Editorial

Many interesting papers have been published over the last three months. The large volume of papers coupled with the start of the new college semester (and the workload it brings) delayed this issue of Killi-Data News. But better late than never—or so I hope!

In this issue Richard van der Laan provides an insightful review of the recent Aphanius papers as to their taxonomic implications and questions. The systematic issues he raises show the importance of the Molecular project: we need to get more samples of the various cyprinodontiforme families to resolve unsettled systematic and taxonomic issues.

In the Next issue Andrei Tatarenkov (and colleagues) will tell us about Kryptolebias distribution and evolution—now that the official research paper has been published. In the paper they have divided the mangrove killifish into three major clades by molecular phylogenetics. They have analyzed several new populations. They have decided to allocate the names bonairensis, hermaphroditus and marmoratus to the clades. Also, Bettina Reichenbacher will also offer some insight into the new, monotypic, genus of killifish discovered in the high Andes of Chile, Pseudorestias lirimensis.

We hope you will enjoy this issue and find something useful you can use in your fishroom or research.

[Tyrone Genade]

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1This species is still regarded as Rivulus in KDI.
Interesting Websites

https://www.youtube.com/watch?v=9mJn9rwiASU  *Oxyzygonectes dovii* spawning. Killimania Costa Rica has posted several videos on this species.

Nathan Lujan reports on the possible collection of a toptype of *Anablepsoides taeniatus*¹: https://www.facebook.com/NKLujan/posts/10105159090872401’. It is not clear if Nathan has *taeniatus* or sp. aff. *limoncochae* which is known from that area.

The type locality of Kjel is stated as Morelia, río Caqueta drainage, southern Colombia. Frans Vermeulen and Ken Normandin have collected *Anablepsoides taeniatus* and have provided some photos. At a nearby location (ICE 2016-02), on the other side of Morelia, they did capture a sp. aff. *limoncochae* which had more broken lines than stripes.

In The News

Killifish have made their way into the news in several articles:

http://www.thewetumpkaherald.com/2017/06/20/doctor-eyes-health-of-local-creek-home-to-threatened-species/ An article profiling long-time AKA member Dr. Joseph Scanlan’s efforts are conservation of Sofkahatchee.


http://www.practicalfishkeeping.co.uk/news/fishkeeping-news/articles/2017/6/19/ryedale-as-reports-good-news-for-clubs Ollie’s first win at an aquarium club show for *Nothobranchius rubripinnis*.


¹ More information is needed to confirm the species identification.

Male and female *Rivulus taeniatus* ICE 2016-03 from a small pool alongside the road just past Morelia, Columbia. Photos by Frans Vermeulen.
Collection location ICE 2016-03 from which *Rivulus taeniatus* were captured. Ken Normandin reports that the adults were easily captured from the open water where they were displaying to each other while the juveniles were found among the plant roots in the shaded areas. The water was crystal clear before Frans and Ken entered the water to collect the fish.

**Review of New Research Publications**

**Systematics, Taxonomy & Distribution**

Neotype designation of *Aphanius iconii*, first reviser action to stabilise the usage of *A. fontinalis* and *A. meridionalis* and comments on the family group names of fishes placed in *Cyprinodontidae* (Teleostei: *Cyprinodontiformes*). Freyhog J; Özuluğ M; & Saç G. Zootaxa, 4294:573–585, 2017. URL: https://www.biotaxa.org/Zootaxa/article/view/zootaxa.4294.5.6

The first part of this work delves into the question of how many species of *Aphanius* there are in the Anatolian lake region (with numerous springs and lakes) and what are the names to be used for the valid species. Akşiray (1948) described many species / subspecies from this region in Turkey, particularly from Lake Eğirdir, and the Konya endorheic basin in Central Anatolia. However, all material examined by Akşiray (1948) is lost and nearly all names were treated as synonyms for half a century, mostly under *Aphanius anatoliae* (Leidenfrost, 1912) or *Aphanius mento* (Heckel 1843), with some species sometimes mentioned in the no longer recognized genera *Anatolichthys* and *Kosswigichthys*. According to the latest reviews of Wildenkamp et al. (1999) and Hrbek et al. (2002) there are but a few valid species of Anatolian killifishes, a situation reflected in Killi-Data. Fortunately, during the last decade there has been an extensive exploration of the Turkish freshwater fish fauna, leading to a much better knowledge of the Turkish fishes (see Çiçcek, Birecikiligil & Fricke 2016, Addenda and errata of: freshwater fishes of Turkey: a revised and updated annotated checklist. FishTaxa v. 1 (no. 2):116–117).

More valid species of *Aphanius* could be recognized from different molecular studies, and these valid species are acknowledged by the authors. Akşiray (1948) figured nine syntypes of *Aphanius iconii*, of which five originate from Lake Eğirdir (Plate 3:21–25), which proved to be a valid species, and four from the surroundings of Konya (Plate 4: 43-46), which are now determined as *A. anatoliae*. With a selection of a lectotype (a male pictured by Akşiray 1948 in Plate 3 as number 23) and the designation of a neotype (to provide a type specimen in alcohol), the name *Aphanius iconii* is now firmly attached to the Lake Eğirdir basin killifish. In the *A. anatoliae* group (sometimes included in the subgenus Anatolichthys Kosswig & Sözer, 1945) the authors recognize the following species as valid: *A. anatoliae* (Leidenfrost, 1912), *A. danfordii* (Boulenger, 1890), *A. fontinalis* Akşiray, 1948, *A. iconii* Akşiray, 1948, *A. maeandricus* Akşiray, 1948, *A. maras-
santensis Pfleiderer, Geiger & Herder, 2014, *A. meridionalis* Akşiray, 1948, *A. saldae* (Akşiray, 1955), *A. splendens* (Koss-wig & Sözer, 1945), *A. sureyanus* (Neu, 1937), *A. transgrediens* (Ermin, 1946), and *A. villwocki* Hrbek & Wildekamp, 2003. Adult male *A. iconii* are distinguished from adult males of all these species by having a black dorsal-fin base followed by a wide, white or hyaline band at about below the middle of the dorsal fin. *Aphanius fontinalis* (synonyms: *Aphanius chantrei altus* Akşiray, 1948 and *Aphanius chantrei litoralis* Akşiray, 1948) is distinguished from *A. sureyanus* by being completely covered by scales (vs. scales reduced in *A. sureyanus*), a questionable difference. Further study is warranted. The authors also confirmed the validity of *Aphanius meridionalis* Akşiray, 1948 (synonym: *Aphanius chantrei parvus* Akşiray, 1948).

The articles contains some nice photos of *A. fontinalis* and *A. meridionalis* (both with a black dorsal fin) and *A. iconii*. A distribution map of the various *Aphanius* species discussed in the text, would have been very useful.

In the second part the authors also review the molecular studies on cyprinodontiform phylogeny and noticed strong disagreements between published family assignment and phylogenies of killifish and livebearers. They propose a new family structure for the killifishes previously placed in the family Cyprinodontidae. Although the revision of the family group Cyprinodontidae is more than overdue, we still need a carefully researched study with sufficient data. The old classification certainly led to some difficult biogeographical explanations, see also Costa 1997, Phylogeny and classification of the Cyprinodontidae revisited (Teleostei: Cyprinodontiformes): are Andean and Anatolian killifishes sister taxa? Journal of Comparative Biology v. 2 (no. 1):1–17. Simply raising the genera to families (as in most families only one recent genus is included!) could be useful for listing the families in alphabetical order, but certainly obscure their relationships. The authors propose the following family classification:

Aphaniidae Hoedeman, 1949: included genus *Aphanius*; according to Parenti (1981:521), Aphaniidae is distinguished from Orestiidae by having a vomer (vs. absent), a cartilaginous mesethmoid (vs. ossified) and an ossified interhyal (not ossified).

Cubanichthyidae Parenti, 1981: included genus *Cubanichthys*; Parenti (1981:519) diagnosed the family Cubanichthyidae (as Cubanichthyinae) by its possession of several rows of teeth in the upper and lower jaw, an enlarged supraoccipital crest, an elongate dorsal process of the autopalatine, a supraorbital sensory-pore pattern consisting of a large third pore, and lacking an ossified ventral limb of the posttemporal.

Cyprinodontidae Wagner, 1828: included genera *Cualac, Cyprinodon, Floridichthys, Jordanella* and *Megupsilon*; Parenti (1981:526) diagnosed the family Cyprinodontidae (as Cyprindo-dontini) by its having a derived form of the attachment of the first vertebra to the skull and the pharyngobranchial teeth being organised in discrete rows.

Orestiidae Bleeker, 1859 (with stem Oresti- assumed by the authors as being in prevailing usage!): included genus *Orestias*; Parenti (1981:526) diagnosed the genus *Orestias* by its lacking the vomer and the first postcleithrum.


Clarity is an advantage of a classification in different families, however, the interrelationships of the different monophyletic families are not clear. The simple usage of different families worked well within the loach suborder Cobitoidei (mostly because there weren’t many serious phylogenetic studies and researches needed a useful and simple system) and soon the carp family Cyprinidae will be split in different families, but here the knowledge on the relationship of the different groups is much better.

In the recent study on the phylogenetic classification of
the bony fishes of Betancur-R et al. 2017 (BMC Evolutionary Biology, 17), the only change made in the Cyprinodontidae was removal of Aphanius to the Valenciidae (but included in a family with a junior family-group name!) and the authors noted the question of the “Pantanodonta”. So, many questions remain. Would it be better to treat the Cubanichthyinae as a subfamily of Cyprinodontidae? What about the superfamily Cyprinodontoidea? Can this larger superfamily still be defined by two synapomorphies: a ventroposterior portion of the dentary slightly expanded (usually forming a concavity anteriorly) and the dorsal process of the maxilla expanded medially, usually with a distinct groove. Which “new” family is more related with the Fundulidae, Poeciliidae, Goeiidae or Profundulidae? Are the Aphaniiidae and Valenciidae to be united in one family (under the correct name Aphaniiidae)? What is the relation with the currently defined groups of Poeciliidae? See the review of Jean Huber in Killi-Data News, volume 2 number 2 (Summer 2017), p. 24. What about the relationship with the Pantanodon-group, or the other lampeyes (currently in Aplocheilichthyinae Myers, 1928 and Procatopodinae Fowler, 1916)? Maybe we have to live a bit longer with the polyphyletic Cyprinodontidae until we can reach a solid scientific solution.

In conclusion, a technical paper on some Anatolian Aphanius species improving our insight in the diversity of the Anatolian killifishes (that maybe contribute to the conservation of these wonderful fishes) and a new idea about the family status of the various groups in the superfamily Cyprinodontoidea.

[Richard van der Laan]

A detailed analysis at the species level of this clarifying post-molecular work leads (1) to accept, in Killi-Data, iconii, fontinalis and meridionalis as revalidated species with (some) molecular differences (not obvious for fontinalis) and with importantly new clear diagnoses of live males (vs. all other components of that group), (2) to accept anatoliae, splendens, sureyanus, and transgrediens as distinct species (not subspecies anymore), but (3) NOT to accept maenandricus and saldae as distinct species because of lack of new diagnoses for them and because of some ambiguity on conservation issues vs. systematics on saldae/splendens (however further studies are needed and this position may be changed in the future).

Aquarium populations have been reallocated to newly defined accepted as valid species.

[Jean Huber]

Plio-Pleistocene phylogeography of the Southeast Asian Blue Panchax killifish, Aplocheilus panchax. Beck SV; Carvalho GR; Barlow A; Rüber L; Hui Tan H; Nugroho E; Wowor D; Mohd Nor SA; Herder F; Muchlisin ZA; & de Bruyn M. PLOS ONE, 12:1–17, 2017. DOI https://doi.org/10.1371/journal.pone.0179557

The authors are endeavoring to understand how repeated cycles of glaciation effected biogeography in Southeast Asia. They set out to discover whether Aplocheilus panchax could serve as a representative species for the study of evolutionary patterns in the area. Analysis of the mitochondrial DNA revealed three major clades which date to approximately 2.6 million years ago. Phylogeographic analysis showed a clear west to east dispersal pattern following by rapid radiation with the most easter population, on Sulawesi, was colonized some 30’000 years ago. Nuclear DNA did not show as structured a pattern of migrations. The authors conclude that recent gene flow has been a key factor in determining nuclear genetic variation patterns.

[Tyrone Genade]


In this paper Costa redescribes N. guentheri and N.
melanospilus drawing on preserved specimens collected by Stuhlmann, Playfair as well as more recent collections. He also reports on the modern names of the original collection. As motivation for the redescription Costa states that “except for a few morphological characters briefly described in old papers, data on taxonomy, morphology and useful diagnostic features of N. guentheri are not available in the scientific literature... morphological characters useful to distinguish N. guentheri and N. melanospilus are not available in the literature, so both species are still misidentified in museum collections.” Costa provides a thorough redescription of both species as well as provides diagnostic criteria.

[ison女性朋友]

Nothobranchius guentheri ZAN 2014-2, about 1.3 km north of Mahonda Mkataleni police station, Kaskazini district, along the main road, just past a bridge over a creek (goo.gl/eghy6g. Photo by Tyrone Genade.


A new (for-killifish) research team (welcome!) from Universidade Estadual de Maringa (State University, located in Maringa city in Parana state, southeastern Brazil) describe a new species named Melanorivulus nelsoni2 (1), with a lack of Ventral fins; according to the authors the new species is only separated from other components of Melanorivulus by male live color pattern like all other known species (today more than 50); the male color pattern is shown in a photo just after collection and indeed it is typical (and standard) of Melanorivulus with irregular red chevrons on sides over a blue background; however in text, the authors state a Caudal fin in male light yellow, with dark brown stripes [bars] which seems unusual in the (sub)genus; the new species is said always (on 13 types?) lacking Ventral fins and girdle (like in pictus and planaltinus, but in those 2 species specimens do show 1 or 2 Ventral fins also); the authors show with phenotypical evidence that the absence of Ventral fins in Melanorivulus species is likely genetically controlled by an allele that, when in heterozygosity, may result in unpredictable phenotypes (they discuss other cases among killifish in much more details than Costa & Brasil, 2008); they also studied a large number of specimens of pictus on the issue of Pelvic fin number (and also on frontal squamation with distinct results from Costa’s) but they do not discuss the large variability of color pattern in pictus; anyway the type locality of nelsoni, in the municipality of Juscimeira, Mato Grosso state, is far from that of pictus (they are at the same latitude, but nelsoni is further west by at least 500 km but congener bororo—with Ventral fins—is also collected not far in Mato Grosso); the new species is dedicated to the late (1937–2011) renown US ichthyologist Joseph S.

2In KDI this species is maintained in the genus Rivulus
Nelson (known as Joe, and so nice, author of the book “Fishes of the World”, 1st edition 1976, 4th edition 2006) and is also separated from congeners in the same region by a deeper body exemplified by higher transversal scale counts vs. all other Melanorivulus species.

[Jean Huber]


This is a new species from the Garukana creek (a tributary of the Rio Preto) near Campinas do Rio Preto village in Santa Isabel do Rio Negro, Amazonas, Brazil. It is distinguished from all other members of *Laimosemion* by its metallic blue stripe which starts in the caudal fin and extends to the mid-body of the fish. The fish were collected from a black water stream with no turbidity. The stream was 1 to 2 cm deep with lots of leaf litter on the bottom of it. Species of characids and cichlids (e.g Apistogramma species) were also present. The water was 25.9°C with pH 4.5 and conductivity 40 µS.cm⁻². The locality was within a seasonal flooded forest. The species is names for José Ramón Garcáa Gil.

[Tyrone Genade]


Nielsen describes two new species: *Melanorivulus canesi* and *britzkei*.

*M. canesi* was collected from Pará state in the Rio Curuá basin (middle rio Xingu basin) near to a place called Vereda at Km 84 on road BR-163. It is diagnosed from other *Melanorivulus* by its metallic green body with 11–12 oblique brown bars which have one or two narrow red oblique lines along with the bars. The stream it was collected from had flowing

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3In Killi-Data.org, this species is still recognized as Rivulus.
4In killi-data.org this is still considered a subgenus of Rivulus.
water and was shallow (0.5-1.5 m in depth) and 2 m wide. The water was clear with grasses growing along the banks. The bottom was composed of sand and mud. The species is named after the environmentalist, Paulo José Ferreira Canes.

*M. britzkei* was collected from Pará state at the headwaters of the rio Braço Norte. It differs from other species in the subgenus by males having metallic yellow on the head and female with bright yellow on the upper and lower portions of the caudal fin. The type location is described as a small (2 × 1 m pool) which was 20–40 cm deep in an almost dry stream bed of sand and plants. This species is named for Ricardo Britzke.

[**Tyrone Genade**]

A *new annual fish of the genus Simpsonichthys (Cyprinodontiformes: Cynolebiidae) from the upper Rio Jequitinhonha basin, Brazil.* Nielsen DTB; Pessali TC; & Dutra GM. *Zootaxa*, 4263:165–172, 2017. DOI http://dx.doi.org/10.11646/zootaxa.4263.1.8

This very nice paper describes a new annual species as *Simpsonichthys espinhacensis* located 22 Km North-west of the small town Olhos D’água near Vereda Volta da Capoeira, tributary of Ribeirão da Areia, Rio Jequitinhonha basin, Minas Gerais, Brazil. The types were found and collected on 08 Feb 2016 by T. C. Pessali and T. A. Barroso. The species was recorded first on 20 Aug 2014 by T. J. Sousa & B. Andreata not far from the type locality of the holotype and are included in the paratype series.

After an informative introduction with clear history of the original genus name *Simpsonichthys* and the complex route in which the genus first was seen as a junior synonym of *Cynolebias*, then recognized as a valid genus, later divided into five subgenera, again later all raised to genus level. Then *Simpsonichthys* was restricted to eight valid species, which finally were recognized to form two clades. Recently, the monophyly of *Simpsonichthys* was questioned by a molecular phylogenetic analysis in which *S. margaritatus* and *S. parallelus* were recovered as more related to *Spectrolebias*.

In the diagnose *Simpsonichthys espinhacensis* is distinguished from all congeners by the frontal squamation A-patterned (versus E-patterned). Besides different color pattern *S. espinhacensis* differs in several morphomeristic features with its closest relatives. Females of *S. espinhacensis* can be distinguished from all congeners by presenting the tip of pectoral fin reaching vertical anterior to pelvic fin (versus vertical at urogenital papillae or anus).

*Simpsonichthys espinhacensis* could be classified as Critically Endangered (CR), according to the International Union for Conservation of Nature due to occurrence of fire, water catchment, construction of embankments, and deforestation for eucalyptus plantations, threatening its habitat.

The name “*espinhacensis*” refers to the name of the close by Serra do Espinhaço, meaning “backbone” mountain, a mountain range with longitudinally slender North-South ori-

This is no doubt a controversial paper. The result therein will inflame the tensions between taxonomic splitters and lumpers. Furthermore, it calls into question previous DNA phylogenies and theories of killifish diversification in South America.

The authors constructed a DNA phylogeny using two mitochondrial genes (COX1 and 16S) and four nuclear genes (ENC1, GLYT1, RHO and SH3PX3). Both the Cynolebiini and Cynopoecilini tribes were well supported. The phylogeny produced was well resolved with high support for all eight Cynolebiini genera previously proposed by Costa based on morphological data. Only Hypsolebias had low support but retained it monophylety as a sister clade. Nematolebias and Xenurolebias emerged as sister clades to the rest of the Cynolebiini. This is confirmed by the phylogeny of Furness et al (2015) and Ponzetto et al (2015). The organization of Hypsolebias clades does not agree with Ponzetto et al (2015) paraphyletic Hypsolebias but it does agree as respect to placing Ophthalmolebias as a sister clade to Hypsolebias. It is important to note that Ponzetto relied on a single mitochondrial gene: ATPase8/6 and his phylogeny includes species that are not present in Costa et al’s and this could explain the variance of the results.

Costa et al places the divergences between Cynolebiini and Cynopoecilini at 19–33 Mya (million years ago) with the eight genera evolving within the last 12–20 Mya. The most surprising result is that Cynolebias is shown as diverging from Simpsonichthys! and being distantly related to Austrolebias. Spectrolebias is shown as basal to Simpsonichthys, Cynolebias, Austrolebias, Ophthalmolebias and Hypsolebias. The ancestral origin is predicted to be the Atlantic Forest coastal lowlands from which the spread into other regions before the uplift of the Central Brazilian Plateau. Costa evokes the hypothesis of Friedman et al (2013) that fish spread from Africa to South America via a now submerged Palaeogene island chain during the Eocene when South American and Africa were only 1000 km apart. This speculative hypothesis is all the more tempting for Kryptolebias appearing in all phylogenies as a bridge between African and South American Cyprinodontiformes. With multiple labs working on Cyprinodontiforme phylogenies it is expected that this will not be the last word on this matter and that more conflicting results will be published and we are a long time away from having a consensus on this matter. So far, it seems that each publication brings us closer to the truth (whatever that may be is still difficult to predict) as well as generating more interesting questions.


Microanatomical diversification of the zona pellucida in aplochelioid killfishes. Thompson AW; Furness AI; Stone C; Rade CM; & Ortí G. *Journal of Fish Biology*, Epub ahead of print. ISSN 1095-8649, DOI http://dx.doi.org/10.1111/jfb.13332

The zona pellucida is the chorion of the egg, the outer barrier. It is formed as four layers by the oocyte, follicle cells

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5 This is also postulated as an explanation for how monkeys and rodents reached South America! See de Oliveira et al. (2009) Paleogeography of the South Atlantic: a route for primates and rodents into the New World? In South American primates (eds Garber et al), pp. 55â€“68. New York, NY: Springer.
of the ovary and glands of the oviduct. The secondary layer, formed by the follicle cells, contains structures such as filaments, protuberances, jelly-coatings or honey-comb patterns that help the eggs adhere to the substrate. Thompson studied the zona pellucida of 84 species by means of 16 characters (defined by the author based on the morphology of surface of the eggs). Most species were South American Annuals. Using these 16 characters the authors constructed a phylogeny. This phylogeny supports the eight Cynolebiini genera of Costa and the distinctness of the Cynopoecilini. Few species of the Neofundulini, Rachoviini and Rivulini were samples for the phylogenetics to be very informative. Simple, uniformly distributed filaments and differentiated rounded bases is reported as the ancestral condition in cynolebiasine fishes. The six Nothobranchius species analyzed produce a phylogeny that supports that of Dorn et al (2014). Very interestingly, the phylogeny places bokermanni-lineage as an ancestor to Ophthalmolebias. Thompson et al show that its zona pellucida of bokermanni is intermediate between that of Simpsonichthys and Ophthalmolebias, having short bases to the palm-tree-like extensions of the zona pellucida compared to long bases in Ophthalmolebias. These palm-tree-like structures are unique to the Ophthalmolebias species and bokermanni. As per Nematolebias the eggs of papiliferus and whitei have different eggs structures. Thompson cautions that the characters examined are highly homoplastic (i.e. deceptive) and the phylogenetic tree produced cannot be replied to give an accurate picture of evolutionary relationships by itself. It is clear, however, that they are of phylogenetic use, revealing differences between species that are otherwise morphologically similar (e.g. papiliferus vs whitei) as well as revealing evolutionary intermediate lineages. Research that is needed is to determine if there is any correlation between the structure of zona pellucida and the substratum the eggs are deposited in.

[Tyrone Genade]


In this very informative paper reporting *Austrolebias nigripinnis* found, so far only known from Uruguay and Argentina, on new locations just across the Uruguay- and Argentina-border in South western Rio Grande do Sul state of Brazil. Authors give in-depth details about species history, spreading and type of habitat and the method of collecting and preservation. *Austrolebias nigripinnis* is endemic in temporary pools and the floodplains alongside both margins of the lower Uruguay-, Paraguay-, Paraná-, and de La Plata basins in Uruguay and Argentina. The presence of the taxon in that area was expected but till the actual capture of the species and publication of this paper not confirmed. Authors propose the species to be placed in the IUCN Red List of critically endangered species for Brazil.

Remarks: On the map(s) (Fig. 1) the spreading of *A. nigripinnis* is demonstrated in detail. However, the northern-most known location at San Javier, Misiones, Argentina is not marked. There *A. nigripinnis* was repeatedly reported and further all along the Uruguay riverbank which forms the Argentinian border with Brazil in the north and more to the south with Uruguay. The new location in this paper, the village Uruguaiana, is situated along the Brazilian bank of the Uruguay River. Due to the flooding of these rivers, interbreeding of populations at both banks of the river can occur and genetic exchange may take place between populations. In both neighboring countries i.e. Argentina and Uruguay, *Austrolebias nigripinnis* is abundant available and may not be endangered at all and the new population occurs in the same geographical area. Therefore, I personally question the need to place the Brazilian sub-population on the IUCN red list as a critically endangered species if we take the guidelines for such proposed placement into account.
The guidelines for using the IUCN Red List Categories and Criteria says following:

“If a subpopulation assessed under the criteria is not isolated (i.e., if it may be exchanging individuals with other subpopulations), its assessments must follow the regional guidelines (IUCN 2003). In addition, it must be a biological subpopulation (i.e., not defined by political or national boundaries). Although the regional guidelines can in principle be applied at any geographical scale, application within very small geographical areas is strongly discouraged. The smaller the subpopulation as a proportion of the global population of the species, the more often the subpopulation will exchange individuals with other subpopulations. Therefore the assessment of extinction risk based on the criteria would become more unreliable...” and further “…the risk of extinction may be over-estimated.”

In fact, the population, presented in this paper, dwells in less suitable biotopes close to rice fields. Biotopes may have being there before the rice fields were established. However, these biotopes may also are artificial biotopes and with the many non-annual, in some cases predatory species, in it like *Astyanax*, *Hoplias*, *Callichthys* and *Pimilodella* mentioned in the paper, annual species do not have much chance on survival anyway.

In overall the paper is good reading, very informative and a professional report on a new finding extending the range of spreading of this beautiful and well known *Austrolebias nigripinnis*.

[Frans Vermeulen]
Sex composition modulates the effects of familiarity in new environment. Lucon-Xiccato T; Mazzoldi C; & Griggio M. *Behavioural Processes*, Epub ahead of print, 2017. ISSN 0376-6357, DOI https://doi.org/10.1016/j.beproc.2017.05.003

*Aphanius fasciatus* (Mediterranean banded killifish) caught in the wild were used for this study into familiarity between fish and its effects on behavior in a new environment. Having dined out on fresh mussels each day the recruits were subsequently paired up with either a familiar fish, or an unfamiliar fish. Pairs consisting of two females, two males or one of each were introduced to an experimental tank with a darkened refuge area. Their behavior was recorded with regard to their boldness, habituation and cohesion by observing how quickly they emerged from cover, reduced rapid swimming and how closely they swam respectively. Females and mixed pairs that were familiar with each other were bolder and habituated more quickly than unfamiliar ones. Females also initially stayed closer. Male pairs however were bolder and habituated more quickly when they were unfamiliar. The authors do not speculate on any underlying causes but highlight the significant difference sex composition has on familiarity when facing a new environment.

[Andy Patel]


The authors describe the micro-structure of the gonads of *A. sophiae*. The fish originated from the Beiza spring system in Denhan village, Kor River basin, Fars Province, Iran. The authors used hematoxylin and eosin staining of tissue sections to study the structure of the ovaries and testes. The development progression of the eggs in the ovaries is described by five stages: (I) oogonia and chromatin nucleolar; (II) perinuclear; (III) cortical alveoli; (IV) vitellogenic; and (V) maturation stages. Notably, they report that during the non-spawning period the ovaries appeared white while during the spawning period they were a dirty yellow gold. Unlaid mature egg diameter was 0.526 mm. Testes were elongated and a milky white. Immature fish had a thin organ while mature fish had large, thick testes. No mention of the effect of the spawning and non-spawning period on testes condition. The development of the spermatozoa is described in five stages. According to the authors, similar development was observed in *A. persicus* and compares well to other species. The authors hypothesize that “batch spawning of an individual in a spawning season is an advantage for this fish that lives in unstable and changeable environments, such as temporary lagoon or very small pool. This strategy of spawning allows relatively large eggs to be laid, which have a greater chance of survival.”

[Tyrone Genade]


This research was presented at the annual meeting of the Experimental Biology Conference held April 22–26, 2017. The objective of the study was to examine the role of small RNAs in long-term vertebrate anoxia tolerance in *Austrofundulus limnaeus*. Embryos at four different developmental stages were exposed to anoxic conditions and the expression levels of small RNAs was examined. Many small RNAs were differentially expressed. 12% of the unique small RNAs were encoded in the mitochondrial genome and 20% of the highly differ-
entiated small RNAs were of mitochondrial origin. Transfer RNAs were particularly enriched and most of the small RNAs were localized to anoxia sensitive tissues such as the brain and heart. Experiments on *A. limnaeus* cells in culture corroborated the whole-embryo experiments. The authors states that the “role in anoxia tolerance may provide novel insight into combating cellular damage induced by heart attack and stroke, two lethal consequences of heart disease in humans.”

[**Tyrone Genade**]

**Embryonic development of the annual killifish *Austrofundulus limnaeus*: An emerging model for ecological and evolutionary developmental biology research and instruction.** Podrabsky JE; Riggs CL; Romney AL; Woll SC; Wagner JT; Culpepper KM; & Cleaver TG. *Developmental Dynamics*, Epub ahead of print. ISSN 1097-0177, DOI http://dx.doi.org/10.1002/dvdy.24513

In this paper Podrabsky et al describes the developmental sequence from egg to larvae of *Austrofundulus limnaeus*. He notes differences between *Austrofundulus limnaeus* and *myersi* as well as discusses the virtue of employing multiple organism models of development to complete our understanding of developmental biology.

Several tables are provided that outline the development events as well as many clear images of developing embryos. Diapause I occurs are Stage 20 where the blastula disperses over the yolk. If the embryo escapes diapause the blastomeres reaggregate. Only 25% of the blastomeres at Stage 19 will participate in forming the early aggregate and go onto to form a new fish. Diapause II occurs at Stage 33. At this stage the embryo is well advanced. *A. limnaeus* differs from *A. myersi* in respect to the number of somites (42 vs 50) at stage 34, pigmentation appears are Stage 36 vs Stage 37; and the rate of trunk growth is slower in *A. limnaeus*. The time of development is discussed, notably is the slow development. *A. limnaeus* takes 32 days to develop to hatching while *Nothobranchius* can take only 21 days. Interestingly, early development (to Stage 12) is control and orchestrated by genes expressed by the mother. If RNA synthesis is inhibited the embryos will still develop up until Stage 12. The decision to enter diapause is up to the embryo, not the parents. Podrabsky writes “If we are to truly understand development, and how it is linked to ecological and evolutionary processes, we must look beyond the current handful of model organisms… Thus it is time we focus our efforts on using the diversity that nature has offered to strengthen our understanding of the basic processes that unify, as well as the unique and specific processes that contribute to the enormous biodiversity that we observe.”

What will be useful to aquarists is the statement that the eggs can be forced out of diapause by incubating the eggs at 30°C under constant light for 48 hours. This might prove useful for the aquarium culture of *Aphyolebias*, *Gnatholebias*, *Micromoema*, *Moema*, *Neofundulus*, *Pterolebias*, *Rachovia*, *Ter rantatos* and *Trigonectes* species whose long incubation times make their captive maintenance problematic. Podrabsky has published a hobbyist version of this paper in *Journal of the American Killifish Association* (reference follows)


[**Tyrone Genade**]

**Characterization and phylogenetic analysis of complete mitochondrial genomes for two desert cyprinodontoid fishes, *Empetrichthys latos* and *Crenichthys baileyi*.** Jimenez M; Goodchild SC; Stockwell CA; & Lema SC. *Gene*, 626:163 – 172, 2017. ISSN 0378-1119, DOI https://doi.org/10.1016/j.gene.2017.05.023

In this paper the authors set out to resolve the question of whether the Empetrichthyidae are a subfamily within Goodeidae or a separate family. To solve this issue the authors...
sequenced the entire mitochondrial genomes of *Empetrichthys latos latos* and *Crenichthys baileyi moapae*.

The *E. latos* were obtained from the Shoshone Ponds and Lake Harriet—two locations into which the fish were introduced as a conservation effort. The introduced fish originated from Corn Creek which is also an introduced population established in the 1970s. The Corn Creek population was established using 29 fish from the original Manse Spring location which was extirpated in 1975 due to ground water over exploitation. A single *C. baileyi moapae* was obtained from Plummer Stream in the upper Muddy River complex, Clark County, NV.

The authors sequences and compared the mitochondrial genomes of both species and found very low variation between the two *E. latos* fish which they attribute to a series of bottlenecks in the original Manse populations compounded by a low founding population in Corn Creek. There was 92.31% identity between the mitochondrial DNA sequences of the two species. The authors compared evolutionary divergence between the two species, Goodeidae, Profundulidae, Fundulidae, Cyprinodontidae and Poeciliidae. The authors, based on work by Kartavstev (2011) that the low average nucleotide divergence between the two species and Goodeidae indicates that *Empetrichthysidae* is a subfamily of Goodeidae. The authors support this with phylogenetic analyses based on the NT and Cyt-b genes. Their phylogenetics corresponds with Reznick et al (2017) that also shows that *Empetrichthysidae* is basal to the Goodeinae. Their phylogeny does indicate that Cyprinodontidae groups with Orestiadin as a sister taxon but *Aphanius* does not.

It is unlikely that any of these fish will ever be introduced the hobby making the *Crenichthys baileyi baileyi* (which is endangered in the wild while other species are more secure) that are in the European hobby especially valuable to killiphiles.


A complex paper describing how the authors have identified a total of 74 nuclear receptors in Mumichogs and a detailed breakdown of the sub-families that each belongs to, comparison with other species and some details on functions controlled.

Cost of tolerance: physiological consequences of evolved resistance to inhabit a polluted environment in teleost fish *Fundulus heteroclitus*. Jayasundara N; Fernando PW; Osterberg JS; Cammen KM; Schultz TF; & Di Giulio RT. *Environmental Science & Technology*, 51:8763–8772, 2017. URL http://dx.doi.org/10.1021/acs.est.7b01913

The Atlantic killifish shows its adaptability in that some populations have developed resistance to a highly toxic pollutant, namely polycyclic aromatic hydrocarbons. But rather than assume that their future is assured the authors investigated whether such adaptation has led to metabolic changes, trade-offs that may affect their ability to deal with other stressors that they encounter. By linking these processes to the genes that control them they have found that the genes have altered and are therefore inherited by offspring even if moved to unpolluted waters. Critical swimming speeds, thermal tolerance, and cost of finding an optimum environment were all shown to be affected by the changes. The authors conclude that rapid adaptation may not mean all is well with a particular population, particularly if secondary stressors come into play.

Taurine protects cardiac contractility in killifish, *Fundulus heteroclitus*, by enhancing sarcoplasmic reticular Ca$^{2+}$ cycling. Henry EF & MacCormack TJ. *Journal of Comparative
A study to evaluate the potential effect of an amino acid (Taurine) on the ability of Atlantic killifish to endure environmental stress. Taurine is abundant in many fish tissues and has a role in maintaining cell volume as environmental salinity levels alter. Obviously this is important in a species found in estuaries. But it also may play a role in keeping the heart pumping when salinity changes are stressing the system. Due to practical issues the positive effect of supplemental taurine could only be recorded in-vitro and in actual fish taurine levels did not alter significantly when subjected to changes in temperature, salinity and oxygen availability. It is not proven that its effect is via the sarcoplasmic reticular pathway.

[Andy Patel]

Ed’s note: taurine is synthesized from the amino acid cysteine. Taurine supplementation has been demonstrated to improve growth and survival of fry (El-Sayed, 2014). A nutritional shortage of cystein and/or taurine could render fry susceptible to osmotic stress caused by large water changes. El-Sayed, A. F. M. (2014). Is dietary taurine supplementation beneficial for farmed fish and shrimp? A comprehensive review. Reviews in Aquaculture, 6(4), 241–255.

The Fundulus diaphanus Species Complex: A Case Study of Convergent Evolution?. Walston TD. Honors Thesis, East Carolina University, 2017. URL http://hdl.handle.net/10342/6299 The aim of the study was to determine whether F. diaphanus from Lake Phelps and F. waccamensis from Lake Waccamaw are independently derived from the ancestral stream of F. diaphanus. The authors samples the lake and and adjacent stream populations of Fundulus and extracted DNA from fin-clips and/or livers. The DNA sequences were compared to a representative Fundulus genome and evolutionary relationships were determined. The Lake Phelps and Scuppernong River populations were most closely related. The Lake Waccamaw population was more closely related to the Lake Phelps population than the Waccamaw River population. The author concludes that the Waccamaw River population diverged from the other three populations before the Lake Waccamaw and Lake Phelps populations.

[Tyrone Genade]

Development of polymorphic microsatellite loci for the design of management and conservation strategies of the critically endangered Barrens topminnow (Fundulus julisia, Williams & Etnier, 1982). Hurt C & Harman A. Journal of Applied Ichthyology, Epub ahead of print. ISSN 1439-0426, DOI http://dx.doi.org/10.1111/jai.13370

A short paper by two of the authors from the other F. julisia paper outlining how they went about identifying a suite of 14 microsatellite markers that could be used as an assessment tool to quantify genetic variability and support ongoing conservation and management efforts.

[Andy Patel]

Genetic diversity and population structure in the Barrens Topminnow (Fundulus julisia): implications for conservation and management of a critically endangered species. Hurt C; Kuhajda B; Harman A; Ellis N; & Nalan M. Conservation Genetics, Epub ahead of print, 2017. ISSN 1572-9737, DOI http://dx.doi.org/10.1007/s10592-017-0984-0

The Barrens Topminnow is found in Tennessee, USA and has been successfully reintroduced in several areas. The authors sequenced mitochondrial DNA to assess the level of genetic variability within original populations as well as those that had been reintroduced. They found that the species has relatively low variability within populations compared to other Fundulus species and evidence that gene flow is restricted since specimens from different drainages have distinct differences. Whilst the reintroduced populations show similar levels of variability to the last three original popula-
tions and demonstrate short term conservation success, the authors conclude that the species remains under substantial threat. Human disturbance of habitats and the introduction of mosquitofish (*Gambusia affinis*) are primarily responsible for the population decline and with such low genetic variability Barrens Topminnow are particularly vulnerable. The authors argue for captive breeding that optimises variability, whilst also maintaining the distinct lineages that are left, to improve future reintroduction efforts. They hope to be able to influence an ongoing review by the U.S. Fish and Wildlife Service and have the species listed under the Endangered Species Act.

[Andy Patel]

**A non-destructive BFCOD assay for in vivo measurement of cytochrome P450 3A (CYP3A) enzyme activity in fish embryos and larvae.** Oziolor EM; Carey AN; & Matson CW. *Ecotoxicology*, Epub ahead of print, 2017. ISSN 1573-3017, DOI [http://dx.doi.org/10.1007/s10646-017-1812-5](http://dx.doi.org/10.1007/s10646-017-1812-5)

This paper describes the development and validation of a new means to conduct environmental toxicology assays for aryl-hydrocarbon pollutants such as PCBs, PAHs and dioxins. The new assays is reported as being cheap, fast, easy and sensitive and preferable over other methods because it allows for in situ responses within live embryos as opposed to test-tube reactions. The authors used eggs of *Fundulus grandis* and zebrafish embryos. These were exposed to low does of 7-benzyloxy-4-trifluoromethyl coumarin to induce the expression of Cytochrome P450 3A (an enzyme that metabolizes xenobiotics). Low does of benzo[a]pyrene and fluoranthene also induced Cytochrome P450 expression. (Benzo[a]pyrene induces the expression of xenobiotic metabolizing gene Cytochrome P450 1A while fluoranthene inhibits the synthesis of 1A.) The authors used these two compounds to determine how selective their assay was for Cytochrome P450 3A activity. The authors we able to demonstrate that their method is selective for measuring the activity of Cytochrome P450 3A and we able to do so without the destruction of the organism.

Useful to aquarists, the authors prescribe a 1 minute wash in 0.3% hydrogen peroxide to sterilize the eggs followed by three rinses with fresh water.

[Tyrone Genade]

**Copper alters hypoxia sensitivity and the behavioural emersion response in the amphibious fish Kryptolebias marmoratus.** Blewett TA; Simon RA; Turko AJ; & Wright PA. *Aquatic Toxicology*, 189:25–30, 2017. ISSN 0166-445X, DOI [https://doi.org/10.1016/j.aquatox.2017.05.007](https://doi.org/10.1016/j.aquatox.2017.05.007)

The soil of mangrove swamps can accumulate copper ions up to 4050 µg/g (dry weight) and aqueous concentration can reach 110 µg/L. Worse still, the highest copper concentration are associated with mangrove swamps close to urban areas. The authors set out to determine how copper toxicity effects the sensitivity of *K. marmoratus* to oxygen deficits (i.e. hypoxia). The authors used the emersion response bioassay in these experiments: when water oxygen levels get too low the fish will emerse. The authos exposed fish to different copper concentrations and then bubbled nitrogen gas through the water, driving the oxygen out of the water. The time it took for the fish to emerse was measured. As expected, fish exposed to high doses of copper emersed sooner than low-dose exposed fish, indicating that the fish began to experience hypoxia at high oxygen concentrations than control fish. The gills were examined for interlamellar cell mass in the gills. It was expected that the fish would response as per trout, which at low does of copper already has thickened gill epithelium (i.e. interlamellar cell mass). Unexpectedly, the copper treated *Kryptolebias* has thinner gill epithelium than high-dose copper treated fish. The thinning of the epithelium is similar to that seen in *Kryptolebias* that have been habituated for terrestrial life (Turko et al). The authors speculate that the copper may have altered oxygen physiology, resulting in the remodelling of the gills to allow for faster oxygen
absorption (the thinner the epithelium the faster the oxygen can cross into the blood). The authors also suspect that, since dissolved organic carbon concentrations were higher in the copper-treated fish water, that the fish were secreting mucus to bind free copper and reduce its toxicity. Mucus would have hindered water flow through the gills, triggering an hypoxia response. The author didn’t observe mucus but the fixation methods used were inadequate for this task. For the fixation of mucus for light microscopy the method of Yukin et al employing glutaraldehyde in DMSO would serve better. Turko et al (2012) J Exp Biol. 215:3973–3980. doi: https://doi.org/10.1242/jeb.074831 Yukin et al (1989) Biol Reprod. 40:661–71 Tyrone Genade

Life history strategies of annual killifish Millerichthys robustus (Cyprinodontiformes:Cynolebiidae) in a seasonally ephemeral water body in Veracruz, México. Domínguez-Castanedo O; Uribe MC; & Rosales-Torres AM. Environmental Biology of Fishes, Epub ahead of print, 2017. URL http://dx.doi.org/10.1007/s10641-017-0617-y

Developmental ecology of annual killifish Millerichthys robustus (Cyprinodontiformes: Cynolebiidae). Domínguez-Castanedo O; Valdesalici S; & Rosales-Torres AM. Developmental Dynamics, Epub ahead of print. ISSN 1097-0177, DOI http://dx.doi.org/10.1002/dvdy.24519

These two papers describe the life history of Millerichthys robustus. The “Life history strategies” paper is more detailed than the “Developmental ecology” paper. In the former paper the author describes the development and life cycle of M. robustus, providing data on growth rate, sex differences, and egg development.

Domínguez-Castanedo et al report that males and females achieve sexual maturity at 3 weeks (based on ovary development). At this age males are almost 3 cm long while females are just over 2 cm. Males are almost 3× as heavy as females at this age. Maximum size is approximate 5 cm for males and this is reached by 10 weeks. Females reach ≈5 cm at 6 weeks of age. The authors hypothesize that this is due to the females investing more energy into egg production and/or female selection for large males. Eggs were collect from the pond sediment throughout the life cycle of the fish. During the time that the ponds were flooded (October–March) only eggs in diapause I were observed. When the pond had dried out (April–June) eggs in diapause II were found. When the rain returned and humidly increased both diapause II an III embryos were observed. It was observed that at 2 weeks into the wet season, when the pond was filled and the fry had hatched, a smaller cohort of fry appears. They authors hypothesize that these are the remaining Diapause II embryos that had quickly developed and then hatched. Austrolebias limnaeus enters Diapause II at 15 days post fertilization and can develop to pre-hatching in 32 days: only 17 days from DII to DIII.

In the “Developmental ecology” paper the authors state that “the isolation in which M. robustus evolved represents a useful model with which to understand certain aspects of diapause evolution in Aplocheiloid fishes. It can be used to compare annual life history strategies between fishes from the other groups in South America and Africa to determine different adaptation in developmental biology research.” I would add that in the comparisons between the different genera exhibiting diapause we would discover the common mechanism. Both papers make for interesting reading. The description of the development in tandem with environmental conditions immediately suggest ways to improve the captive maintenance of South American Annuals. The combination of increased heat and humidity can be used effectively to control development, and this careful control of development might serve to prevent belly-sliding. It has long been reported that a brief wetting of peat containing South American Annual eggs and its repeat incubation for a short time results in a
large and healthy hatch of fry. This is born out by the field observations of *M. robustus*.

[Tyrone Genade]

The Environmental Biology of Fishes paper is remarkable and pioneering and its observation on the link between diapause state and seasonal periods (wet, drought, humid) are worth scrutiny by ecologists and embryologists specialists for Africa and South American annual fishes.

[Jean Huber]

**Life stage dependent responses to desiccation risk in the annual killifish *Nothobranchius wattersi***. Grégoir AF; Philippe C; Pinceel T; Reniers J; Thoré ESJ; Vanschoenwinkel B; & Brendonck L. *Journal of Fish Biology*, Epub ahead of print. ISSN 1095-8649, DOI http://dx.doi.org/10.1111/jfb.13385

In this paper the authors strove to determine how the desiccation of ephemeral habitats of *N. wattersi* effect maturation time and reproductive fitness. The authors hypothesized that both juvenile and adult fish confronted with decreasing water levels will response by maximizing their reproductive output and predicted that juvenile fish will mature sooner and that adult fish will increase fecundity. The authors investigated the trade-offs between maximizing reproduction and other life history traits.

For their experiment the authors bred *N. wattersi* in their laboratory for several generations and then, on hatching a large group of fry, they divided the fry into two groups. The first group was reared in water of 10 cm depth and the second group was reared in water of 4 cm deep until adulthood. Fish of both groups were then transferred to tanks of 20 cm deep and setup for spawning. Then, half the fish from each group were then exposed to a second water level drop to 4 cm. The water level was dropped in 2 cm intervals.

No difference was observed with respect to male maturation nor in body size. Juveniles exposed to the water drop did not produce more eggs but adults did. There was also no change in egg size between the groups. Total egg production was negatively correlated with female lifespan, confirming other studies showing a decline in female fertility with age. Adults exposed to a drop in water level had a 19% shorter lifespan compared to controls and it was females that were dying faster between the two experimental groups (28% shorter lifespan).

The data show that juveniles are not responsive to a drop in water level but adults are. There was no early maturation in response to declining water level but there was a strong response to increase female fecundity that came at the expense of lifespan. This has important implications for aquarists. In the hobby it has been widely reported that lowering the water level stimulates spawning of killifish. This study confirms our anecdotal observations but reveals that there is an unaccounted cost to increased spawning: shorter lifespans of our fish.

[Hatching Date Variability In Wild Populations of Four Coexisting Species Of African Annual Fishes. Reichard M; Blažek R; Polačik M; & Vrtílek M. *Developmental Dynamics*, Epub ahead of print, 2017. ISSN 1097-0177, DOI http://dx.doi.org/10.1002/dvdy.24500

The Reichard lab team here investigate four coexisting species of *Nothobranchius* (*furzeri*, *kadleci*, *orthonotus* and *pienaari*) to see if hatching is synchronous across species as well as across regions and individual pools in the years 2010, 2012 and 2013. The degree of synchronisation was not consistent. Between the four species *N. kadleci* hatched significantly earlier than the other three species. Hatching dates didn’t significantly vary across regions but some individual pools showed greater variation and in a small number of sites multiple cohorts of fish were identified and mean hatching date also differed between the years, ranging from 26th January to 17th February.
Of particular note was that most eggs (81%) hatched between 0 and 20 days after pools filled, an adaptation believed to be a safeguard in case pools didn’t sufficiently fill when first inundated. Again there is a lengthy discussion in this paper that defies over-simplification. The authors conclude that a combination of environmental factors and variation in embryonic ability to respond to environmental cues provide a safeguard against unpredictable rains.

[Andy Patel]

**The role of energetic reserves during embryonic development of an annual killifish.** Vrtílek M; Polačík M; & Reichard M. *Developmental Dynamics*, Epub ahead of print. ISSN 1097-0177, DOI http://dx.doi.org/10.1002/dvdy.24528

*Nothobranchius furzeri* (Turquoise killifish) features in this study of the impact females can have on their offspring’s earliest development. The results challenge the idea that the amount of energetic reserves stored in an egg correlate to the length of time the embryo takes to develop. The notion that older females produce embryos that take longer to develop is also brought into question. Their findings suggest that diapause has much more control over the duration of embryonic development and that any increase in energetic reserves is more likely geared towards survival rates after hatching. The authors found large differences in the size of energetic reserves provided between females and the experimental details reveal the exacting nature of this piece of research. That suggests some benefit to providing larger energetic reserves, but not necessarily at the embryonic stage. Another step in detailing the intricate nature of an annual killifish life cycle.

[Andy Patel]

**Intermittent food restriction initiated late in life prolongs lifespan and retards the onset of age-related markers in the annual fish *Nothobranchius guentheri*.** Wang X; Du X; Zhou Y; Wang S; Su F; & Zhang S. *Biogerontology*, 18:383–396, 2017. ISSN 1573-6768, DOI http://dx.doi.org/10.1007/s10522-017-9699-3

This paper by Wang et al describes the effect of intermittent fasting on aging biomarkers of *N. guentheri*. First, some terminology. Calorie and Dietary restriction differ in that the former is an average reduction in calories consumed per day while dietary restriction is a reduction of a particular nutrient or total nutrition without causing malnutrition. Intermittent fasting is a form of dietary restriction by which the animal is fed and fasted in regular cycles without a change in calories per day for those days the animal is fed.

The authors fed *N. guentheri* for five days with the same amount of food as the control group and then for two days fed the fish a quarter of the normal quantity of food. The experiment was started when the fish were 36 weeks of age (9 months) and then samples to compare the control and experiment groups were taken at week 36, 38, 42, 46 and 50. Median lifespan in the fasting group increased from 47 to 51 weeks. During this time there was a significant decrease in body mass among the fasted fish but no change in body length was observed. (Markofsky (1976) showed that *N. guentheri* approach maximum size between 35 and 42 weeks of age.) When comparing 38 and 46 week old fish the amount of aging biomarkers (lipofuscin, carbonylated, alondialdehyde) continued to increase with age in both populations its increase was slowed in the fasting group. The activity of the antioxidant enzymes glutathione peroxidase (repairs oxidized lipids), catalase and superoxide dismutase (quenches oxygen radicals) also decreased with age but were relatively higher in fasted fish. The expression of SIRT1 and FoxO3a dropped with age but at a slower rate in fasted fish. On the other hand the expression of Insulin-like growth factor 1 (a cellular receptor that senses nutritional status and signals cell growth) was lower in the fasted fish as would be expected from decreased calorie intake.

What is really special about this paper is that the au-
The authors didn’t only samples fish at different weeks but also at different days. They fasted fish over the weekend and then samples on Monday, Tuesday, Wednesday and Friday and also showed that the values of the biomarkers varied with the number of days from the fasting period. The authors state that “late-onset intermittent fasting may induce an increased lifespan via a combined action of the anti-oxidant system and insulin/insuline-like signalling pathway in aging fish,” and “…even late onset intermittent fasting regimes can reduce body weight, retard the onset of age-related markers, and prolong lifespan.” The 5:2 diet\(^7\) of five days eating normally/liberally (1800-2400 calories per day) and two days fasting (500–600 calories per day) has become popular as of late and many anecdotal health benefits have been reported. Studies on humans have, for example, found expression changes in SIRT3 (Wegman et al, 2015) and improvement in various health-markers, such as blood pressure and blood glucose levels, in individuals whose health was poor (Wei et al, 2017). The \textit{N. guentheri} data compliments the human data (and anecdotal claims) and it is encouraging to know that intermittent fasting is not only of benefit to one’s health but could extend human lifespan.


[Tyrone Genade]

\textbf{Oogenesis, vitellogenin-mediated ovarian degeneration and immune response in the annual fish \textit{Nothobranchius guentheri}}. Liu T; Liu S; Ma L; Li F; Zheng Z; Chai R; Hou Y; Xie Y; & Li G. \textit{Fish & Shellfish Immunology}, 66:86–92, 2017. ISSN 1050-4648, DOI https://doi.org/10.1016/j.fsi.2017.05.015

This is a very interesting paper that elaborates on the link between the reproductive and immune systems in \textit{N. guentheri}. The authors note that the habitability of embryos is lower for eggs obtained from aged fish compared to young fish. They then compared 6 and 12 month old fish with respect to the number of oocytes at different developmental stages in the ovaries; senescent-associated-\(\beta\)-galactosidase and lipofuscin staining in the tissue (markers of biological age); expression levels NF-\(\kappa\)B and IL-8 (immune function markers) as well as vitellogenin in the liver and ovaries. The authors report an increase in both undeveloped and atretic (degenerative) oocytes with age as well as an increase in associated-\(\beta\)-galactosidase and lipofuscin while liver and ovary vitellogenin decreased. Immune stimulation increased the number of immature and atretic oocytes as well as the intensity of senescent-associated-\(\beta\)-galactosidase and lipofuscin in both the liver and ovary the expression of NF-\(\kappa\)B and IL-8 increased in aged fish. Also, upon stimulation of the immune system there was a greater response in the aged fish. Conversely, vitellogenin expression decreased with age and there was no significant increase followed by immune stimulation as observed in young fish.

The authors don’t define what is meant by “low habitability”. We now know that the age of the maternal fish effects the duration of diapause (Polačík et al, 2017) so is this increased duration of diapause? Or are they referring to delayed hatching (Martin & Podrabsky, 2017) or an inability of mature embryos to hatch? This is an interesting question. For the hobbyist the take home message is that sick or old fish will yield poor quality eggs. Spawning younger fish would yield more healthy fry.


\[\text{Tyrone Genade}\]
Resveratrol inhibits age-dependent spontaneous tumorigenesis by SIRT1-mediated post-translational modulations in the annual fish *Nothobranchius guentheri*. Liu T; Ma L; Zheng Z; Li F; Liu S; Xie Y; & Li G. *Oncotarget*, Epub ahead of print, 2017. URL https://dx.doi.org/10.18632/oncotarget.19268

This is a wonderful paper. It takes *N. guentheri* from the promise of a model that can be used to study the development of age-related tumors & cancer to being a model that can be used to explain how tumors & cancer come about in the course of aging; and as a bonus shows resveratrol exerts its anti-cancer effects in *Nothobranchius*. There is far too much in this paper to effectively summarize so I will briefly state the methods and key findings.

The authors used *N. guentheri* for their experiments and fed the experimental group resveratrol at 200 µg per gram food. They then sampled fish at 6, 9 and 12 months of age. Livers were collected and weighed and then either fixed for histology or their proteins extracted for immunoblotting (for protein quantification) or used for protein activity assays. The authors observed that resveratrol protected the liver against age-related degeneration. They could trace this positive effect to the activity of SIRT1 enzyme acting on the Ras/PI3K/AKT cellular signaling pathway. A decline in the activity of this pathway resulted in lower concentration of acetylated-FoxO1, phospho-FoxO3a and Bcl2 and a higher concentration of DLC1, Bax, Caspases 3, 7 and 9. The former proteins are anti-apoptotic while the latter are pro-apoptotic meaning that the former preserve cells while the latter kill cells. Cancer cells are apoptosis-resistant so the resveratrol was sensitizing the tumor cells for apoptosis. DLC1 and the FoxO proteins are down-stream targets of SIRT1 enzyme and the Ras/PI3K/AKT pathways. SIRT1 decreased the activity of the Ras/PI3K/AKT pathway while increasing its activity on its direct down-stream targets.

This paper demonstrates the power of *Nothobranchius* for cancer and pharmacological research.

**Acute and chronic sensitivity to copper of a promising ecotoxicological model species, the annual killifish *Nothobranchius furzeri*.** Philippe C; Grégoir AF; Janssens L; Pinceel T; Boeck GD; & Brendonck L. *Ecotoxicology and Environmental Safety*, 144:26 – 35, 2017. ISSN 0147-6513, DOI https://doi.org/10.1016/j.ecoenv.2017.05.047

The authors identify the need for compounds to be tested for their long-term affect on ecosystems and point out that the long lifespan of traditional vertebrate models are inadequate for the task. In this paper the authors present their data, which they believe makes a strong case for the use of *N. furzeri* for eco-toxicology experiments. This work draws on earlier experiments by Shedd et al who used *N. guentheri*. Philippe et al note the male aggression in the latter species and report that this isn’t as much a problem in *N. furzeri*. The authors exposed *N. furzeri* fry to varying concentrations of copper cations (an environmental toxin that is well studied and for which there is abundant vertebrate data for comparison) to determine the Lethal Dose needed to kill 50% (*LD* 50) of the population within 96 hours as well as the effect of lower, sub-lethal, doses on lifespan.

The authors report an *LD* 50 of 38.49 µg/L in hard water (400 mg CaCO 3/L) which was comparable to that for *N. guentheri*. This value was ≈8 times lower than for Rainbow trout in hard water (but rainbow trout are just as sensitive in soft water); and ≈4 times lower than for zebrafish in soft water. Fish exposed to 19.38 µg Cu/L had median survival of 32 days compared to other experimental concentrations which had 97 day median survivals. The experiments were unable to determine effects on growth, fertility and fecundity due to small sample sizes. Increasing copper concentration did seem to delay male and female maturation.

On initial exposure tissue copper content correlated with
the copper dose. Fish that survived the sub-lethal doses seemed to adapt to the copper content of the water and showed little difference in tissue copper content between the experimental groups. There was a small but significant correlation between copper concentration and the tissue abundance of the protein metallothionein, which binds, sequesters and helps in the elimination of metal toxins.

Copper toxicity in the fish manifested as a loss of buoyancy where after the condition of the fish quickly deteriorated and the fish died. Effects on growth were observed. No histopathology was done to determine the effects on individual tissues nor were any behavioral assays performed. It is an open question as to whether low dose copper exposure has adverse effects that were not observed in these experiments. A very interesting question is whether chronic exposure of adults has any effect on the offspring. A major issue with the paper is that the experiments were performed at a low temperature (22°C) and that the fish were raised under cramped conditions. Otherwise, this is a good paper that affirms the potential for these fish in eco-toxicology studies.


[Andy Patel]

Chromosomal organization of four classes of repetitive DNA sequences in killifish Orestias ascotanensis Parenti, 1984 (Cyprinodontiformes, Cyprinodontidae). Araya-Jaime C; Lam N; Pinto IV; Méndez MA; & Iturra P. Comparative Cytogenetics, 11:463–475, 2017. ISSN 1993-0771, DOI https://doi.org/10.3897/compcytogen.v11i3.11729

The authors used classical and molecular cytogenetic methods to study chromosome organization. They focused on four classes of repetitive DNA sequences (histone H3, U2 sn RNA, 18S rDNA and 5S rDNA) of the chromosomes of Orestias ascotanensis. All individuals analyzed had 48 diploid chromosomes. The authors were able to discern the structure of the chromosomes and map the four repetitive DNA sequences to specific locations on chromosomes. No doubt this study is a prelude to a larger study of Orestias chromosome evolution and we can expect very interesting results from this research group.

[Andy Patel]
Longitudinal variability in lateral hydrologic connectivity shapes fish occurrence in temporary floodplain ponds. Couto MTB; Zuanon DJ; Olden DJD; & Ferraz DG. Canadian Journal of Fisheries and Aquatic Sciences, 0:null, 0. DOI https://doi.org/10.1139/cjfas-2016-0388

In this very interesting work a group of Brazilian scientists describe in detail how annual flooding (The “flood pulse Concept” ) of the larger Amazon rivers and smaller creeks leaves temporary pools and how these pools have effect on the fish that live in these environments. The aim in this study is to review the main findings related to the forming of temporary pools and point future paths to better understanding and conservation of these environments, yet little studied, from the Amazon.

They did recognize several types of rivers and streams, which generate different conditions and frequency of flooding. Other than believed so far, there is a relation between the fish population that inhabit the channels and the ones that inhabit puddles and pools. Temporary pools may be invaded by fish species that remain there after the flooding but others seek these refugee places by themselves and live there for some short period or even over a long period until pools dry up. The rapid filling and drying dynamics, as well as the low concentration of oxygen in the water make the temporary pools not a potentially ideal environment for most species of fish from streams. However, some species have adapted to these conditions and field experience shows that most species dwelling in pools show resistance to conditions of hypoxia by various types of accessory breathing. The work mention in this behalf members of the families Erythrinidae, Lebiasinidae, Callichthyidae, Trichomycteridae, Hypopomidae, Gymnotidae, Rivulidae and Symbranchidae. Authors describe in this behalf the special ability of Anablepoides micropus8 (Rivulidae) that can survive between moist leaf litters for days when pools dry out. In this study it is recognized that pools provide shelter during flooding and thereafter, to many species that normally live in the creeks and mean channels, some of them only in their juvenile state, others as long as the pools contain water and that the number of species in the temporary pools is found higher than in the close-by creeks. To ignore the existence and value of these temporary pools would mean neglecting over 50% of the number of fish in a given site. In the last chapter “Challenges for Conservation” authors point out that because of urbanization and agricultural expansion, in addition to the increase in infrastructure works and many other human activities listed in the Amazon region, the integrity of aquatic ecosystems is at risk and the need to look more carefully to the delicate relationship between the fish and the environments in the Amazon terra firme and prevent future loss of aquatic biodiversity.

[Frans Vermeulen]

Ed’s Note: This paper was foreshadowed by an earlier, more detailed account: Thiago Belisario d’Araújo Couto, Helder Mateus Viana Espírito-Santo, Rafael Pereira Leitão, Douglas Aviz Bastos, Murilo Sversut Dias & Jansen Zuanon, Os peixes e as poças: o uso de áreas marginais alagáveis por peixes de igarapés amazónicos. Boletim Sociedade Brasileira de Ictiologia, No 116:31-40, 2015.

The evolution of vertebrate eye size across an environmental gradient: phenotype does not predict genotype in a Trinidadian killifish. Beston SM; Wostl E; & Walsh MR. Evolution, Epub ahead of print. ISSN 1558-5646, DOI http://dx.doi.org/10.1111/evo.13283

Beston et al are interested in how ecological factors affect the evolution of eye size. The authors tested the influence of increased predation on eye size in R. hartii. The authors hypothesized that if increased eye size enhanced antipredator response then eye size would increase and, conversely, if vi-

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8In Killi-Data.org this species is still regarded as Rivulus.
usually orientated predators target eye pigmentation then eye size would decrease as predation pressure increases.

The authors collected *R. hartii* from high-, low- and no-predation habitats of three streams and measured eye size. Some fish were introduced to aquaria to found experimental populations. These captive populations had their eyes measured in the second generation. The authors also compared eye size between populations from low- and high-light habitats to determine if lighting was a confounding factor.

It was found that eye-size was inversely correlated with predation intensity and that this correlation was stronger in females than males (i.e. female had smaller eyes than males). The authors also observed that eye-size varied with the river of origin. Lighting of wild populations did not effect eye size. For captive fish the difference between high- and no-predation populations was consistent across generations. They also observed that eye-size was inversely correlated with food supply.

The authors conclude that there is a genetic component to the difference in eye size and that the decrease in eye size is best explained as a response against predation risk. The results are discussed in respect to the paper on brain size Kotrschal et al (2017) where males had smaller brains than females and conclude that brain and eye size evolution are distinct. The authors close by hypothesizing that small eye size is selected for in high predation environment and that large eye size may be beneficial in sites that lack predators due to high densities, low food availability, and the benefits associated with optimal foraging. This is a very interesting paper and well worth reading.


**What have we learned on aging from omics studies?** Cellerino A & Ori A. *Seminars in Cell & Developmental Biology*, Epub ahead of print, 2017. ISSN 1084-9521, DOI https://doi.org/10.1016/j.semcdb.2017.06.012

In this paper Cellerino & Ori review transcriptomic research and show the consistent findings as pertaining to aging research. The review focuses on the results obtained from *Nothobranchius* research. They distill several key questions that need to be addresed to fully capitalize on transcriptomic research to explain organ aging and instruct the design of health-span promoting interventions.

**Posters & Conference Abstracts**

**Physiological Plasticity and Tolerance of Fundulid Killifish Species to Aquatic Hypoxia.** Hoffman R; Borowiec B; Scott G; Adams C; & Galvez F. *The FASEB Journal*, 31:719.17, 2017. URL http://www.fasebj.org/content/31/1_Supplement/719.17.abstract

This research was presented at the annual meeting of the Experimental Biology Conference held April 22–26, 2017. The authors hypothesized that species from hypoxia-prone environments are more tolerant to hypoxia than species that live in stable environments; as well as that fish from more
variable environments would show a greater scope for physiological acclimation responses. The authors used Lucania goodei and parva as well as Fundulus confluentus and rathbuni for their experiments. They acclimated the fish to either constant hypoxia or diurnal cycles of intermittent hypoxia. F. confluentus, an estuarine killifish, demonstrated a greater hypoxia tolerance than F. rathbuni which inhabits freshwater. Both Lucania species had similar baseline hypoxia tolerance. Gradual hypoxia acclimation improved hypoxia tolerance but no clear patterns were visible between the species.

[Tyrone Genade]

**Understanding and modeling aging.** Brunet A. The FASEB Journal, 31:256.3, 2017. URL http://www.fasebj.org/content/31/1_Supplement/256.3.abstract

This research was presented at the annual meeting of the Experimental Biology Conference held April 22–26, 2017. Brunet points out that our understanding of aging-biology is rudimentary on account of the complexity of the aging process which defies general rules in biology. She reports that her lab has developed a high throughput screening platform to model diseases and screen for aging pathways that can be effected by chemical compounds. She is also using Caenohabditis elegans and mice and human cell cultures to identify genetic and epigenetic mechanisms that regulate lifespan. She reports that “his approach has already generated new insights on the epigenetic regulation of aging. Our work has the promise to transform our understanding of why aging is at the heart of so many human diseases.”

[Tyrone Genade]

**Lessons from Fundulus melanophores: integrative approach in biology education.** Hong SM. The FASEB Journal, 31:576.44, 2017. URL http://www.fasebj.org/content/31/1_Supplement/576.44.abstract

This research was presented at the annual meeting of the Experimental Biology Conference held April 22–26, 2017. The authors describe an undergraduate course that employs physiological color changes as a teaching model of physiological concepts and principles. The authors employed Fundulus heteroclitus because their dermal melanophores are a well characterized useful model system due to the fast time course highly visual physiological responses can be ascertained.

[Tyrone Genade]

**Interesting research on other fish**

**Complexities of gene expression patterns in natural populations of an extremophile fish (Poecilia mexicana, Poeciliidae).** Passow CN; Brown AP; Arias-Rodriguez L; Yee MC; Sockell A; Schartl M; Warren WC; Bustamante C; Kelley JL; & Tobler M. Molecular Ecology, Epub ahead of print. ISSN 1365-294X, DOI http://dx.doi.org/10.1111/mec.14198

In this paper Passow et al report results from a transcriptome investigation of Poecilia mexicana adaptation to hydrogen sulfide. Hydrogen sulfide is more toxic than cyanide. It inhibits cytochrome oxidase enzyme in the mitochondrial. If this enzyme is inhibited aerobic respiration grinds to a halt and the organism quickly expires due to a lack of ATP-energy to sustain physiological processes.

The authors sought to find out how gene expression varies among populations from different habitats. and whether there are expression changes in common among fish from similar habitats. Fish were samples from nonsulfidic and sulfidic surface and cave habitats. Most expression differences correlated with organ type. Exposure to an environmental stressor elicited unique expression differences among the organs. Exposure to sulfide had a greater influence on expression patterns than cave-habitat. The transcriptome annotations provide a spring board for investigating physiological mechanism pertaining to adaptation to extreme environments.

[Tyrone Genade]

This paper describes the physiological effect of increasing ammonia concentrations on Oryzias melastigma. Fish were exposed to ammonia concentrations ranging from 0.1 to 1.1 mmol/L (approximately 18.7 ppm total ammonia). Fish displayed a reduction in growth, feed intake and total protein content. Growth rate was reduced from 0.3 mmol/L ammonia (5.1 ppm total, ≈ 0.03 ppm at pH 7.5). After two weeks the tissue ammonia concentration between the fish raised at different ammonia concentrations was the same, indicating that the fish acclimated to the environmental ammonia concentration. It took 8 weeks for the tissue ammonia to reach that of control fish, in a zero ammonia experiment. Fish showed decreased amino acid metabolism as well as overall energy metabolism (as measured by three metabolites). The authors state that the fish “cope with sublethal ammonia…[by] reducing the ammonia production and increasing ammonia excretion. This is noteworthy to us hobbyists that seek to maximize the growth of our fish and keep them reproductively fit. If fish compromise feeding and energy production to cope with ammonia then this will directly effect the reproductive output of our fish as well as stunt fry growth.

[Genade: Killi-Data News (Fall)]

Predation pressure shapes brain anatomy in the wild. Kotrschal A; Deacon AE; Magurran AE; & Kolm N. Evolutionary Ecology, Epub ahead of print, 2017. ISSN 1573-8477, DOI http://dx.doi.org/10.1007/s10682-017-9901-8

The guppies (Poecilia reticulata) of Trinidad have been studied for many years. Here the authors investigate the combined effects of predators on female guppy brains. Females were chosen as their brains are more accessible due to differences in cranial anatomy. Fish were collected from multiple sites where the number of predator species was recorded along with biomass. The four main predators included two cichlids, wolf fish and shrimps. Existing research associating particular regions of the brain with specific functions/abilities were utilised to identify which predators might influence the size of particular regions in the guppy brain. Amongst their findings some noteworthy ones are:

(a) telencephalon size increases with increased biomass of prawns (the telencephalon controls learning and memory) who take longer to eat their prey so being able to learn from observing other guppies being eaten is beneficial to the individual

(b) optic tectum size increases with increased biomass of wolf fish (the optic tectum processes visual signals) and being able to see the camouflaged and secreted predator more quickly is a bonus.

The paper is a fascinating read and discusses far more than can be covered in a short review. In short predators are a selective pressure on brain size, increasing the size of some areas and decreasing the size of others. Of note is that larger brains are not always an advantage and did you know that the hippocampus of cab drivers increases with experience?

[Genade: Killi-Data News (Fall)]

Thermal stress, thermal safety margins and acclimation capacity in tropical shallow waters—An experimental approach testing multiple end-points in two common fish. Madeira C; Mendonça V; Leal MC; Flores AA; Cabral HN; Diniz MS; & Vinagre C. Ecological Indicators, 81:146 – 158, 2017. ISSN 1470-160X, DOI https://doi.org/10.1016/j.ecolind.2017.05.050

Not directly related to killifish but this research emphasises the potential impact of climate change. Two species of marine fish found in the intertidal zone Abudefduf saxatilis (Sergeant Major) and Scartella cristata (Molly Miller) were
evaluated for their ability to adapt to increasing temperature. Despite their known abilities to move rapidly across a thermal gradient as the tide rises and falls both species were affected in the short term with reduced size and bodyweight. In the longer term it is anticipated that these species would adapt and survive but may already be under stress in warmer years. The authors highlight the need to monitor intertidal habitats closely. (Reviewer note: The implications for species from more stable environments are likely to be much higher).

[Andy Patel]

There are many other interesting papers which our small editorial team haven’t had the chance to review. Here follows a short selection.

**Sex-specific evolution during the diversification of live-bearing fishes.** Culumber ZW & Tobler M. *Nature Ecology & Evolution*, Epub ahead of print, 2017. ISSN 2397-334X, DOI [http://dx.doi.org/10.1038/s41559-017-0233-4](http://dx.doi.org/10.1038/s41559-017-0233-4)
