



# KILLI-DATA INTERNATIONAL

## *Killi-Data News*

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### Editorial

Over the last three months several interesting and exciting papers have been published. By now most of you have heard the *Nothobranchius* fish poo news emanating from the Valenzano lab. That paper is reviewed and certainly has repercussions for the health of our captive fish. Polaçik et al have published interesting data with ramifications as to how we breed and incubate annual killifish.

The big news in this issue is the paper from the Reznick lab which Jean Huber reviews. The contents of that paper goes to the heart of the question of just what a killifish is. The phylogenies from Reznick et al may fundamentally transform our understanding of the origin and evolutive timing of Cypriodontiformes (including killifish). Once again, there are several papers on *Fundulus heteroclitus* on topics of genetics, hybrid zones, evolution and environmental toxicology.

Again, there are new species of *Melanorivulus*-group and I think we can expect more species. There are three articles on the embryonic development of South American Annual fish: *Austrofundulus*, *Austrolebias* and *Millerichthys*. Martin & Podrabsky review the concept of developmental arrest and define the terms diapause, delayed hatching etc. . . as well as review the knowledge of diapause learned from *Austrofundulus*.

I hope you enjoy this issue and put the information to some use in your fishrooms and/or research projects.

[Tyrone Genade]

## Interesting Websites

**Andrew Thompson** published a very interesting video of *Rivulus (Anablepsoides) holmiae* spawning above the water level in mops: <https://www.facebook.com/groups/1585735854987933/permalink/1984903501737831/>. Having trouble spawning your *Rivulus* species? Maybe you should drain the tank.

**Glednanda Gledison Fernanda** has again published more YouTube videos of South American killifish and their biotopes: [https://www.youtube.com/channel/UCglwiywhb\\_bh\\_iQBV\\_fPPgA](https://www.youtube.com/channel/UCglwiywhb_bh_iQBV_fPPgA).

## In The News

**UTA student receives NSF grant for research into ecological drivers of eye, brain size**  
[https://www.eurekalert.org/pub\\_releases/2017-04/uota-usr042517.php](https://www.eurekalert.org/pub_releases/2017-04/uota-usr042517.php)

A student from University of Texas, Shannon Beston, has won a National Science Foundation grant to study the ecological drivers of eye and brain size. The subject of the research: *Rivulus hartii*.

**Evolution's Quick Pace Affects Ecosystem Dynamics**  
<http://www.the-scientist.com/?articles.view/articleNo/49258/title/Evolution-s-Quick-Pace-Affects-Ecosystem-Dynamics/>

This news article reviews the work of the Reznick lab and concerns the rapid evolution of guppies transfer to predator-free streams. It goes on to discuss other evidence for rapid evolution in response to ecological change.

**Update on popular fish model of development**  
<https://www.sciencedaily.com/releases/2017/05/170509084050.htm>

The work of Jason Podrabsky is discussed. There is a nice photo comparing young and old, male and female, *Austrofundulus limnaeus*.

## Review of New Research Publications

### Research Report

**George Maier Fund Grant Review, 2004–2016** Nunziata, C. *Journal of the American Killifish Association*, 50:33–64, 2017.

The March-April issue of JAKA isn't a book but doesn't really fit into the other categories below. In this issue Charles Nunziata reviews the research output over the last twelve years which were funded by the AKA's George Maier Fund. Topics reported on are:

- Watter's *Nothobranchius* fish habitats in the Great Limpopo Transfrontier Park.
- Hrbek's *Rivulus* of Venezuela
- Normandin et al's Distribution of *Brachyrhaphis* and *Rivulus* in Panama.
- Thompson's evolution of diapause.
- Bartons' Jaw morphology and tooth structure of *Cyprinodon* of the Bahamas.
- Brennan's local adaptation of *Fundulus heteroclitus*.
- Turner's characterization of growth hormone genes.
- Collier's biogeography of *Rivulus isthmensis*.
- Burge's study of *Fundulus heteroclitus* and *luciae*.
- Gumm's hybridization between *Cyprinodon variegatus* and *rubrofluviatilis*.
- Stallsmith's survey of *Rivulus* in Panama.
- Nagy & Cotterill's inventory of *Nothobranchius* in Katanga province.
- Gatto's study of wetland species.
- Styga's research into saltatorial locomotion of killifish.
- Benignos' report on habitat restoration and conservation

of endangered Mexican pupfish.

The summaries are brief but well illustrated and references to the published research papers are given. [Tyrone Genade]

## Systematics, Taxonomy & Distribution

**Ichthyofauna of Mundaú river basin, Ceará State, Northeastern Brazil.** Teixeira FK; Ramos TPA; Paiva RECd; Távora MA; Lima SMQ; & Rezende CF. *Biota Neotropica*, Epub ahead of print, 2017. URL <http://dx.doi.org/10.1590/1676-0611-bn-2016-0174>

Among the Brazilian museums, if the 3 major (Rio de Janeiro, Sao Paulo and Porto Alegre) which hold more than 95% of the type-species of killifish are not taken into account, recent dynamics have emerged in order to sample in detail the fish fauna in their given territory aiming at cataloging the fishes of a basin. Usually killifish are not the main target of those extensive collections because they are not riverine fishes.

Sometimes the research and sampling team is also interested in Killifish and that gives the opportunity of attractive findings. This is the case of Telton Ramos, Universidade Federal da Paraíba. He is (with S. Ramos) at the origin of the discovery of *Cynolebias parnaibensis* (in 2009), and of *Rivulus parnaibensis*, in his territory. Recently in a collaboration with Universidade Federal do Ceará, new killifish samples were obtained by him (with R. Paiva, F. Keilo, and S. Ramos) in Ceara state, Mundaú river basin which is located at Center-North Ceará State and occupies a total area of 2,227 square km, including Estuário do Rio Mundaú Environmental Protection Area. The collected cyprinodontiformes fishes were identified as *Rivulus cearensis* (3 localities, placed by them in *Anablepsoides*) [a species related to *urophthalmus*], a yet un-named *Simpsonichthys* sp. (*Hypsolebias* group, currently under study) and a livebearer *Poecilia sarrafae* (described by

Bragança & Costa, in 2011). Those findings are important because they extend the distribution of *Rivulus cearensis* (its distribution record is increased by about 77 km west, to the lower portion of Mundaú river, in Itapipoca) and they push to revise its conservation strategy which was described as critically endangered by Costa & Vono, in 2009. Together with collections by European aquarists in 2010 of the species (one of them being Didier Pillet), this means, a good (but rare) news, that the species cannot be considered as critically endangered anymore, even if Telton Ramos remains cautious asking (pers. comm.) that more precise studies should still be performed to know if it is even of *cearensis*. [Jean Huber]

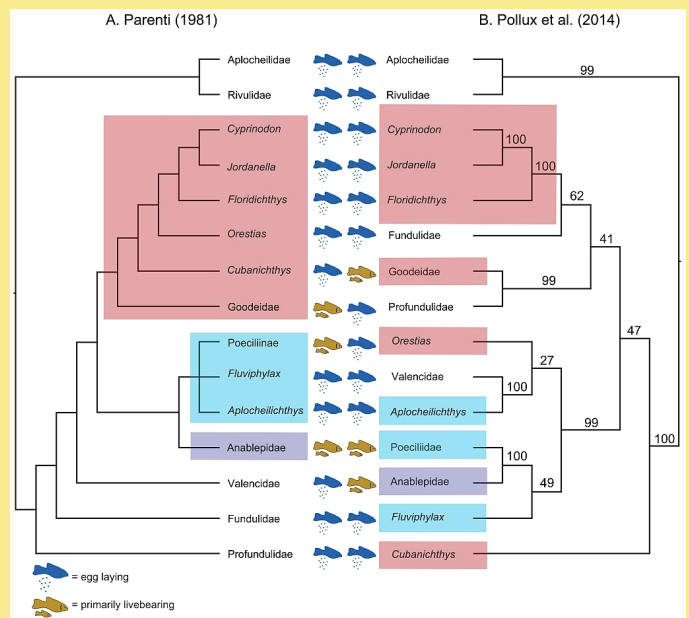
**The origin and biogeographic diversification of fishes in the family Poeciliidae.** Reznick DN; Furness AI; Meredith RW; & Springer MS. *PLOS ONE*, 12:1–20, 2017. URL <https://doi.org/10.1371/journal.pone.0172546>

This is a major work, not only for poeciliins (livebearers) but also for (oviparous) killifish because, for the latter group, it raises important questions with findings that are more in line with recently disclosed killifish fossils (that will need to be tackled soon), and, for the former it proposes a time calibrated molecular tree of many species (and most species groups in livebearers including goodeids). The molecular knowledge on poeciliins is greatly improved with more species studied and more sequences, all time calibrated. Notably one issue is strongly confirmed: *Xenodexia* (viviparous) and *Tomeurus* (oviparous) are very primitive in poeciliins (then a previous hypothesis, as strongly derived, based on osteological cladistics is now considered as obsolete. . . again molecular data and osteological data do not live easily together), but both genera are very much biogeographically apart!

Results in poeciliins are surprising for at least 4 lineages: (1) *Priapichthys* where three groups of species are scattered in the tree pushing to a necessary revision and rediagnosis of

the genus, if valid (and identification move of some species such *darienensis* or *panamensis*, currently as congeners), (2) *Brachyrhaphis* is also heterogenous and a similar systematic treatment to *Priapichthys* is needed, (3) *Cnesterodon* belongs to the same big branch as *Poecilia*, but not *Phalloptychus* and *Phalloceros* although they are vicariant (then the vicariant morpho-osteologically based *Cnesterodontini* tribe is not founded), (4) likewise *Heterophallus* belongs to the same branch as *Gambusia* and is not easily distinguished and might not deserve separate status. Outside poeciliins, there are a number of oddities (but that is not the focus of the paper) that should push to reevaluations, such as (1) in goodeid livebearers, *Xenotoca* is also heterogenous with a congener, *variata*, completely displaced, or at upper group levels, (2) poeciliins and anablepsids are sister groups (a major finding) and more related to each other than to *Fluviphylax* (the next close relative), emphasizing the initial vicariance pattern, if their direct ancestors were not marine fish, then next related to African lampeyes and Eurasian *Valencia* (*Aplocheilichthys* s.l. are most closely allied with Valencidae, and again initial vicariance might be a key because there are fossils related to *Pantanodon* that are close to *Valencia* ancestors), and next again, *Orestias* are related to the above clade-group and not at all to Cyprinodontidae (as thought today) which would push to a major reshuffling of the family-subfamily levels in cyprinodontiformes, (3) the generic definitions by molecular evidence are not at ease (3 examples : *cubensis* and *pengelleyi* in 1 genus or 2 depending on authors are separated by at least 25-35 MYA, *Tlaloc* and *Profundulus* are separated by at least 35 MYA whereas new (2016) molecular data had proposed not more than 20 MYA, *Pseudepiplatys* (*annulatus*) and *Aphyoplatys* (*duboisii*) are separated by at least 30 MYA and still for Collier (2009, 2016) they are not generically separated from *Epiplatys* (however not all researchers relate generic validity to shallowness vs. deepness of molecular divergence, far of it). The authors stress those oddities but they do not go further (at this time) this may be

regretted because there is a strong need to develop diagnoses in line with molecular data (and up to now, everybody failed unless by using only characters that a priori fit with molecular data... which is “already” not bad but unsatisfactory).



Comparison between phylogenies of Parenti and Pollux et al. (Nature 513, 2014, <http://dx.doi.org/10.1038/nature13451>). Based on the data from Helmstetter et al. (Nature communications 7, 2016, <http://dx.doi.org/10.1038/ncomms11271>), Aphanini are a sister taxon to Valencidae where in Parenti's phylogeny the Aphanini are grouped with the Cyprinodontini. Image by Mark Springer of Pollux et al. Figure included by Tyrone Genade.

Finally the paper is also important because the timing method is new for cyprinodontiformes (if I am not mistaken it has been recently used for other groups of fishes): it uses more ancient fossils outside cyprinodontiformes as multiple calibra-

tions to get the timing of diversifications of all groups down the tree (starting with molecular data processed by maximum likelihood as the method of phylogenetic reconstruction, the time tree is being built using another program called PAML). Lower level diversifications of poeciliins are consistent (but actually quite erratic with today's picture) with dispersal over the Aves Land Bridge (proto-Caribbean arch) between South America and the nascent Greater Antilles (around 45 MYA), as anticipated by previous authors. However the upper time diversification results are surprising (but again rather in line with new papers on other groups of fishes, e.g. for cichlids or osteoglossiformes, but not only, far of it). The surprise herein with cyprinodontiformes is that the upper diversifications are not congruent with plate tectonics along Gondwana split (around 130 - 110 MYA) notably the split of present Africa and South America - and the difference is not small, by at least 20 MYA. For example, the diversification of poeciliins + anablepsids is dated minimum 39.9 MYA, maximum 71.2 MYA, around 65 MYA in the tree (based on undescribed poeciliid fossils that are known from the Lumbrera Formation in Argentina), or, the first diversification which produced the first cyprinodontiformes is dated about 78 MYA in the tree (at that time tectonics show a full and wide separation of Africa and America, since long (remember that early molecular works on cyprinodontiformes by Meyer et al., Collier et al., Hrbek et al., back in the early-mid 90ies were mimicking plate tectonics to explain the diversifications in groups (then without fossil calibrations) and obviously that was their (erroneous?) basis for splitting families (see also Huber, 1998, for lower groups). However (1) the new groupings in the present work can be seen as consistent with biogeography (poeciliins + anablepsids + *Fluviphylax* are exclusively New World taxa, and the African *Aplocheilichthys* is not part of poeciliins according to the new results) and (2) the big issue is the date of the much upper node hypothesized as 165.2 MYA maximum for *Clupecocephala* (but could be much earlier, at

least 215 MYA instead) which influences everything down the branches. By comparison of the molecular tree with branch diversifications and present biogeography, the authors propose a series of scenarios, mostly by dispersal (through sea, because of those fish broad tolerance for brackish and saltwater environments), for the distribution of present genera with a very complex (and poorly explained by tectonics) set of migration patterns between north and south America (both ways) and to/from central America with the proto-Caribbean arch as an intermittent and variable platform/path of exchange, much before present (and recent) central American bridge (Mexico to Panama).

In conclusion, a very attractive work that, no surprise, pushes to more questions than to settled issues at the groups levels from upper to lower branches and which leaves unresolved (but somewhat improved) the critical role of central America including Caribbeans islands (West Indies) since the beginning of continental divide as food for (long, never ended) thoughts,

[Jean Huber]

*Ed's note: This paper is available free online.*

**New record of the mangrove rivulid *Kryptolebias hermaphroditus* Costa, 2011 (Cyprinodontiformes: Cynolebiidae) in the Pará state, northern Brazil.** Guimarães-Costa A; Schneider H; & Sampaio I. *Check List*, 13:2093, 2017. DOI <http://dx.doi.org/10.15560/13.2.2093>.

The authors present photographic evidence for *Kryptolebias hermaphroditus* (sensu Costa) at Ajuruteua beach, Pará State, Brazil. DNA was sequenced and its identification as *hermaphroditus* confirmed. The authors note that this is the most northerly location of this species in Brazil, being 1350 km from Rio Grande do Norte (the previously most northern location). Specimen are known from Guyana (Tatarenkov, pers comm.) and it was expected that specimens would be found between Guyana and Rio Grande do Norte.



**Two new species of *Melanorivulus* (Cyprinodontiformes: Cynolebiidae) from Rio Verde drainage, Upper Rio Paraná basin, Brazil.** Volcan MV; Klotzel B; & Lanés L. *Zootaxa*, 4236:82-94, 2017. DOI <http://dx.doi.org/10.11646/zootaxa.4236.1.4>.

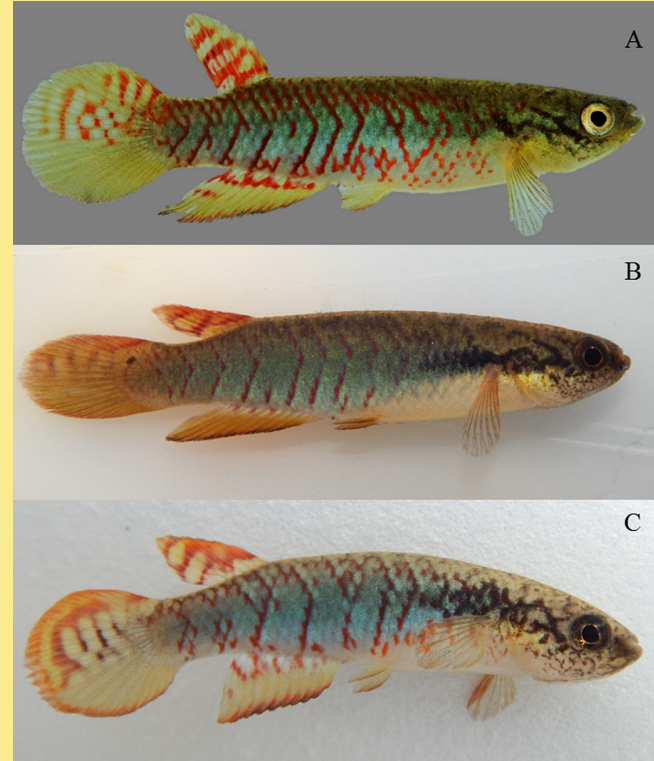


*Melanorivulus nigropunctatus*, living males (above) and female (below) from Água Clara, Mato Grosso do Sul, Brazil. Photo by MV Volcan.

The authors describe two new species of the genus *Melanorivulus*<sup>1</sup> from middle Rio Verde drainage, upper Rio Paraná basin, Mato Grosso do Sul, Brazil. These species, considered endangered, are endemic to Cerrado Biome, considered one of the world's biodiversity hotspots. The main threats

<sup>1</sup>In the KDI database *Melanorivulus* is considered a subgenus of *Rivulus*.

to Cerrado's biodiversity are related to intensive monoculture of grains and extensive farming of livestock. Despite these threats and the area's importance for biodiversity conservation, the Brazilian Cerrado has few protected areas.



*Melanorivulus ofaie*, living males from Ribas do Rio Pardo, Mato Grosso do Sul, Brazil. Photo by MV Volcan.

Both new species are members of the *Melanorivulus pictus* clade, this "assemblage" is diagnosed by having particular osteological feature (ventral process of angulo-articular ves-

tigial) and males coloration (flanks intense greenish blue or greenish golden to purplish blue above anal fin base).

*Melanorivulus nigropunctatus*, is known from wetlands of a small drainage tributary of right side of the Rio Verde and differs from all other species of the genus, as specific name clearly say, by possessing black dots over the head and body in both sexes additionally the pectoral fins in males are orange with a dark grey margin. Additional features can be used to separate this species from the other of this assemblage are reported (frontal squamation, length of caudal fin. . .).

*Melanorivulus ofaie*, is found in a similar environment, but at the opposite margin of the Rio Verde. This species is distinguished from other members of this genus by males coloration presenting flank greenish blue to light blue, with seven to nine oblique chevron-like red bars, ventral portion of head whitish with dark brown spots, dorsal fin yellow with two to three transverse broad red oblique stripes and distal region red, anal fin light orangish yellow, basal area light blue with short red bars and distal portion with a dark red margin, and caudal fin yellow or orangish yellow with three to four vertical red bars in the dorsal and middle portions, sometimes with a orange distal margin. Additionally can be distinguished by caudal fin length, number of scales in transverse series, anal fin origin position. . .

A nice addition in an already rich genus and many other species will be described in the area (as suggested on the text).

[Stefano Valdesalici]

**Pattern of genetic differentiation of an incipient speciation process: The case of the high Andean killifish *Orestias*.** Guerrero-Jiménez CJ; Peña F; Morales P; Méndez M; Sallaberry M; Vila I; & Poulin E. *PLOS ONE*, 12:1–19, 2017. DOI <http://dx.doi.org/10.1371/journal.pone.0170380>

The fish of the genus *Orestias* ranges from Lago Lascha, Peru, to Salar de Ascotán, Chile. There are 44 described species of which 23 inhabit Lake Titicaca and 21 inhabit rivers,

lagoons, lakes, wetlands and salt pans of the Altiplano Plateau. In this paper the authors investigate the genetic diversity between different populations of four species (*O. chungarensis*, *laucaensis*, *parinacotensis* and *piacota*) found in and around the Lauca National Park. These four species are a distinct chromosome lineage but cannot be differentiated meristically or molecularly. The authors hypothesize that this is due to incomplete sorting, that is, that the fish species have not yet fully speciated and represent a incipient allopatric speciation. The authors resolve various genetic groups, representative of the four species based on various genetic markers. The genetic groups coincide with the fragmentation of the aquatic system. The authors also show that there is mixing between the different genetic groups.

[Tyrone Genade]

***Nothobranchius* fishes of the Kibasira Swamp.** Watters BR. *Journal of the American Killifish Association*, 49:131–153, 2016.

In this paper Brian Watters describes the habitats and fish *Nothobranchius* species found in the Kibasira Swamp. This swamp is found in the Kilombero Valley. Several rivers flow through this flood plain. The rivers associated with this swamp drain into the Kilombero River and then into the Rufiji River. Watters reviews *Nothobranchius* collections and habitats in this area beginning with Rosenstock's collections in 1989. Four species of *Nothobranchius* are found at various locations in the swamp: *geminus*, *kilomberoensis*, *lourensi* and *lucius*; as well as *Micropanchax kongoranensis* and sp. aff. *maculatus*. The article is full of photos and provides a map of the area.

[Tyrone Genade]

## Killifish Biology: Ecology & Physiology

**Community assembly in *Nothobranchius* annual fishes: Nested patterns, environmental niche and biogeographic history.** Reichard M; Janáč M; Polačik M; Blažek R; & Vrtílek M. *Ecology and Evolution*, 7:2294–2306, 2017. DOI <http://dx.doi.org/10.1002/ece3.2851>

This paper is a detailed evaluation of community assembly of African annual killifishes of the genus *Nothobranchius* (Cyprinodontiformes) which are small fishes associated with annually desiccating pools. Previous research showed three distinct but overlapping evolutionary groups (O, F and R-clades) distributed across 168 communities across the genus range. The authors concentrated on understanding the finer-scale processes that evolutionarily have led to particular local community assembly using both biotic interactions (other species presence/absence) data and seven environmental variables using both univariate and multivariate statistics as well as niche modeling.

Specifically, they asked: 1) is the distribution of the three clades geographically congruent and is the distribution of species within each clade allopatric?; 2) are local *Nothobranchius* communities composed of a predictable subset of regionally present species and is the assembly of local communities driven by positive or negative associations among species?; and 3) is the presence of particular species in local communities explained by local environmental factors?

Overall, historical and local effects interact to shape African *Nothobranchius* species community assembly and define abrupt limits of species distributions. The authors argue that, at the level of clades of closely related species, co-occurrence patterns arise from specific associations with environmental drivers rather than from biotic interactions. Environmental factors associated with altitudinal and precipitation gradients apparently drive geographic distribu-

tion. Finally, the authors note that the species with the most widespread populations had also the highest relative abundance in local communities but sister species never co-existed. These data further suggest a major role for geographic isolation in speciation; and potential for competitive exclusion among co-existing sister species. [Mark Peterson]

**MicroRNA miR-29 controls a compensatory response to limit neuronal iron accumulation during adult life and aging.** Ripa R; Dolfi L; Terrigno M; Pandolfini L; Savino A; Arcucci V; Groth M; Terzibaszi Tozzini E; Baumgart M; & Cellerino A. *BMC Biology*, 15:9, 2017. DOI <http://dx.doi.org/10.1186/s12915-017-0354-x>

MicroRNAs (miRNA) are a class of small RNA sequences that have been demonstrated to regulate the translation of certain genes into proteins. miR-29 has been shown to regulate aspects of the aging process in various organisms, in particular, aspects of neurodegeneration. In this paper the authors tested the hypothesis that miR-29 plays a role in regulating iron accumulation in the brain. They showed that with age the expression of miR-29 increased in *N. furzeri* and regulated genes reported to change expression in response to aging. They also showed that iron accumulated in the mitochondria of the fish with age. By reducing miR-29 expression the authors showed that the fish accumulated iron faster and that neurodegeneration advanced at a faster rate. The authors showed that miR-29 inhibits the production of the protein IRP2 (a protein that binds and transports iron). With less miR-29 iron accumulated faster and to a greater extent in the brains of *N. furzeri*. The authors put forward a model where age-related iron accumulation causes cellular damage, which then stimulates miR-29 expression which in turn represses IRP2 expression leading to slower iron accumulation in the tissues. This model is in concord with earlier work by Baumgart et al (*Cell Syst.* 2, 122–132, 2016) showing that mitochondrial dysfunction predicted lifespan.



In fish, iron is absorbed through the gastrointestinal tract and gills. As an excess supply of iron can be toxic and its accumulation may cause aging-related pathology preventing excessive iron toxicity would be beneficial to the health of our fish. Iron is normally not soluble in water as it auto oxidizes to  $\text{Fe}^{3+}$  which will precipitate with hydroxide ions in the water. The pH would have to drop below 3 for  $\text{Fe}(\text{OH})_3$  to dissolve. The  $\text{Fe}^{2+}$  is very soluble in water and dosing aquaria with such iron could lead to toxicity issues over time. Acute toxicity is observed around 200 ppm which is unlikely in aquaria. There is little research on the effects of chronic exposure and what doses are dangerous. Fish need 30–170 mg Iron per kg of fish (dry weight) to remain healthy. Insect larvae are reported to contain 60 mg iron per 100 g. It is likely that iron is available in surplus in fish diets and there is probably little we can do about this. Limiting iron concentrations in the water might be more beneficial but this doesn't seem like a matter to worry about.

[Tyrone Genade]

**Parallel evolution of genes controlling mitonuclear balance in short-lived annual fishes.** Sahm A; Bens M; Platzer M; & Cellerino A. *Aging Cell*, Epub ahead of print, 2017. DOI <http://dx.doi.org/10.1111/accel.12577>

Sahm et al used genomic data to determine if any and what genes have been under positive selection in the course of *Nothobranchius* evolution. They compared six *Nothobranchius* species: *furzeri*, *kadleci*, *orthonotus* (what they refer to as *kuhntae*), *pienaari*, *rachovii* and *korthausae*. The genome of these species was compared to *Aphyosemion striatum*, *Xiphophorus maculatus*, *Poecilia formosa* and *Fundulus heteroclitus* among other non-cyprinodonts. From this comparison they showed that there was selection for genes involved in the biogenesis and maintenance of mitochondria in all *Nothobranchius* species. Several genes under positive selection were also found which were associated with diapause and aging in *Nothobranchius*. Many of the genes under positive selection

were also under positive selection in long-lived mammals. This work is in concord with previous experiments on *N. furzeri* where the measure of mitochondrial biogenesis and maintenance predicted lifespan while the stimulation of the mechanisms controlling mitochondrial biogenesis and maintenance increased lifespan (Baumgart et al *Cell Syst.* 2, 122–132, 2016).

[Tyrone Genade]

**Maternal source of variability in the embryo development of an annual killifish.** Polačik M; Smith C; & Reichard M. *Journal of Evolutionary Biology*, Epub ahead of print, 2016. URL <http://onlinelibrary.wiley.com/doi/10.1111/jeb.13038/full>

This is a fascinating paper with implications for *Nothobranchius* hobbyists and researchers alike. Polačik et al set out to test two hypotheses: (1) that paternal effects or (2) maternal effects are the primary source of variability in the duration of embryo development of *N. furzeri*. The authors proceeded to mate each of three female fish with three males. Each pairing was replicated twice for three matings per pairing. Egg yield, fertilization success and escape from diapause were monitored. Egg numbers increased with each replicate. This is predictable given the growth in the fish allows it to develop and lay more eggs. Surprisingly, fertilization success also increased with each replicate. Escape from diapause as well as fertilization success was determined to be due to maternal influence only. Eggs laid early on in the female's lifespan escaped diapause sooner than eggs laid later on during the fish's life. In each spawn there was always a percentage of slow developing embryos that took months to escape diapause. The implication for hobbyists is that eggs laid in the youth of the fish are likely to develop faster; as well as that an early wetting of developed embryos could produce fry from only one or two females from a group. To maintain genetic diversity fry from later rewettings of the peat would be needed. However as eggs laid later in the life of the fish were more prone to develop slowly this means that there could be great variability

in incubation time for a particular species; and that these later-laid eggs would develop more uniformly, providing for better continuity of genetic diversity of captive populations.

[Tyrone Genade]

**Regulation of Life Span by the Gut Microbiota in The Short-Lived African Turquoise Killifish.** Smith P; Willemssen D; Popkes ML; Metge F; Gandiwa E; Reichard M; & Valenzano DR. *bioRxiv*, Epub ahead of print, 2017. DOI <http://biorxiv.org/content/early/2017/03/27/120980>

Since its publication this paper has been [making headlines](#) and for very good reason! The paper describes experiments conducted in Dario Valenzano's laboratory. The authors compared the diversity of intestinal bacteria between young and old fish raised in their lab and collected in the wild. They observed that with age the diversity of bacteria shrinks and bacteria associated with illness began to dominate the microbiome of the fish. They proceeded to treat middle-aged fish with a range of broad spectrum antibiotics to kill off their endogenous bacteria. The antibiotic-treated fish were then exposed to the intestinal bacteria of young fish to reinoculate their intestines. This resulted in the extension of the lifespan of the fish given the young-fish intestinal bacteria. Surprisingly, those fish treated with the bacteria but not exposed to young-fish bacteria also lived longer than the control fish that were not treated with antibiotics. This showed that simply removing the "bad" bacteria was enough to extend lifespan, but the extension of lifespan was greater when the middle-aged fish were given young-fish bacteria. The authors also examined the guts of the fish to determine what proteins are expressed in the gut as the fish ages. As the fish aged they began to express genes associated with inflammation and cancer. Giving the middle-aged fish bacteria from young fish partially restored gene expression to that of healthy young fish—but not enough for complete rejuvenation. Young fish given bacteria from old fish did not live shorter lives. This suggests

that it isn't the bacteria themselves that are shortening the lives of the fish but the intrinsic aging of the fish that allows the bad bacteria to proliferate; but that providing a healthy bacteria community (that provides metabolic support and which are not injurious to the host) allows for the fish to heal itself, even in old age. The authors identified four bacteria genera that could be of particular benefit and state that their "approach could provide a key to slowing aging and retarding the onset of age-associated diseases by specifically targeting the" gut microbiome. This paper marks the starting point for a very interesting and profitable line of *Nothobranchius*-aging research.

[Tyrone Genade]

**Non-canonical aging model systems and why we need them.** Valenzano DR; Aboobaker A; Seluanov A; & Gorbunova V. *The EMBO Journal*, Epub ahead of print, 2017. DOI <http://emboj.embopress.org/content/early/2017/03/20/emj.201796837>

In this paper the authors argue for the importance of using models of aging other than mice, fruitflies, nematodes and yeast. The authors discuss the potential of *Nothobranchius furzeri*, the planarian (*Schmidtea mediterranea*) and the naked-mole-rat (*Heterocephalus glaber*). The advantages of these three species are highlighted and it is argued that each model organism has virtues and that a broad selection of model organisms needs to be studied to better understand aging through comparative genomics.

[Tyrone Genade]

**Morphological changes during diapause stages in the embryonic cortex of the annual killifish *Millerichthys robustus* (Cyprinodontiformes: Cynolebiidae) under natural conditions.** Domínguez-Castanedo O; Rosales-Torres AM; & Uribe MC. *Ichthyological Research*, Epub ahead of print, 2017. DOI <http://dx.doi.org/10.1007/s10228-017-0582-7>

In this work, presented on March 2017 in the magazine "The Ichthyological Society" of Japan authors present a remarkable study to the changes in egg cortical structures of

annual fish species in general but with the focus on the Mexican annual taxon *Millerichthys robustus*.

It is not the first report on what happens inside the killifish egg during their development and the time needed to be ready for the next rainy season. It is however the first time that scientists looked in-depth to what this process is doing with eggs taken directly from their natural habitat at different locations, time frame's and stages of development and for that this study is unique. The scientific approach revealed very interesting knowledge and results that let us better understand how eggs protect themselves against dehydration, extended or interrupted dry periods and other environmental stressed situations. These results and conclusions are giving answers to all of us which may not fully understand how the changes inside the egg envelope do have effect on their survival.

Eggs were collected during three periods of the year in temporary pools under flood, drought and wet conditions. During these different periods eggs were found in diapause I, II and III and were measured in size, thickness of cortical structure and the presence of filaments. Changes were precisely monitored, photographed and described including changes in the space between egg outer shell and inner membrane (the perivitelline space). Research demonstrates that annual fish embryos respond to their exposure to seasonal environmental variations with dynamic structural changes that are fundamental for their survival. This work is good reading and interesting for all, scientists and hobbyists, who wonder how these adaptations benefit the capability for killifish to survive in their harsh environments.

[Frans Vermeulen]

*Ed's note: this paper is Open Access.*

*An important paper to be complemented by Dominguez, O.C., Valdesalici, S. & Rosales, A.M.T. 2017. Developmental ecology of annual killifish Millerichthys robustus (Cypripinodontiformes: Cynolebiidae). Developmental Dynamics, doi:10.1002/dvdy.24519, just published, analyzed in the next*

*K-D N issue, which should be duplicated with African and South American annuals to show if this is species specific or not (remember that according to molecular data annualism has emerged during palaeo-history several times independently). [Jean Huber]*

**Gastrulation in an annual killifish: Molecular and cellular events during germ layer formation in *Austrolebias*.** Pereiro L; Loosli F; Fernández J; Härtel S; Wittbrodt J; & Concha ML. *Developmental Dynamics*, Epub ahead of print, 2017. DOI <http://dx.doi.org/10.1002/dvdy.24496>

Pereiro et al describe the gastrulation of two *Austrolebias* species (*charrua* and *bellottii*). The authors employed bright field and time-lapse confocal microscopy as well as genetic manipulations to label specific cell types and fates. In non-annual species development proceeds from fertilization to a spherical ball of cells, a blastula, which then develops a cell mass at one pole which becomes the gastrula. During gastrulation the cells in the egg typically reorganize to form the embryo through the concerted movement of cells to form different embryonic structures. In annual species the cell mass doesn't form, instead the blastula splits into an external enveloping cell layer and a deep layer of blastomeres and the eggs enters Diapause I. (A third layer, common to all organisms, the internal yolk syncytial layer envelops and fuses with the yolk.) In annual fishes the deep layer reagggregates to form the embryo but embryogenesis proceeds very differently. In non-annual fish the embryo forms by the reorganization of cells at the one pole. This reorganization is called epiboly and can proceed by various means, most typically there is a migration of cells into the embryonic cell mass, this is called internalization. This is not observed in *Austrolebias*. Instead gastrulation occurs without epiboly, where the early-reagggregated cell mass appears to be part of the extraembryonic tissues that don't form part of the embryo. The cells that formed the embryo appeared from beneath the early-reagggregate and were not expressing the cell markers

expressed by cells of the reaggragate. The authors report that gastrulation proceeds from a compressed blastopore-like structure that develops by the reorganization of individual cells rather than by internalization of cells. The uniqueness of this process generates many questions as to the mechanism and regulation of development. [Tyrone Genade]

**Hit Pause: Developmental arrest in annual killifishes and their close relatives.** Martin K & Podrabsky JE. *Developmental Dynamics*, Epub ahead of print, 2017. DOI <http://dx.doi.org/10.1002/dvdy.24507>

In this paper Martin & Podrabsky define the terms used in the scientific literature for developmental arrest. They note that each form is accompanied with specific physiological and molecular changes. The terms and their physiological definition are summarized in Table 1 of the article. The diverse forms of developmental arrest are defined as quiescence, embryonic diapause and delayed hatching. The authors point out that quiescence can be environmentally induced quickly and the embryos exit this state rapidly. Conversely, diapause takes several days to weeks to induce, is driven by an internal mechanism, involves a profound suspension of metabolic activity and that the embryo only exits diapause on specific environmental cues. The authors retain Wourm's three stages but note that the three stages can be facultative or obligate and whether a specific stage is facultative or obligates varies from species to species. Diapause I is described as being shallow and easily broken; diapause II is mostly facultative and is induced environmentally. Diapause III is differentiated from delayed hatching in that in the former metabolism is depressed while in delayed hatching the fish still has an active metabolism. Hypoxia is reported as a reliable trigger for hatching of eggs in a state of delayed hatching while embryos in Diapause III are largely unresponsive. While annuals typically employ Diapause III in the resting embryo state,

<sup>2</sup>Technically, these fish from Maine would be the subspecies *macrolepidotus*.

non-annuals employ delayed hatching. As consequence fully developed non-annual eggs do not wait long to be wet before they begin to die. For certain species this delay can last a few days while for others, such as *Kryptolebias marmoratus* Dangriga, it can last 40 days! [Tyrone Genade]



Photo of *Austrofundulus limnaeus* by Jerry Waskilak, accessed from [http://img.kil.fish/1/LIM/.00-1-Copr\\_1997\\_Jerry\\_Waskilak-477x237x24\\_29088.jpg.meta/](http://img.kil.fish/1/LIM/.00-1-Copr_1997_Jerry_Waskilak-477x237x24_29088.jpg.meta/).

**The Landscape of Extreme Genomic Variation in the Highly Adaptable Atlantic Killifish.** Reid NM; Jackson CE; Gilbert D; Minx P; Montague MJ; Hampton TH; Helfrich LW; King BL; Nacci DE; Aluru N; Karchner SI; Colbourne JK; Hahn ME; Shaw JR; Oleksiak MF; Crawford DL; Warren WC; & Whitehead A. *Genome Biology and Evolution*, 9:659, 2017. DOI <http://dx.doi.org/10.1093/gbe/evx023>

The large number of scientists credited is indicative of the work involved in this study. The authors have sequenced the full genome for *Fundulus heteroclitus*, using specimens from the northern most part of its range<sup>2</sup>. Despite the fact that the southernmost populations have been identified as genomically more diverse, the sequenced genome nevertheless shows extremely high genetic diversity (98th percentile for



vertebrates). Comparisons with known genomes from 9 other fish species and humans show that *F. heteroclitus* possesses a higher number of similar (orthologous or analogous) genes than found in the other fish genomes. The study also looked at the degree of variability amongst particular gene types and found that functions such as neurological and cytoskeletal function have lower variability than some other functions. They also find that variability in non-coding sections is higher than the coding sections suggesting greater capacity for regulation of gene expression. And high vs low variability is not restricted to particular regions of the genome. The authors conclude that the high variability between individuals is instrumental in the success of *F. heteroclitus* to quickly adapt to changes in environmental conditions. They also argue for greater use of *F. heteroclitus* as a model for the study, given the detailed and rigorous nature of their genomic sequencing and the ecological/physiological knowledge already available, of individual gene interactions with the environment.

[Andy Patel]

**Intrinsic reproductive isolating mechanisms in the maintenance of a hybrid zone between ecologically divergent subspecies.** McKenzie JL; Bucking C; Moreira A; & Schulte PM. *Journal of Evolutionary Biology*, Epub ahead of print, 2017. DOI <http://dx.doi.org/10.1111/jeb.13055>

This is a study of ‘hybridisation’ between the two subspecies of the Atlantic killifish *Fundulus heteroclitus*, also known as the Mummichog. *F. heteroclitus* is a well-studied model and the authors have analyzed multiple factors in investigating how a hybrid zone is maintained. The subspecies *F. h. heteroclitus* is found in the southern section of the North American Atlantic seaboard and *F. h. macrolepidotus* in the northern. The State of New Jersey is home to the crossover area. The authors point out that previous studies have focused primarily on salinity and temperature as keys to this divide. They instead use DNA analysis of both mitochondrial and

nuclear material to evaluate the results of breeding experiments (both in vitro and in vivo) between distinct populations from New Hampshire and North Carolina. Coupled with population and environmental data from 8 sites in the hybrid zone they identify multiple interacting factors that might be responsible for the stability of the two sub-species and the ongoing maintenance of the hybrid zone. These include differences in mate choice, microhabitat preference and hybrid viability as well as environmental factors. [Andy Patel]

**When evolution is the solution to pollution: Key principles, and lessons from rapid repeated adaptation of killifish (*Fundulus heteroclitus*) populations.** Whitehead A; Clark BW; Reid NM; Hahn ME; & Nacci D. *Evolutionary Applications*, Epub ahead of print, 2017. DOI <http://dx.doi.org/10.1111/eva.12470>

The attentive reader will note that four of the authors appear in “The Landscape of Extreme Genomic Variation in the Highly Adaptable Atlantic Killifish”. This research unsurprisingly adds support to the use of *Fundulus heteroclitus* as a model species. The ability of different populations to survive pollution from a number of chemicals is studied to assess the speed with which they can adapt. Pollutants that act on specific regulatory pathways can be attributed to particular genes, enabling research to focus on changes at those loci. But as they point out adaptations to increase tolerance to a pollutant may have other indirect fitness effects so it is not simply a case that individuals with the greatest tolerance will be the most successful. Once tolerance has been established further adaptations may be necessary to re-establish an optimal balance of adaptability to other stressors (e.g. competition). The specific genes, or combination thereof, associated with those stressors is not as well understood. So fitness consequences and time scales are more difficult to track. Whether any adaptations can be reversed once a pollution threat is over also raises questions. Variation between populations and between

generations is noted. The interdependence of the full array of genes and how/whether they are expressed presents difficulties in trying to determine how a species might (be able to) adapt to other pollutants. All three papers on *F. heteroclitus* point to the adaptability of this species but this paper highlights that evolutionary mechanisms have limits. Even where, as with the atlantic killifish, evolution is fast and repeated, it may still have limits. [Andy Patel]



Photo of *F. heteroclitus* from the vicinity of Georgetown, South Carolina. Photo from <https://en.wikipedia.org/wiki/Mummichog#/media/File:Mummichog.jpg>.

**Resistance to polycyclic aromatic hydrocarbon toxicity and associated bioenergetic consequences in a population of *Fundulus heteroclitus*.** Lindberg CD; Jayasundara N; Kozal JS; Leuthner TC; & Di Giulio RT. *Ecotoxicology*, Epub ahead of print, 2017. DOI <http://dx.doi.org/10.1007/s10646-017-1775-6>

Di Giulio has previously discussed the trade-offs evolved by *F. heteroclitus* in adapting to polycyclic aromatic hydrocarbons (PAHs) (e.g. see Riley et al, <http://dx.doi.org/10.1177/0192623316636717>). The authors examined a previously unstudied population from the site of the Republic Creosoting industrial plant on the Elizabeth River, Maryland.

The response to PAH toxicity and energy metabolism were compared to fish from the Kings Creek location. The fish from Republic Creosoting site were resistant to PAH induced cardiovascular deformities. The Republic Creosoting fish had an elevated basal oxygen consumption rate as embryos but juveniles had lower maximal metabolic rates and reduced aerobic scope compared to the Kings Creek fish. The authors note that the cost of PAH resistance is altered metabolism; and that this could predispose the fish to being vulnerable to other environmental and anthropogenic stressors.

[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. DOI <http://dx.doi.org/10.1111/jai.13370>

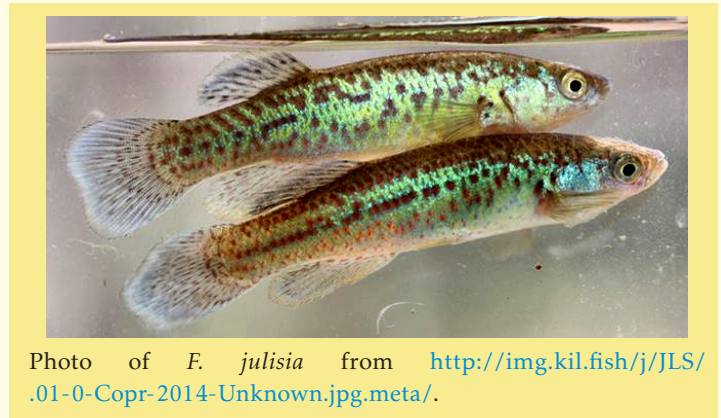


Photo of *F. julisia* from <http://img.kil.fish/j/JLS/.01-0-Copr-2014-Unknown.jpg.meta/>.

*Fundulus julisia* populations have declined dramatically due to the loss of pristine habitat and the introduction of *Gambusia affinis*. This has led to the loss of 12 of 14 natural

populations since the 1980s. The authors identified 14 microsatellite loci (genetic markers) that can be used to assay genetic diversity within populations. Applying these markers to the two remaining populations showed structuring of genetic variation and differing levels of heterozygosity among the populations. [Tyrone Genade]

**Embryonic-only arsenic exposure in killifish (*Fundulus heteroclitus*) reduces growth and alters muscle {IGF} levels one year later.** Szymkowitz DB; Sims KC; Castro NM; Bridges WC; & Bain LJ. *Aquatic Toxicology*, 186:1 – 10, 2017. DOI <http://doi.org/10.1016/j.aquatox.2017.02.020>

Arsenic was recently in the news. It was **reported** to be a contaminant in rice and could be present in rice at toxic concentrations. While these reports might be over-blown, arsenic is a real danger. Chronic exposure to low levels of arsenic in drinking water has been linked to decreased birth weight, weight gain and skeletal muscle function. The authors set out to develop *F. heteroclitus* as a model to study the effects of arsenic exposure. They began by exposing only the embryos to arsenic and studying its effect on muscle growth, insulin-like growth factor signaling. Muscle samples were taken from the fish at various ages: 16, 28, 40 and 52 weeks. Significant effects on condition factor (mass/volume) were observed at the 16–40 week time points but not at the 52 week time point. Embryos kept at high arsenic concentrations (200 and 800 ppb) had elevated insulin-like growth factor receptor expression compared to unexposed fish. This increased expression was maintained to 40 weeks of age. At 52 weeks of age only the fish that had been incubated in the 800 ppb dose retained any difference in insulin-like growth factor receptor expression. Skeletal muscle expression of insulin-like growth factor was also elevated throughout the 52 weeks in the 800 ppb group. The authors conclude that arsenic has long-term effects from acute, early-life exposure. [Tyrone Genade]

**Nekton Use of Flooded Salt Marsh and an Assessment of Intertidal Creek Pools as Low-Tide Refuges.** Allen DM; Ogburn-Matthews V; & Kenny PD. *Estuaries and Coasts*, Epub ahead of print, 2017. DOI <http://dx.doi.org/10.1007/s12237-017-0231-4>

Based on 67 sampling dates over a nine year period, Allen et al. (2017) quantified use of high- and low-tide quantitative samples from salt marsh habitat (dominated by *Spartina alterniflora*) in South Carolina. Their study focused on marsh use by resident and transient fishes and decapod crustaceans. They noted that 8 of the 64 quantified taxa always ranked in top 10 in abundance in both high- and low-tides collections and the majority of resident taxa (not transient taxa) were positively correlated with the area of marsh flooded. However this relationship did not apply to the most abundant resident the mummichog, *Fundulus heteroclitus*, which is known to retreat to subtidal refuge habitat particularly for larger sizes. In contrast, some transients taxa used flooded marsh while others did not and some transients retreated to downstream pool habitat at low tide but other left the marsh completely. These data illustrate the underappreciated value of intertidal pool habitat as low-tide refuges in meso-tidal salt marsh.

[Mark Peterson]

**Urban Land use Affects Resident Fish Communities and Associated Salt Marsh Habitat in Alabama and West Florida, USA.** Wedge M & Anderson CJ. *Wetlands*, Epub ahead of print, 2017. DOI <http://dx.doi.org/10.1007/s13157-017-0902-8>.

Baited minnow trap samples from 23 micro-tidal salt marshes (dominated by *Juncus roemerianus*) divided among three urbanized tidal creeks and three reference tidal creeks in Alabama and west Florida ecosystems were examined for relationship to a host of terrestrial and aquatic variables, including salinity, marsh slope and sediment cadmium concentration. Data revealed that total fish abundance and biomass were significantly lower in urbanized creeks compared to

reference marsh creeks. Additionally, multivariate statistics also revealed that urbanized and reference fish assemblages were clearly separated in multivariate space and correlated these environmental variables like salinity, marsh slope steepness and cadmium concentration. These data further support earlier research on urbanized marsh habitat in terms of fish assemblage structure and driving metrics that influence how well these urbanized marsh habitats can support a 'reference' fish assemblage, many of which are commercially important.

[Mark Peterson]

**Influence of euthanasia method on blood and gill variables in normoxic and hypoxic Gulf killifish *Fundulus grandis*.** Larter KF & Rees BB. *Journal of Fish Biology*, Epub ahead of print, 2017. DOI <http://dx.doi.org/10.1111/jfb.13316>

In this paper the results of experiments of four euthanasia methods are reported. The four methods are MS-222 (tricaine), clove oil, rapid cooling and blunt trauma. Parameters assayed were euthanasia-induced hypoxia, haematocrit, plasma cortisol, blood lactate, blood glucose, gill damage and energetic status (the ratio of ATP to ADP). Fish killed with MS-222 had higher haematocrit and lower gill damage and ATP to ADP ratio. The methods did not effect hypoxia levels. MS-222 euthanasia fish lost equilibrium faster than by other methods except blunt trauma. There was no difference in time to death, haematocrit, cortisol, lactate or glucose concentrations. The authors conclude that their experimental conditions matter more than the choice of euthanasia method.

[Tyronne Genade]

**Nine co-localized cytochrome P450 genes of the CYP2N, CYP2AD, and CYP2P gene families in the mangrove killifish *Kryptolebias marmoratus* genome: Identification and expression in response to B[ $\alpha$ ]P, BPA, OP, and NP.** Puthumana J; Kim BM; Jeong CB; Kim DH; Kang HM; Jung JH; Kim IC; Hwang UK; & Lee JS. *epub ahead of print*, Epub ahead of

print, 2017. DOI <http://doi.org/10.1016/j.aquatox.2017.03.022>

Cytochrome P450 enzymes play important roles in the metabolism of xenobiotic compounds (such as antibiotics or pollutants). The CYP2 family of genes is involved in the metabolism of fatty acids. Some of its substrates, such as arachidonic acid, play important endocrine roles. These genes have been shown to be up-regulated by exposure to environmental pollutants such as benzopyrene (B[ $\alpha$ ]P), bisphenol A (BPA), 4-octylphenol (OP) and 4-nonylphenol (NP). As an androdioecious fish species wherein almost 60% of hermaphrodites transform into secondary males, *K. marmoratus* could prove useful in assessing the effects of various environmental pollutants on sexual development. The authors set out to determine whether *K. marmoratus* had CYP2 genes, where they are located in the genome and how their expression varies during development and in response to xenobiotics. They found 9 CYP2 genes as well as evidence for gene duplications and inversions. The gene expression of the CYP2 genes varied during the course of normal development. Exposure to each of the pollutants resulted did not effect the expression of all genes equally. *Km-CYP2N22* was sensitive to B[ $\alpha$ ]P; *Km-CYP2N18* and *-19* were sensitive to BPA; *Km-CYP2AD12s* was sensitive to OP; and *Km-CYP2P20* was sensitive to NP. The authors discuss the gene evolution of the *Km-CYP2* genes and their structure and function relative to what is known for *Fundulus heteroclitus*. They note that the differential expression of the CYP2 genes during development as well as their different sensitivities to the various pollutants suggest that these genes play critical roles in the fish. The role of these genes in endocrine function is discussed in the light of previous experiments wherein *K. marmoratus* was exposed to pollutants and steroids.

[Tyronne Genade]



## Posters & Conference Abstracts

**Behavioral effects of carbaryl and temperature on killifish *Fundulus heteroclitus*.** Maze KL & Dalman N. Project presentation, *University of Northern Georgia*, 2017. URL <http://digitalcommons.northgeorgia.edu/ngresearchconf/2017/biology/1/>

As yet unpublished paper, presented at the University of North Georgia research conference. The researchers looked at the effect of a widely used pesticide, carbaryl and found that it reduced foraging ability and that this increased with higher temperature. But fish at lower temperatures had higher levels of carbaryl in their body tissue. This nicely demonstrates how a pollutant can have far more effect than just toxicity. [Andy Patel]

## Interesting research on other fish

**Colorful invasion in permissive Neotropical ecosystems: establishment of ornamental non-native poeciliids of the genera *Poecilia/Xiphophorus* (Cyprinodontiformes: Poeciliidae) and management alternatives.** Magalhães ALB & Jacobi CM. *Neotropical Ichthyology*, Epub ahead of print, 2017. ISSN 1679-6225, URL [http://www.scielo.br/scielo.php?script=sci\\_arttext&pid=S1679-62252017000100204&nrm=iso](http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1679-62252017000100204&nrm=iso)

The authors are well-versed in investigating species invasion and in this paper look at the issue of guppies, platys and mollies that have been introduced into small headwater creeks close to an ornamental aquaculture facility in Brazil. They examine the ability of these common aquarium fish to establish themselves in such an environment as the relatively low native fish species diversity makes it particularly vulnerable to even small disturbances. In line with their expectations the oviparous ornamentals demonstrated distinct survival

advantages over the natives due to their more frequent reproduction and extended breeding season. The paper articulates a number of ways in which these non-native invaders may be detrimental and argue for more effective conservation of headwaters in Brazil. Astonishingly, given Brazil's ongoing measures to prevent over-exploitation of native fish for the aquarium export trade, there is insufficient regulation of aquaculture facilities for the domestic trade. Breeding ponds are regularly drained, directly into the river system, with no filtering mechanism to prevent escapees. The invasive species are therefore able to continually recruit, placing sustained pressure on native stocks that are already threatened by habitat degradation. [Andy Patel]

**Characterization of appetite-regulating factors in platyfish, *Xiphophorus maculatus* (Cyprinodontiformes Poeciliidae).** Pitts PM & Volkoff H. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 208:80–88, 2017. DOI <http://doi.org/10.1016/j.cbpa.2017.03.018>.

Appetite is regulated by an interplay of several peptide hormones such as cholecystokinin (CCK), cocaine-and-amphetamine-regulated transcript (CART), orexin and neuropeptide Y (NPY). CCK and CART suppress appetite while orexin and NPY stimulate appetite. To find out how appetite is regulated in platyfish they were injected with CCK and/or orexin and their feeding behavior and food intake analyzed. CCK reduced food intake and searching behavior while orexin increased searching behavior but did not affect food consumption. The tissue distribution of these hormones was assayed and found to have widespread distribution in brain and several peripheral tissues (e.g. intestines and gonads). Fasting decreased the expression of CCK and CART and increased orexin mRNA expression in the brain. No effect was observed on NPY expression. There were also no sex-specific differences. The authors conclude that these hormones might have several physiological roles in platyfish. [Tyronne Genade]

**Three-dimensional computer graphic animations for studying social approach behaviour in medaka fish: Effects of systematic manipulation of morphological and motion cues.**

Nakayasu T; Yasugi M; Shiraishi S; Uchida S; & Watanabe E. *PLOS ONE*, 12:1–17, 2017. DOI <https://doi.org/10.1371/journal.pone.0175059>

The authors present a technology to make three-

dimensional computer graphics of medaka fish which can be used to study behavior. These models could be displayed to real fish and elicit behavior responses but only if the model displayed normal behavior itself. The authors discerned that the induction of natural social behavior was dependent on the models presenting natural behavior. [Tyrone Genade]

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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.

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