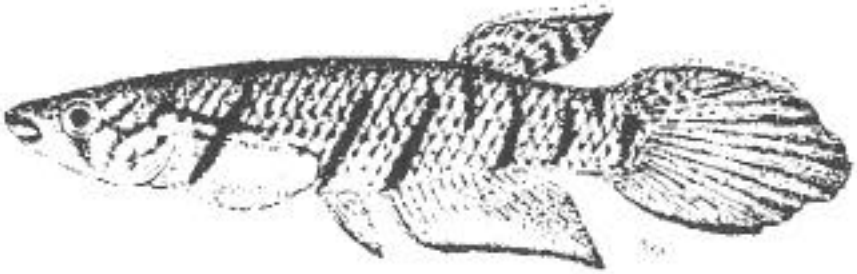
Journal

of the

American Killifish Association



Epiplatys togolensis
(Taylor Creek, Niger Delta, southeastern Nigeria)

including
KILLIE NOTES

March / April - Volume 31, No. 2, 1998

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with Killie Notes

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EDITORIAL

I won't flatter myself and even hint that I am the first person that has ever noticed how appropriate the abbreviation for the American Killifish Association is. For years, many of us have struggled to keep up on the "name game", trying to apprise ourselves of the current nomenclature for the many species of killifish. So it is little wonder, that in some quarters, AKA is interpreted as "also known as"... as in *alias.*

Not that I am contending that there is some sort of cosmic coincidence here, but you have to admit that it certainly is Freudian.

During the coming years we will certainly see much more clarification of the classification of killifish. Modern technology has offered many new techniques and tools to help unravel the complex relationships that have been the topic of hot debate among hobbyists for decades. Application of the new methods will not end all arguments and conjecture, but it will certainly enhance our understanding of these fish. Hopefully a better understanding will lead to better preparing the AKA to assist killifish in surviving throughout the world.

Through better understanding the genetic codes, physiology and environment that lie hidden at times to the mere hobbyist, we may indeed be able to discover a vital link that fortifies the chances of species survival. There has to be more to being an AKA member than just having access to "pretty fish". When we acquire a rare, or new fish, there has to be a better reason than just because we had the opportunity. Our motivation must always stem from an altruistic desire to do our own part to ensure the place of these fish in the world.

In the long run, it may not be important to the fish whether they are known as *elberti* or *bualanum, cameronense* or *halleri, schmitti* or *liberiense, coeruleum* or *sjoestedti, Aphyosemion* or *Fundulopanchax.* It should be important to us, however, that they are not "also known as" extinct.

Brent D. Kelley

Correction: In the January/February 1998 issue of JAKA, the printer mistakenly reversed the photographs on page 12. We apologize for any confusion this may have caused.

Journal of the American Killifish Assoc. 31(2) 35-41 (1998)

Forensic Phylogenetics of Rivulus

Glen E. Collier, Mike Espinoza and William J. Murphy

Identification of new imports of *Rivulus* has historically been problematical at best and hopeless at worst. This was the joint result of not knowing the exact geographical origin of most commercial imports and the fact that many of the older original description of *Rivulus* species were extremely brief. Often the morphometric differences within groups of related *Rivulus* species were small to none. Further, given the area of South and Central American occupied by *Rivulus* and the proliferation of new names over the past decade as the genus has been studied more extensively (see Huber, 1992 for a review), we have probably not yet come close to seeing all extant species of *Rivulus.* So in the absence of reliable locality data for the imports and a lack of knowledge of the life colors of the species described decades ago, attempts to identify them with certainty was often a fool's errand.

One name that has been attached to aquarium strains of *Rivulus* over the past 30 years is the name *Rivulus holmiae.* Al Klee in 1966 attempted to identify two imports then going around the hobby as *Rivulus* U-l and *Rivulus U-2.* The slight morphometric differences between these two strains led to them keying out as *R. holmiae* and *R. micropus,* respectively, in both Regan's 1912 key and Hoedeman's 1961 key to the genus *Rivulus.* Klee concluded that these identifications were "correct" in light of our current knowledge of the micropus complex [of the genus *Rivulus].* Whether he was correct or not, the name stuck. *Rivulus holmiae* was the name attached to strains reported on by John Miles (1972) and Ken Stoops (1994). It is a name that still appears with some regularity in the fish and egg listing of the AKA's Business Newsletter.

Another name that has appeared in recent years is *Rivulus sp.* Mikkelson, a name which sometimes is unfortunately and erroneously translated into *Rivulus "mikkelsoni".* This is an import of unknown origin that was found in a pet shop by David Mikkelson, son of former AKA Chairman Al Mikkelson. There has not been a formal description using the name *"mikkelsoni",* hence this usage is incorrect. Nevertheless, it is an attractive large *Rivulus.*

Over the past few years, we have accumulated DNA sequence data as a means of assessing relationships within and between groups of killies (Murphy and Collier, 1996: Murphy and Collier, 1997: Murphy, 1997). Part of this work included DNA sequences from four populations of known origin from two locations along the north coast of Venezuela (DCT87-5, DCT88-28), Margarita Island (OL-2) and Trinidad (Fig. l). The Venezuelan populations had previously been considered to be *Rivulus bondi* (Thomerson and Taphorn, 1987; Collier, 1990) while the island populations were considered to be *Rivulus hartii* (Thomerson, pers. comm.). However, the karyotypes of these populations are very similar (Fig. 2), the differences in DNA sequences are minimal (see below), and they are cross-fertile (Thomerson, pers. comm.). The conservative approach then would be to consider these populations of a single wide spread species. Since *R. hartii* is the older of the two names, it takes precedence.

Both aquarium strains of *Rivulus "holmiae"* and *Rivulus "mikkelsoni"* are phenotypically very similar to the four *R. hartii* populations we had sampled. Thus, comparing the DNA sequences of these strains to the populations of known origin provided a means to assess their identity and possible place of origin. DNA has become a powerful tool in forensic investigations of crimes. In this case, we are using information from DNA in conjunction with phylogenetic analysis to identify strains of unknown origin. Hence the term forensic phylogenetics seems appropriate for this study.

We obtained aquarium strains of both *"R. holmiae"* and *"R. mikkelson'"* from Liz Hutchings. This particular strain of *"R. holmiae"* was obtained from a pet store by John Claremont and the name *R. holmiae* was suggested as appropriate. DNA was isolated from small samples of muscle from the caudal peduncle and used as template DNA for the amplification of a portion of the mitochondrial genes for cytochrome b and 12S RNA as reported earlier (Murphy and Collier, 1996). These two segments of DNA were sequenced and compared to the corresponding sequence from the four populations of *R. hartii* (DCT87-5; DCT88-28, OL-2 [Margarita Island] and Trinidad). Homologous sequences from the related species *R. deltaphilus*

(JET88-10) and R. sp. Rio Supamo were used as outgroups. [DCT and JET refer to the field collection numbers of Dr. Donald Taphorn and Dr. J.E. Thomerson, respectively.] In a study of a large number of *Rivulus* species (Murphy, 1997), *R. deltaphilus* is one of a small group of species very closely related to *R. hartii.* Thus, it is an appropriate outgroup for this study.

The sequence data consists of 360 base pairs of part of the gene that encodes the protein cytochrome b and 318 base pairs of part of the gene that encodes the large ribosomal RNA (12S RNA). These gene segments are often used in similar works of molecular systematics of many groups of vertebrates including fishes. These two segments accumulate changes at different rates; with the gene for cytochrome b accumulating change more rapidly. Of the 124 variable sites among the 678 total sites, 87 were from the cytochrome b sequence while 37 were from the 12S RNA gene segment. The pattern of change was similar for each of the individual segments, but analysis of the combined sequence generated trees of slightly greater statistical reliability.

The fish currently known in the hobby as *Rivulus holmiae*
Photograph by M. Addicott

Another *Rivulus* with an identity problem is *R. micropus*, previously designated as *Rivulus* U-2
Photograph by M. Addicott

The number of specific differences between taxa are shown in Table 1. There were only 7-11 differences between the sequences of Trinidad *R. hartii* and *"R. holmiae"* and *"R. miklelsoni"*. Greater differences were seen between the island taxa and the mainland taxa suggesting greater genetic differentiation. However, this greater divergence has not been sufficient to result in post-

mating isolating mechanisms. This is inferred from the fertility of interpopulation hybrids produced in captivity. The Rio Supamo population is as similar to *R. deltaphilus* (JET88-10) as the island *R. hartii* populations are to each other. This suggests that the Rio Supamo fish is conspecific with the nominal *R. deltaphilus.*

TABLE 1.

Matrix of the number of sequence differences1 and genetic distances2 among six samples of *R. hartii* and two outgroup taxa.

"mikkelson"	"holmiae"	hartii Trinidad	hartii OL-2	hartii JET88-28	hartii DCT87-5	deltaphilus JET88-28	Rio Supamo
	7	11	18	48	52	85	87
0.011		6	15	44	47	83	85
0.017	0.009		13	43	46	84	85
0.027	0.023	0.019		44	53	88	90
0.075	0.069	0.069	0.067		47	80	86
0.082	0.074	0.084	0.072	0.074		73	73
0.143	0.137	0.146	0.138	0.131	0.118		12
0.139	0.141	0.149	0.141	0.142	0.118	0.018	

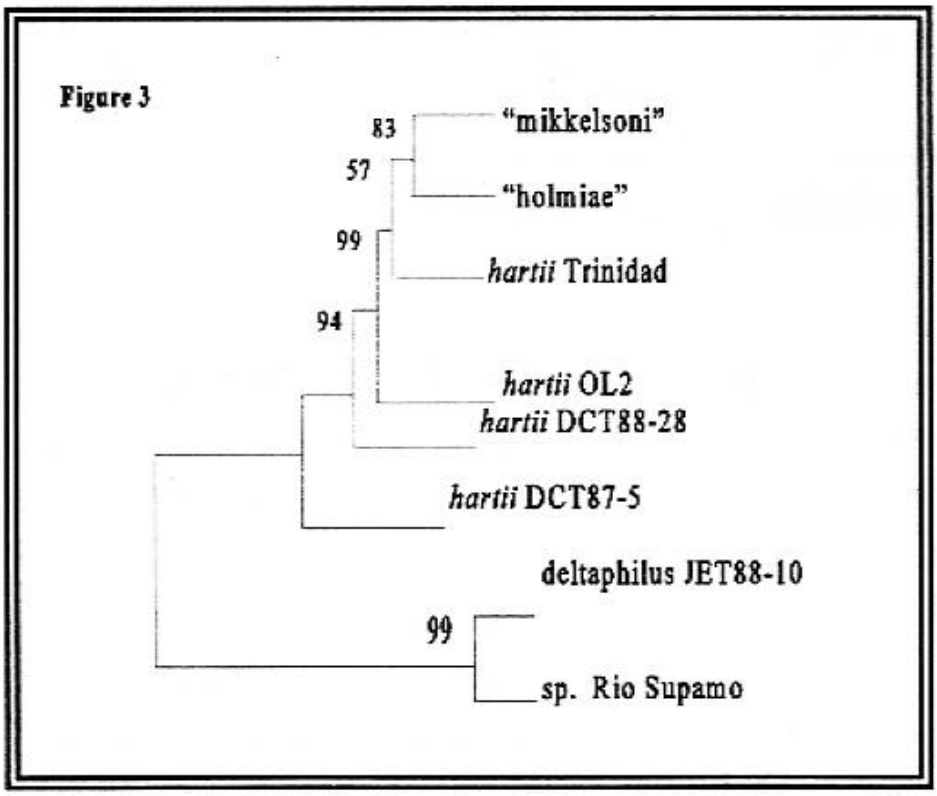
1 Number of differences (above the diagonal) for each pairwise comparison of 678 bp of the genes for cytochrome b and 12S RNA.

2 Genetic distance (Kimura 2-parameter; below the diagonal)

Karyotype of *R. hartii* (DCT97-5, DCT88-28, OL-2, Trinidad). All four populations are 2n=44

These differences were used in two different ways to estimate the phylogeny of these taxa. In one approach, the differences between each pairwise comparison are converted into an estimate of the total number of genetic changes that have occurred since the two taxa last shared a common ancestor. The matrix of all pairwise genetic distances are then used to search for a tree with an arrangement of species that minimizes the total genetic distance over the whole tree. One efficient method of finding such trees is called the Neighbor Joining method (NJ)(Saitou and Nei, 1985). In the second, each position in the aligned sequences are compared and those for which at least two taxa differ from the rest are considered phylogenetically informative. Trees are then searched for which minimize the number of changes necessary to explain the current sequences. This approach results in what is called the maximum parsimony (MP) tree.

The same topology (branching pattern) is found by both methods of analysis (Fig. 1). The reliability of such trees can be estimated by several statistical approaches. In one approach each position of each sequence is resampled from the pool of the sample of sequences in the data set, and the resample data is assessed to see if the same branching pattern is obtained. Such "bootstrap" values are an indication of the reliability of a particular node. In another kind of analysis unique to NJ trees, the interior branch length confidence level (Pc values) can be calculated. This measure essentially is the level of confidence we have that the interior branch lengths are different from zero. Both of these measures of reliability are high for all parts of this tree.



Neighbor Joining Tree for four populations of R. hartii, two commercial imports and related outgroup. The numbers represent the Pc values for the respective nodes. Maximum parsimony trees for these data generate a single most parsimonious tree of identical topology.

These data support the close relationship of the four populations of hartii. Clearly *"holmiae"* and *"mikkelsoni"* are both a part of this group. This was not too surprising given the similarity in size and coloration of these strains. What is striking is that the differences between these aquarium strains and the wild population of *R. hartii* from Trinidad are so small that it is highly likely that both of the aquarium strains are also originally from Trinidad. Given the volume of tourists and commercial shipments in and out of Trinidad relative to the other three areas, this is not a surprising result. It *is* surprising that it should be so evident in the molecular data.

Map of coastal Venezuela and adjacent islands showing the locations of the populations sampled.

Does this approach offer a means of identifying other aquarium strains of killies of unknown or uncertain origin? In this case, the "unknown" aquarium strains were phenotypically similar to a commonly imported species *Rivulus.* Further, we had sequence data from a range of known wild populations for this particular taxa. As our database of DNA sequences grows (we now have information for over 200 species of Aplocheiloidei), we can more easily identify the nearest relative of any "new" species or import. In some cases where the tree is well understood, there should be additional cases were such forensic phylogenetics will prove informative.

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Journal of the American Killifish Assoc. 31(2) 42-44 (1998)

Breeding *Aphyosemion herzogi*

by Aaron Kaminski

One thing I have found since I have entered the killifish hobby several years ago is that there are several things which make a killifish desirable to us. Among them are the following. The fish must be attractive (and to each his own on this one). It must be rare. There must be some amount of prestige associated with it's keeping and/or breeding. In my opinion, *Aphyosemion herzogi* meets all of these.

My main reason in wanting this fish was it's reputation for difficulty, sometimes I seem to have a glutton for punishment. Everyone I talked to about this fish had different opinions on it, most seeming to point to the fact that it was difficult to breed, and not always easy to keep. As is par for this hobby, everyone seems to have a somewhat different perspective on the matter (often a good thing in this hobby).

My actual purchase of the original pair was a chance one. The fish were in the auction at the Elkhart, IN killifish show. I decided this was to be one of the fish I wanted, and lucked out in getting them for a fairly reasonable price.

I feel one of the reasons why we lose our fish is failure to acclimate well. If the fish die within the first few days of getting them home, this may often be the case. Hoping to avoid this, my fish were acclimated to my water via a slow drip, and fared well from the beginning.

The tank I kept the pair in was a standard 2 1/2 gallon, filled to the top despite recommendations that a shallow setup was necessary. Filtration was a corner box filter with both floss and gravel, but a sponge filter could have served the purpose just as easily. I placed a floating mop and a bottom mop to allow the fish to choose whichever they preferred.

Perhaps I should detail my water chemistry a little here. Since I fool around a lot with many of the small bubble nesting bettas, I keep quite a bit of RO water on hand, so I decided to use this as my water of choice. I added a little tap water back, to make the water hardness between 10-20 ppm. This was filtered over peat to bring the pH to about 6.0. Water of this softness necessitates frequent water changes to keep the pH stable, since it has little buffering capacity, so I found myself changing water as often as every other day.

The maintained temperature was between 70-76 degrees F, and didn't prove to be quite the limiting factor I had expected it to in terms of breeding success.

The foods I offered were all live, and *A. herzogi* takes all of the standard killie foods. I fed mostly daphnia, but also things like black worms, white worms, mosquito larvae, brine shrimp, fruit flies and whatever else I could get my hands on. I generally fed once or twice per day, depending on my schedule. The daphnia were nice since they would survive even in this soft water until eaten, so I would often put enough in to last the whole day.

Now for the eggs. After about a week, I was finding eggs in the top mop, near the float on the mop. Very few were found in the bottom mop. Gone for me were the myths of shallow water, peat fibers and bottom mops. Egg predation may be the reason for no eggs in the lower part of the mop and the bottom mop, but I, for some reason, doubt this since I would find fry in the tank which are left alone by the parents.

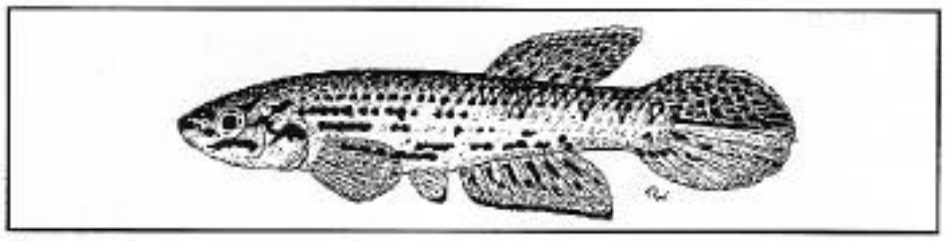
I would pick the eggs every other day and place them into a small covered container with some methylene blue. I know there are better anti fungal medication, but I remove the bad eggs daily anyway, the dye just lets me gage viability better than clear water.

The fry hatched for me in a little longer than two weeks. For the most part they are large enough to accept baby brine, so foods to offer pose no real problem. they do however seem sensitive to water quality. My losses were heavy if I didn't change the water frequently. Growth isn't overly rapid, but by six weeks many males could readily be sexed. The sex ratio was fairly close to even as well.

Aphyosemion herzogi has long been known as a difficult fish
Photograph by L. Mackowiack

Although there is much discussion about breeding, no one argues about its beauty and coloration
Photograph by M. Lehrman

I feel the limiting factors with this fish may be twofold. First being water quality/chemistry. In soft water my fish were willing breeders, producing a steady but somewhat small supply of eggs. When I kept them in somewhat harder water, the supply of eggs diminished. The live foods I offer may have also played a role, but since no experimentation was done, this is only a guess.



Aphyosemion herzogi (Médouneu, northern Gabon)
Sketch by Ruud Wildekamp

Overall, if and when this fish becomes available, I'd recommend interested parties give the species a try. You may find, as I did, that its reputation for difficulty is not totally deserved.

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Plancterus zebrinus, Sitting Bull Falls, New Mexico Location

Ken McKeighen

I first collected the killifish, *Plancterus zebrinus*, in 1980 at Palo Duro Canyon State Park which is located south of Amarillo, Texas. This region is typical high plains and grasslands. The Prairie Dog Town Fork of the Red River cuts a panoramic canyon through Triassic and Permian rocks. The high plains regions of Texas and New Mexico are virtually treeless and the climate is semi-arid.

I discovered the Sitting Bull Falls location in 1983 while I was staying in New Mexico. The population I have now was collected in 1996 during an excursion into this area in southeastern New Mexico. Several adults and young were set up in a small pond where they have been successfully established since that time.

The *Pl. zebrinus* in this article is found in an isolated stream in Last Chance Canyon which is in the Guadalupe Mountains. This is the only mountain dwelling population of *Pl. zebrinus* that I am aware of. The stream is part of the Pecos River drainage and it empties into the main river just above Loving, New Mexico. Due to the arid conditions in this area, the stream is dry for most of its length. Like the *Cyprinodon* species in the Southwest, this population has been isolated from the main river since the end of the last glacier period.

On a map, Sitting Bull Falls and Palo Duro Canyon appear to be fairly close, and geographically these areas are only a few hundred miles apart. However, by river, the separation is thousands of miles including several hundred miles of shoreline of the Gulf of Mexico. The Pecos River empties into the Rio Grande east of the west Texas town of Langtry while the Prairie Dog Town Fork of the Red River drains into the Mississippi River near Simmesport, Louisiana.

Sitting Bull Falls is about forty miles west of the Pecos River. It is a little over an hour's drive from Carlsbad, New Mexico, which is northeast of Sitting Bull Falls. It is situated in the Lincoln National Forest and has a small picnic area and hiking trails, but overnight camping is prohibited at this time.

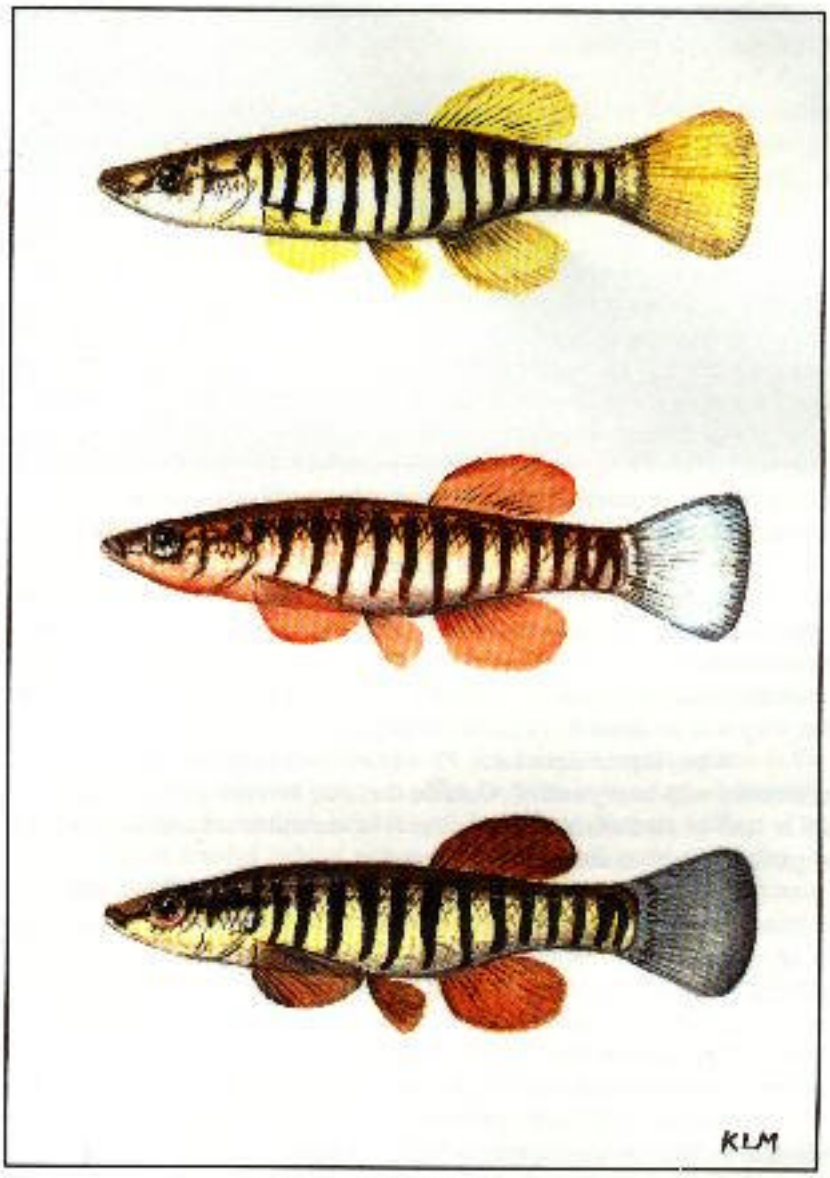
The geologic age of the Guadalupe Mountains dates back to the Permian period. The rock in this region is primarily limestone and sandstone. The falls itself was formed by a collapsed cave system which creates water flowing both over and through the falls itself. A small cave can be entered about halfway up the falls.

The stream has a lush growth of reeds, cattails, and small trees which line the banks. The humidity generated by the water splashing over the falls has a natural cooling effect on the immediate area. The plant growth and cliffwalls make this location shadier than any other *Pl. zabrinus* locations that I have visited. Leopard frogs *(Rana pipiens)* and a shiner *(Notropis sp.)* share the habitat with the *Pl. zabrinus*, although the shiners are found in the pools at the base of the falls while the *Pl. zebrinus* are found in the stream flow below the pools. The stream flows for about half a mile below the falls before it disappears underground.

The coloration of the Sitting Bull males differs from males from the Texas panhandle and Oklahoma by being a generally "duskier" fish. The pectoral, ventral, and anal fins are a dark orange, almost black in some males, while the Palo Duro Canyon males sport a powder blue to white caudal. The back and bars of the Sitting Bull Falls males are a blackish and the sides are a pleasing golden color. The Palo Duro Canyon males are reddish on the back and bars and the sides are silver to white. The females of both locations are marked similarly to the males but lack the bright colors in the fins and on the body. The colors described are for active males... nonspawning males are not as highly colored.

Pl. zebrinus can be fairly tough to keep in an aquarium environment. The adults in a confined area can be quite aggressive with their tankmates. They will also turn predatory and cannibalistic on smaller tank inhabitants. The aquariums employed should be as large as possible with lots of floating plants and hiding places. *Pl. zabrinus* do well with other families of fish, and

can even get along with smaller cichlid species such as Convict Cichlids *(Cichlasoma nigrofasciatum).*



Plancterus zebrinus kansae
Plancterus zebrinus zebrinus, Palo Duro Canyon, S.P. Texas
Plancterus zebrinus zebrinus, Sitting Bull Falls, New Mexico

Color illustrations by the author

I have never been successful in spawning *Pl. zebrinus* in an aquarium. The smallest aquarium that I ever kept these fish in problem free was over one hundred gallons in size. This was the Palo Duro Canyon population, and unfortunately I was not trying to spawn them at the time. It became quite apparent that males were defending territories in the open areas, but I never observed any young, but then again, this was a community tank.

The only requirements for the water, whether aquarium or pool, are that it must be very clean and with a neutral to slightly alkaline pH. The water from Sitting Bull Falls flows through limestone, so it is moderately hard with a pH just slightly on the alkaline side. Water changes are an absolute must with *Pl. zebrinus* as they will waste away when the water is dirty or becomes acid.

I've had fantastic success with *Pl. zebrinus* from both locations when I have kept them in small ponds outside. This system also allows for a more natural approach to keeping these fish. The natural light and steady supply of insects seems to be a positive factor in the health of this

species. The adults stay in the open areas of the pool unless disturbed and frightened. When that happens, they will dash for cover, only to reappear a few minutes later. Males have loose territories when spawning, although when not spawning the fish will congregate in schools of a dozen or so individuals. I've not had a chance to visit Sitting Bull Falls in the winter, but I've kept *Pl. zebrinus* from this location outside successfully year around. The temperature in Los Lunas, New Mexico, I must admit, is nowhere near as cold as some areas of North America, but it can get down to zero degrees (Fahrenheit) and if two inches of ice forms, it has been a tough winter. Daytime temperatures do generally get above freezing even during the coldest part of winter. During the coldest parts of the year, these fish will bury themselves in the bottom sediment, but when the sun comes out can be found warming themselves in the shallow areas where the ice has melted.

The *Pl. zebrinus* started coloring up in early May. Spawning was observed by this author. Spawning took place with the eggs being deposited on the bottom, in plants, and along roots of water plants. Although I have not had a chance to visually check the incubation time, it's probably safe to assume they take seven to ten days to hatch. The newly hatched fry can be seen stalking small crustaceans and other minute creatures along the water's edge. The young start swimming in the more open areas when they are about three quarters of an inch long. They reach spawning size by the next summer when they attain a body length of about two and a half inches. At two year's old, they will be about three inches in length.

Plancterus zebrinus kansae
Photograph by Tony Terceira

When kept in aquariums, *Pl. zebrinus* will consume all killie foods associated with heavy eaters. Outside they can be seen eating insects that fall or land on the surface. Aquatic insect larvae and small crustaceans such as gammarus also make up part of their diet.

Pl. zebrinus have been a most interesting killifish to keep, although in an aquarium they demand plenty of room, lots of light and heavy feeding. In an outdoor environment they have proven to be prolific, hardy and colorful. Their natural behavior in a pond environment has been very rewarding, so I would recommend *Pl. zebrinus* for those that enjoy keeping large, robust killifish that are a challenge to maintain at their peak. If you have plans to work with this fish, be sure you have large quarters for them.

Because of the arid conditions of west Texas and southeastern New Mexico, several isolated species of killifish have evolved along the Pecos River drainage including *Cyprinodon pecoensis, Cyp. bovinus,* and *Cyp. elegans.* It is interesting to note that *Cyp. pecoensis* at Bitter Lakes Wildlife Refuge near Roswell, N.M. is separated from the Pecos River by a series of steps in the outflow of the lakes. The lakes are about a half mile from the Pecos. It helps put the isolation of Sitting Bull Falls in perspective.

Due to its extreme isolation from the Pecos River, I feel there is a possibility that the Sitting Bull Falls location of *Pl. zebrinus* may indeed be a new subspecies. They should be kept separate from other locations of *Pl. zebrinus,* just to preserve their unique coloration. Another factor to consider is how much their habitat has shrunk since the end of the Pleistocene. I contend that the duration of their isolation from the Pecos River populations is a major consideration in

bestowing the subspecies status.

Furthermore, I would like to see this location given a place in the AKA species maintenance program. The location is in a very vulnerable position due to the limited habitat and increasing human traffic. The combination of limited habitat, unique coloration and rarity make this an excellent candidate for the program. Anyone interested in pond maintenance of the Sitting Bull Falls location of *Plancterus zebrinus* should contact the author.

Author's note:

The *Plancterus zebrinus* location at Palo Duro Canyon was extirpated in 1994 when the city of Amarillo, Texas began dumping sewage runoff into the Prairie Dog Town Fork of the Red River. All fish species were killed off including *Notropis lutrensis* which was sympatric with *Pl. zebrinus.*

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46 Cuerro Lane
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Journal of the American Killifish Assoc.31(2) 50-54 (1998)

Epiplatys infrafasciatus rathkei and the "Natural" Way

Gregory J. Niedzielski

The fishes of the genus *Epiplatys* have always been favorites of mine. The first killie I kept (more years ago than I care to remember) was *Epiplatys dageti monroviae.* Ever since then I have almost always had some *Epiplatys* or other swimming in my tanks. While not as colorful as some other killies, they have a quiet beauty and attractive markings. Their even temperaments and outgoing dispositions make them excellent community tank fish. Over the years I have kept (and bred) such species as *Epiplatys roloffi, Epiplatys chaperi* and *Epiplatys olbrechtsi,* but recently I have been working with an old favorite under a new name, *Epiplatys infrafasciatus rathkei.*

A stunning male *Epiplatys infrafasciatus rathkei*
Photograph by A. Castro

Killie aficionados of long experience know this fish as *Epiplatys sexfasciatus rathkei,* named for Father Karl-Heinz Rathke, an African missionary. However, an article recently published by Wildekamp (1997) reclassified several fish that were previously known as *Epiplatys sexfasciatus,* moving some of these fish into *Epiplatys infrafasciatus* and *Epiplatys togolensis.* Hence we no longer speak of *E. sexfasciatus rathkei* as a valid species. The correct name is now *Epiplatys infrafasciatus rathkei,* or, if we follow Wildekamp's view that *"rathkei"* is not a valid subspecies, *Epiplatys infrafasciatus* "Rathkei". Just to confuse things further, Scheel's Atlas of Killifishes of the Old World (1990) puts all Epiplatys species into *Aplocheilus,* although no one seems to be taking this massive reclassification seriously. The recent work of Murphy and Collier (1997) on the molecular phlyogeny of the killifishes indicates that the Indo-Malaysian genera of *Pachypanchax* and *Aplocheilus* are genetically very distant from *Epiplatys,* providing further evidence against "lumping" schemes.

Epiplatys is an African genus of killies, inhabiting streams, swamps and rivers in equatorial West Africa. *Epiplatys* are often found in association with *Aphyosemion* and *Fundulopanchax* species, as well as African barbs and small cichlids. *E. infrafasciatus* and *E. sexfasciatus* are found in Nigeria, Equatorial Guinea and Gabon (Wildekamp, 1996), to the east of the Dahomey Gap. According to Radda (1970), the type locality of *rathkei* is Kumba in west Cameroon. Langton (1996) records two collections of this fish, one in 1970 by Alfred Radda and Walter Haefelin and another in 1989 by Eberl, Legros and Vlijm. I do not know which collection my fish come from, but in the absence of any of the collection numbers used by Eberl et al. on the bag they came in my guess is that they are from the earlier one.

There is quite a bit of variability in coloration and pattern among the *E. sexfasciatus-E.*

infrafasciatus-E. togolensis types. Some, such as *E. sexfasciatus* "Mbebe", and *E. infrafasciatus* "Bipindi", are very colorful and attractive, while others are more somber. All have an olive or tan body with several prominent vertical black bars on the flanks, and all have greater or lesser amounts of green iridescence on the flanks and red and yellow markings in the unpaired fins. *E. infrafasciatus rathkei* is one of the more attractive of these fishes. It has a tan ground color with a green iridescence to the body, with a bright red dot on each scale. The green iridescence is not as prominent as it is in some other populations of *E. infrafasciatus.* The male has a concentration of green color just behind the gill covers, where the "wound" marking would be in an *Aphyosemion occelatum.* Both the male and female *rathkei* exhibit six vertical bars along their sides. The unpaired fins in the male are yellow and orange with red marginal bands. The tail in particular is prominently marked. They are a large fish, and rathkei typically attain a length of two to two and a half inches at maturity.

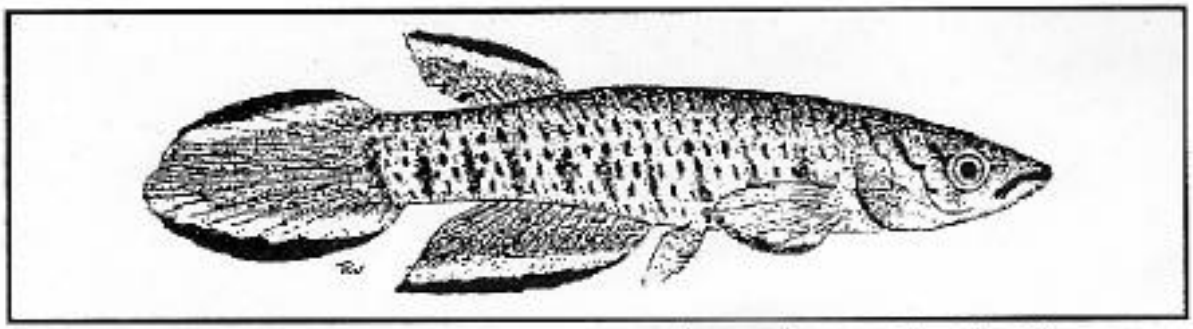
I acquired my first pair of *E. infrafasciatus rathkei* at the 1995 American Killifish Association Convention in Buffalo, New York. They actually arrived a few days after the convention, in a box from the Scandinavian Killifish Society that had been delayed by the airlines. Despite their long journey (the box had been in transit for over a week by the time I got it), they were in good shape and quickly recovered. They were placed in a five and a half gallon aquarium with a mop, a sponge filter and a tightly fitted cover to prevent the sort of airborne adventure that *Epiplatys* are so fond of. Here they thrived on a diet of frozen brine shrimp and blood worms, with the occasional treat of live blackworms. They particularly appreciated wingless fruit flies, once they figured out what they were and that they could be eaten. As with most of my killies, large or small, I fed them a little bit of freshly hatched brine shrimp everyday, the importance of which will be seen later on. In general this fish prefers floating food, and is one of the few killies I have kept that actually seems to enjoy flake food. They will eat food that has fallen to the bottom of the tank only with reluctance, as their instincts evidently tell them that "real" food floats.

Like most *Epiplatys, rathkei* are generally very friendly fish. They often swim to the front of the tank when someone approaches, looking for food, although they also spend a fair amount of time lurking in the plants and mops in the tank. They are also excellent jumpers, and will avail themselves of any opportunity to jump out of the tank. When startled, they may attempt to jump out of harm's way, often hitting the cover of the aquarium with a resounding thwack. They make good community tank fish, being large enough to take care of themselves but not exhibiting too much aggressive behavior towards their tankmates.

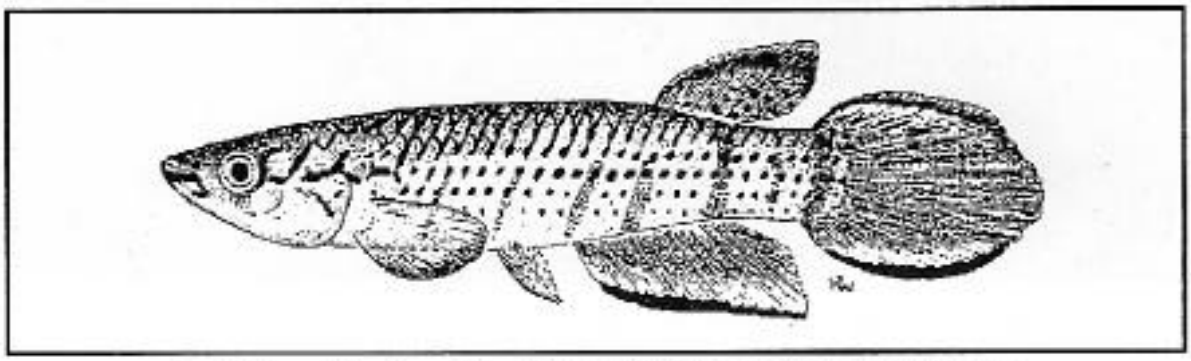
I keep this fish in water that is hard and alkaline, with a pH of 7.4 and a hardness of about 150 ppm. To this I add 1/2 teaspoon of salt per gallon. The temperature varies from the low to upper seventies. They do very well under these conditions, and when I set up that first pair from Denmark they almost immediately began laying eggs. They deposited eggs in the mop and on the sponge filter. Some eggs always seemed to show up underneath the sponge filter, which I have observed to happen with other large *Epiplatys* as well. I have never figured out how the eggs get there as I never see fish under the filter. I regard this as being one of the "Great Mysteries Of The Aquatic World", right up there with "Why I Cannot Keep *Bualanum* Alive".

The fish are quite productive, and a well-conditioned pair of *rathkei* can lay upwards of thirty eggs a day. At first I picked the eggs from the nylon mop every day. I placed the eggs in water to which a small amount of methylene blue had been added. I had very poor results using this method. The eggs were very small and delicate, and broke easily. Most of the eggs died after a few days as indicated by dye uptake. The few fry that hatched were belly- sliders and died after a day or two. The fish were very prolific, but out of at least a hundred eggs I did not keep a single fry alive for more than a week at a time. Needless to say, this got very frustrating after a while, and eventually I stopped picking eggs altogether.

The fish had other plans, however, and kept merrily laying eggs in the mop, under the sponge filter, and in general everywhere except the glove compartment of my car. One fine day I was siphoning some water out of the tank when a flash of movement caught my eye. I shined a light into the tank, and there was a small baby *rathkei*, no more than a quarter of an inch long, clinging to the side of the aquarium. I peered deeper into the tank and saw several more, and quickly reached for a net to remove the parents to another tank. The fry had evidently been feeding upon the baby brine shrimp fed to the parents, as mentioned above. With the brine shrimp as a food source they were able to grow quite nicely. The adults apparently do not cannibalize their fry to any great extent, and out of that small, almost bare five and a half gallon tank I raised over thirty *rathkei* fry.



Epiplatys infrafasciatus from Lake Barombi Ba Mbu near Kumba (the type locality which Scheel gives for *rathkei*)



Epiplatys infrafasciatus from Kribi in southern Cameroon
Sketches by Ruud Wildekamp

This has become my method of choice for raising this fish. I move the parents from tank to tank every month or so, and commence feeding the fry with baby brine shrimp. After a month or two the fry can be moved to larger quarters for growing out. So far, this has been a very successful method for me, and I have been able to raise and distribute reasonable numbers of this fish. I have also bred larger groups in ten and twenty gallon tanks, although whether or not this results in significantly larger amounts of progeny I have not been able to determine.

Epiplatys sexfasciatus (Makondo) can be extremely colorful fish
Photograph by L. Mackowiack

I suppose if there is any lesson to be learned from this, it is that not all killies like to have their eggs handled. There is still a place in this hobby for "old-fashioned" spawning methods, i.e. putting the fish in the breeding tank and leaving them alone. In the early years of the hobby, before anyone had heard of nylon mops or peat moss, all killies were spawned in this manner, with varying degrees of success. While often used for very small species, even large fish such as *E. infrafasciatus rathkei* can be successfully propagated using this technique. Sometimes, natural is the best way.

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Journal of the Amencan Killifish Assoc. 31(2) 55-56(1998)

The Silent Hatchery

Mounir Gemayel

When I first started keeping fish I lived in a one-bedroom apartment and shared my sleeping quarters with my aquatic wards. Soon, with a steadily increasing number of tanks I became concerned about the noise generated by the many filters, air pumps and air stones around me, so when I came around to hatching my first brine shrimp eggs, I chose to do so the silent way by using flat trays and no aeration. I continue to do this today even though the fish now have their own quarters in my basement. There are many advantages to this method over the vigorous aeration method. First, you don't need an air supply which preserves the precious pressurized air to run the sponge filters in the tanks. Second, there is no annoying continuous bubbling sound and no corrosive salt spray around the hatching area. If you are using glass, it eliminates the potential for breakage, and also those unstable inverted plastic cones that require a frame and special plumbing. This method also cuts down on the need for replacement air stones.

Canning Utah brine shrimp eggs
Photograph by C. Grimes

The tray separates the hatched nauplii very effectively from the empty egg shells and the unhatched eggs while the yields remain essentially the same as with aeration.

If you want to try this method, then you may want to modify the basic tray hatching technique to suit your particular needs. I use two trays that alternate. One is a 15"x6"x2" drawer organizer, while the other is a 16"x10"x2" tray out of a laundry tub. Ideally the trays should be made out of thick opaque plastic (enamel trays, while perfectly opaque, chip easily and are eventually corroded by the salt brine). The divider consists of a piece of thin plastic cut to fit the width and inserted into two- 2" sections of the plastic clip (the type used as back rib on plastic folders) that are siliconed to the inner sides of the tray and serve as guides for the divider which is inserted and adjusted to height of 1/4" above the tray bottom.

The trays are positioned above ground level near a window and the salt solution is poured to a level of 3/4 of an inch. The brine shrimp cysts are sprinkled over the surface of the longer section of the tray leaving a few empty spots here and there. This section is then covered with a double layer of aluminum foil. After 48 hours at 72°F, a large number of the eggs will have hatched and the nauplii will have swum under the divider to congregate in the shorter, uncovered section of the tray. These are siphoned into a net and fed to the fish, after which the siphoned water is carefully poured back into the tray. On the third day the process is repeated, but this time I remove the aluminum foil and tilt the tray slowly over a sink to pour off the floating egg cases. What remains in the tray is a dense orange mass of dead and dying nauplii that I transfer to the

shrimp net and feed to the fish. This is eagerly accepted by most fry and growing fish. If the orange mass smells foul, then it has been collected too late and should be discarded.

I then remove the divider and wash the tray before restarting with a new batch of eggs. These two trays, together with my microworm cultures, are all that I have ever used to feed the many killifish and non-killie fry that I have raised successfully over the years. Still, what I appreciate the most is their silence.

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A Treatment for *Camallanus*

Charles H Harrison

The first time I saw this parasite was in a Convict cichlid back in 1970. It was a stranger to us then, but since that time, I have lost several fish to this parasite. The following article has been written for the killifish enthusiast, but it certainly applies to all the tropical fish hobby.

Camallanus is an intestinal parasitic infection. This malady is due to a nematode or parasitic worm which is found in wild fresh and salt water fishes throughout the world. The infestation is characterized by, among other things, the presence of tiny red thread structures protruding from the anus of the infected host fish. Close inspection of the victim shows a swollen and irritated vent area. The worms actually protrude 1/3 to 1/2 inch from the anus of the infected fish. This is about one fourth to one third of the worm's length. The worms are red from the victim's blood in their gut.

As with most parasites, there are two main body functions of this worm... reproduction and nourishment. The attachment inside the fish's gut is by what appears to be a simple row of long hooks. Closer examination reveals a burr like structure. Attachment to the intestinal wall causes considerable damage to the host's intestines.

The remainder of the body of the worm is dedicated to reproduction. Microscopic larvae from this parasite are constantly being produced and dumped into the water. A secondary host is necessary to carry on the life cycle. But, once established in the copepods found in most of our tanks, the nematode infection is easily transferred from hobbyist's aquarium to aquarium with nets, feeding utensils, water change devices and simple aspiration of splashes and spray from one tank to another. This carries the infection rapidly around the hobbyist's fish room in a matter of days.

The early signs of the parasite's presence are typically unnoticed. The victims show a minor swelling of the belly and may go off their food. Usually the fish have no problem laying eggs until the infection has reached the stage of protrusion from the anus. In the end, the anus and reproductive organs of the fish become so irritated and inflamed that secondary infection sets in and swelling causes enough hemorrhaging for the victim to bleed to death or die from internal bacterial infection. In addition to this obvious outside appearance, the worm has been found in the body cavity of infected hosts. This may explain some of the mysterious deaths one sees in fishes which have been successfully treated for the nematode. The adult worm has died inside the body cavity and decayed inside the host fish.

We Now Seem to Have Found a Sure Cure! Ken Laidlaw, <kl@jach.hawaii.edu> made a posting on the KILLIE-List a couple of months ago in response to someone who announced that they "had a fish with worms sticking out from its anus."

Ken said he had used a substance known as levamisole to kill these awful creatures. I wrote to him and he back to me and we came up with a solution for an infection I have found in some of my fish. On Thursday, 26 Sep 1996 08:13:48 -0400 (EDT) I received this posting:

"Hi, Charles, Here as promised is the recipe for *Camallanus* treatment.

1. Add 1.5 milliliter per 7.5 liter of 7.5% Levacide (levamisole hydrochloride) to each tanks to be treated. Mix the drug in a liter of water before pouring over the tank surface. If you obtain another strength of the drug (e.g. 1.5%, 3%) then just adjust dosage as required. For 1 .5% add 1 ml in 1.51 of tank water.

2. After 24 hours perform 100% (as much as possible) water change whilst vacuuming the gravel. The water change is not required due to toxicity but to remove the possibility of any offspring in the gravel.

3. No further treatments should be necessary.

Good luck and please let me know how you get on. Feel free to pass this information to whoever you like.

Regards, Ken L"

Well, I went to work to find the medicine. Actually as a chemist, I went to work to find the compound which Ken talked about, and I did. One of our dog friends is a Vet tech, and has been active enough with her group to be able through networking to come up with several large pills about the size of the end of your thumb. The pills weighed 3000 mg each. The activity of each was stated at 185 mg per pill. This didn't sound very logical, but I went on this assumption for dosage in my tanks- one and a half pill per 4 gallons of water. You couldn't see through the tank! Egad, what a mess!

Inadvertent introduction of parasites jeopardizes prized killies like this *Pterolebias zonatus*
Photograph by D. Straker

Intensity of color and spreading of fins is a sign of good health in killies
Photograph by T. Terceira

The evening of the next day the worms protruding from a trio of Gularis were over half gone. I netted out one of the females and examined her vent for the pests. The worms fell out into the net. The individual worm was about I cm long, red and transparent. The head end was somewhat bigger around than the rest of the body. The gut was red and visible with simple hand lens magnification.

I was elated. These fish had come through a lot and they were some of the nicest Blue Gularis I have had in several years. I really wanted to see them survive. We had success. This was not the first time I had come across this parasite. Several years ago I got some fish from a fish store that had come in with some plants from Florida. The little fish gave this parasite to my fish room and in a matter of a week, I wound up flushing three 30 gallon tanks of various tetras and killies. I am very glad to have found a cure.

The actual story goes like this: There were several bags in a fish shipment to SLAKA. Several specimens showed the outward signs of the parasite. Trevor Meyer and I shared most of the fish of this shipment. Several adults which were stressed by the shipping were showing the effects of the parasite. But, this time we had relief, the Levamisole had done the trick and eliminated the worm completely. I wish I had known of this chemical back when I lost all those *C. adloffi* and Black Tetras. That was devastating!

Back to the story. Trevor was about to flush the victims of this infestation. His specimens were smaller (*A. cognatum* and *A. fulgens)* and even more stressed than my Blue Gularis and *Fp. deltuense.* I gave him three of those pig pills and told him to:

Rx Crush one of them into as fine a powder as possible and dissolve the powder in a cup of tank water. Add this mess to the 2 1/2 gallon drum bowl the *A. cognatum* were in. Add some air with an air stone or tubing and feed them with live food. Change their water after a couple of days and let's see what happens.

Well, the worms vanished! The fish are up eating like crazy and spawning again three days after the treatment. Trevor is happy, the fish are saved, and SLAKA is moving forward saving fishdom and the hobby from those awful parasites which hang from their victims' anus and slowly kill them from any number of things.

Since the treatment has worked so well, I decided to get some of this compound in its pure form and keep it around for others as well as myself. A medical chemical company which we deal with sells levamisole hydrochloride in 50 gram containers. I split this up into plastic bags to contain 5 grams each. The treatment dosage is 1.5 ml of a 7.5% solution in 2 gallons of water. If one dissolves one of these five gram packets in 3 ounces of water, that's about a 5% solution. Two milliliters of this 5% solution will treat 2 gallons of tank water. Eighteen to twenty drops makes a milliliter or about one medicine dropper full. One ml per gallon. This provides a 15 milligrams per liter treatment bath.

This 5 gram packet will stay good for at least a year if it is kept dry. The solution is good for ninety days. Five grams will treat 100 gallons of tank water. It is hard to overdose with this chemical. The fish in our study showed no side effects at all. Close examination of the tank water showed no effect on the protozoa or other tank flora either. The only thing which seemed susceptible was the parasites. The broad spectrum anthelmintic action of this chemical makes it a welcome drug in my fish room.

The "Complete Book of Dwarf Chichlids" by Hans-Joachim Richter, published in 1989 by TFH, reports,

"An illness that almost always appears only with wild-caught fishes and that one at first does not regard as an illness, is the infestation with nematodes. Only after some months can one observe the at first just barely a few millimeters-long nematodes hanging out of the anus. With increasing growth, these can extend up to 10 millimeters outside of the body. It is generally a question of worms when we observe such an infestation. Despite the use of the most diverse measures, I was not able to control them. One should therefore remove and destroy infected fishes at the first sign of infestation. The following symptoms apply to a nematode infestation: emaciation with complete loss of appetite, rubbing, and slimy, whitish excrement. Whereas one can already suspect a *Camallanus* infection by the worms hanging out of the anus, other nematodes can be detected only through the microscopic examination of the excrement or the intestinal contents. Treatment is possible using food animals soaked in niclosamide.

Other authors have stated that the nematode infection is impossible to cure and fishes

should be destroyed and the tanks which have contained them should be sterilized."

The actual transfer of the nematode from fish to fish must occur in stages. There are five stages of development required to molt in the end into the reproducing adult. An intermediate host is required. The usual one identified is the cyclops. Other intermediate hosts have been conjectured - daphnia have not been ruled out. The worm may reach several inches in length. Those normally found in our tanks in our fish would kill the host long before they reached full size.

The attachment of the creature to the fishes' gut is from pinching from long structures of the head of the nematode. These longitudinal ribs grasp the folds of the gut and are capable of considerable damage if pulled from the host either by the aquarist or another fish looking for a wormy meal.

Considering the treatment and testing we have been able to accomplish, it would appear that we could recommend a prophylactic swim in a concentrated solution of levamisole to help rid the fishes of suspected infestations of various nematodes which includes the *Camallanus.* One should observe the victim for several days after treatment, however, as the full impact of the infection may not be seen immediately. Dead worms inside the fish may cause future mortality. The treatment dosage which is recommended for cattle and sheep ranges from 2 milliliters of 5 % solution per 100 pounds of body weight for cattle and 2 ml of a 1.4 % solution per 50 pounds of body weight for sheep. There is a statement on the package of levamisole which indicates that cattle and sheep which are maintained under conditions of constant exposure or renewal exposure should be re-treated within two to four weeks after the first treatment. This re-treatment of the affected tank and or victims sounds like a very good idea to aid in the complete removal of the parasite. Cattle are not to be treated with 48 hours of slaughter for food which would indicate that you should not eat your killies after you treat them with this stuff unless you first destroy the chemical.

Levasole (levamisole hydrochloride) is a broad spectrum anthelmintic and is effective against the many nematode infections in cattle and sheep. I maintain a supply of this chemical if it can not be found at your local drug store.

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The Manual of Fish Health, C. Andrews. A. Exell, N. Carrington, Tetra Press, 1988 (pp 176,80).
Parasitic Worms of Fish, H. Williams, A. Jones, Taylor & Francis, 1994 (pp l39,40, Fig. 2.33.1-9

Additional information regarding the nasty worm has come from Marty Greenwell of the J. G. Shedd Aquarium in Chicago, private communications. (Thank you Marty.)

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Killifish in the Community Tank
Some Observations

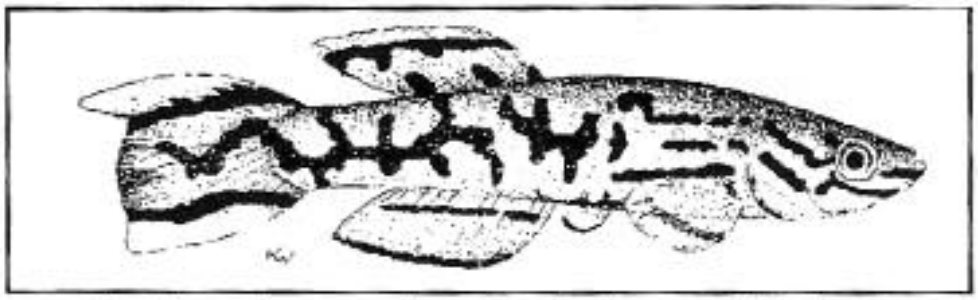
Jim Warner

While most of my killies are in breeding set-ups in the fish room, occasional impulse buys and home bred juveniles end up in one or another of my four community tanks which are roughly divided into fish of maximum size: under 5 cm., 5-7 cm., 7-10 cm., and over 10 cm.

I have found that *Aphyosemion australe, Chromaphyosemion bitaeniatum* and *Chromaphyosemion spendopleure* are all well behaved with most small fish (e.g. Cardinal Tetras, Cherry Barbs, Bumblebee Gobies, etc.) In a four foot tank at 79°F, though the *australes* did take exception to the *Poppendata furculata,* which I put down to the wagging tail of this fish while holding station in flowing water.

Aphyosemion gabunense and *A. striatum* are less well behaved, nipping any fins which come within reach, and, in the case of *A. gabunense,* pursuing the unfortunate victim for some distance. Since my *A. striatum* have always tended towards the upper reaches of the tank, most of the other fishes were spared the continued harassment, but I found that marble hatchetfish didn't have much of a tail left after a week or so in their company.

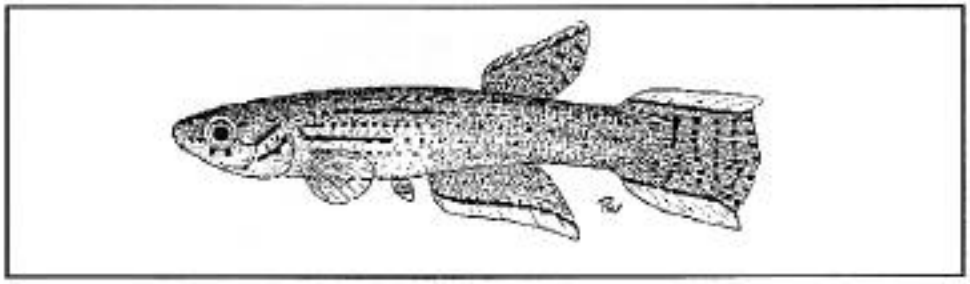
Surprisingly, *Fundulopanchux spoorenbergi* and *Pachypanchax playfairii* juveniles (5 cm. and 3.5 cm. respectively) did not cause too much trouble in this tank, though the *Pachy. playfairii* have now been moved to a tank with larger fish as a precaution.



Fundulopanchax spoorenbergi, Aquarium import 1974, Variety A
Sketch by Ruud Wildekamp

But now one of my early big mistakes, *Epiplatys zimiensis* is in this same tank. I introduced two pairs approximately 4.5 cm. in length, and all seemed fine for a couple of months. Then the bodies started to appear. Being a beginner, I didn't catch on until one evening I noticed three *Ep. zimiensis* lurking with intent at the bottom of the tank... an odd place to see them. I then noticed a flurry of activity under a piece of bogwood and on investigation found the other *Ep. zimiensis* shaking a male guppy vigorously by the tail! Needless to say, the culprits were moved on to play with the big boys after that.

In a two foot tank for very small species (e.g.. Spotted Rasboras, Gelius Barbs, Sparkling Gouramis, etc.) I have kept the following species at various times (without aggression); *Aphyosemion dargei,* juvenile *A. calliurum, Micropanchax macrophthalmus* and *Pseudoepiplatys annulatus.*



Aphyosemion calliurum (Edea, western Cameroon)
Sketch by Ruud Wildekamp

In another two foot tank, along with a pair of *Melanotoenia bosemanni* and a flag cichlid, I am currently keeping *Pachy. playfairii* males (4 cm.), *A. marmoratum* males (4 cm.) and *A. striatum* females (3 cm.) in apparent harmony.

The most embarrassing combination was putting a male Siamese fighting fish in a tank witch already contained a pair of *Fundulopanchax gardneri* Biassa. It took thirty seconds for them to notice the newcomer with the long tail and for the next minute they chased it relentlessly around the tank (in spite of being half its size) until I could rescue it! The image of a tattered, shell-shocked betta being lifted to safety in a net with the male *Fp. gardneri* hanging onto its tail like a dog with a bit of rope will serve as a constant reminder that one must carefully consider community tank mates.

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