SYMPOSIUM

Twenty-Four Years in the Mud: What Have We Learned About the Natural History and Ecology of the Mangrove Rivulus, *Kryptolebias marmoratus*?

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Synopsis Although first described in 1880, *Kryptolebias marmoratus* avoided scientific scrutiny until 1961, when it was identified as the only known selfing hermaphroditic vertebrate. The subsequent intense interest in this fish as a laboratory animal, continuing to this day, might explain the paucity of wild collections, but our collective knowledge now suggests that the inherent difficulty of wild collection is more a matter of “looking in all the wrong places.” Long thought to be rare in the mangroves, and it is rare in certain human-impacted habitats, *K. marmoratus* can be quite abundant, but in microhabitats not typically targeted by ichthyologists: ephemeral pools at higher elevations in the swamp, crab burrows, and other fossorial or even terrestrial haunts. Field studies of this enigmatic fish have revealed almost amphibious behaviors. During emersion these fish tolerate extended dry periods. In water, they are exposed to temperature extremes, high levels of hydrogen sulfide, and depleted dissolved oxygen. Finally, their catholic diet and a geographically variable sex life completes a portrait of an unusual animal. A clearer picture is emerging of adult life, with initial population density estimates now known and some indication of high population turnover in burrows, but juvenile habitat and adult oviposition sites remain unknown.

Range and nomenclature

The neotropical cyprinodontid fish, mangrove rivulus (*Kryptolebias marmoratus*) has an astounding geographical range, in fact the widest distribution of any inshore-dwelling coastal fish species in all of North, Central, and South America—about 52° of latitude and 91° of longitude (assuming that *K. marmoratus* and *K. ocellatus* are synonymized as per some authors). This basically encompasses the entire tropical and subtropical eastern Atlantic basin (Huber 1992; Taylor 2000).

Originally described from Cuba (Poey 1880), the species has since been slowly documented as far north as central Florida, USA, north through the Yucatan Peninsula and Bay of Campeche in Mexico, along almost the entire Central American and northern South American coastline, and likely south to the mouth of the Amazon River. However, records east and south of Venezuela are sparse (Huber 1992).

The species designation on the South American continent remains confused. The International Commission on Zoological Nomenclature (ICZN, Case no. 2722, 1992) gave precedence to *Rivulus marmoratus* and relegated *Rivulus ocellatus* to the former’s synonymy. However, recent molecular evidence supports separation of the two species (Tatarenkov et al. 2009, 2010), and suggests that the “*ocellatus*” lineage is found as far north as Panama and southern Cuba. Biogeography theory might suggest that *K. ocellatus* is found from southern Brazil north to the mouth of the Amazon River, while *K. marmoratus* is found further north. A third taxon, *K. marmoratus bonairensis*, the “subspecies” from the Netherlands Antilles is now generally disregarded, and recategorization of the Brazilian species continues (Costa 2011). However, more extensive collections subjected to morphological/meristic and molecular analyses are required over the entire South American coastline before the nomenclatural
issues can be resolved. Recent collections just south of the Amazon River do confirm the presence of a Kryptolebias sp. (M. Barletta, personal communication).

North of the confusing South American picture, K. marmoratus is presumably found throughout all of the Antilles and Bahamas, including the island of Barbados, far to “windward.” This remarkable and widespread distribution is clearly a tribute to “waif” dispersal and the evolutionary advantage of the ability to find a population from a single progenitor. The ultimate proof of the latter supposition would be documentation of the presence of the fish on the island of Bermuda, the most distant extension of the tropical Atlantic and the neotropical mangrove ecosystem. However, the fish has yet to be recorded there, and in 2007 a brief survey within the very limited mangrove habitat did not produce this fish (D. S. Taylor, unpublished data).

In spite of its extensive distribution, K. marmoratus has been frequently described as “rare”: “It is undeniable that (Rivulus) marmoratus is rare and little known in the wild . . . ,” by Harrington and Kallman (1968). Serious students of K. marmoratus in the field now recognize that this is overstated, but even so the state of Florida has listed K. marmoratus as a “Species of Special Concern.” Kryptolebias marmoratus is also a Federal “Candidate Species” for listing by the NMFS (National Marine Fisheries Service) (Fed. Reg. 62:(134) 7/14/97), but there is no current Federal listing, nor does any other nation list the species. The current listing for Florida is being reevaluated, but it has been pointed out that in at least some portions of the State, the species has been adversely impacted by development and by hydrologic alteration of tidal wetlands (Taylor 1999).

**Habitats**

As the common name “mangrove rivulus” implies, K. marmoratus is affiliated closely with and spends its entire life cycle within mangroves, and the distribution of the fish tracks closely with that of the red mangrove, Rhizophora mangle. Only one other fish is a true mangrove-affiliate, the poeciliid Gambusia rhizophorae (Davis et al. 1995). In fact, stable isotope data for K. marmoratus from Everglades, Florida, and Twin Cayes, Belize indicated a “strong” mangrove signal (C. C. McIvor, unpublished data).

Part of the presumed rarity of K. marmoratus is due to looking in all the “wrong” places. Conventionally minded ichthyologists are at a disadvantage, and the fish must be sought within a suite of micro-habitats within the mangroves. The list is rather incongruous: intermittently dry shallow, stagnant pools; crab burrows (specifically, those of Cardisoma guanhumi, great land crab, and Ucides cordatus, mangrove land crab); temporally flooded swales; ditches (including anti-mosquito ditches); inside/under logs and mangrove leaf litter; solution holes; empty beer cans and coconuts; and even several meters inside anchialine cave systems (Taylor 2000; Taylor et al. 2003, 2008). A common denominator among these micro-habitats includes their being either fossorial, intermittently dry, or having adverse water quality conditions that preclude the establishment of other species of fish. Perhaps Harrington and Rivas (1958) have aptly summarized the conditions favored by K. marmoratus in their description of some collections from anti-mosquito ditches in east central Florida: “water . . . turbid to opacity, and the ditch bottoms were mostly treacherous, malodorous, marly muck . . . .” Such a description might not inspire most ichthyologists, and this passage alone may account for the paucity of K. marmoratus in collections.

The specific identification of crab burrows (C. guanhumi, U. cordatus) as a focus of K. marmoratus habitation has expanded the extent of collections, both geographically and numerically, thereby revising the impression of the fish’s rarity (Davis et al. 1990; Taylor 2000). The “fossorial” nature of K. marmoratus figures prominently in its natural history, and we may be underestimating the use of smaller burrowing crabs (e.g., Uca spp.), which are as yet impossible to sample. These may serve as refuges during low water. Kryptolebias marmoratus have frequently been observed entering Uca spp. burrows when dropped during field collection or during very low water (D. S. Taylor, unpublished data). In addition, McIvor and Silverman (2010) and Richards et al. (2011) collected large numbers of K. marmoratus in the intertidal mangroves during tidal flooding at both Shark River, Everglades, FL and Tampa Bay, FL, where the only refuges during low water appear to be smaller crab burrows (Uca spp. and mud crabs [Eurytium limosum]). Larger burrowing crabs (C. guanhumi and U. cordatus) are not found in any significant numbers from Florida Bay up the western coast of Florida (personal observation). Similarly, at the northern extent of the fish’s range on the eastern coast of Florida (Volusia County), the only fossorial habitats available at low water are
burrows of *Uca* and *Eurytium* and rotten logs. However, *K. marmoratus* at their northern range on this coast has presented other puzzles, including an apparent “outlier” population that is disjunct from those to the south by 105 km (Taylor 2000). In addition, the most recent collection from this region (2009) was obtained from a benthic sample by scraping the fouling growth on the pontoons of a floating restaurant (C. Macfie, personal communication).

**Environmental physical parameters**

**Temperature**

*Kryptolebias marmoratus* is perhaps more tolerant of temperature extremes than are some other tropical species and it has been collected over a wide thermal range (7–38°C) (Taylor et al. 1995); there is evidence that the fish can survive 5°C when emersed (out of the water) (Taylor 1999). Extreme cold has resulted in documented cold mortality at the northern extreme of the fish’s geographic range (central Florida), a hazard common to all tropical fish species found at the limits of their ranges (Taylor 1993).

Falling temperature seems to induce emersion in a laboratory setting (Huehner et al. 1985), but the adaptive value of this is unclear, and no comparable study looking at the effects of increasing temperature has been carried out. Clearly, fossorial niches (e.g., crab burrows) offer a thermal refuge during cold events. During a cold event in Florida, water temperature inside *Cardisoma* burrows remained at 16°C, while nearby shallow pools were 10°C, a temperature fatal to many tropical fishes (Taylor, 1992; Gilmore et al. 1978). However, if these types of fossorial refuges are not available, and, in fact emersed fish are more resistant to low temperature, the laboratory observations of increase in emersion with falling temperature (Huehner et al. 1985) may indicate that such emersion has survival value.

Low temperature is probably the primary factor limiting northern distribution at the extremes of range in Florida, but ranges do not appear to be equivalent on both coasts. A search for *K. marmoratus* on the western coast of Florida (offshore islands of the Cedar Key area) was completed at the equivalent latitude (29° 05’) of the known northern limit on the eastern coast. No specimens were found at Cedar Key, although the habitat appeared ideal, and this may reflect lower temperature regimes on the western coast of Florida (D. S. Taylor, C. C. McIvor, and W. P. Davis, unpublished data).

**Salinity**

*Kryptolebias marmoratus* is euryhaline and has been collected in the wild at salinities of 0–70 ppt (Kristensen 1970; Taylor et al. 1995), and in the laboratory juveniles can tolerate 70–80 ppt (Taylor 2000). While it is noted that *K. marmoratus* can be reared and will reproduce in fresh water (Huber 1992; Lin and Dunson 1995), collections at very low salinities in the wild probably reflect temporary conditions following heavy rainfall (unpublished data). In natural habitats where there is a gradient from pure fresh water to saline mangroves (e.g., Everglades, Florida), *K. marmoratus* were not found except in areas with salinity >10 ppt (D. S. Taylor and W. P. Davis, unpublished data). Taylor (1999) reported that attempts to introduce *K. marmoratus* into isolated freshwater pools in the field were unsuccessful, except in one instance where the site intermittently dried and no species of fish were present. The abundance of freshwater invertebrate predators may also prevent recruitment of juvenile *K. marmoratus* (D. S. Taylor, unpublished data).

In contrast, extreme salinities are a common feature of mangroves during drought or periods of reduced tidal inundation. Adaptation to hypersaline conditions in teleosts is partially contingent upon activity of chloride cells. King et al. (1989) found that the chloride cells in the opercular epithelium and opercular skin increased in size (up to 39–100% larger) in *K. marmoratus* raised at 100 and 200% seawater versus fish raised at 1% seawater. The overall number of chloride cells, however, did not increase at elevated salinities. This study also reported that in laboratory “transfer” studies, *K. marmoratus* could adapt to 114 ppt seawater.

**Hydrogen sulfide**

Hydrogen sulfide (H$_2$S) is widespread in marine environments and it is well-known that it is toxic to fish (Bagarinao and Vetter 1989); it is common in surface and porewaters of mangrove systems. Anyone venturing into the mangroves is familiar with the characteristic “rotten egg” smell. In addition to direct toxicity, H$_2$S quickly scavenges any available dissolved oxygen, presenting a double challenge to aquatic life. Hydrogen sulfide is likely a key driver of emersion behavior (see below) in *K. marmoratus*, but there are few field studies on the concentrations of H$_2$S in *K. marmoratus* microhabitats. Abel et al. (1987) measured H$_2$S at 0.4–0.7 ppm in a
one-time survey of inundated mangrove pools that contained *K. marmoratus* in southwestern Florida. These data may actually represent more “benign” conditions, as Carlson et al. (1983) found maximum H$_2$S levels of 51.1 ppm in pore water on Floridian conditions, as Carlson et al. (1983) found maximum H$_2$S levels of 51.1 ppm in pore water on Floridian mangrove islands. Rey et al. (1992) measured H$_2$S in pore water as high as 1.64 ppm in mangrove/salt-marsh wetlands in east central Florida. In a very limited study of H$_2$S within *U. cordatus* burrows in Belize, concentrations varied from 0.59 to 10.59 ppm. Concentrations did not vary significantly between surface water in the burrow and water at 8 cm depth, but when incoming tides flooded the burrows, H$_2$S levels dropped quickly. In general, burrows further from the oceanic open water exhibited higher levels of H$_2$S (D. S. Taylor, unpublished data). While the levels of H$_2$S observed in Belize are likely not sufficient to be fatal to *K. marmoratus* (tolerant estuarine species had a 96 h LC$_{50}$ of 17.8–23.8 ppm [Bagarinao and Vetter 1989]), avoiding elevated levels may be a constant challenge for *K. marmoratus*.

During low-water periods, *K. marmoratus* within crab burrows and stagnant pools frequently develop a white coating on the skin and fins (Taylor 2000; Taylor et al. 2004). If specimens displaying these features are captured and placed in fresh seawater, the coating disappears and attempts to scrape the material off the fish and preserve it have been unsuccessful (D. S. Taylor, unpublished data). It was previously suggested (Davis et al. 1990) that this feature was mucus produced in response to adverse water quality, but it seems more likely that it is the actual growth of a sulfur-oxidizing bacterium (*Beggiatoa* sp.). This bacterium is frequently seen coating the surfaces of water or substrate in high sulfide mangrove settings and has also been seen growing on the epidermis of poeciliid fishes living in H$_2$S enriched artesian flows from wells in Florida (D. S. Taylor, personal observation).

**Ammonia**

Frick and Wright (2002a) investigated ambient ammonia levels in Belizean *U. cordatus* burrows known to contain *K. marmoratus*. They found that ammonia averaged about 1 mmol/L, a level greater than that typically found in most other aquatic habitats, fresh or saline. Since they determined that *K. marmoratus* can tolerate levels >10 mmol/L, the observed levels would not appear to significantly stress the fish but, as with H$_2$S, lack of tidal/rainfall flushing might allow levels to elevate further. The pH of the water in burrows was also measured in their study, and found to be lower than in the adjacent open ocean (pH 7 versus pH 8). However, these lower pH values may mitigate potential toxic consequences of ammonia. The lower pH observed in burrows may be the result of H$_2$S production or result from the metabolic activities of resident crab(s) and/or fish (Frick and Wright 2002b). Further complicating the chemical “soup” found within crab burrows is the reduced tolerance of fish to ammonia at lower salinities (Frick and Wright 2002b), and, as noted, salinities can drop drastically following heavy rainfall. An extensive array of physiological adaptations is clearly required for *K. marmoratus* to thrive in the mangroves.

**Dissolved oxygen**

Other than H$_2$S, another ubiquitous feature in mangroves is the common occurrence of low dissolved oxygen (DO). *Kryptolebias marmoratus*, as noted, have frequently been collected under conditions of very poor water quality, and have been documented to survive hypoxic conditions (<1.0 ppm) in the field (Dunson and Dunson 1999). While, conditions this extreme may be uncommon and usually occur at night, a series of 149 DO readings in flooded *K. marmoratus* habitat in Belize over several days on two different years found a mean DO of 2.39 ppm (D. S. Taylor, C. C. McIvor, E. Reyier, and W. P. Davis, unpublished data). However, extremes of low DO can occur in isolated mangrove pools in Belize, where a 1-day survey of several pools containing *K. marmoratus* at very low-water found DO ranging from 0.16 to 0.98 (D. S. Taylor, P. Molloy, and M. Fish, unpublished data). Finally, DO was measured across a suite of six different mangrove habitats (as described by Feller et al. [2002]): fringe, transition, dwarf red, creek, pond, and sinkhole. These data indicate that the sinkhole habitats (where *K. marmoratus* are regularly found) had the lowest DO, followed by the transition zone (the higher elevations where *K. marmoratus* would typically be found in burrows and pools). This parameter partially explains the paucity of other fish species in the internal mangroves. Finally, it has only been recently determined that hypoxia (0.2 mg/L) is known to induce emersion in the laboratory (Regan et al. 2011).

Survival in environments with low DO may require fish to be physiologically adapted for surface aerial respiration (SAR), in which the oxygen-enriched
surface layer of the water is utilized in respiration. *Kryptolebias marmoratus*, with its flattened head, upturned mouth and small size (Lewis 1970) exhibits the physical characteristics allowing SAR. In addition to these features, an enhanced capillary network is found in the dorsum of the epidermis, within 1 μm of the surface. This feature is not found on the ventral surfaces (Grizzle and Thyigarajah 1987). These capillaries are most dense on the nape, decreasing posteriorly until becoming absent past the dorsal fin. All ages/sizes of fish illustrated this same pattern (Grizzle and Thyigarajah 1987). Partial emersion, whereby *K. marmoratus* place themselves at the water’s edge/surface, typically lodged against/under debris, is frequently seen in the field, as well as short-term bouts of SAR while actively swimming and skimming the surface (personal observation). The anatomical features described, therefore, are clearly adapted for these behaviors. The importance for this species of contact with the air/water interface is emphasized by repeated observations of fish “drowning” if retained in traps beneath the water, often only for very short periods. Such fish display classic symptoms of anoxia, with flared, pale gills, but the influence of H₂S in these mortalities is unknown (Davis et al. 1990; D. S. Taylor, personal observation).

**Diet**

There are limited studies of the diet of *K. marmoratus* in the wild, but this fish is clearly a predator, with various terrestrial and aquatic invertebrates forming the bulk of the diet. Harrington and Rivas (1958) provided the first insights into its diet, although from the guts of only two specimens from mosquito ditches, and found mosquito larvae, a snail, a crab, and an adult stylops (an insect parasite). Kristensen (1970) supplemented this information, reporting that *K. marmoratus* from the Netherlands Antilles consumed gastropods, various crustaceans, dipterans, formicids, and juvenile fishes. Huehner et al. (1985) collected emersed fish in the Florida Keys and analyzed the guts of 21 specimens; they found mosquito larvae, fish parts, polychaetes, copepods, and miscellaneous insects. Both Kristensen (1970) and Taylor (1988) indicated that *K. marmoratus* is cannibalistic. The most comprehensive study of diet to date, 111 specimens collected from the burrows of *C. guanhumi* in east-central Florida, found that 60% of guts were empty (Taylor 1992). This relatively low frequency of feeding may indicate a paucity of food resources in the marginal, intermittently dry microhabitats where the fish is found. Periods of flooding by rainfall or tidal inundation could provide enhanced feeding opportunities and also allow *K. marmoratus* priority feeding, as other fish species would be moving from sources of permanent water, for example estuarine water itself or permanent ponds/creeks within the mangroves (Taylor 2000). Of the guts containing food items, 41% contained items of terrestrial origin (dominated by unidentified insect parts) and 92% contained items of aquatic origin (mostly polychaete parts and gastropods). It appears likely that the latter dietary items originated within the crab burrows themselves, as during the study period the 111 specimens were likely confined to the burrows, due to lack of tidal/rainfall flooding (Taylor 1992). However, since the infauna of crab burrows is largely unstudied, the exact origin of these food items cannot be determined. In addition, although it is known that *K. marmoratus* will leave the water to capture food and return to the water to consume it (Huehner et al. 1985; Taylor 1990), the extent of this behavior is unknown. It is clear that to some degree crab burrows serve as pit-traps for insects, and some of the predation on insects by *K. marmoratus* has been observed at night (Taylor 1992). During flooding events it is likely that fish range some distance from their burrows for feeding, and several fish have been observed attacking a moth that had fallen onto the water’s surface (D. S. Taylor, personal observation).

The saltmarsh mosquitoes, *Aedes taeniorhynchus* and *Ae. sollicitans*, are known to oviposit in some of the microhabitats occupied by *K. marmoratus* (e.g., intermittently dry pools and mosquito ditches, as larvae are frequently observed here (D. S. Taylor, personal observation; Ritchie and Johnson 1986). It is therefore likely that mosquito larvae are a frequent dietary item. Both laboratory and field studies have confirmed heavy predation by *K. marmoratus* on saltmarsh mosquito larvae (Taylor et al. 1992). In the laboratory, *K. marmoratus* of 40 mm standard length (SL) consumed up to 80 larvae in 24 h, and even juvenile fish (1-day old) ate a maximum of 21 newly-hatched larvae (Taylor et al. 1992). These feeding rates meet or exceed those of well-known mosquito larvae (*Gambusia* spp.) (S. A. Ritchie and E. Johnson, unpublished data). Yet, natural populations of *K. marmoratus* never seem to reach the densities seen in larvivorous poeciliids (*Gambusia*), so
their direct contribution to mosquito “control” is perhaps limited.

**Emersion**

Emersion is well-known among *K. marmoratus* and some of its congeners (approximately 100 species; Huber 1992) and is a comprehensive term that includes both active movement out of the water (wiggle/flipping across a damp substrate) or the more torpid state achieved during more long-term emersion (Taylor 1990). Kristensen (1970) was the first to describe emersion in *K. marmoratus*, where he documented specimens from the Netherlands Antilles emersed within damp algal mats and also moving overland as follows: “When their habitat dries up they are able to find their way back to the water by jumping, crawling, and wiggling through wet pebbles and mud, covering distances of dozens of meters.”

As noted, one physical driver of emersion is excessive H₂S. Although data are limited, Abel et al. (1987) determined that in the laboratory 50% of *K. marmoratus* left the water at an H₂S concentration of 0.12 ppm, a level extraordinarily low compared to known field levels (D. S. Taylor, unpublished data). The duration of emergence in the laboratory was correlated with the concentration of H₂S; and at lower concentrations, fish tended to ventilate at the surface more but did not emerse. No effect of hypoxia on emergence was noted (Abel et al. 1987; Taylor 1990), although neither of these studies looked at the low levels (0.2 mg/L) now known to induce emersion (Regan et al. 2011).

Harrington and Rivas (1958) reported that *K. marmoratus* was the “last” species to be affected by rotenone, a toxicant targeting the gills, compared to 28 other fish species taken in “association” with it. This apparent resistance to rotenone is more likely the result of emersion as a means of avoiding it. Intra-specific aggression can also lead to emersion in both the field and laboratory (Taylor 1988, 1990), although neither of these studies looked at the low levels (0.2 mg/L) now known to induce emersion (Regan et al. 2011).

Huehner et al. (1985) were among the first to look at the details of emersion behavior in the field and laboratory. They were also the first to note an apparent knowledge of terrestrial landscapes by the fish. When recently emersed fish in the field were exposed by turning over logs, they would flip up to 0.5 m away with some accuracy, to disappear down crab burrows or burrow into leaf litter. Taylor (1990) also demonstrated this in a laboratory microcosm containing crab burrows; there fish were free to move overland between two burrows separated by 8 cm of mud. Fish appeared highly motivated to switch burrows, one fish doing so six times in 24 h. When a plexiglass barrier was erected in the mud between the two burrows, this movement ceased, but time-lapse videography showed repeated attempts to cross the barrier. Further, when a small hole was subsequently drilled in the barrier at the
surface of the mud, the fish found the opening and resumed movement. Some of the activity occurred at night (Taylor 1990).

In their limited laboratory study of the effects of temperature on emersion, Huenher et al. (1985) found that fish preferred being in water at 25°C, moving to land as temperatures dropped to 19–20°C, but even then fish would temporarily return to water. The authors speculated that emersion during dry-down prevents the concentration of K. marmoratus at the edge of the mangroves, where predation is greater. Re-flooding would then result in immediate resumption of aquatic activity, within the original occupied territory. Fish that emersed for >12 h were less responsive to mechanical stimulation, requiring prodding before movement. In many cases in the wild, emersion is clearly the result of drying of the habitat, often over an extensive period. Taylor (1990) found that K. marmoratus can survive at least 66 days out of the water in the laboratory. The study animals were emaciated (31.4% weight loss), but upon reflooding they immediately resumed feeding and normal activity.

Taylor et al. (2004) described two patterns of emersion in Belizean K. marmoratus: (1) under debris (including sheets of plastic and lumber), leaf litter, inside logs, and (2) onto the sides or mouths of crab burrows. The former cases are probably long-term and occur when temporary pools have dried or water quality within crab burrows has degraded. Emersion within a crab burrow was of shorter term, and fish often reentered the water several times within an hour. The incidence of this short-term emersion in burrows seemed to increase at night, when it is likely that water quality declines (D. S. Taylor, personal observation).

Multiple fish are known to emerge inside rotting mangrove logs on the forest floor, resulting in very high concentrations of animals (Taylor et al. 2004, 2008). This phenomenon (termed “log packing”) occurs inside logs that have been galleried by beetle larvae and termites, which subsequently fall into shallow pools in the mangroves. The galleries are 10–20 mm in diameter in many cases riddling the logs, and are full of a sawdust-like frass, the byproduct of insect activity. These logs remain spongy and water-saturated for a considerable period of time after dry-down (D. S. Taylor, unpublished data). How and when fish enter the logs is unknown, but it likely occurs as the pools near final dry-down. Some preliminary laboratory data show that fish will enter artificial “logs” in aquaria as water levels decline, but often waited until the log was out of the water, necessitating traveling across a “dry” substrate (D. Bechler, personal communication). However, in the wild, fish may traverse a considerable distance once inside the logs, with some individuals being found 20 cm from obvious entrance holes. The densities observed inside these logs are remarkable: 100 individuals inside a section 1.5 m long and 9 cm in diameter (Taylor et al. 2004). Individuals are closely packed together, exhibiting enhanced mucus production (D. S. Taylor, unpublished data), but become active immediately upon being exposed, either flipping out of the galleries or attempting to slither further into the interior (Taylor et al. 2008).

Cessation of intra-specific aggression when fish are emersed has been noted in this species, which can be quite aggressive in water (Taylor 1990; Huenher et al. 1985). There may also be as-yet undocumented physiological benefits to emersion in groups with close physical contact, for example reduced metabolic rates (Taylor et al. 2008). Laboratory rearing in “community” tanks (many individuals of a common lineage in the same tank) has also revealed differences in emersion behavior between “clones”, with some clones emersing more frequently and others emersing in compact groups of multiple individuals. This behavior is being further investigated (D. Bechler, personal communication). However, the clear survival value of emersion is very apparent in the field when other fish species (e.g., poeciliids) have been found dead in drying pools and crab burrows known to contain K. marmoratus, with K. marmoratus emersed above the floating corpses (Taylor 1990; Abel et al. 1987).

Predation

Few instances have been documented of predation on K. marmoratus. There are two records of predation by wading birds (Wood Stork, Mycteria americana and Tricolored Heron, Hydranassa tricolor) as determined from regurgitates (Taylor 1999). But overall, wading birds do not seem to frequent the small ponds and heavy mangrove cover where K. marmoratus is found (D. S. Taylor, personal observation), with the exception of smaller species like the green heron (Butorides virescens). Given the extremely secretive and fossorial nature of the fish, it is hard to envision intense avian predation (Taylor 1999). Kristensen (1970) proposed that predation by
other fish is probably minimal, as *K. marmoratus* is so secretive and cryptic. Estuarine piscine and crustacean predators (e.g., snapper, barracuda, and blue crab) do move into *K. marmoratus* habitat during periods of heavy tidal flooding (personal observation) and there may be incidental predation on *K. marmoratus* then. McIvor and Silverman (2010) documented several potential predators in association with *K. marmoratus* in Everglades, FL mangrove lift-net collections, including a few goby species, *Cichlasoma urophthalmus*, *Belenesox belizanus* (these two species were also documented in Belize mangroves [Taylor et al. 2007]), and *Strongylura notata*.

Three species of eleotrids, *Guavina guavina*, *Dormitator maculatus*, and *Eleotris pisonis* have been documented inhabiting crab burrows (Taylor 2001), and all three are known to prey upon other fishes (Teixeira 1994; Taylor 2001). However, the only direct evidence of predation on *K. marmoratus* is in one instance when *E. pisonis* was found to prey on them in pits of brackish water (Kristensen 1970). However, where *G. guavina* was present in large/deeper *U. cordatus* burrows in Honduras, which normally might have contained numerous *K. marmoratus*, none was found. These same *Guavina*, however, freely preyed upon *Gambusia* sp. presented on a baited hook (Taylor 2001).

A more significant predator may be the mangrove water snake, *Nerodia fasciata compressicauda*, mis-identified as the Atlantic saltmarsh snake (*N. f. taeniata*) by Taylor (1990), which is seen in crab burrows and mangroves in Florida and Belize. Predation on *K. marmoratus* by this species has been confirmed in Belize (D. S. Taylor, unpublished data).

**Parasitism**

*Kryptolebias marmoratus* has never been noted to display any of the “classic” teleost skin diseases (“Ich” or other white-spot diseases) in either laboratory or field populations (Taylor 1999). Rarely, in Floridian populations an unidentified fungal pathogen was seen on the body and fins (Taylor 1999), and a digenean trematode was seen in a Brazilian museum specimen (D. S. Taylor, unpublished data; E. H. Williams, personal communication). Whether the species is innately more resistant to external parasites than most other species, or the exposure to parasites is not as frequent in the marginal habitats that the fish occupies, remains to be seen. However, a recent study (Ellison et al. 2011) demonstrated that among Belizean *K. marmoratus* with varying rates of genetic heterozygosity, more heterozygous fish had fewer internal parasites than did homozygous fish. However, all fish were infected with one of three different parasites: bacterial cysts on the gills, *Trichodina* (a protozoan), or acanthocephalans.

**Competition**

As noted, other species of fish are not commonly sympatric with *K. marmoratus*, presumably due to the demanding physical regimes of their preferred microhabitats. Only a few other species (e.g., *Gambusia* spp., *Pecilia* spp., *Fundulus* spp., *Cyprinodon* spp., *Adenia xenica*, and *D. maculatus*) have been collected in any significant number in the intermittently flooded swales/potholes/ditches where *K. marmoratus* may be found. One exception to this was McIvor and Silverman (2010), where greater diversity was found in riverine forests along Shark River, Everglades. However, most, if not all of these fishes, leave with receding tides or do not survive periodic dry-down (Abel et al. 1987; Davis et al. 1990; Taylor 1990; D. S. Taylor, unpublished data). Conversely, *K. marmoratus* is rarely taken in mangrove habitats with permanent water. No studies, either field or laboratory, of competition between *K. marmoratus* and these sympatric species have been conducted, and the reason(s) that *K. marmoratus* is not more often found in permanent waters is unknown (Taylor 1999). Dunson and Dunson (1999) found a similar situation (relatively few sympatric species present) on the western coast of Florida, south of Tampa Bay. In their field collections of *K. marmoratus*, they noted that at the higher elevations *K. marmoratus* occupies, abiotic conditions are too extreme to allow other competitively superior small fishes (e.g., *Gambusia*) to survive. McIvor and Silverman (2010) studied *K. marmoratus* in the Shark River, Everglades National Park, FL and found 21 other species of fish species “associated” with it, but only six species (including *K. marmoratus*) made up 90% of their bottomless lift net catch and two species (*K. marmoratus* and a palaemonid shrimp) comprised 75% of the catch.

A congener, *Rivulus tenuis*, is commonly found sympatric with *K. marmoratus* in crab burrows/potholes on mainland Belize. *Rivulus tenuis* is a dioecious, freshwater form (Huber 1992), but was found in salinities up to 10 ppt (Taylor et al. 2004) in 2000
at Dangriga, Belize. The potential competitive interactions of these two species merits further attention. Up to four *R. tenuis* were found in a single crab burrow along with one *K. marmoratus*. At the same site, up to ten *K. marmoratus* were collected along with 13 *R. tenuis* (Taylor et al. 2004). However, near this same site in 2006, salinities were slightly higher (14–16 ppt) and 60 *K. marmoratus* were taken with no evidence of *R. tenuis* (D. S. Taylor, P. Molloy, and M. A. Fish, unpublished data).

**Population density/movement**

In some locales, *K. marmoratus* is no longer considered “rare,” with a single crab burrow having produced 26 individuals (Taylor 1990) and catches from other burrows and potholes sometimes in excess of ten individuals not uncommon (Taylor 1992; D. S. Taylor, unpublished data). In a preliminary survey of *K. marmoratus* on a Belizean offshore cay (Twin Cayes) transition mangroves (an area of predominately black mangroves [*Avicennia germinans*]) inland from the fringing red mangroves), Taylor et al. (2007) erected small fiberglass screen enclosures during low tide (the sites then being dry and all *K. marmoratus* presumably underground). The enclosures were then removal–trapped to depletion with cup traps (small plastic cups fitted with a funnel for sampling within crab burrows or very shallow water [Taylor 1990]) at each tidal flooding. Four areas were enclosed, varying in size from 0.75 to 2.2 m². Although complete integrity of the sites could not be assured, as there may have been underground connections via crab burrows, the density of *K. marmoratus* varied from 7 to 26 fish/m², remarkable numbers when considering that the transition is basically intertidal. At another Belizean cay (Calabash Cay), four other enclosures (0.80–2.41 m²) produced from 2.5 to 13.4 fish/m² (D. S. Taylor, unpublished data).

McIvor and Silverman (2010) utilized a modified 6 m² bottomless lift-net in riverine mangroves in the Everglades. In their extensive study, nets were deployed 189 times at three locations over 7 years, and 21 species of fish were taken, numerically dominated by *K. marmoratus*. Overall, density of *K. marmoratus* was 0.39 fish/m². *Kryptolebias marmoratus* were most abundant at the higher elevation site with the least tidal flooding, and with lower density of fishes in general and least overall diversity of fish. Conversely, *K. marmoratus* were least abundant where tidal flooding was greatest, that is, at lower elevations (C. C. McIvor, personal communication).

Further north in Florida, in the Tampa Bay area, Richards et al. (2011), noting a lack of data linking microhabitat variables to abundance of *K. marmoratus*, utilized trench traps sunk in the forest floor and found that *K. marmoratus* was the numerically dominant species of fish in mosquito-ditched intertidal mangroves. *Kryptolebias marmoratus* were more abundant further away from permanent water-filled mosquito ditches. Higher catches were associated with greater amounts of leaf litter. No association was found between number of fish and abundance of crab burrows (fiddler crabs and mud crabs: *Uca* sp. and *E. limosum*). Since no fish were observed among the leaf litter at low tide, it is assumed that they were retreating down crab burrows at that time. The density of burrows was high (24–256/m²), and it may be that burrow density is not limiting, so no correlation between density of burrows and abundance of fish was seen (Richards et al. 2011; C. C. McIvor, personal communication).

The movement of individual *K. marmoratus* in the field remains largely unstudied. In Belize, 14 marked (unique fin-clip) fish were released in their burrows of origin. Repeated cup trapping (in burrows and on the surface of the sediment) over several days within an area of several meters adjacent to capture sites resulted in only two marked recaptures, although a total of 81 fish was taken in the recapture effort. One marked fish had moved a maximum of 7 m from burrow of origin in 5 days, while the other had moved 1.5 m. However, the sizes of the fish captured from the same burrows over time were noted, and there was little repeatability in size, indicating a high turnover of occupants, while the low recapture rate again attests to high population density (D. S. Taylor, P. Molloy, and M. A. Fish, unpublished data).

**Oviposition/habitat of juveniles**

In a laboratory setting, observations in the crab burrow “microcosm” described by Taylor (1990) suggested that wild fish strand their embryos, a trait rather common among the genera *Rivulus* and *Kryptolebias* (Huber 1992), and in fact, much of this behavior may occur at night (Taylor 1990). However, repeated searches for embryos in the field largely
have proved fruitless, with only a few embryos found on the sides of crab burrows and several secreted within leaf litter at the exposed margins of stagnant pools (Taylor 1999; D. S. Taylor, unpublished data).

*Kryptolebias marmoratus* embryos will develop fully without standing water, provided they are kept damp (Taylor 1990). Embryos apparently enter a “diapause” when fully developed, holding at full development and then finally hatching when flooded. This has been observed both in the laboratory (personal observation) and in the field, where newly-hatched *K. marmoratus* appeared immediately after reflooding of dried mangrove pools where adult fish were known to be present (Ritchie and Davis 1986).

Very large (40–55 mm SL) wild gravid hermaphrodites in both Belize and Florida have been known to release large numbers of viable embryos (approximately 50 or more) in “mass spawnings” within collection containers after capture (Taylor 2000; D. S. Taylor, unpublished data). While this may be a response to the stress of capture, the prospect for deposition of large numbers of embryos in a single oviposition event in the wild seems likely. However, where oviposition occurs, remains one of the more enduring mysteries of the fish’s natural history; given the apparent density of many populations, there must be many embryos present.

In the tropics, it is safe to assume that reproduction occurs year-round, except for prolonged droughts and/or low tides. We have seen reproduction in the laboratory immediately following capture at all times of the year in Belize (W. P. Davis and D. S. Taylor, unpublished data). In the sub-tropics (e.g., Florida), there is some data on *K. marmoratus* gonads that support a hiatus in reproduction during cooler weather. Taylor (1993) examined 111 gonads from phenotypic hermaphrodites from east-central Florida. Most fish (*n* = 108) contained developing or mature eggs within the ovotestes; but fish taken during late winter (February) had eggs that were smaller (<0.25 mm diameter) and fewer in number (40–60), while in April to May eggs were larger (>1.0 mm) and more numerous (up to 75 total).

Outcrossing, in which males presumably fertilize infertile eggs laid by hermaphrodites, is documented to occur frequently in some locales in Belize, and with less frequency in other populations (Lubinski et al. 1995; Mackiewicz et al. 2006b). Except for the offshore cays of Belize, males are rare, but even in populations in which males are very rare or unknown, a certain amount of heterozygosity exists, suggesting possible hermaphrodite/hermaphrodite crossings (Mackiewicz et al. 2006c). Hermaphrodite/male outcrossing has been documented in the laboratory (Harrington and Kallman 1968; Mackiewicz et al. 2006a) and, given the general difficulties of observing this cryptic animal in its complex natural environment, it is not surprising that there has been no visual confirmation of outcrossing (or oviposition) in the field. Given that oviposition may take place out of the water, there are obvious complications to outcrossing. Nevertheless, observations in aquaria, in which wild Floridian hermaphrodites were presented with brightly colored, wild Belize male fish, suggest that the innate behaviors for outcrossing are there. When the male fish approached the hermaphrodites near spawning “mops” at the top of the aquarium, the two sexes displayed many of the classic killifish courtship/spawning behaviors: recurved bodies coupling closely and vibrating (D. S. Taylor, unpublished data). Such behavior is rather incongruous, given that male fish are unknown from these Floridian populations and the general intraspecific highly aggressive tendencies normally seen between individuals in aquaria.

The embryos are not the only early life-history stage that is little-known, as few juveniles (<10 mm SL) have been found in the wild. Harrington and Rivas (1958), using rotenone to collect in mosquito ditches in east-central Florida, have the most documented success with 22 very small juveniles (7.5–11.1 mm SL) having been taken. These collections occurred in August, implying that oviposition and hatching occurs earlier in the summer. Taylor (1999) reported about a dozen small fish (7–15 mm SL) taken from crab burrows in autumn and winter in east-central Florida. Given the propensity for cannibalism in this species, the fact that no fish smaller than 7 mm SL has ever been taken from a crab burrow suggests that juveniles may largely be occupying as-yet unidentified micro-habitats (Taylor 1990). Of the 161 total specimens taken by Richards et al. (2011) from Tampa Bay, size ranged from 7 to 35 mm SL (*X* = 16.8 mm), including one “cohort” of 25 individuals (7–11 mm SL) taken at the end of June, again, likely a result of oviposition in early summer. Overall, specimens from Tampa Bay were notably smaller than those from two other Floridian populations examined: Shark River, Everglades, and east-coast Florida (Richards et al. 2011; Taylor 1992). This reduced size was attributed to the lower temperatures encountered at more...
northerly latitudes (Richards et al. 2011). In fact, a wide-ranging examination of the meristics/morphometrics of 12 populations ranging from Florida to Honduras, and including two populations from Brazil, documented a marginal increase in size at more southerly latitudes (Taylor 2003).

Age and growth

Growth of laboratory-reared fish has been extensively documented (Sakakura and Noakes 2000; Grageda et al. 2004), but only one study (Dunson and Dunson 1999) has looked at the growth of caged wild fish, placed in different small enclosures at varying depths in the water within the mangroves. They concluded that growth was related both to temperature and incidence of hypoxia, and inversely correlated with the number of fish in enclosures. *Kryptolebias marmoratus* was also noted to grow more slowly when placed with species other than its own (Dunson and Dunson 1999).

*Kryptolebias marmoratus* have been aged only in a laboratory setting. Sakakura and Noakes (2000) confirmed the presence of daily otolith increments up to 60 days age. They advocated taking this technique to the field for aging wild fish, but this work remains to be done. There are similarly no data on the longevity of fish in the wild, but *K. marmoratus* can be long-lived in the laboratory, with one specimen noted to survive 8.2 years (D. S. Taylor, unpublished data).

Conclusion

*Kryptolebias marmoratus*, with its unique physiological attributes and bizarre reproductive/mating system, has long been a source of fascination as a laboratory organism and genetic model, and it is in the forefront of research in these areas. However, insights into its natural history and ecology have not come easily, as this animal lives in a complex, multi-dimensional, cryptic habitat that is widely variable in its environmental and physical features. We can now characterize the types of microhabitats in which the mangrove rivulus might be found in the field, and with this knowledge dedicated field biologists may now began to unravel more of its elusive life in the wild.

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