rotten egg smell of hydrogen sulfide, and yes, the buzzing of hundreds of mosquitoes. I had given up trying to get a "biting count", and was disgusted that I had again missed the opportunity to discover where the mosquitoes had come from. I had stood in this very spot two weeks before, and there were none, but since then the tide had risen sharply, flooding the mangrove swamp and apparently hatching the myriad of saltmarsh mosquito eggs secreted in the mud. I had missed finding the larvae, a critical part of any control effort. Frustrated, I jammed my dipper, the long handled scoop which is the main tool of the mosquito larvae sleuth, into a small puddle. No larvae, of course, but four small fish darted frantically about in the dipper. Always interested in fish, I glanced at them and could not immediately identify them, so I placed them in a bucket.

Sometimes it is the smallest things that are life-changing: this simple act, placing the four little fish in a bucket, has led to over 10 years of research and insights into the remarkable life of a very unusual fish. It turns out that the fish were specimens of the mangrove rivulus, Rivulus marmoratus. Rivulus were very well known to ichthyologists, but very seldom collected- at this time, fewer than 50 had been taken in Florida, a state thoroughly sampled for fishes, and although known from Brazil to Florida, they were equally scarce elsewhere. The "well known" part was due to a very unusual sex life: this is the only vertebrate which is a selfing, functional hermaphrodite- they clone themselves. Adult fish have a complex reproductive organ known as an ovotestis, and internally self-fertilized eggs are laid which hatch into exact genetic duplicates of the parent. Oddly enough, a few pure male rivulus, which differ from the "herms" in having a

bright orange/red tint, had been caught in the wild, but their function was unknown- sexual reproduction had never been documented, but more on this later.

Needless to say, I was soon back in the swamp, peering at the small puddle where I had collected the fish. But no common puddle this- I immediately recognized it as a water-filled land crab burrow. With a quantum leap in collecting technology, I plunged a small net into the murky depths of the burrow, and withdrew it with 5 more rivulus. A few more dips, and I had a total of 13. I was floored to realize that I had just collected more rivulus in one location than anyone ever had previously.

### **MORE THAN A HOLE IN THE GROUND?**

After gently placing the fish back in the burrow, I left, wondering if this association was mere coincidence. The land crab (*Cardisoma guanhumii*) is a large blue crab found throughout the shorelines of the tropical Atlantic, but it is actually a terrestrial crab. It digs burrows in saltmarshes and mangroves and sometime inland for some distance. The burrows are dug to the depth of groundwater, typically about 2-3 ft. deep, and the crab uses the pool of water to moisten those gills.

I began to suspect that the “scarcity” of rivulus had more to do with “looking in all the wrong places”, and I was right. I checked crab holes far and wide, in every saltmarsh and mangrove swamp to which my job took me. In the ensuing years, I have collected a few thousand rivulus, and not just in Florida: with the help of colleagues William P. Davis and Bruce J. Turner, I have found them in Belize (where the landlord is a different crab- the mangrove land crab *Ucides cordatus*), and also in Honduras and the Bahamas. The relationship seems well established.

A better mousetrap usually follows any prototype. My first blind stabs with the net were a fluke, I discovered. Most burrows were too deep or twisted to sample so easily. A solution was slow to come, but when it did, it was a revelation derived from boyhood memories. As a child in Florida, I had spent countless hours catching the native minnows in ditches and canals by using a stick with a thin fishing line and a small hook baited with an earthworm. My eager

quarry would grab the ends of the worm, to be lifted from the water into my waiting hand. Somewhat embarrassed, and afraid of being seen by reputable scientists, I decided to try the technique, and it was instantly successful. I guess if I were living in a crab hole I would be half starved also. The rivulus eagerly nail the worm, but I am glad that most people have more sense than to wander into mangrove swamps. Picture this: a grown man, mud spattered, sweating and squatting in the mire, fishing in a crab hole. Not a pretty sight to the casual onlooker.

### **FISH OR AMPHIBIAN?**

Early in my burrow-peering days, I found that a quiet approach to a rivulus-containing burrow would reveal the fish out of the water, stuck to the side of the burrow, well above the water line. This behavior, known as emersion, is also found in other members of the genus Rivulus, and apparently it offers the fish a means of avoiding poor quality water, specifically high levels of hydrogen sulfide, a common toxic component in mangrove marshes. During these aerial jaunts, the fish becomes quiescent and respire through an extensive capillary network in the skin. The adaptive value of emersion was dramatically revealed to me once, when I came upon a crab burrow in which about two-hundred small minnows had been stranded by receding tides. Not as hardy as rivulus, the minnows began to die and rot, and I observed several rivulus emersed above the rafts of floating corpses, waiting for conditions to improve. A week later, when all trace of the dead fish was gone, only live rivulus were left in the burrow.

In the mangroves of Belize, my colleagues and I sometimes found rivulus in temporary pools in the mangrove swamp, and we assumed that they would retreat to adjacent crab burrows when the pools dried. It turns out that there were other housing options too. In the tropics, termites attack dead tree limbs and excavate tunnels and galleries inside. We found a pool that had recently dried, around which lay a number of dead mangrove branches. When we broke open the rotten branches, we discovered that dozens of rivulus were inside, filling the galleries like so many hibernating salamanders. This damp, secretive habitat allowed them the opportunity to survive until the next flooding event. Oddly enough, in aquaria rivulus are highly aggressive to each other, yet in the confines of the branches, they were packed as tightly as peanuts in a

can.. not the place to be aggressive, apparently.

I investigated emersion further by building a tall glass aquarium, filling it with the gooiest saltmarsh mud I could find and then digging two “burrows” by hand next to the glass walls, the “ant farm” effect for rivulus. I planted some vegetation, scattered some mangrove leaves about and placed two fish in the tank. Their antics amused me for over a year, and I discovered that they would readily travel ‘overland’ to reach a desired burrow, and could even figure out how to cross through a plexiglass barrier with a small hole drilled in it. They seemed to be equally at home on land as in the water.

### **THE BIRDS AND THE BEES**

My cross-sectional tank also offered another insight. I had puzzled for a long time over where the fish laid their eggs, and there was no information on this in the scientific literature. In spite of extensive searching in the field, I had never found eggs, but my two captive fish soon began laying eggs, not an unusual occurrence for the fish in captivity, but not in the water. The eggs were all deposited on the mud, most often in the corner of the tank furthest from the burrows, and usually at night. I was equally intrigued to find that the eggs would develop fully on the mud surface, and then would hatch when sprinkled with water. The young fish would wiggle under a leaf after hatching, but the few that reached the burrows were promptly eaten by the adults. These events inspired further egg-searching in the field, and I have now found a few, out of the water of course, but the reproductive behavior of the fish still remains largely a mystery.

Speaking of sex, I have already alluded to the mysterious presence of male fish in an hermaphroditic species. Why would nature find this necessary? We know that male fish regularly show up in laboratory colonies of rivulus, and rearing the developing eggs at lower temperatures produces a high percentage of males, but low temperatures are not a common phenomenon in the tropics. So my colleagues and I were shocked in our initial collections in Belize to discover that fully 25% of the population was male, and this high ratio has now persisted for over a decade. A few stray males have shown up in Florida, and a couple from the

Bahamas, but something strange was going on in Belize, because here the fish is reproducing sexually. Apparently, in the presence of these flashy males, the herms somehow suppress internal self-fertilization (a fascinating physiological problem in itself!) and lay unfertilized eggs, which are then fertilized externally by the males. Of course, in the murky confines of a crab burrow (not the most romantic of settings), this has never been observed, but my colleague Bruce J. Turner has determined that this is taking place by looking at DNA fingerprints of Belize fish. When a wild Belize fish produces self-fertilized eggs in captivity, the offspring are not genetically identical. At some point in prior generations, sexual reproduction occurred. Cloning has taken a back seat here. Why?? We don't know.

As they say, much remains to be done, but I thank my lucky stars that I did not discard that dipper full of muddy water on that fateful, mosquito-ridden day 13 years ago. I still stalk the mangroves and look for other miracles. And there are others out there, to be sure.