



# KILLI-DATA INTERNATIONAL

## *Killi-Data News*

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## Editorial

This is the start of Killi-Data News' second year. In this first issue of the year we have the usual review of research publications as well as input from Martin Reichard on his lab's *Nothobranchius* research.

Martin is responding to my reviews of his lab's work in the previous edition. I am serious about making sure the content in this newsletter is reliable but I erred in the previous edition and Martin has written extensively to correct my mistake in the section "Erratum". This reply is welcomed and owed to readers. I must confess that I don't know everything and my area of scientific expertise is mostly restricted to histology and protein chemistry. When I comment on subjects outside of my area of expertise I tread on unfamiliar ground and am not much more expert than someone randomly selected off the street. For this reason we still need to expand the pool of reviewers for Killi-Data News.

I am happy to report that Andy Patel and Manuel Zapater Galve have volunteered to review papers for us. Andy brings with him expertise in animal behavior and Manuel in physiology and ecology. We still need reviewers to eco-toxicology.

Andrei Tatarenkov is reviewing a paper on *Kryptolebias*. Andrei is the expert on these fish and right now there is a need for expert opinion on these fish. It was hoped that Andrei would tell us a bit about *Kryptolebias* phylogenetics and address the issue of the ranges of *K. hermaphroditus* (sensu Costa) and *marmoratus*. As it turns out Andrei et al have a

manuscript in production and have decided to hold off until after the paper's publication to comment on this subject.

There has been an appeal by Prof Ryan Earley for help with a large evolutionary ecology study he and collaborators are undertaking. His letter follows in the next section.

There aren't a lot of papers this quarter due to the cut-off for inclusion in the newsletter. We had decided to run from December first up until February first for the three months of the Spring quarter. As consequence this issue is a bit thin but already there has been a flurry of papers published from the second of February. The next issue will be much thicker.

[Tyrone Genade]

## Letters to KDI

From Prof Ryan Earley of the University of Alabama, [rearley@ua.edu](mailto:rearley@ua.edu).

"Thank you again for agreeing to help us reach out to your readership in an effort to accumulate ecological data for our macroevolutionary examination of terrestrial jumping in Rivulidae! Our goal is to accumulate data for any species within the genera *Aphyosemion*, *Rivulus*, *Laimosemion*, *Cynodonichthys*, *Atlantirivulus*, *Melanorivulus*, and *Anablepsoides*. With these data, we will parameterize adaptive macroevolutionary models to determine which ecological characteristics have been important to the evolution of terrestrial locomotion (i.e. tail-flip jump) and related morphology in Rivulidae. I've CC'd my PhD student (Joe Styga, [jmstyga@crimson.ua.edu](mailto:jmstyga@crimson.ua.edu)) and collaborator (Jason Pienaar, [jason.pienaar@ua.edu](mailto:jason.pienaar@ua.edu)), who are driving the bus on the evolutionary analyses. The data that are of particular interest to us include:

- Measurements/observations of water level and/or tidal flux (i.e., how ephemeral is that habitat?)
- Flow rate

- Oxygen concentration
- Water temperature
- Salinity
- CO<sub>2</sub>/pH levels
- Notes on water borne pollutants
- Turbidity
- Abundance data (of 'target' species and any other species in or around their habitat)
- Feeding behavior (especially observations regarding terrestrial prey capture)
- Predator escape behavior (e.g. jumping onto land to avoid being eaten)
- GPS location (if available)
- Observations of eggs (location i.e. in water or out of water? number?)
- Habitat description (aquatic habitat, types and density of plants adjoining habitat, relationship to human activities, etc.).
- Description of capture location
- Description of terrestrial environment (muddy, leaf litter, rocky etc)

"However, this is not an exhaustive list. Any other information or ideas of other potential environmental factors contributing to variance in jumping performance in the family would be greatly appreciated. Although GPS data would be helpful, we should convey to your readership that this is not a requirement to participate in our study since this may be proprietary to their collecting operations. It would be highly valuable to have GPS information. If participants want to help but are wary about providing the data, we would have no problem indicating that GPS information will be kept strictly confidential and that we would not visit any of the sites without being accompanied by the person providing the data. We look forward to working with you and your readers!"

## Erratum

### Response to KD-News 1:4

Scientific papers use language that sometimes reads difficult to layman. This is because many of the terms used have specific meaning and using them makes the scientific communication shorter. The cost is that without a proper background, science writing may be hard to follow. In other cases, scientists would love to be more direct in their language, but scientific scrutiny requires strong evidence to support that something is indeed happening. Observations have to be verified by experiments, experiments have to be properly executed and then replicated etc. On top of that, all scientific papers pass through rigorous and tough reviews when at least two (but often more), anonymous colleagues double-check everything that is done and written in the article and require critical revisions before the article can be published. At the end, the text becomes full of diversions from the main story, additional clarifications, and refined and more cautious versions of original statements. This makes scientific articles indeed hard to read and may lead to misunderstanding of the content. Do not (always) blame the authors! More importantly, never blame people who have the hard job of translating the text to a more straightforward language! Things always tend to get lost in translation.

I would like to take this opportunity to provide some clarification of the summaries of recent killifish papers from my lab. For anybody who is interested in reading the primary scientific work, all is free to download as PDF on my website ([www.reichardlab.eu](http://www.reichardlab.eu)). I hope to be able to summarize outcome of our continuing work on killifish.

**Nezhybová, V.; Reichard, M.; Blažek, R.; Ondračková, M. (2017) Metazoan parasites of African annual killifish (Nothobranchiidae): abundance, diversity and their environmental correlates. Biotropica, DOI:10.1111/btp.12396.**

This paper basically describes natural parasite infections of *Nothobranchius* killifishes in Mozambique. We dissected fish directly in the field, using individuals collected from 21 populations of four species across relatively large area of the region that we regularly visit since 2008 during our long-term research to understand the evolution and ecology of those fishes.

Many fish parasites have direct cycles and are transmitted from one fish to another. Other fish parasites have more complex strategies and change their hosts during the life cycle. They often start with a free-living stage that hatches from the parasite egg and actively seeks an invertebrate (such as planktonic crustacean or snail) to infest. In that host, it multiplies by cloning itself into thousands copies. After growing to another infectious stage, it leaves its invertebrate host and search for a new one (or gets eaten with its host), to complete a next developmental step. In aquatic habitats, the second host is often a fish. In fish, the parasite develops further and then waits to be transmitted to the final host, be it predatory fish (catfish, for example) or a bird (heron, kingfisher etc.). It is the predator that is the final host of most such parasites. Only there, the egg production through mating between male and female parasites occurs, with the eggs leaving the host with its feces. And in annual fishes, those parasites with the indirect, complex cycle prevail massively. Naturally so, direct transmission from fish to fish is not possible when the habitat dries out.

Our study found that *Nothobranchius* are very heavily infested with parasitic flukes (trematodes) and serve as their second host, waiting to be eaten by a piscivorous bird. So, the parasite found in the fish are not really the worms pictured in books, but their earlier developmental stage (named metacercarium), a tiny bundle encysted in some fish organ. Killifish can be heavily infested indeed and we found poor little fish with hundreds of larval flukes in their muscles. Those larval flukes form the major part of *Nothobranchius* parasite fauna.

The important thing is that those parasites pose absolutely no threat to your fish breeding colony when incidentally imported from the wild. You would need a heron in your fish breeding system, and that heron would have to defecate into your tanks where, on top of all that, abundant planktonic crustaceans and snails must range.

Other *Nothobranchius* parasites were much rarer. Nematode worms (rarely observed in wild fish) could perhaps be imported to captivity and infect other fish, but cestodes (also observed in wild fish) have also complex life cycle that cannot be concluded in your fish room. Virtually all parasites were internal (endoparasites)—infecting internal organs. Ectoparasites (infecting skin, fins or gills) were not found despite being very common in most fish in permanent waters. As I said, this is understandable—they would not survive the dry period.

On the other hand, our study looked exclusively at multicellular parasites and fish were certainly infected by smaller bugs (microparasites) such as various viruses, bacteria and microsporidia. These would go undetected in our dissections, as they need a different method for discovery and quantification. I would argue that microparasites are likely much stronger threat to the hobby—but our study cannot tell anything about their natural occurrence.

The rest of the study basically shows that parasites infecting *Nothobranchius* fishes have wide spectrum of hosts overall. None of them really specialize on a single species and it is likely that they would also infest other small fish, such as cyprinids and alestids, should they live in the same pools. More species of parasites were found in larger pools with a rich community of invertebrates. But the absolute numbers of individual parasites, unlike the number of parasite species, were not predicted by any of the environmental characteristics we recorded. Instead, we think that a large number of flukes were found where infected birds simply defecated most, in a truly random way. An interesting find is that one of the fluke species infects killifish brain and makes them to adver-

tise themselves to birds—jumping from the water, resting flat in water lily leaves and avoid escaping bird attack. We look at that phenomenon in details and will hopefully be able to tell more (remember that an observation must be followed by experiments in scientific scrutiny!).

**Blažek, R.; Polačik, M.; Kačer, P.; Cellerino, A.; Řežucha, R.; Methling, C.; Tomášek, O.; Syslová, K.; Terzibaszi Tozzini, E.; Albrecht, T.; Vrtílek, M.; Reichard, M. (2017) Repeated intra-specific divergence in lifespan and ageing of African annual fishes along an aridity gradient. *Evolution*, DOI:10.1111/evo.13127.**

This paper summarized our 5 years long study to understand ageing process in annual killifish from the evolutionary perspective - with general implications for ageing science. You may have noted that annual killifish, especially African *Nothobranchius* and *Nothobranchius furzeri* in particular, serve as widely used model organisms in studies into understanding aging. To make these applied studies relevant to other organisms (including human), a lot of assumptions must be fulfilled. One potential caveat is that short lifespan does not equal rapid aging. The first assumption is that short life is associated with rapid aging, demonstrated as progressive declines in physical, physiological and cellular functions. Such declines in body functions must, in turn, be mirrored in the increase in mortality with age. As I said, short lifespan does not necessarily mean that aging is rapid. To simplify, mortality is composed of non-aging and aging parts. The non-aging part expresses the probability of the fish individual to survive over, say, a next month. It may be very high (95% of so) or quite low (30%) but if this percentage remains the same over the entire lifespan, it should not be called aging (it is rather called baseline mortality). It may produce fish with long or short lifespans. The key thing is that probability of death remains the same across all ages.

The aging part of the mortality indicates how this percent-

age changes with age. So, young adult fish, be it killifish for our example, may have high survival probability to the next month, say that 90%, over the six months of its life and then this probability to survive drops, to 70%, 50%, 25%, and then even 10% over successive months. This dramatic decline in the probability to survive to a next month is the real aging, in this particular case expressed demographically (or mathematically, in a sense). So, human live long lives but age rapidly; human survival probability is very high over most lifespan but declines dramatically after a certain age limit is reached.

The take home message is that short life does not necessarily mean rapid aging. The short lifespan may mean high risk of mortality overall, this has nothing to do with aging in the sense of a terminal decline in organism performance (which you may call health or susceptibility to functional failure). To conclude, annual killifish may die at a high rate without aging—this is observed in many organisms, for example many small birds living in your garden! Other assumptions follows, such as that aging pattern should be determined by genetic background, be it by the genes themselves or though the modes how the genes are regulated to express their function. At the end, it would be great to have a species that is comparable to aging in humans, this is what most people ultimately want to understand. But lets cut that story short for now.

In our study, we asked whether short-lived *Nothobranchius* killifish age rapidly, how the decline in survival rate is related to their health and how presumed rapid aging is related to the other aspects of their life history. So—first, *Nothobranchius* indeed do age rapidly, the risk of their death increases dramatically with age. Second, populations from relatively drier regions of particular species distribution (where the pools last predictably shorter time before they desiccate) age quicker than populations from a wetter part of their distribution. This clearly applied for 3 out of 4 studied species, with *N. orthonotus* being the exception with no difference in lifespan

and aging between the two contrasting regions. Third, fish health deteriorated with age, and more rapidly so in fish from the dry-region populations. Studied fish suffered from liver and kidney tumors, with quite a high incidence. The burden of metabolic waste arising from fighting external challenges (named oxidative stress) in fish organs (brains, heart and liver) increased with age. This rapid functional aging, and differences between the populations from dry and wet regions, was evident across all 4 study species.

Reproduction also become less efficient with age—problems with fecundity (egg production) and with sperm quality (and hence fertilization) are perhaps most apparent. Here, the outcomes were mixed. The number of eggs did not dramatically decrease with age and, in the longest-lived of the study species—*N. pienaari*—it rather increased. This is because fish fecundity increases with body mass and most fish keep growing throughout their lives, at least in terms of body mass. However, when we recalculated fecundity per unit of body mass, fecundity actually did decline with age. Fertilization success was also lower at the older age. Importantly, although this is not apparent from the final version of the paper, the declines were often terminal, with little change over the lifespan and abrupt decrease in fecundity and fertility at the really old age.

Fish that live short lives are expected to sexually mature quickly and reproduce as soon as they can—to be able to reproduce at all. This phenomenon is apparent when comparing species with contrasting lifespans. However, we wanted to know whether it applies also to differences between populations of the same species. We were surprised to see that the trend was very weak (valid only for *N. kadleci* and *N. pienaari*). Maybe, quickly growing species such as *N. furzeri* develop at such a rapid rate that they push the absolute limit with no easy way to further speed up the juvenile growth. Finally, we also did not find any difference in metabolic capacities and behavior of fish from the dry and wet regions.



To sum it up, we showed that (1) short lifespan in *Nothobranchius* killifish is related to rapid aging, (2) differences in lifespan are related to differences in functional problems, (3) these parameters are concerted and genetically underpinned, and (4) life history of those fish is perhaps already at the maximum and cannot be speeded up further. Summed up, *Nothobranchius* killifish age as we would expect from a normal vertebrate and are indeed good models for aging science.

[Martin Reichard]

## Interesting Websites

Killifish RS' Youtube channel again has many interesting videos. Recent videos show the habitat of *Spectroplebias costai*, new collections of *Pituna poranga* and *Maratecoara lacortei* as well as *Campellolebias chrysolineatus* male display behavior. The youtube channel can be accessed at [https://www.youtube.com/channel/UCglwiywhb\\_bh\\_iQBV\\_fPPgA](https://www.youtube.com/channel/UCglwiywhb_bh_iQBV_fPPgA).

[Tyrone Genade]

## In The News

### CRISPR, A Molecular Technique, Moves Science Forward In Woods Hole

[http://www.capenews.net/falmouth/news/crispr-a-molecular-technique-moves-science-forward-in-woods-hole/article\\_c92c152f-6a5d-504b-a553-fe2642e42841.html](http://www.capenews.net/falmouth/news/crispr-a-molecular-technique-moves-science-forward-in-woods-hole/article_c92c152f-6a5d-504b-a553-fe2642e42841.html)

CRISPR, the new method for genetically modifying organisms, is again being directed at killifish. This time the target is pollution resistant *Fundulus heteroclitus*. Dr Mark E. Hahn, senior scientist at the Woods Hole Oceanographic Institution to understand the function of specific genes in *F. heteroclitus* to find out how they control fishes' responds to chemical pollutants in the environment.

[Tyrone Genade]

### Combating iron in the brain: Researchers find anti-aging micromolecul

<https://www.sciencedaily.com/releases/2017/02/170214094040.htm>

New, unpublished results on how research of *N. furzeri* aging is illuminating how it is that iron accumulates in the brains of aging organisms is reported.

[Tyrone Genade]

### CRUDE HEALTH Mutant fish deformed by Deepwater Horizon oil spill shine light on how air pollution affects humans

<https://www.thesun.co.uk/news/2915723/mutant-fish-deformed-by-deepwater-horizon-oil-spill-shine-light-on-how-air-pollution-affects-humans/>

This new paper article reports on the effect of the Deepwater Horizon oil spill and its effects on, for example *Fundulus* embryos. The author draws parallels between the effects of the oil spill on the fish and humans exposed to atmospheric pollutants. The newspaper article refers to the paper by Brette et al Scientific Reports 7:41476, 2017, <http://www.nature.com/articles/srep41476>.

[Tyrone Genade]

### O pequeno rei da adaptabilidade. um peixe que vive no seco, respira pela pele e se auto-reproduz

[http://www.brasil247.com/pt/247/revista\\_oasis/280336/O-pequeno-rei-da-adaptabilidade-Um-peixe-que-vive-no-seco-respira-pela-pele-e-se-auto-reproduz.htm](http://www.brasil247.com/pt/247/revista_oasis/280336/O-pequeno-rei-da-adaptabilidade-Um-peixe-que-vive-no-seco-respira-pela-pele-e-se-auto-reproduz.htm)

This article (English translation of the title: The little king of adaptability. a fish that lives in the dry, breathes through the skin and self-reproduces) is a review of *Kryptolebias* biology emphasizing the adaptability of the fish to their environment. Google Translate does a good job of translating the article.

[Tyrone Genade]

## Review of new research publications

### Book Reviews

**The aging gap between species.** Ioviță A. (self published), 2016.

<https://www.amazon.com/Aging-Gap-Between-Species/dp/1517484812>

In this book the author reviews the aging rate and processes between species. In particular, several fish (including *Nothobranchius*) are compared to other species. I have not had the opportunity to read this book and can't comment on its contents.

[Tyrone Genade]

### Systematics, Taxonomy & Distribution

**The *Rivulus* of Guatemala: *Rivulus tenuis* Meek 1904, *Rivulus godmani* Regan 1907, and *Rivulus degreefi*, sp. nov.** Collier GE. *Journal of the American Killifish Association*, 49:34–47.



Male (above left) and female (above right) *Rivulus tenuis* Taco Talpa. Photo by Frans Vermeulen.

The author analyzed the cytochrome B, 12S and 16S rRNA gene sequences of fish ascribed to *Rivulus tenuis*. The resulting phylogeny revealed the existence of three distinct genetic lineages. Fish of the Alta Verapaz area and adjacent areas

of Mexico separate out from *R. tenuis* which is restricted to lowland coastal streams of southern Mexico, Yucatan and Belize to northwestern Honduras. The Alta Verapaz populations correspond with *Rivulus godmani* Regan 1907 which had previously been synonymised with *tenuis*. Collier resurrects *Rivulus godmani* Regan 1907 as a distinct species.



*Rivulus godmani* Tamarandito. Photo by José Poves ©2017.

A new species, *Rivulus degreefi* is described, coming from small puddles adjacent to the Rio Oscuro of the Rio Polochic Valley in Guatemala. (The location is variably stated as Rio Oscuro or Rio Oscura in the text of the paper. Google maps identifies a Rio Oscuro draining into Lago de Izabal.) The new species is differentiated based on adult coloration. Males have distinct irregular rows of orange spots along their sides. The caudal fin has a broad light margin and thick black sub-marginal band along both the upper and lower edge. Females have brown spots giving rise to a reticulated pattern. The new species is named for Jaap-Jan DeGreef who collected the type specimens.

[Tyrone Genade]



Male (above) and female (below) *Rivulus degreefi* Rio Oscuro, Guatemala. Photo by Anthony C. Terceira ©2017.

**Three new species of the killifish genus *Melanorivulus* from the central Brazilian Cerrado savanna (Cyprinodontiformes, Aplocheilidae)** Costa WJM. *ZooKeys*, 645:51–70, 2017. DOI: <https://doi.org/10.3897/zookeys.645.10920>.

The greatest diversity among species of *Melanorivulus*<sup>1</sup> endemic to the Cerrado is concentrated in the central-western Brazilian plateaus, which range in altitudes from 400 to 1,100 m above sea level (asl), in the Caiapó mountain range

(Costa 2012). This area is drained by the upper tributaries of the Rio Araguaia, flowing north and belonging to the Amazonas-Tocantins river system, and the upper Paraguai and Paraná river basins, flowing southwest and south, respectively, and belonging to the Paraná-Paraguay-Uruguay river system. During a recent expedition to this area, three new species were collected, one from the upper Araguaia basin and two from the Paraguai basin. This new species belong to the assemblage called *M. dapazi* species group, endemic to the Paraguai basin, that can be easily recognized by a dark reddish brown stripe on the distal margin of the anal fin in males. Micro-morphological and proportional differences within this assemblage, as well as caudal fin coloration, can be used to differentiate the species: *Melanorivulus ignescens* is distinguished from all other species of the *M. dapazi* group by having the anal fin, in adult males, bright reddish orange (vs. yellow in *M. dapazi*, *M. flavipinnis*, and *M. regularis*). *Melanorivulus flavipinnis* differs by the presence, in males, of seven or eight narrow red bars on the caudal fin, irregularly shaped and sometimes interconnected (vs. less in number or absent in other species of this group). *Melanorivulus regularis* is distinguished by the presence in males, of five or six dark reddish brown, regularly shaped and never interconnected bars on the caudal fin (vs. seven or eight narrow red bars in *M. flavipinnis*; four or fewer short rudimentary bars, sometimes absent, in *M. dapazi*; bars always absent in *M. ignescens*). All the new species are known only from the respective type localities. *M. ignescens* from a small stream tributary to the Rio Bandeira, Rio das Garças drainage, upper Rio Araguaia basin. *M. flavipinnis* from a small stream tributary to the Rio Anhumas, Rio São Lourenço drainage, Rio Paraguai basin. *M. regularis* from Ribeirão da Sobra, an upper tributary of the Rio Itiquira, Rio Paraguai basin. Costa (2016) discussed the importance of using live colour pattern characters to diagnose

<sup>1</sup>KDI considers *Melanorivulus* as a subgenus of *Rivulus*.



species and species groups of *Melanorivulus*, showing high congruence with molecular data. Particular attention was given to patterns involving the caudal fin, which contained a high concentration of phylogenetically informative characters, useful to delimit most species of the *M. zygometes* group. It shows that colour patterns documented from live fish is an accurate tool to recognize species of the *M. dapazi* group.

#### Literature cited:

Costa, Ichthyological Exploration of Freshwaters 23:211–218, 2012.

Costa, Ichthyological Exploration of Freshwaters 27:107–152, 2016.

[Stefano Valdesalici]

***Anablepsoides chapare*, a new killifish (Teleostei: Cyprinodontiformes: Cynolebiidae) from central Bolivia.** Valdesalici S & Gil J. *Aqua, International Journal of Ichthyology*, 23:11–18, 2017. URL: [http://aqua-aquapress.com/product/23-1\\_anablepsoides-chapare/](http://aqua-aquapress.com/product/23-1_anablepsoides-chapare/)

*Anablepsoides chapare*<sup>2</sup>, is here described from a small stream in the Isiboro River drainage, tributary of Mamoré River, Amazon basin, central Bolivia. It is a member of the *A. limoncochae* species group and differs from all the other species of this assemblage by its rounded caudal fin, by males having five thin longitudinal stripes of same width extending from caudal-fin base up to humeral region, by dorsal fin with white coloration at proximal portion and light blue pelvic fins (and other minor morphological differences). *A. chapare* seems to be more related to *A. christinae* and *A. luitalmiae* (by coloration and morphology) both occurring at the Rio Madeira basin. *Anablepsoides chapare* is the only formally described species of the genus occurring in Bolivia apart from *A. beniensis*, which is also known from the Rio Madeira basin, but clearly differs from this latter species, belonging to a different

<sup>2</sup>KDI considers *Anablepsoides* as a subgenus of *Rivulus*.

species group. *Rivulus corpulentus* Thomerson & Taphorn is herein transferred to the genus *Anablepsoides* and considered as belonging to the *A. limoncochae* species group by the presence of scales on chin and contact organs on body scales and an oblique stripe on middle of the dorsal fin.

[Stefano Valdesalici]

**Redescription of *Nothobranchius lucius* and description of a new species from Mafia Island, eastern Tanzania (Cyprinodontiformes, Aplocheilidae).** Costa WJ. *Zoosystematics and Evolution*, 93:35, 2017. DOI: <https://doi.org/10.3897/zse.93.11041>

This review may come across as being confrontational and negative and I would like to avoid such a misunderstanding. I value Costa's work and believe he has and is still developing many valuable tools for taxonomic analysis of killifish. This paper is no exception. The strength of these tools is gaged by how well they stand up to criticism and scrutiny. It is the job of scientists to ask difficult questions of the methods and results. This is not simply to dismiss the result but to strengthen the result in the eyes of other scientists. The harsher the criticism the method and results survive the more certain we can be of the validity of those results.

In this paper Costa describes a new species, *Nothobranchius insularis*, and redescribes *N. lucius* to exclude the new species. The *N. melanospilus* group is also redefined to include: *elongatus*, *hengstleri*, *insularis*, *interruptus*, *jubbi*, *krammeri*, *lucius*, *makondorum* and *melanospilus*.

*N. insularis* is diagnosed from *N. lucius* based on it having smaller premaxillary teeth compared to the inner maxillary teeth; *N. lucius* has an inner row of teeth that are inward facing; *N. insularis* has a subtruncate caudal fin (vs rounded caudal fin); the females have vertically elongated dark dots arranged in oblique rows (vs round dots arranged in horizontal rows); dark gray dots on the unpaired fins in females that

are restricted to the basal portion (vs extending over most of the fin); as well as shorter caudal, pectoral and pelvic fins. *N. insularis* has three neuromasts in the posterior section of the anterior supraorbital series vs two in *N. lucius*. Differences in the length of the jaw are also described.

Costa discusses errors regarding fin ray counts in the Wildekamp et al description and notes the possible reason for these errors. The small sample size for the new descriptions and that only two populations of the fish were examined raise concerns about the validity of the new species. How many of each species were used for the jaw comparison isn't clear—especially for the differences in dentition which seems an important diagnostic character. The nature of the female color pattern is also in question. Photographic evidence (pages 11 and 12) from both locations shows variation with respect to color and shape of the spotting as well as the pattern of spotting. Also, there is variation in the shape of the tail and jaw length. Populations from the Mbezi and Ruhoi River basins were not examined by Costa. *N. lucius* is a highly variable species and many of the diagnostic criteria given by Costa could be intrapopulation variation present in both species. In the phylogeny of Wildekamp et al the three specimens (from the Ifakara, Kiziko and Mafia Island) group together with the Mafia and Mbezi fish shown to be more closely related to each other than to the Ifakara specimen. In the phylogeny of Dorn et al there is no specimen from the Kilombero-Rufiji River area but the relationship pattern is inverted, with the Kiziko and Kinungamkele (from the Mbezi-Triangle, Watters, *in production*) fish being basal to the Mafia Island population whereas the Mafia specimen is basal in the Wildekamp et al paper. The Kiziko TAN RB 05-47 population sampled by Dorn et al has the same elongated body form as *lucius*. The phylogenetic analysis suggests a close relationship. If the Mafia Island fish are distinct then they should form a branch apart from Kilombero-Rufiji and Mbezi-Triangle fish (which would group together on their own branch).

There are limits to the DNA phylogenetics. Very few indi-

viduals were sampled for the phylogenetics and it isn't clear how variable the DNA is within and between populations. More intense sampling may reveal that the Mafia Island fish described as *N. insularis* are genetically distinct. A detailed study of more fish and more populations from multiple river basins is needed to affirm *N. insularis* as a distinct species. Behavioral data and principle component analysis would also be helpful in this matter. The jaw structure and dentition is a very interesting character and confirming it as a diagnostic character would be very useful in later research to discern cryptic species. (The fish of the *makondorum* species also show high interpopulation variation: could there be cryptic species hidden among the populations?). While the *Nothobranchius* community was very skeptical of the description of *ruudwildekampi* the osteological (Reichenbacher et al) and behavioral research (Reichard et al) affirmed *ruudwildekampi* as a distinct species. Similar research for *N. insularis* would help affirm the position of this species as a distinct species from *lucius* and validate the anatomical methods employed by Costa. These methods can then be used on other species groups.

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Note: while Killi-Data does not follow immediately and automatically generic changes, Killi-Data considers automatically a new species as valid until further scientific evidence and new studies are encouraged on that new species.

[Tyronne Genade]



*N. insularis* Mafia Island TAN 02-4, Brian R. Watters © 2002



*N. insularis* Mafia Island TAN 02-5, Brian R. Watters © 2002



*N. lucius* Ifakara TAN 95-4, Brian R. Watters © 1995



*N. lucius* Luhule River TAN 02-8, Brian R. Watters © 2002



*N. lucius* Lupiro TAN 00-15, Brian R. Watters © 2000

An array of photos of female fish of *N. insularis* and *lucius*. The Ifakara, Lupiro and Narubungo populations are from the Kilombero Valley area; the Luhule River populations are from the coastal part of the “Mbezi Triangle”. Note the arrangement and shape of the spots on the flanks of the females. There is no evidence of a pattern of oblique rows or horizontal spots being typical of either species. Similarly there is no pattern or difference in the density of the spots in the unpaired fins. There is considerable inter- and intrapopulation variation in the pattern and color of spotting and these are probably not useful characters for separating the two species. All photos are of wild caught fish.





An array of photos of male fish of *N. insularis* and *lucius*. There is no obvious difference in the shape of the caudal fin. Male morphology is variable with some populations more elongate than others. For example, compare TAN 00-13 with TAN 02-24, both from the Kilombero River drainage from sites that are close to one another. Similar variation is apparent in the fish of the Mbezi Triangle based on photos of the males of the Kiziko TAN RB 05-47 and Kinungamkele TAN RB 05-48 as well as Ruhoi River TZN 09-10 populations. All photos are of wild caught fish.



## Killifish Biology: Ecology & Physiology

**Catálogo ilustrado de los especímenes tipo de peces cubanos II (Osteichthyes, clase: Actinopterygii: Cyprinodontiformes, Gadiformes, Lampridiformes, Mugiliformes, Myctophiformes, Ophidiformes).** Faloh-Gandarilla I; Alvarez-Lajonchere L; García-Machado E; Gutiérrez De los Reyes E; Orozco M; Cortés R; Alfonso Y; Lemus E; Corrada Wong RI; Chevalier-Monteaigudo P; Osoria RP; & Álvares IL. 2016. URL: <http://revistas.geotech.cu/index.php/poey/article/view/145>.

This paper lists the following species of killifish as being present on the Island of Cuba: *Fundulus saguanus*, *Cubanichthys cubensis*, *Rivulus cylindraceus*, *Riv. berovidesi* and *Kryptolebias marmoratus*. The following information is provided for each species: Synonyms, Common names, Holotype, Paratype, Depositary Institution, Locality of collection (with latitude and longitude), Collection date, Title of the paper where it was originally published, Original reference and Other references. A list of livebearers is also included.

[Tyrone Genade].

**Life history of the Mediterranean killifish *Aphanius fasciatus* in brackish water habitat of Algerian low Sahara.** Guezi R; Chaoui L; & Kara MH. *Environmental Biology of Fishes*, Epub ahead of print, 2017. DOI: <http://dx.doi.org/10.1007/s10641-017-0578-1>

The authors set out to understand the life history of this species in the Algerian low Sahara. They collected almost 1868 specimens and determined age by scalimetry. The populations were composed of 6 age classes. Spawning is reported as occurring only between February and July. The fish mature at 45 mm.

[Tyrone Genade]

Lake Ayata is located near the national road No. 3 about 6km after Djamaa in the direction of the town of Touggourt. The site has an area of about 155ha with an average altitude

of 31m. It is delimited by the following geographical coordinates: Longitude 33°29'17"N and 33° 29'48"N; Latitude 05°59'10"E and 05°59'37"E (Figure 1). The site is located near the town of Sidi Amrane (Daïra of Djamaa) 150km west of the wilaya (department) of El Oued (Souf). Other populations of *A. fasciatus* are known from springs in the Maghreb (i.e. including Tunisia and Algeria) and some taxa have even been described and are today in the synonymy of *fasciatus*, like *A. desioi* and *A. thermarum*.

[Jean Huber]

**Length-weight relationships, population structure and body condition of *Aphanius anatoliae* (Cyprinodontidae) and *Pseudophoxinus ninae* (Cyprinidae) living in Karaevli Lake (Burdur-Turkey).** İnnal D; Aksu M; & Giannetto D. *Review of Hydrobiology*, 9:73–83, 2016. URL: <http://www.reviewofhydrobiology.org/page/pdf.asp?pdf=9-2/9-2-1-Full.pdf>

The authors surveyed populations of *Aphanius anatoliae* of Karaevli Lake in Lake District Region in south-western Anatolia. They captured 107 individuals and report a size range of 2 to 5.3 cm and that the fish were in good condition. Length-weight relationships were calculated for the fish. The authors note that the lake itself dries out during the course of the year and the fish are then restricted to springs that feed the lake. The fish are imperiled by road building for peat extraction.

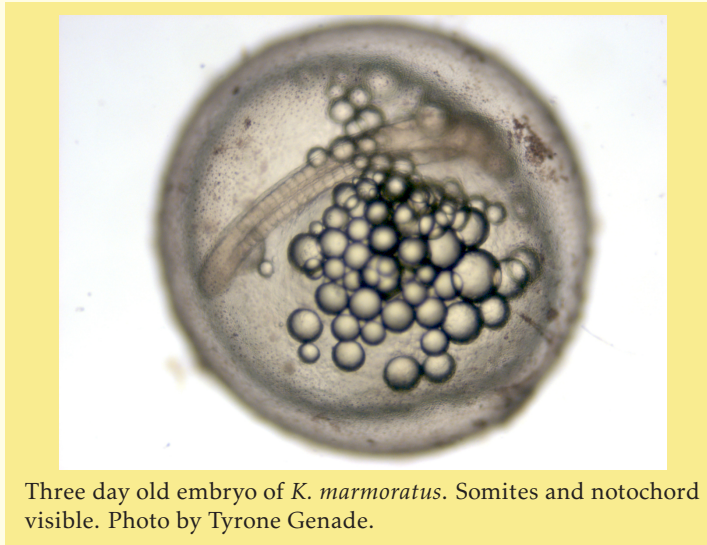
[Tyrone Genade]

**The use of *Kryptolebias marmoratus* eggs as an educational tool for embryology education.** Genade T. *Bioscene*, 42:26–31, 2016. URL: [http://www.acube.org/files/9314/8477/0940/Bioscene\\_December\\_2016\\_issue.pdf](http://www.acube.org/files/9314/8477/0940/Bioscene_December_2016_issue.pdf)

The author describes the use *Kryptolebias marmoratus* in the class room to teach embryology. He notes that the embryos are large and can be viewed by means of a light microscope without need to harm the organism. Eggs are easily obtained

from captive fish and are easy to display to students. Embryological features common to early human development (and the development of other vertebrate phyla) are readily visible in the *K. marmoratus* embryos: early brain development, eye development, somites, limb buds, blood circulation and pigment cell migration. Embryos also twitch when exposed to light and the beating heart is readily viewable. Use of the embryos in a class room has proven rewarding, students marvel at observing embryological development and are more eager to consider the common embryology and evolution of chordates.

[Tyrone Genade]



Three day old embryo of *K. marmoratus*. Somites and notochord visible. Photo by Tyrone Genade.

**Diversity, distribution, and significance of transposable elements in the genome of the only selfing hermaphroditic vertebrate *Kryptolebias marmoratus*.** Rhee JS; Choi BS; Kim J; Kim BM; Lee YM; Kim IC; Kanamori A; Choi IY;

<sup>3</sup>In an early draft of this paper, which was published online, the strain used by Jae-Seong Lee was stated as originating from Panama. This error was corrected during peer review.

Schartl M; & Lee JS. *Scientific Reports*, 7:40121, 2017. URL: <http://www.nature.com/articles/srep40121>

Mangrove rivulus *Kryptolebias marmoratus* and its closely related sibling *K. hermaphroditus* are unique among vertebrates in that they reproduce predominantly by self-fertilization. Repeated selfing leads to the loss of heterozygosity, so that many fish in natural populations are isogenic (i.e. have identical homologous chromosomes). Progeny of such isogenic fish are genetically identical among themselves and to their parent. This feature attracted attention of experimental biologists, because it allows a complete control of genetic background. The growing popularity of mangrove rivulus as a model species stimulated sequencing of its full genome independently by two research groups. One version of the genome (strain RHL from San Salvador Island, Bahamas) was presented by Joanna Kelley and coauthors in 2016. Another version, reviewed here, is produced by a team led by Jae-Seong Lee using a strain of unknown origin (SK)<sup>3</sup>. Apparently, this is the same strain that was used to generate the first complete mitochondrial genome of *K. marmoratus* in 2001 by the senior author. In order to determine the geographic origin of the strain, I compared it to collections of *Kryptolebias* from the Caribbean and Brazil using several mitochondrial genes. The SK strain groups with *K. marmoratus* from Florida. (It definitely does not belong to a distinct *Kryptolebias* lineage inhabiting southern Cuba, Turks and Caicos, Puerto-Rico, and Panama, that is more related to *K. hermaphroditus* than to *K. marmoratus*). Haploid genome size of the SK strain is estimated to be 680 million base pairs (Mb), which is similar to the estimates of 654 Mb and 642 Mb by Kelley et al. (2016) and by Mesak et al. (2015), respectively. The ultimate goal of a genome assembly is to produce continuous nucleotide sequences that would correspond to chromosomes of an organism. However, genome assembly is an inherently complex

procedure, typically resulting in numerous fragments (contigs and scaffolds) each representing just a fraction of a chromosome. Genome of the SK strain is quite advanced in this regard, being assembled into 3072 scaffolds varying in size from about 4 kb to nearly 12 Mb. Half of the *K. marmoratus* genome is represented by scaffolds that are at least 2.2 Mb long, as measured by statistic N50. Sola et al. (1997) found that haploid chromosome number in *K. marmoratus* is 24. This number is consistent with the number of linkage groups on genetic map as determined by Kanamori et al. (2016) using molecular markers. The availability of the genetic map makes arrangement of the scaffolds along the linkage groups a relatively easy task. Over 98% of markers used to construct the genetic map could be assigned to scaffolds. The sum of the sizes of scaffolds mapped to a particular linkage group gives an estimate of the chromosome size, which ranged from 18 Mb to 47 Mb. One complication, however, is that many scaffolds are mapped to more than one linkage group, making their arrangement uncertain. A strong feature of the presented genome is its detailed annotation. Overall, the authors identified 20954 genes, 643 tRNAs, and various other features of the *K. marmoratus* genome. One interesting observation is that the *K. marmoratus* genome is riddled with diverse transposable elements (TEs), or ‘jumping’ genes, amounting to nearly 27% of the genome of this fish. It is thought that selfing (and other forms of ‘virgin’ birth) are not conducive to the maintenance and spread of TEs. Possible explanations for the observed richness of TEs include their potential adaptive significance and occasional outcrossing promoting the horizontal TE transfer. It might be added that self-fertilization in mangrove rivulus is evolutionary recent and thus the richness of TEs may be a vestige of the past reproduction by cross-fertilization. Overall, the study by Rhee and co-authors is a valuable addition to the growing genomic resources available for *K. marmoratus*.

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[Andrei Tatarenkov]

**Increased juvenile predation is not associated with evolved differences in adult brain size in Trinidadian killifish (*Rivulus hartii*).** Beston SM; Broyles W; & Walsh MR. *Ecology and Evolution*, Epub ahead of print, 2017. DOI: <http://dx.doi.org/10.1002/ece3.2668>

The authors set out to determine if predator-prey relationships between *Rivulus hartii* and guppies effects brain size of the *R. hartii*. The authors hypothesized that *R. hartii* would have smaller brain sizes as they allocated resources away from brain growth to body growth to escape predation from the guppies. The brains of *R. hartii* from environments with guppies were compared to those without guppies. The fish were captive raised to exclude confounding factors associated with food supply in the wild. Brain size was found to vary from population to population but not with the presence of guppies. The authors attribute the lack of changes in brain size from exposure to high-predation guppy environments to the lack of any adaptive response by the fish to the presence of the guppies. Conversely, the presence of large predators did effect brain size as well as behavior (Walsh et al 2016). As the guppies only prey on the *R. hartii* fry the authors put forward that mortality experienced early in life may not alter selection on adult brain size.

**Literature cited:**

Walsh et al. Proceedings of the Royal Society B, 283, 20161075. [Tyrone Genade]



Male *Rivulus hartii*, Rio Copei, Isla Margarita. Photo by Frans Vermeulen ©2017.

**Are rodlet cells reliable biomarkers in *Fundulus heteroclitus* (L.)?** Kramer C; Hongach S; Antonov K; & Qama E. *J. Morphol*, 33:205–214, 2016. DOI: <http://dx.doi.org/10.4322/jms.096815>.

Rodlet cells are a poorly understood type of cell that is believed to play a role in responding to disease and environmental insult. Kramer et al used two populations of *F. heteroclitus*: one from a highly polluted site and one from a less polluted site. Rodlet cells were counted on the gills of each population. Rodlet cell numbers did not correlate with parasite burden nor did they correlate with the sex of the fish. No difference was found between the sites. The authors conclude that rodlet cells are not informative biomarkers of the response to parasites and environmental stressors.

[Tyrone Genade]

**Sequence and functional characterization of hypoxia inducible factors, HIF1 $\alpha$ , HIF2 $\alpha$ a, and HIF3 $\alpha$ , from the estuarine fish, *Fundulus heteroclitus*.** Townley IK; Karchner SI; Skripnikova E; Wiese TE; Hahn ME; & Rees BB. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, Epub ahead of print, 2016. DOI: <http://ajpregu.physiology.org/content/early/2016/12/27/ajpregu.00402.2016>

The authors report the identification and characterization of four heat inducible factors (HIF): 1 $\alpha$ , 2 $\alpha$ a, 2 $\alpha$ b and 3 $\alpha$ . The authors report that these transcription factors play a role in oxygen sensing and the activation of other transcription factors. They show that the cellular level of HIF $\alpha$  mRNA varied from organ to organ. The HIF 2 $\alpha$ b protein is reported to lack an oxygen-sensing and transactivation domain and its function is undetermined. Analysis of the HIF DNA sequences from several populations of *F. heteroclitus* reveal multiple DNA polymorphisms and could explain reported population differences to hypoxia.

[Tyrone Genade]

**Relative Contributions of Copper Oxide Nanoparticles and Dissolved Copper to Cu Uptake Kinetics of Gulf Killifish (*Fundulus grandis*) Embryos.** Jiang C; Castellon BT; Matson CW; Aiken GR; & Hsu-Kim H. *Environmental Science & Technology*, 51:1395–1404, 2017. DOI: <http://dx.doi.org/10.1021/acs.est.6b04672>.

Jiang et al exposed embryos of *F. grandis* to dissolved copper and copper oxide nanoparticles at varying pHs and in the presence of three types of natural organic matter at 0.1 to 10 mg Carbon per liter. These conditions influence copper uptake by the embryos. The found that the copper uptake was determined primarily by the concentration of the copper in the solution; the natural organic matter and pH had little effect.

[Tyrone Genade]



**Mitochondrial genotype and phenotypic plasticity of gene expression in response to cold acclimation in killifish.** Healy TM; Bryant HJ; & Schulte PM. *Molecular Ecology*, Epub ahead of print, 2016. DOI: <http://dx.doi.org/10.1111/mec.13945>.

This study was conducted with two subspecies of *Fundulus heteroclitus*: *F. h. macrolepidotus* is the northern one, extending from New Jersey to Canada and *F. h. heteroclitus*, from New Jersey to Florida. At a location where both subspecies can be found together (Allens Fresh Run, Maryland, USA) fish were captured and classified after their genotype into northern or southern subspecies. All the fish were held at 15 °C and adjusted at a 12 hours daylight—12 hours darkness light period and 20 ppt salinity. 5 fish from each subspecies were held in 110 L glass tanks, 4 tanks set at 15 °C and 4 at 5 °C. The experimental conditions lasted for 4 weeks, then the fish were euthanized and samples of lateral skeletal body musculature were preserved in liquid nitrogen until analysis. 14,784 genes were analyzed for changes in expression as a result of differences in acclimation temperature and mitochondrial genotype. Results: Acclimation to cold temperature had large impacts on gene expression. More nuclear genes increased in expression than decreased in response to cold acclimation (5460 genes up-regulated and 1746 down-regulated) compared to the fish kept at 15 °C. Six mitochondrial genes were differentially expressed at 5 °C and all of them were down-regulated. Discussion: In killifish, cold acclimation is associated with up-regulation of genes involved in RNA splicing, proteasome function and DNA repair, which is consistent with a cellular stress response. Genes involved in oxidative phosphorylation are down-regulated, which suggest metabolic suppression. Cold temperatures reduce the flexibility of biological proteins such as DNA, RNA and proteins, which impacts molecular functions and secondary structures. The up-regulation of genes with repair functions may indicate responses to counter the effects of low temperatures. Up-regulation of genes involved in RNA processing and splicing is an indicative of

responses that alter splicing patterns in the cold. In general, when ectothermic organisms adjust metabolic processes as a result of thermal acclimation, there are two overall strategies of response: (1) thermal compensation, which results in changes in biological rates that are in the opposite direction of thermodynamic effects, or (2) inverse compensation, which results in changes in rates that are in the same direction as thermodynamic effects. Decreases in temperature cause an exponential reduction in biological reaction rates. Thus, increased expression of metabolic genes at low temperatures is potentially consistent with thermal compensation to offset the decrease in reactions rates caused by low temperatures, whereas decreased expression is potentially consistent with inverse compensation (i.e., metabolic suppression), which could act as an energy saving mechanism during periods of low food availability, such as during the winter. Interestingly, the directions of change in metabolic gene expression are variable among different species, and several studies have reported increases in the expression of metabolic genes at low temperature, consistent with thermal compensation, whereas other studies have observed decreases in the expression of metabolic genes, consistent with metabolic suppression. Conclusions: Cold acclimation causes a dramatic change in the muscle transcriptome of killifish, inducing a cellular stress response that is accompanied by an overall reduction in the expression of genes involved in oxidative phosphorylation. The majority of intraspecific variation in gene expression in killifish is due to regulatory differences associated with the nuclear genome, although genotype × environment effects are potentially more common than fixed effects of variation in mitochondrial genotype.

[Manuel Zapater Galve]

**A miRNA catalogue and ncRNA annotation of the short-living fish *Nothobranchius furzeri*.** Baumgart M; Barth E; Savino A; Groth M; Koch P; Petzold A; Arisi I; Platzer M; Marz M; & Cellerino A. *bioRxiv*, Epub ahead of print, 2017. DOI: <https://doi.org/10.1101/103697>.

Small non-coding RNAs are becoming more and more important in the study of disease and aging. Baumgart et al present a catalog of small non-coding RNA for *N. furzeri*. They demonstrate that the RNAs they have identified are indeed expressed in *Nothobranchius*. The catalog is compared to that of zebra-, rice-, pufferfish and sticklebacks. This catalog will go on to be instrumental in studying the aging and pathology *Nothobranchius* fish. [Tyrone Genade]

**Trophic plasticity, environmental gradients and food-web structure of tropical pond communities.** Schalk CM; Montaña CG; Winemiller KO; & Fitzgerald LA. *Freshwater Biology*, Epub ahead of print, 2016. DOI: <http://dx.doi.org/10.1111/fwb.12882>.

The authors describe food-web structure of temporary pond communities in the Gran Chaco ecoregion of south-eastern Bolivia. The ponds (N = 13) were surveyed at the beginning and the end of the rainy season and varied along gradients of canopy cover, pond size, and hydroperiod. Stable isotope ratios of carbon ( $^{13}\text{C}/^{12}\text{C}$ ) and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) were used to quantify the spatiotemporal dynamics of the food webs. Carbon isotopes provide insights as to the basal sources supporting consumers and nitrogen isotopes make inferences on trophic positions. Tadpoles and macroinvertebrates within these ponds exhibited high trophic variability, with multiple taxa occupying more than one trophic position across space or time. In addition to tadpoles and macroinvertebrates, several species of fishes were collected, including the killifish *Neofundulus ornatipinnis*. With the exception *N. ornatipinnis*, which has a life history adapted to ephemeral aquatic habitats, the fish species collected from these Chacoan ponds would

have colonized them during intervals when they had surface water connections with the nearby stream. *Neofundulus ornatipinnis* was collected from one pond and did not co-occur with the other fish species. On average, *N. ornatipinnis* was a carnivorous (average trophic position = 3.5), but like tadpoles and macroinvertebrates, had large within-taxon variation in trophic level during the early rainy season with a trophic position ranging from 2.5 (omnivorous) to over 4 (carnivorous). In the late season, trophic position was 3.5, but only one individual was collected, our small sample sizes render this generalization speculative. In these dynamic aquatic habitats, consumers display considerable trophic plasticity, consuming diverse resources opportunistically dependent upon availability. While this was most prevalent and greatest in tadpoles and macroinvertebrates, it was also observed, to a smaller degree, in *N. ornatipinnis*. The flexible trophic strategy of consumers may explain the lack of a relationship between food-web vertical structure and environmental gradients in Chacoan ponds.

[Christopher M. Schalk, personal synthesis focused on *Neofundulus*, January 2017, for K-D News]

***Campellolebias brucei* Vaz-Ferreira & Soerr, 1974.** Nielsen DTB. *Journal of the American Killifish Association*, 49:56–59, 2016.

Dalton Nielsen relates the taxonomic history of this species as well as described its habitat and the peril it faces.

[Tyrone Genade]

**Maintaining *Campellolebias brucei*.** Katz D. *Journal of the American Killifish Association*, 49:60–64, 2016.

In this paper Dan Katz described how he has been able to maintain this species for over 15 years without interruption. Dan Katz describes how he breeds the fish and rears the offspring in his fishroom. The author maintains his fish in 10 gallon aquaria with copious Java moss for subdominant

males and females to hide in. Water of pH 6.4 is used. The dissolved solids are variable (the author uses well water). The temperature is kept between 20 and 21 °C. The author experiences fish deaths above 24 °C. He feeds mostly live grindal worms and newly-hatched *Artemia* nauplii. The author uses peat containers for spawning but reports finding many eggs amongst the mulm at the bottom of the aquarium. The color of the eggs is variable from white to dark brown. He incubates the eggs in a cool room for two to five months and hatches the eggs by placing them in a 1 L screw-cap bottle or jar filled to 1 inch from the brim. This is then submersed to at least 9 inches deep.

[Tyrone Genade]

## Theses & Dissertations

**Effects of Salinity and pH Change on the Physiology of an Estuarine Fish Species, *Fundulus heteroclitus heteroclitus*.** Tietze SM. Masters thesis, *Georgia Southern University*, 2016. URL: <http://digitalcommons.georgiasouthern.edu/etd/1518>

The author sets out to determine whether *F. h. heteroclitus* can handle multiple stressors simultaneously. Experiments were performed wherein the fish were exposed simultaneously to changes in pH and salinity. Wild caught fish from Sapelo Island, Georgia, were used. Six fish from each experimental group were sampled and analyzed for cortisol levels and oxygen consumption. There was no change in cortisol levels but the resting metabolic rate of the fish did increase in response to low pH. The author notes that the exposure of *F. h. heteroclitus* to low pH in natural environments (and I guess in the aquarium) may compromise overall fitness as more energy is invested in physiological acclimation instead of system maintenance.

[Tyrone Genade]

**The Effects of 17alpha-ethinylestradiol in the Live-Bearing Fish *Heterandria formosa*.** Jackson L. Doctoral thesis, *University of Louisiana at Lafayette*, 2016. URL: <http://gradworks.umi.com/10/16/10163310.html>

Jackson exposed least killifish to three doses of 17alpha-ethinylestradiol and assayed its effect on development, growth, reproduction, survival and population dynamics. Time to maturity increased and brood size decreased. The length of females declined while that length of males increased. 17alpha-ethinylestradiol exposure affected gonad and liver development in the fish: males had delayed sperm maturation and severe intersex (where eggs and sperm were produced in the same male fish); and females had delayed egg maturation. These effects were dose dependent. Chronic exposure to low doses resulted in reduction in population size and growth rates as well as female biased sex ratio. 17alpha-ethinylestradiol enters the environment through the use of oral contraceptives and has been reported at concentrations of 1 ng/L (Wedekind, *BMC Biology* 12:10, 2014). Jackson used a chronic exposure of 5 ng/L. Whether the same effects will be seen at 1 ng/L is an interesting question.

[Tyrone Genade]

## Interesting research on other fish

**The Genome of the Trinidadian Guppy, *Poecilia reticulata*, and Variation in the Guanapo Population.** Künstler A; Hoffmann M; Fraser BA; Kottler VA; Sharma E; Weigel D; & Dreyer C. *PLOS ONE*, 11:1–25, 2016. DOI: <http://dx.doi.org/10.1371/journal.pone.0169087>.

The genome of the guppy has been published. The fish used to construct the genome came from the Guanapo drainage. The genome has a size of 731.6 Mb pairs and consists of 22 autosomes and an X-chromosome (the male chromosome was not sequenced). 10 wild caught male fish were analyzed and a measure of genetic variation determined. 5

million single nucleotide polymorphisms (sites in the DNA that were different between fish) were identified. The authors note that the genome of the guppy will be very useful for investigating adaptation to different predation regimes. This genome will also be useful for those studying aquarium populations and the variation in color, behavior, aging etc. . . between the populations.

[Tyrone Genade]

**Impacts of the antidepressant fluoxetine on the anti-predator behaviours of wild guppies (*Poecilia reticulata*).**

Saaristo M; McLennan A; Johnstone CP; Clarke BO; & Wong BB. *Aquatic Toxicology*, 183:38–45, 2017. DOI: <http://dx.doi.org/10.1016/j.aquatox.2016.12.007>.

The authors build on previous research into the effects of a widely prescribed medication that can persist for long periods in aquatic environments and alter fish behaviour. This is now

documented across a number of species including the killifish *Aphanius dispar*. In focusing on initial predator response, but also the after effects the paper contrasts the effects of a selective serotonin reuptake inhibitor (SSRI) on guppies with those on humans. Comparison of changes noted in other animal groups is attributed to possible experimental differences (Reviewers note: though this may be a more fundamental difference in behavior and the results may highlight discrepancies in our understanding of anxiety vs stress). However this is a limited study with regard to actual behavioural changes but does confirm that the highly conserved vertebrate serotonergic system makes fishes susceptible to pharmaceutical contamination of natural waters through animal and human urine. Of particular interest for ichthyologists is the fact that the research made use of a wild population of guppies, caught in Queensland, Australia.

[Andy Patel]



Genade: Killi-Data News (Spring)

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## How to join Killi-Data International

Killi-Data presents top quality information on Killies or oviparous Cyprinodontiformes fishes, in a Data Base, with full ichthyological, ecological and historical coverage of each taxon with all details of systematics, morphology, genetics, patterns of each species and, with nearly 3000 color photos and maps for over 1100 valid species (male and female); as well as with all aquarium information for maintenance and breeding of each species.

Killi-Data is now nested in non-profit association "K-D-I" for services to their members (access to Data Base, Killiflash and PDFs of K-D-S journal) for a modest fee (a once-off € 10 Euro registration fee, + € 6 per year) with all revenues redistributed in grants (for researchers and collecting aquarists).

Email [editor@killi-data.org](mailto:editor@killi-data.org) for help.