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Editorial

Valued readers, it is with a heavy heart that I inform you that this is the last issue of Killi-Data News. The good news is that we will be back as Killifish Research Review.

The dissolution of Killi-Data International created a problem: how can the newsletter of a defunct organization live on without that organization? But other additional problems were building in the background. The first issue numbered 15 pages. The previous issue was 28 pages. The number of killifish related papers is increasing while time on our end (the editorial team) is running out. It takes a lot of time to read the papers and put all the contributions together into the newsletter. Were it not for the people writing us to inform us that they appreciate our work we would probably give up. So we intend to continue in a new format. We are starting the journal afresh on Elsevier's (formerly Bepress's) Digital Commons platform which will enable us to publish reviews and summaries ad hoc, with open access and operating as a journal with a modicum of peer review.

At this time we don't yet have a link to the new journal page but this will be mailed out when we launch early next year. In the interim we hope you, the readers, will help us and come on board in a reviewer or contributor capacity. We need people with expertise in physiology, molecular phylogenetics, taxonomy, toxicology and ecology. As we are rebranding as a killifish review journal we are after review articles on killifish-related research. Submissions will be much appreciated.

There is a diverse assortment of papers reviewed in this issue. The special edition of Developmental Dynamics on annual fish has provided a lot of material (some of it was given in the previous issue). We draw your attention to Podrabsky and Arezo's editorial which lays out the need for research on annual fish. We also have two papers that are relevant to your health: the effect of glyphosate on *Aphanius* and arsenic on *Fundulus*. We also have new descriptions and molecular systematics work. We are fortunate to have Bettina Reichenbacher reviewing the recent description of the new killifish genus, *Pseudorestias*.

We hope you enjoy this issue and catch up with us in the new year at our new home on the Digital Commons.

[*Tyrone Genade*]

Erratum

On page 57 of the previous issue I refered to "*Austrolebias limnaeus* enters Diapause II at 15 days post fertilization and..." that should be *Austrofundulus limnaeus*.

In The News

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

http://www.alraimedia.com/ar/article/local/2017/10/30/801302/nr/nc A story about *Aphanius* Kuwait's Jahra Reserve and efforts to conserve it and its biodiversity.

http://www.news-gazette.com/news/business/2017-10-22/wired-becky-fuller.html Becky Fuller's research on *Lucania goodei* has made the news.

https://www.israel21c.org/tiny-fish-swims-to-israel-to-help-unlock-mystery-of-aging/ Itamar Harel has started a *Nothobranchius* research lab in Jerusalem.

Review of New Research Publications

New Books

Intermittent Rivers and Ephemeral Streams: Ecology and Management. Editors: Datry T, Bonada N and Boulton A. Elsevier Science & Technology Books, 2017, ISBN: 978-0-12-803835-2. 622 pages. DOI https://doi.org/10.1016/B978-0-12-803835-2.09997-6.

This book has several chapters of interest to killifish hobbyists and researchers:

Chapter 4.10: Genetic, Evolutionary, and Biogeographical Processes in Intermittent Rivers and Ephemeral Streams by Bonada N, Carlson, S, Datry, T, Finn, DS, Leigh, C and Lytle DA, Monaghan MT, Tedesco PA.

Chapter 4.5: The Biota of Intermittent Rivers and Ephemeral Streams: Fishes by Kerezsy A, Gido K, Magalhães F and Skelton PH.

Ichthyofauna of the Itimbiri, Aruwimi, and Lindi/Tshopo Rivers (Congo basin): diversity and distribution patterns. Decru E; Vreven E; Danadu C; Walanga A; Mambo T; & Snoeks J. *Acta Ichthyologica et Piscatoria*, 47:225–247, 2017. URL https://www.aiep.pl/volumes/2010/8_3/txt/txt_02.php

This is a catalog of ichthyofauna collected from the Itimbiri, Aruwimi, and Lindi/Tshopo Rivers. It lists many species, including 19 species of killifish.

[Tyrone Genade]

Systematics, Taxonomy & Distribution

Morphological and taxonomic descriptions of a new genus and species of killifishes (Teleostei: Cyprinodontiformes) from the high Andes of northern Chile. Arratia G; Vila I; Lam N; Guerrero CJ; & Quezada-Romegialli C. *PLOS ONE*, 12:1–36, 2017. DOI https://doi.org/10.1371/journal.pone.0181989.

This is a major work dedicated to the description of a new killifish genus and species from the southern Chilean Altiplano of the Andean mountains. Its name is *Pseudorestias lirimensis*, and it comes from the wetlands and streams of the Chancacolla River at about 4,000 m height (a.s.l.). In previous work, *Pseudorestias lirimensis* had been tentatively assigned to *Orestias* cf. *agassii*. However, numerous differences in the morphological characters, coloration and karyotype provide compelling evidence that *P. lirimensis* is completely different from all other species of *Orestias*. Its autapomorphies comprise unique configurations of several structures, i.e. (i) the Meckel cartilage, (ii) the basibranchials, (iii) the second pharyngobranchial, (iv) the ceratohyal, and (v) the dorsal as well as the (vi) anal radials. In addition, *P. lirimensis* displays strong sexual dimorphism, with females being nearly twice as large and long than males, while males show absence of neuromast lines and reduced squamation on the head (amongst others). The authors provide detailed and thorough descriptions of *P. lirimensis*, together with excellent drawings and photos. They suggest that the emergence of the new genus and species may have resulted from Miocene-Pliocene vicariance events and the isolation of the Chancacolla River ecosystem since then.

Moreover, this work contributes to solve the debate whether a sister-relationship exists between the Old-World killifish *Aphanius* and the New-World killifish *Orestias*. Based on morphological evidence, such a relationship was proposed by Parenti (1981, 1984), and has also been adopted in Nelson et al. (2016). A different phylogenetic interpretation was published by Costa (1997) based on a data matrix comprising 65 morphological characters. According to his analysis, *Orestias* would be sister to a large clade comprising *Aphanius* + New World Cyprinodontini, which is actually more in line with the disjunct biogeography of *Orestias* and *Aphanius* and additionally confirmed by recent molecular-based work (e.g. Reznick et al. 2017). Arratia et al. (2017) provide now a new list of characters diagnosing the Orestiini, containing *Orestias* and *Pseudorestias lirimensis* as sole members, and thus contribute to a much improved understanding of this tribe.

As it is the case for many endemic killifish species, also *Pseudorestias lirimensis* must be considered as critically endangered. This threat is not only because of its small habitat and reduced distribution, but also due to water demands and climate change in the region. Its recognition as a new genus and species will hopefully help to develop new conservation strategies for the southern Altiplano, from where, besides *P. lirimensis*, also six endemic species of *Orestias* are known.

[Bettina Reichenbacher, Department of Earth and Environmental Sciences, Ludwig-Maximilians University, Munich, Germany.]

Deep and concordant subdivisions in the self-fertilizing mangrove killifishes (*Kryptolebias*) revealed by nuclear and mtDNA markers. Tatarenkov A; Lima SMQ; Earley RL; Berbel-Filho WM; Vermeulen FBM; Taylor DS; Marson K; Turner BJ; & Avise JC. *Biological Journal of the Linnean Society*, 122:558–578, 2017. DOI http://dx.doi.org/10.1093/biolinnean/blx103.

This is a high impact paper. It calls into question current understanding of the fishes we call *Kryptolebias hermaphroditus* (sensu Costa, 2011) and *marmoratus* and sets the stage for multiple descriptions of new species in a cryptic species complex and the reinstatement of old species long ago synonymised with *K. marmoratus*.

The authors analyzed 734 specimens from 33 locations from Florida, USA to Fazenda River, Brazil. *K. caudomarginatus* (sensu Costa) was not included in this study. This study restricted itself to the topic *K. hermaphroditus* and *K. marmoratus*. 33 nuclear microsatelite DNA sequences were studied in addition to regions of the mitochondrial cytochrome oxidase subunit I and cytochrome B genes. Microsatellite, cytochrome oxidase subunit I and cytochrome B data revealed three major clades (Northern, Central and Southern). The Northern and Central Clades are both derived from the Southern Clade, which itself is hypothesized to have its origin in northern South America. Within the Southern Clade are three smaller groupings: two around the north east corner of Brazil and the third around Rio de Janeiro. Within the central clade there are again three groups: the Antilles, Bonaire- Aruba and Panama. In the Northern Clade the Honduras-Belize clade separates out from the Florida-Bahamas clade. These sub-clades have lower support than the three major clades.

Photo of male *K. marmoratus* Dangriga and hermpahrodite *K. bonairensis* by Frans Vermeulen©

There is a dearth of specimens from northern Brazil through to Venezuela, also most of the Caribbean islands haven't been sampled making it difficult to delineate the borders between the species. The authors adopt a conservative analysis of the data, interpreting it as support for *K. marmoratus*, *K. hermaphroditus* (sensu Costa, 2011) and *K. bonairensis/heyei*. The authors adopt the name *bonairensis* for the Central Clade because the island of Bonaire has been sampled. The taxon *heyei* (from the Island of Saona, Dominican Republic) is older than *bonairensis*. Should samples from the Island of Saona group with the Central Clade it would have to be given the name *heyei* or the Antilles Clade be separated out from the Bonaire-Aruba Clade as a distinct species. The authors discuss at great length the decision of whether to go with one, two or three species. They write " The decision on taxonomic status of the three identified lineages of *Kryptolebias* is thus somewhat subjective and largely determined by the adherence of a researcher to one or another school of thought, BSC or PSC. Anecdotally, even within the small group of co-authors of this study, we could not come to consensus on this subject." The authors are adamant on the data for the Southern Clade supporting a one species hypothesis of *K. hermaphroditus* only. The authors conclude that "Our finding of two highly distinct Caribbean lineages—Northern and Central—raises intriguing questions. Are there significant (but hitherto unrecognized) biological differences between the lineages? Are they (or were they ever) in direct contact? Does introgression or displacement occur in putative contact zones? The reclusive *K. marmoratus* is still far from revealing all of its secrets." Indeed, this paper is probably the beginning for systematic *Kryptolebias* research.

As an aside, Guy Tiriault of the Island of Guadeloupe, was generous enough to supply us with samples of *Kryptolebias* from the island of Guadeloupe. These have been entered into the DNA bank and samples have already been sent to Dr Andrei Tatarenkov and analyzed, identifying them with the Central Clade. Live fish of this collection are being distributed

as *Kryptolebias* sp. cf. *bonairensis* Bon Gout GT-15. *I* had settled on this designation based on the clear genetic separation of these fish from *K. marmoratus* and *K. hermaphroditus*. *I* am not committed to the *bonairensis* name for these, and suspect that in the end *heyei* will win out for the Antilles clade. I leave this matter to the professional taxonomists to settle. Also, fish from Puerto Rico are also of the Central clade and are **not** *marmoratus*. If you collect *Kryptolebias* while on holiday in South/Central America or the Caribbean, please preserve a specimen for DNA analysis!

[Tyrone Genade]

***Aphanius kruppi*, a new killifish from Oman with comments on the *A. dispar* species group (Cyprinodontiformes: Aphaniidae)** . Freyhof J; Weissenbacher A; & Geiger M. *Zootaxa*, 4338:557–573, 2017. DOI http://dx.doi.org/10.11646/zootaxa.4338.3.10.

This is a very important paper in the field of *Aphanius* research and should be read by hobbyists that keep *Aphanius* as well. This paper presents a description for *Aphanius kruppi* as well as provides an identification key for the *Aphanius dispar* species group. It also presents a DNA phylogenetic scheme for the species group which raises the very important question of just what is *A. dispar*?

A. kruppi is named for Faheed Krupp for his many valuable contributions to the explorations of the freshwater fishes of the Middle East. The specimens used to describe the species were obtained on a hobbyist collection trip to Oman in 2015 that were raised by the Zoo of Vienna. The fish comes from the Al Mudayrib spring and Wadi Bani Khalid at Sayh al Hayl (both sites are in Wadi al Batha which drains into the Arabian Sea north of Al Ashkharah). *A. kruppi* has a stable and distinct phenotype that allows it to be easily discerned from other species. It differs from *sirhani* in females haveing 7–12 short bars on its flank and males lacking a black dorsal-fin margin and 5–10 irregularly set and shaped bands of spots or

Photos of male and female *Aphanius kruppi* from Al Mudayrib (top) and Wadi Bani Khalid (bottom), Oman. Photo taken from Freyhof et al©.

blotches as well as three black bars on the caudal fin. It differs from *A. dispar, richardsoni* and *stoliczkanus* by having black of dark gray bars on the flank and a diamond-shaped

Photos of male and female *Aphanius* cf. *kruppi* from Al-Hoota, Oman (top) and *Aphanius stoliczkanus* fromWadi Fanja, Oman (bottom). Photo taken from Freyhof et al©.

or vertically-elongate black or brown blotch at the base of the caudal-fin of females. The dorsal and anal fins are short relative to these three species. It differs from *A. ginaonis* by having 9–14 brown or grey bars on the flank. It differs from *A. furcatus* by having a truncate caudal fin and the body that is completely covered with scales (*A. furcatus* has a partially scaleless body). *A. stiassnyae* does not have the upturned lower jaw. The authors consider *A. kruppi* as a threatened species due to its small distribution and very small populations.

Photo of male and female *Aphanius* cf. *dispar* from Atlit, Israel. Photo taken from Freyhof et al©.

The molecular analysis of the cytochrome oxidase I gene produced a phylogeny with strong support but which did not align with the current conception of the species. *A. dispar* breaks into three distinct clades (Egypt-Israel, Eritrea and Saudi Arabia). *A. kruppi* and *A. stoliczkanus* seem to have hybridized at Khor Kalba nature reserve (United Arab Emirates), Fanja and Al Hoota, Oman (which shows an intermediate color pattern between the species), but in general *A. stoliczkanus* has a discrete and wide range. It is unlikely that

A. dispar exists in Iran, instead, these are probably *stoliczkanus* but DNA samples are only available from the Iraq populations and not Iranian populations. *A. ginaonis* was only sampled from one location (Geno, Iran) and no surrounding *stoliczkanus* were sampled so it is unknown just how widely both species extend in Iran.

It is clear, after reading this paper, that the taxonomy of the *Aphanius* species from Egypt to Iran is largely unsettled. We don't know where Rüppell collected his samples of *Aphanius dispar* so extensive collection along the Red Sea and southern Israel are badly needed so as to redescribe of this species. More samples are needed from the area between Oman and Pakistan so as to better delineate the species. I expect many interesting research papers will grow out of Freyhof et al's description of *A. kruppi*.

[Tyrone Genade]

Editor's note: In this paper Freyhof et al. revalidate previous synonym *stoliczkanus* with a new definition and raise *richardsoni* to full species status and it is acknowledged in Killi-Data, however more research is needed as to the final identification of many populations of *dispar* s.l. in other regions and countries, including Iran, previously identified as *Aphanius dispar dispar*.

[Jean Huber]

Let's jump in: A phylogenetic study of the great basin springfishes and poolfishes, *Crenichthys* and *Empetrichthys* (Cyprinodontiformes: Goodeidae). Campbell DC & Piller KR. *PLOS ONE*, 12:1–21, 2017. DOI https://doi.org/10.1371/journal.pone.0185425

This new comprehensive molecular study by 2 US researchers from Southeastern Louisiana University focuses on components of subfamily Empetrichthyinae (only 2 genera, with a relict distribution in southern central USA). This new study is very much welcome for the species belonging today to *Crenichthys* (*baileyi albivallis*, *baileyi baileyi*, *baileyi grandis*, *baileyi moapae*, *nevadae*, *baileyi thermophilus*) and *Empetrichthys* (*latos concavus*, *latos latos*, *merriami*, *latos* Pahrump, with all but *latos latos* being extinct now). The authors analyze the relationships between those closely related taxa and assess the validity of the subspecies designations given by Williams and Wilde (1981). The new molecular results are

Photo of a pair of *Crenichthys baileyi baileyi* by Frans Vermeulen©

notably solid because the 2 authors study 19 populations covering the entire distribution (and several specimens of each) and use the present state of the art of molecular techniques (nuclear sequences, mitochondrial sequences, haplotypes and microsatellites markers) and they conclude that the subspecies designations of Williams and Wilde are not valid for *Crenichthys*, that rather a conservative approach suggests there are two species within *nevadae* and two species within *baileyi* but that no structure is found for *latos*. However the 2 authors do not go beyond that by naming the new molecular species based on the distinct population of *nevadae* (the northern clade) and by proposing a stable new systematic structure for *baileyi*, probably because their results are somewhat heterogeneous between mitochondrial and nuclear data and because they could not yet propose new diagnoses in line with

their results (etc.). In total, after a comparison of subresults according to independently nuclear data, mitochondrial data and haplotypes data, the 2 authors suggest that there are 2 to 4 distinct species in *Crenichthys*, and not 2 species, one with 5 subspecies as previously, i.e., *nevadae*, the new un-named aff. *nevadae*, *baileyi* and possibly *moapae*, while *thermophilus* and *albivallis* seem identical (and then each other synonyms and junior synonyms of *baileyi*). To go further (Kyle Piller pers. comm. November 2017), the authors plan additional studies (with more genetic results combined with new morphological data) and those future studies are looked forward to, not only because of the conservation status of those species, but also because the genetic distances between the discovered lineages are low within *Crenichthys* based on *cytb*, however, this may be a trait common across the family Goodeidae and a result of the recent estimated divergence time for *Crenichthys*.

Additional note: a question related to this publication and the subspecies concept has been raised to Killi-Data and answered in last November at: http://www.killi-data.org/faq.php#Q(48):

[Jean Huber]

***Nothobranchius cooperi* (Teleostei: Cyprinodontiformes): a new species of annual killifish from the Luapula River drainage, northern Zambia**. Nagy B; Watters B; van der Merwe P; Cotterill F; & Bellstedt D. *African Journal of Aquatic Science*, 42:201–218, 2017. DOI http://dx.doi.org/10.2989/16085914.2017.1372270.

Nagy et al describe a new species of *Nothobranchius*. This work is the product of a longterm study of *Nothobranchius* species in Zambia, and follows on from the description of *N. sainthousei* from the same river system: the Luapula River drainage. Keen readers of the *sainthousei* paper would have noticed that the *rosenstocki* populations were very genetically different. In this paper *rosenstocki* is split into *rosenstocki* and *cooperi*.

The new *N. cooperi* refers to those populations from Mansa (ZAM 97-2, ZAM97-3, ZAM 07-8, ZM 12-7) as well as Samfya. *N. cooperi* was fist discovered by John Rosenstock and Jorgen Sunesen in 1989 in a small stream (the Lwingishi River, 36 km east of Mansa). (On this same trip they discovered a female fish in the Luapula drainage near Kapalala which is today *rosenstocki*.) This species can be distinguished from *sainthousei* by a solid orange-red anal fin with irregular light blue zone close to its base; and from *rosenstocki* by having a prepelvic length of 48.8–51.9% SL (vs 45.1–49%) and head depth of 75–77% HL (vs 78–84%) as well as a caudal peduncle length 1.2–1.3 times it depth compared to 1.4–1.5 for *rosenstocki* and *sainthousei*. The species could be discerned by a combination of molecular phylogenetics, color pattern as well as principal component analysis of meristic characters.

The habitat is described as a stream system composed of multiple channels, flowing through dense grasses, roadside ditches and residual pools. The water was always flowing but the fish favored slow-flowing to still, densely vegetated regions in the habitat. The pH was 6.18–6.35 and total dissolved salts were less than 10–16 ppm and the water had a temperature of 24–26°C. Other species of fish were present: *Enteromius* barbs, *Ctenopoma* species as well as two *Micropanchax* species. The conservation status of the species is given as Vulnerable.

The species is named for Dr Barry Cooper who participated in the collection of this species in 2007 (along with Brian Watters, Otto Schmidt, Bill Bishopp, Pieter Kearney, Johan Jordaan and Johan Bornman), the specimens collected on that trip being pivotal to the phylogenetic analysis that revealed this species. A report of the 2007 collecting trip is given by Otto Schmidt (Auf Killi-Jagd nach Sambia. DKG-Journal 31 (4): 79-91, August 1999 and translated into English and published: 2008. Zambia revisited, 2007, Journal of the American Killifish Association 41:3–18).

[Tyrone Genade]

From top to bottom: *N. cooperi* Mansa ZAM 97-3; *N. rosenstocki* Kapalala ZM 12-9; *N. sainthousei* Mweshi ZM 12-19. Photos by Béla Nagy©).

Two new seasonal killifishes of the *Austrolebias adloffi* group from the Lagoa dos Patos basin, southern Brazil (Cyprinodontiformes: Aplocheilidae). Costa WJ; Cheffe MM; & Amorim PF. 2017. URL http://www.senckenberg.de/root/index.php?page_id=16562#VZ67-2

This very detailed paper by Costa, Cheffe and Amorim describes 2 new *Austrolebias* species from the Lagoa dos Patos basin, in southern Brazil. The first is: *Austrolebias pelotapes* sp. nov. which is closely related to the other new species *Austrolebias pongondo* sp. nov.

The clade comprising the two new species is supported as being more closely related to *A. adloffi* than to *A. nigrofasciatus* that is endemic to the same area.

A. pelotapes n. sp. is distinguished from all other species of the *A. adloffi* group by having the urogenital papilla base attached by a thin membrane to the anterior margin of the anal fin in males.

Austrolebias pelotapes seems to be the smallest species of the *A. adloffi* group, not reaching 35 mm SL (vs. maximum adult size between 43 and 45 mm SL). *Austrolebias pelotapes* is distinguished from *A. pongondo* by the former having 14–19 neuromasts in the supraorbital series (vs. 20–21) and 22 –23 neuromasts around orbit (vs. 24–27) and other features you may find in the description.

Type locality: Brazil, Estado do Rio Grande do Sul: Município de Pelotas: temporary pool in the Sanga Funda drainage, tributary of Arroio Pelotas, 31°43'46"S 52°19'07"W; Collected by L. Lanés et al., 5 Nov. 2005.

Austrolebias pongondo n. sp. is distinguished from all other species of the *A. adloffi* group, except *A. pelotapes*, by a combination of characters showing a transverse row of small spots on the middle portion of the dorsal fin in males (vs. transverse row absent in all other species), unpaired fins with a single row of light blue spots on their basal portion in males, and with a distinctive dark gray zone on the posterior portion of the dorsal and anal fins and some other features mentioned

in the description.

Type locality: Brazil, Estado do Rio Grande do Sul: Município de Pelotas: swamp at the road to Torotama island, Arraial, Povo Novo district, 31°55'54"S 52°14'42"W, Collected by: M. Cheffe et al., 21 Aug. 2001.

One figure shows the Maximum likelihood tree of relationships among species of the *Austrolebias adloffi* group. Two tables show meristic data of both new taxa. One map shows Geographical distribution of the group. Photos show life color of *A. pelotapes* and preserved state. *A. pogondo* only in preserved state.

The paper is good reading and interesting is the discussion in which, at the end, the need for clarifying the relationship between *A. salviai*, *A. reicherti*, *A. charrua* and *A. minuano* is recommended as a future study before synonymies being formally made.

[Frans Vermeulen]

A new annual fish of the genus *Austrolebias* (Cyprinodontiformes: Rivulidae) from Rio Camaq a basin, Laguna dos Patos system, Brazilian Pampa. Volcan MV; Gonçalves AC; & Lanés LEK. *Zootaxa*, 4338:141–152, 2017. DOI http://dx.doi.org/10.11646/zootaxa.4338.1.7.

The authors describe *Austrolebias camaquensis*. It was discovered in a temporary pool adjacent to the Arroio Santo Antônio (a tributary of the Rio Camaquã). The pool is close to highway BR-471, 30°58'25"S52°39'47"; and is within the Canguçu muncipality, Rio Grande do Sul State, Brazil. It can be distinguished from other members of the *Austrolebias alexandri* species group (except for *paucisquama* and *juanlangi*) by having well-defined bright blue bars on its flanks in males. It differs from *paucisquama* by having contact organs on the first rays of the pectoral fin and a greater number of scales along the caudal peduncle (14–16 vs 12). Its dorsal fin origin is anterior to the vertical through the anal fin origin in males compared to *juanlangi* as well as having fewer neuromasts in the post otic series, shorter dorsal fin base length and in females, having irregular spots arranged on the anterocentral portion of the flanks.

This species was found in the Pampa domain (a large grassland) in pools that dry out in the dry season. It is found along both sides of the Rio Camaquã in small, shallow pools located in grassland areas of farms. The plant life was mostly emergent and submerged plants. Other species of fish were also captured: *Austrolebias bagual*, *Callichthys callichthys*, *Rhamdella eriarcha*, *Corydoras paleatus* and *Cheirodon ibicuiensis*. The water is tinted brown but transparent, pH 5.71, salinity 0.04 ppt, conductivity 0.08 μS/cm, temperature 16.6°C, dissolved solids 39 g/L and a depth of 25.2 cm. The sex ratio of the species ranged from 1:1 to 1:2.4 males to females. The locations where the fish occur are already modified by man (rice fields are being cultivated, alien tree species have been planted and their is mining upstream). The authors recommend that this species be listed as critically endangered under caegory CR B2ab (ii, iii) of the IUCN.

[Tyrone Genade]

Description of two endangered new seasonal killifish species of the genus *Cynolebias* from the São Francisco River basin, Brazilian Caatinga (Cyprinodontiformes, Aplocheilidae). Costa WJEM. *Zoosystematics and Evolution*, 93:333–341, 2017. DOI https://doi.org/10.3897/zse.93.20906.

In this paper, two new species of the genus *Cynolebias* are described from temporary pools of the Verde Grande River drainage, São Francisco basin, northeastern Brazil. Described are *Cynolebias elegans* sp. n., a member of the *C. gilbertoi* group, is considered as the smallest species of the genus, reaching about 38 mm of standard length. The second is *Cynolebias gorutuba* sp. n. belongs to the *Cynolebias zeta*-clade, a group of large species supposedly feeding on smaller sympatric seasonal killifishes. The latter differs from other species of the group by the female color pattern, relative position of

dorsal fin and vertebrae, and cephalic neuromast pattern. Both species were not found in recent collecting trips, after their habitats had been drastically modified, and are possibly highly endangered if not already extinct.

The author mentions two seasonal killifish generations per year in most of the Caatinga region. The first one during the first rains (after the longest dry period), generally between November and January, and the second one between February and April. The author gives a clear overview of the history of the genus and its individual taxa. Amazingly all 16 now known species of *Cynolebias* from the Caatinga, except one, are described after 1990 due to recent in-depth sampling of the temporary pools in that semi-arid area. The discussion focuses on the increasing rate of biodiversity loss and the high risk of extinction of organisms in the Caatinga area mainly due to the high levels of specialization of these organisms. The two new species provide a good example of two extreme divergent morphological patterns within the genus *Cynolebias*. The small size and delicate morphology of *C. elegans*, with relatively long fins in males, contrasts with the robust aspect and short fins of *C. gorutuba*, which is a member of CZC (i.e. the *Cynolebias zeta* Clade), with species often reaching over 100 mm SL or more and which predate on the smaller *Hypsolebias* ssp they live with.

The name *Cynolebias elegans* refers to the Latin elegans (elegant, fine), an allusion to the distinctive general appearance of the new species, with males combing relatively slender body with long unpaired fins. The name *Cynolebias gorutuba* refers to the Gorutuba River floodplains, the type locality of *C. gorutuba*. This paper is not only of great value for scientists and hobbyists but certainly for environmentalists and politicians who set the future for the region and must decide how to protect the endangered ecosystem of the semi-arid Caatinga. Very good reading!

[1] KDI regards *Melanorivulus* as a subgenus of *Rivulus*.

[Frans Vermeulen]

A new pelvic-less species of *Melanorivulus* Costa (Cyprinodontiformes: Cynolebiidae), with a discussion on the pelvic-fin absence in killifishes. Deprá GDC; Silva HP; & Da Graça WJ. *Zootaxa*, 4300:111–124, 2017. URL http://www.mapress.com/j/zt/article/view/zootaxa.4300.1.6/0

In this paper the Brazilian authors describe a new species named *Melanorivulus nelsoni* which is found in a stream draining into the upper rio Paraguai basin, in the municipality of Juscimeira, State of Mato Grosso, Brazil.

It is distinguished from all other congeners, except *M. pictus* and *M. planaltinus* by the absence of pelvic fins and girdle. The genus *Melanorivulus* overall does not show pelvic-less taxa except for *M. pictus* and *M. planaltinus* which show missing pelvic fin(s), one side or both, occasionally. Besides describing the new species in the present paper authors studied the pelvic-less occurrence within the genus *Melanorivulus*. Authors present a clear history of the genus *Melanorivulus*, erected by W. Costa 2011 formerly in the genus *Rivulus*[1], and describes the difficulties of determining the identity of samples if no life color information is available and when material is available in preserved state only.

The two species *M. pictus* and *M. planaltinus*, both occasionally show missing pelvic fins, occur in nearby localities in the vicinity of Brasília, although in distinct river basins (respectively, upper rio Paraná and upper rio Tocantins).

Melanorivulus nelsoni, in which all available specimens lack both, the pelvic fins- and the girdle, was recently discovered in the upper rio Paraguai basin, about 800 km from Brasília and is described in the present paper. Additionally, the authors discuss the possible genetic and evolutionary mechanisms behind the absence of pelvic fins in *Melanorivulus* and other Cyprinodontiform genera. *M. nelsoni* is named in honor of Joseph Nelson (1937–2011), who published on the

absence of pelvic-fins in bony fishes earlier in 1989, and also in recognition for his pivotal contributions to the knowledge of fish diversity as a whole.

The paper is very informative and well written, with clarifying tables(1), maps (1) and several photo clusters of specimen showing life colors, photo's of museum material and photo's of the *M. nelsoni* habitat.

A single point of criticism: The discussion around the occurrence of pelvic-less species in the genus *Melanorivulus*, like in the new species *M. nelsoni* but also occasionally in *M. pictus* and *M. planaltinus* and other Cyprinodont fishes, is of great interest and, in my opinion, complex enough to bring such in a separate paper and not include it in the description of this new taxon. Nevertheless the paper is excellent reading for scientists and hobbyists.

[Frans Vermeulen]

Killifish Biology: Ecology & Physiology

Effect of conservation of fish in formalin and ethanol on length-weight relationships and condition factor in *Tlaloc labialis* (Günther, 1866). Anzueto-Calvo MJ; Velázquez-Velazquez E; Matamoros WA; Cruz Maza BGA; & Nettel-Hernanz A. *Journal of Applied Ichthyology*, 33:1184—1186. DOI http://dx.doi.org/10.1111/jai.13461.

Fixation of fish with formalin and ethanol affects the vital statistics of the preserved fish. The formalin stiffens tissues and dulls colors while the ethanol, as well as washing out certain colors, also dehydrates the fish. This makes it difficult to use preserved fish for determining lengths, weights and condition factors. In this paper Anzueto-Calvo et al set out to determine how much the mass is lost due to preservation of *Tlaloc labialis*[2].

Freshly collected fish were fixed in formalin for 72 hours and then moved to ethanol for various periods of time where after the weight and length of the fish was measured and compared to the initial measures of the freshly collected fish. Fomalin fixation caused a 5.78% loss of mass and 2.53% loss in standard length. A month in ethanol caused an ≈30% in mass. After 2 years the fish had lost 33.74% of the mass and the fish's standard length had reduced 3.47%. As consequence, freshly fixed fish give an over estimate of condition factors while long-term fixed fish will give an under estimate.

This study provides a useful benchmark for future studies of preserved specimens of *T. labialis*. These experiments would need to be repeated for each species to be able to use preserved specimens to determine condition factors. The author advised against comparing freshly collected and preserved specimens.

[Tyrone Genade]

[2]KDI lists this species as *Profundulus labialis*, *Tlaloc* is considered a subgenus of *Profundulus*.

Do not eat your kids: embryonic kin recognition in an amphibious fish. Wells MW & Wright PA. *Behavioral Ecology and Sociobiology*, 71:140, 2017. ISSN 1432-0762, DOI 10.1007/s00265-017-2360-y, DOI https://doi.org/10.1007/s00265-017-2360-y

An intriguing title that proves to be more a statement than a warning to future fish generations. The mangrove rivulus (*Kryptolebias marmoratus*) are the investigated parents, due to their ability to self-fertilize and produce isogenic strains that can be easily differentiated. Long-term captive populations originating from the USA (Florida) and Belize were utilized. Individual adults were kept well fed or starved for two weeks (to see if hunger played any role) and were then placed in a container. An embryo was subsequently added and observations made on the time taken before the individual investigated, the number of actual investigations and the number of nips taken. The embryos were either from the parent, from the same strain or from a different strain.

What they found was that no fish cannibalized its own embryos but some did cannibalize those from the same strain and an increased number cannibalized those from other strains. Interestingly there were differences between the two populations and the authors speculate on possible causes, indicating areas for further research. The authors conclude that *K. marmoratus* adults can identify their own kin and differentiate on that basis when 'considering' cannibalism. An interesting paper that provides far more insight than can be reproduced here. The same volume of the journal features an editorial: Kin recognition and filial cannibalism in an amphibious fish Joachim G. Frommen; Behav Ecol Sociobiol (2017) 71:141 DOI 10.1007/s00265-017-2369-2 that highlights the value of the work as a first step in understanding filial cannibalism.

[Andy Patel]

Plasticity of skin water permeability and skin thickness in the amphibious mangrove rivulus *Kryptolebias marmoratus*. Heffell Q; Turko AJ; & Wright PA. *Journal of Comparative Physiology B*, Epub ahead of print, 2017. DOI https://doi.org/10.1007/s00360-017-1123-4.

It is known that *Kryptolebias marmoratus* is able to respire through its skin. While emersed the fish are able to increase their body water content even though they are unable to drink and are exposed to the air. The authors sought to find out what changes occur to the skin tissue over time as the fish is emersed, and how this explains it ability to both respire and gain water volume when emersed.

Fish were acclimated to 15‰ or hypersaline 45‰ water and then acclimated to air exposure on filter paper dampened with water of 0.3, 15, 45‰. Fish spent most of their time with their ventral skin in contact with the moist substrate but there was no particular skin area that permitted more water to pass across it into the fish. Freshwater acclimated fish had higher water influx across the skin but after 7 days of emersion this effect vanished. Skin thickness varied both with salinity and air exposure. The dorsal skin thickness was unaffected by salinity but got thicker in response to air exposute. The ventral epidermis was thicker in response to increasing salinity and didn't change when exposed to the air but the dermis did get thinner in response to emersion.

The authors conclude that the fish are able to manage their water balance by changing their skin thickness. Emersion increased skin water flux and the authors hypothesize that the fish are able to take up water when emersed due to the thinner ventral dermis.

[Tyrone Genade]

Skeletal stiffening in an amphibious fish out of water is a response to increased body weight. Turko AJ; Kültz D; Fudge D; Croll RP; Smith FM; Stoyek MR; & Wright PA. *Journal of Experimental Biology*, 220:3621–3631, 2017. DOI https://doi.org/10.1242/jeb.161638.

A very interesting paper but first watch this: https://www.

youtube.com/watch?v=mKxRe0hAQmg! To move on land fish had to stiffen their skeleton for two reasons: the first is that they needed firm support to propel their bodies and the second is that out of water they lose the buoyant supporting environment the water offerend and need to evolve a stiffer skeletal structure to maintain their body shape. A big question is whether weight-responsive bone is a fundamental characteristic of bone or an early vertebrate evolved trait during the invasion of land. The goal of Turko et al's study was to determine whether there is a cellular response by the skeleton of of amphibious fish in response to mechanical load when out of water or when the effect of gravity was artificially reduced.

Two experiments were performed. (1) Fish were acclimated for 7 to 14 days out of water, and then half were reacclimated to water, and then the tensile strength of their gill arches (a skeletal tissue) was examined. (2) Fish were kept in an apparatus designed to reduce the gravitational force on them (in this case to 0.06 of what we experience). Both water and terrestrial animals were exposed to microgravity. They made use of Picrosirius Red staining and Transmission Electron Microscopy to assess changes in bone density; as well as proteomics to assess changes in gene expression in the bone of the gill arches.

The gill arches of terrestrial-acclimated fish resisted more force than gill arches of fish kept in water. This resistance increased with time out of water, and decreased to normal levels when reacclimated to water. There were changes in the abundance of various proteins in response to terrestrial acclimation, in particular to the levels of collagen and laminin and other bone-related proteins. Histological analysis revealed an increase in collagen deposition and structure but no changes were readily visible in the amount of calcium deposition in the bone. Fish exposed to reduce gravitation force didn't undergo any detectable modification to their skeletal structure or skeletal strength.

The lack of a response to microgravity showed that the fish were responsive to the body weight on the gill arches and not a side-effect of reduced buoyancy by emersion. The changes in collagen structure with little change in calcium deposition indicates that the fish stiffen their gill arches by changes in the interactions between the collagen protein fibers. While we may think that our bones are strong because they are hard, i.e. made up of calcium phosphate crystals, they are actually strong because of the collagen matrix in the bone. Without this matrix the bone will crumble under pressure. That this fish, from an order of chiefly fully-aquatic species, can adapt its skeleton to increased load when in a terrestrial state is interpreted, very cautiously, by the authors as evidence for a weight-responsive bone being a fundamental characteristic of vertebrate bone.

[Tyrone Genade]

Environmental calcium regulates gill remodeling in a euryhaline teleost fish. Platek A; Turko AJ; Donini A; Kelly S; & Wright PA. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, 327:139–142, 2017. ISSN 2471-5646, DOI http://dx.doi.org/10.1002/jez.2079.

This is an interesting paper with interesting implications for the aquarium hobby. The gill is the site of more than just gaseous exchange, it plays an important role in nitrogenous waste excretion, acid-base regulation, osmoregulation and ion regulation. For gaseous exchange the gill tissues need to be thin, but for regulation of ions it needs to be thick. For example, it is reported that goldfish under normal oxygen or cool conditions will reduce their gill surface area to advantage ion regulation; while under low oxygen or warmer conditions they will increase their gill surface area to increase gaseous exchange at the expense of ion regulation. It is also known that calcium ion concentrations affect gill surface area, in particular the thickness of the layer of cells between the gill epithelium (exposed to the environment) and the endothe-

lium (layer of cells that make up the blood vessels). This layer is called the interlamellar cell mass and is observed in the channel between the gill lamellae. Platek et al hypothesized that the gill surface area of *K. marmoratus* would be modulated by water hardness.

K. marmoratus were kept in control water (hard well water) or acclimated to low-calcium water (reconstituted RO water) or high calcium water (reconstituted RO water with extra calcium chloride added). After 7 days of acclimation fish were killed and fixed and their gills examined histologically. As expected, the fish in the soft water had increased interlamelar cell mass and therefore decreased gill surface area.

It is discussed in the paper that the move to soft water could be stressful to the fish and the effect could be mediated by increased cortisol. Even we, when stressed, will respond by retaining cation such as calcium and sodium. As these affects are also brought about by changes in temperature and oxygen availability it would be interesting to find out how our cool stream inhabiting *Aphyosemion* respond to to oxygen levels and temperature. One of the symptoms of heat stress in these fish is wasting, which would be expected from high cortisol levels and/or low oxygen levels. If our *Aphyosemion* respond to warm water by increasing gill surface area then they would struggle to maintain their internal cation levels, causing them additional stress. This is an interesting avenue for research. Also, how the kidneys respond to the move from hard to soft water would also be interesting to know. In freshwater fish the principle task of the kidneys is blood filtration, salt retention and water excretion. The authors note that *K. marmoratus* often inhabit ephemeral pools in the mangrove forests and that the fish have to rapidly cope with changing water conditions. Fish from more stable environments might not be able to adapt as fast as *K. marmoratus*. This is also an interesting tie-in with *Nothobranchius* research. Does the increasing dissolved solids of the pond signal the fish that the end is near and they have to increase reproductive rate at the expense of their other tissues?

[Tyrone Genade]

Population dynamics of *Melanorivulus rossoi*, a restricted geographic distribution killifish species. Severo-Neto F & Volcan MV. *Environmental Biology of Fishes*, Epub ahead of print, 2017. DOI https://doi.org/10.1007/s10641-017-0695-x.

The authors set out to characterize the distribution and temporal variation in body size, sex ratio, density, length-weight relationship and condition factor of *Melanorivulus rossoi*[3] in its natural habitat. As their motivation, the authors note that many of the recently described *Melanorivulus* have restricted distributions and very little is known about them other than they are vulnerable to extinction due to the small ranges. In this case, *M. rossoi* is threatened by landscape modification for agriculture.

Google Earth satellite images were used to look for suitable habitat for this species. The potential sites were surveyed and, finally, a convenient site was chosen for monthly monitoring. Specimens were anesthetized with eugenol (0.1 mg/L) before their vital statistics were taken. Specimens were returned to the wild once they had recovered from the eugenol. Suspected predators were sampled (i.e. *Hoplias malabaricus*, *Synbranchus marmoratus* and *Gymnotus* species) and their digestive systems inspected for *M. rossoi*.

The paper contains a wealth of information and is interesting reading.

[Tyrone Genade]

Annual killifishes as model systems for advancing understanding of evolution and developmental biology. Podrabsky JE & Arezo M. *Developmental Dynamics*, 246:778–778, 2017.

[3] KDI regards *Melanorivulus* as a subgenus of *Rivulus*.

ISSN 1097-0177, DOI http://dx.doi.org/10.1002/dvdy.24594

This paper is the editorial to the Developmental Dynamics special issue "Annual Killifishes as Model Systems for Advancing Understanding of Evolution and Developmental Biology". It is noteworthy for spelling out the need for an understanding of ecological and evolutionary consequences of altered developments; and a growing need to expand development studies in the lab to more species. The authors note the multiple lineages present in the Cyprinodontiformes and the varied developmental trajectories.

[Tyrone Genade]

Study of the chorion of seasonal and non-seasonal Africa and Neotropical oviparous Cyprinodontiforme fishes. Messaddeq N; Hergueux J; Weickert JL; & Romand R. *Environmental Biology of Fishes*, Epub ahead of print, 2017. DOI https://doi.org/10.1007/s10641-017-0698-7.

The study by Messaddeq et al. compares egg structure between the eight species and the laboratory model, *Danio rerio* (zebrafish). Eggs were imaged using scanning and transmission electron microscopy. Both seasonal (*Nothobranchius patrizii*, *Nothobranchius guentheri*, *Spectrolebias filamentosus*[4], *Notholebias vermiculatus*, and *Austrolebias luteoflammulatus*) and non-seasonal (*Fundulopanchax gardneri*, *Aphyosemion striatum*, and *Anablepsoides*[5] *rubrolineatus*) species were examined. Overall, the authors find that the structure of the egg envelope is highly variable between species, but much more complex and ornate in killifishes than zebrafish. In addition, the egg envelopes are made of multiple layers that vary in electron density, and different species possess envelopes with differing thickness and numbers of layers. The authors report that egg structure is not necessarily correlated with ecology or evolutionary relationships of the killifish species and state that further studies are needed to find such correlates. The manuscript provides a number of beautiful, clear photos that will be useful in describing the morphological diversity of killifishes for years to come.

[Andrew Thompson]

Gene expression during delayed hatching in fish-out-of-water. Thompson AW; Hayes A; Podrabsky JE; & Ortí G. *Ecological Genetics and Genomics*, 3-5:52–59, 2017. DOI https://doi.org/10.1016/j.egg.2017.09.002.

Fish-out-of-water provide unique opportunities for scientists to study the evolution and molecular mechanisms of stress response and adaptation to harsh environments.

This study, which is rather technical, provides insight into how delayed-hatching embryos of *Aplocheilus lineatus* react to aerial incubation and suggest that delayed hatching is a phenomenon distinct from the diapause stages of related annual species. Similar patterns of gene expression are shared among *Aplocheilus* and other egg stranding and amphibious fishes.

The methods of retrieving data are explained in detail and a table with longest recorded pre-hatching delays in 43 species from 8 different families is provided. Color figures show results of experiments but the reader is referred to the web version of this article for better view.

I think this paper is for specialists only, specialists that like to understand more about the processes of delayed hatching, diapauses and stress related environments that play a role in the survival techniques of our annual- and non-annual Killi fishes.

[Frans Vermeulen]

Females of the annual killifish *Austrolebias reicherti* (Cyprinodontiformes: Rivulidae) recognize conspecific mates based upon chemical cues. Blengini FR; Tassino B; & Pas-

[4]KDI considers *Spectrolebias* to be a subgenus of *Simpsonichthys*.

[5]KDI regards *Anablepsoides* as a subgenus of *Rivulus*.

sos C. *Behavioural Processes*, Epub ahead of print, 2017. DOI https://doi.org/10.1016/j.beproc.2017.08.007.

In this paper Blengini et al report on experiments to determine whether *Austrolebias reicherti* females recognize mates based on chemical cues. The authors setup an aquarium that was subdivided into three sections. In this aquarium female *reicherti* were exposed to male *A. charrua* and *reicherti*. These two species are found sympatrically in the Río Cebollatí system but for the most past have a parapatric distribution. The authors selected fish from parapatric locations. They could either detect the fish of the opposite sex by sight or by smell. Without exposure to the smell of the males the female *reicherti* would associate with the male *reicherti* just as often as the male *charrua*, while if the female *reicherti* could use her sense of smell she would select the correct mate, providing strong evidence that these two species can distinguish each other by scent. Data on female *charrua* mate choice wasn't reported.

[Tyrone Genade]

Incubation media affect the survival, pathway and time of embryo development in Neotropical annual fish *Austrolebias nigrofasciatus* (Rivulidae). da Fonseca AP; Volcan MV; & Robaldo RB. *Journal of Fish Biology*, Epub ahead of print. DOI http://dx.doi.org/10.1111/jfb.13504.

This is a very useful paper to hobbyists (as well as scientists working with annual killifish). It compares the effects of different incubation mediums on eggs survival and development.

The authors used distilled water (with or without powdered coconut husk, i.e. coco peat), Yamamoto's solution and Yamamoto's solution with antibiotics (penicillin & streptomycin). For substrates the authors used cotton wool or coconut husk or no substrate at all. The eggs were stored in hermetically sealed containers at 20°C in the dark. Survival and development was monitored at regular intervals.

Yamamoto's solution and coco husk gave the best results at 8 days (98.7% survival). Adding antibiotics gave a poorer a result (91.7%). Distilled water and water with coco husk gave the worst results (72% survival). Eggs on Yamamoto's solution dampened cotton wool developed faster than those in Yamamoto's solution dampened coco husk. All eggs that exitted diapause developed uniformly in liquid medium. 50% of eggs placed in coco-husk water did not exist diapause II. Eggs kept in liquid medium had more eggs in diapause II at 100 days from laying than those which were moved onto damp medium. Almost 80% of the eggs placed in coco husk water were still in diapause II after 100 days.

The authors speculate that something in the coco husk interferes with embryogenesis. They interpret that the poor survival of eggs in distilled water was due to the proliferation of fungi in the water. The authors conclude that Yamamoto's solution is an ideal medium for egg incubation of *A. nigrofasciatus*. The solution is as follows: 0.75% NaCl, 0.02% KCl and 0.02% $CaCl_2$. It is interesting that da Fonseca et al are able to induce diapause with coco-husk as effectively as Arezo (next article, below) was by keeping the eggs in peat in the presence of the adults. In my experience with *N. furzeri*, eggs spawned in sand and left with the parents will fully develop in three weeks. Could it be the peat that is inducing diapause and not the parents?

[Tyrone Genade]

Annual killifish adaptations to ephemeral environments: Diapause I in two *Austrolebias* species. Arezo MJ; Papa NG; Berois N; Clivio G; Montagne J; & De la Piedra S. *Developmental Dynamics*, 246:848–857, 2017. DOI http://dx.doi.org/10.1002/dvdy.24580.

Arezo et al provide an information-rich review of our current knowledge of the regulation of Diapause but their main focus was on characterizing diapause I at morphological and molecular levels. As a prelude to this they began by evaluat-

ing the induction of Diapause I under laboratory and natural conditions. To do so they set out to establish morphological features of development of *Austrolebias viarus* and *Austrolebias charrua*.

In brief, because the work was highly technical, eggs were induced into diapause I through one of two incubation methods. In one experiment freshly collected eggs were either incubated in Yamamoto's Solution away from the parent fish, or sealed in a porous mesh inside glass bottles with peat moss sealed on the bottom with the parent fish. Oxygen levels were 0.02 g/L in the bottles and all eggs were kept at 19°C under a natural photoperiod. Eggs were checked for development on a regular basis. RNA was extracted at specific developmental stages: epiboly, diapause IA (cells in dispersed configuration but rounded in shape), diapause IB (loosely arrayed rounded cells in close proximity), reaggregation (exiting diapause I).

100% of eggs kept with the adults entered diapause while 21% of the eggs kept in Yamamoto solution entered Diapause I. *A. viarius* embryoes, compared to *A. charrua*, were more prone to enter IA than IB. Embryos were monitored for 30 days. The authors observed that the highest mortality occured in the blastula and gastrula stage. Viability was between 59% in Yamamoto's solution and 45% for embryos kept with the adults. The alternative diapause state, IB, is reported by Arezo et al for the first time in this paper (but they note that it may have been noticed by Levels et al in *N. korthausae* embryos) and they speculate that this could create more developmental diversity, and thus responses to environmental cues. It is also observed that *A. charrua* exhibits high genetic variation compared to *A. viarius*. RNA quantities varied with developmental state, first declining as the embryos enter diapause, and then increasing above the levels at epiboly as the embryo reaggregates.

This paper is very useful to hobbyists eager to control embryonic development of *Austrolebias* embryos. If the goal is rapid development and a quick second generation then eggs must not be left with the adults for any length of time. On the other hand, if you want to maintain lots of different species but have few tanks, then by keeping the eggs with the adults the eggs can be forced into diapause I. The study by da Fonseca (in this issue of KD-News) suggests that simply moving the eggs from the peat water onto peat, exposed to the air, stimulates the eggs to develop to diapause II, and the research on *Millerichthys* (in the previous issue) suggests that emersion and redrying of diapause II eggs (and raising the temperature) stimulates development to diapause III.

[Tyrone Genade]

Stem cells distribution, cellular proliferation and migration in the adult *Austrolebias charrua* brain. Torres-Pérez M; Rosillo JC; Berrosteguieta I; Olivera-Bravo S; Casanova G; García-Verdugo JM; & Fernández AS. *Brain Research*, 1673:11–22, 2017. DOI https://doi.org/10.1016/j.brainres.2017.08.003.

This paper continues previous work by the authors to identify and characterize the neurogenic niches in the brain of *Austrolebias charrua*. In this paper they use a double-labeling method to label fast and slow-cycling neurogenic cells to differentiate the stem cells from the progenitor cells.

Using a combination of CldU and IdU they identified fast cycling cells, slow cycling cells (i.e. the stem cells) and migrating cells in the brain of *A. charrua*. The migrating cells were observed moving out radially or tangentially from the stem cell niches. 10% of the labeled cells in all regions of the brain were identified as stem cells. They used a 3D modeling technology to digitally reconstruct the brains of the fish with locations of the labeled cells.

[Tyrone Genade]

Influence of temperature on age-related lipid peroxidation in a short-lived vertebrate (*Nothobranchius furzeri*). Milinkovitch T; Lefrançois C; Durollet M; & Thomas-Guyon

H. *Fish Physiology and Biochemistry*, Epub ahead of print, 2017. DOI https://doi.org/10.1007/s10695-017-0439-z.

Previous research had shown an age-related accumulation of lipid oxidation products (i.e. lipofuscin) in *N. furzeri*. In this paper Milinkovitch et al investigate the effect of temperature on the development of lipid oxidation products (lipid peroxides).

The authors used the MZM 04-10 strain from the Chefu Rivier system and maintained them at either 22.1 or 25.6°C and sacrificed fish at 7, 18 and 30 weeks of age. They then set about measuring the amounts of malondialdehyde content of liver and muscle tissue. With age there was an increase in malondialdehyde in muscle tissue, with a sudden increase at 30 weeks. There was no difference with temperature in muscle tissue samples. In the liver malondialdehyde levels dropped from 7 to 18 weeks of age before increasing again at 30 weeks. The fish kept at 22.1°C had more malondialdehyde in their liver at 30 weeks compared fish kept at 25.5°C.

The authors observe that their findings "disagree with the idea that oxidative stress varies positively with temperature." Liu et al showed the *Austrolebias bellottii* have a much faster metabolism at cooler temperatures (Growth, 1975 39:337–343) so an increase in liver metabolism and free radicals at lower temperatures isn't unexpected but this is still a surprise result. Terzibasi et al showed an age-related increase of lipofuscin in the from 9 week old liver of this strain (PloS One, 2008 3:e3866) used by Milinkovitch so we should expect to see an increase in lipid peroxidation with age but this was not observed in the liver and muscle. The authors speculate that the lower temperatures cause a decrease in oxidative stress in the lysosomes which generate lipofuscin. The disparity in results serves as an interesting departure point for further experiments into the physiology of *Nothobranchius*.

[Tyrone Genade]

Dietary resveratrol increases mid-life fecundity of female *Nothobranchius guentheri*. Lee Y; Drake AC; Thomas NO; Ferguson LG; Chappell PE; & Shay KP. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, Epub ahead of print, 2017. DOI https://doi.org/10.1016/j.cbpc.2017.10.006.

In this paper Lee et al report on the effect of resveratrol supplementation on female fecundity. The authors fed fish resveratrol and assayed egg production and egg fertility at 14 and 20 weeks (as well as keeping track of the total number of eggs laid over 30 weeks). The fish fed resveratrol laid more eggs over their lifespan and had higher egg fertility. At 20 weeks the resveratrol fed fish had larger ovaries and more mature oocytes. Protein analysis of the ovaries for the relative abundance of NAMPT and SirT1 revealed an increase in NAMPT in the resveratrol fish but not difference in SirT1 abundance.

NAMPT, Nicotinamide phosphoribosyltransferase, is an enzyme involved in the regeneration of NAD^+ which is needed to maintain metabolism and Sirtuin activity, and accordingly, the increase NAMPT activity could be extrapolated to an increase in Sirtuin activity. The lack of SirT1 increase is at odds with the work of Liu et al (Oncotarget. 2017, 8:55422–55434) which found an increase in SirT1 expression in the liver in response to resveratrol. It is already reported that the different tissue of *N. guentheri* age differently (Dong et al, Fish Physiol Biochem 43:309, 2017, DOI 10.1007/s10695-016-0288-1). Now it is reported that different tissues respond to resveratrol in different ways and this discovery needs to be taken into account when designing experiments to elucidate how resveratrol affects lifespan.

[Tyrone Genade]

The regeneration capacity of caudal fin in the common tooth-carp, *Aphanius dispar* (Rüppell, 1829)(Teleostei: Cyprinodontidae). Zeinali F & Motamedi M. *International Journal of Aquatic Biology*, 5:321–327, 2017. URL http://

ij-aquaticbiology.com/index.php/ijab/article/view/375/0

This is the first investigation of fin regeneration in *Aphanius*. The authors used what they call *Aphanius dispar* from Bandar Abbas city in southern Iran. They kept the fish at three different temperatures: 23, 25 and 28°C and at a pH of 6.8 to 7. The fish were fed a freeze dried live food and dry fish food. Fish were anesthetized with clove oil (180 ppm) for 3 minutes and then the caudal fin was cut down to the caudal peduncle. Fin regeneration was observed daily. Fin regeneration was fastest at the higher temperature in both males and females. The authors note that due to the short lifespan of the fish this species could be used to study regeneration in aging.

[Tyrone Genade]

Evaluation of glyphosate toxicity on Arabian killifish, *Aphanius dispar* collected from southwestern Saudi Arabia. Messaad IA & Al Zailaie KA. *Global Journal of Science: Frontier Research*, 1:43–49, 2017. URL https://globaljournals.org/GJSFR_Volume17/4-Evaluation-of-Glyphosate-Toxicity.pdf.

Glyphosate, more popularly known as RoundUp, is in the news for its possible link to cancer. Messaad & Al Zailaie exposed *Aphanius dispar* to varying concentrations of glyphosate and studied the consequences.

The authors experimented with several concentration ranges: 0, 60–89, 90–119, 120–149 , 150–179, 180–209 and 210–240 mg/L. At the low dose the fish stopped schooling and became hyperactive with 20% mortality after 96 hours of exposure. All fish died at 250 mg/L doses. 96 hour LC_{50} was calculated as 115.25 mg/L. Histological study revealed abnormalities in the liver and gills of fish exposed to a quarter of the LC_{50} dose.

For comparison, nile tilapia (*Oreochromis niloticus*) have an LC_{50} of 1.05 mg/L and *Tilapia zilli* is 211.80 mg/L. In the USA glyphosate as been detected at 0.27–1.5 mg/L (http://cdn.intechopen.com/pdfs/12592.pdf). There is little data on the long-term effects of glyphosate on animals. The authors recommend that glyphosate be regulated near aquatic environment.

[Tyrone Genade]

Variations in fish body and scale shape among *Aphanius dispar* (Cyprinodontidae) populations: insights from a geometric morphometric analysis. Khosravi A; Golmakan MS; & Teimori A. *Capsian Journal of Environmental Sciences*, 15:113–123, 2017. URL http://cjes.guilan.ac.ir/article_2368.html

This is an interesting anatomical study of *Aphanius dispar*. The authors were interested in the environmental and genetic influence on fish morphology. They chose to study *Aphanius dispar* because it inhabits a broad range of habitats, shows a high tolerance to ecological and environmental changes and morphological variation from location to location. Also, many populations are geographically isolated and some are translocations from small founder populations implying high genetic divergence between populations.

The authors collected fish from a man-made canal within Bandarabbas City, the Shur River (a brackish environment) and a man-made pool in Kahnuj city. Scale morphology was studied using seven landmarks and a multivariate analysis to visualize shape changes mathematically was performed whereby they could compare the shapes between populations. Morphological differences were observed between the populations and between the sexes and variance in morphology could be accounted for by the comparison of specific landmarks. This data could be used to predict genetic relatedness.

The authors note that changes in the fin and scale shapes would effect the hydrodynamic power and swimming ability of the fish. The authors conclude that the variation in body shape and scales among the populations are probably the consequence of ecological conditions, in particular, to water flow.

[Tyrone Genade]

The role of habitat choice in micro-evolutionary dynamics: An experimental study on the Mediterranean killifish *Aphanius fasciatus* (Cyprinodontidae). Angeletti D; Sebbio C; Carlini A; Strinati C; Nascetti G; Carere C; & Cimmaruta R. *Ecology and Evolution*, Epub ahead of print. DOI http://dx.doi.org/10.1002/ece3.3540.

The authors set out to sample *A. fasciatus* from different microhabitats within the same location to determine if there is any genetic divergence within the populations.

Fish were sampled in normoxic and hypoxic environments and then compared the behavior between the fish under normoxic and hypoxic conditions. The authors then analyzed six allozyme loci that were found to be highly divergent between populations in previous studies. They found a connection between the glucose-6-phosphate isomerase-1 locus and the fish's preference for and behavior under hypoxic or normoxic conditions. The authors state that this is evidence that individual behavioral traits and underlying genetics could lead to nonrandom distribution of genotypes within a population, with some genotypes favoring certain microhabitats.

Accordingly, habitat choice could then be a significant driver of micro-evolutionary dynamics and even speciation. This observation could also affect molecular phylogenetics were few studies have been performed to determine whether the genes for sequencing have any effect on behavior or physiological selection in the environment.

[Tyrone Genade]

Towards the conservation of a critically endangered species, *Aphanius farsicus*: embryogenesis and development. Vahed NS; Esmaeili HR; Masoudi M; & Ebrahimi M. *Environmental Biology of Fishes*, Epub ahead of print, 2017. DOI https://doi.org/10.1007/s10641-017-0691-1.

The authors describe the embryonic development of *Aphanius farsicus*. This is the one of the first descriptions of embryogenesis of *Aphanius* species.

Fish were collected from the Maharlu Lake Basin, southwestern Iran and bred at Shiraz University. The fish were fed *Artemia* and *Daphnia* as well as artificial dry food. Methylene blue was added to the water (0.4 g/L) of the aquaria to deter fungus. Fish were bred in groups of 1 male to 3 females in tanks at 24°C using green spawning mops. The authors describe unfertilized eggs, fertilized eggs (1.4 mm in diameter), blastodisc formation, cleavage through the 2-cell stage to the blastula formation, gastrulation, embryogenesis and then through to hatching. The entire process took 162 hours (≈7 days). The authors note that there was no sign of diapause in the development of the embryo. The photo micrographs are beautiful.

[Tyrone Genade]

Phylogeography and population genetic analyses in the Iberian toothcarp (*Aphanius iberus* Valenciennes, 1846) at different time scales. Gonzalez EG; Cunha C; Ghanavi HR; Oliva-Paterna FJ; Torralva M; & Doadrio I. *Journal of Heredity*, Epub ahead of print, 2017. DOI http://dx.doi.org/10.1093/jhered/esx076.

Secondary freshwater fish species inhabiting fluctuating and extreme environments are susceptible to changes in dispersion, effective population size, and genetic structure. The Iberian toothcarp *Aphanius iberus* is an endemic cyprinodontid of the Iberian Peninsula restricted to brackish water of salt marshes and coastal lagoons on the eastern Spanish Mediterranean coast. The genetic structure, diversity and historical demography was studied analyzing mitochondrial and nuclear markers across its distribution range. The results indicated a subtle levels of phylogeographic structuring. This, combined with substantial mitochondrial diversity, suggest that Pleistocene glaciations had a lesser effect on the demographic structure of its populations than was the case for Iberian fresh-water species with similar distribution. Other test showed a relatively high degree of mitochondrial differ-

entiation, which could be explained by historical isolation of a population. Conversely, significant genetic differentiation, following an isolation-by-distance pattern was detected with microsatellites, suggesting extensive habitat fragmentation over the past hundreds of years. At a smaller geographical scale, habitat fragmentation, probably due to human activity, appears to have resulted in substantially reduced migration and increased genetic drift, as shown by expanded genetic differentiation of populations.

[Stefano Valdesalici]

Shoal sex composition affects exploration in the Mediterranean killifish. Lucon-Xiccato T & Griggio M. *Ethology*, Epub ahead of print. DOI http://dx.doi.org/10.1111/eth.12654.

A paper by the same authors was reviewed in the last issue of KDN and appears based on the same experimental methods involving *Aphanius fasciatus*. In this paper the authors go further in analysing the results and highlight that females seem to have a far greater effect on the behavior of a mixed sex shoal than males. In making comparison to studies on other taxa the authors discuss different selective pressures on the sexes, the effect of individuals on group behavior and in particular how this might impact on exploration. They urge more use of groups of fish to investigate exploratory behavior pointing out that many studies have previously focused on individuals only. A practice that may well prove less than satisfactory.

[Andy Patel]

Assessment of genetic diversity of an endangered toothcarp, *Aphanius farsicus* (Teleostei: Cyprinodontiformes: Cyprinodontidae) using microsatellite markers. Yaripour S; Esmaeili HR; Gholamhosseini A; Rezaei M; & Sadeghi S. *Molecular Biology Research Communications*, Epub ahead of print, 2017. DOI https://doi.org/10.22099/mbrc.2017.24404.1246.

Photo of a male *Aphanius fasciatus* from Capu Biancu FRN 10-12. Photo by Béla Nagy©.

Aphanius farsicus Teimori, Esmaeili & Reichenbacher, 2011 is an endemic fish restricted to the Maharlu Lake basin in Fars Province, southwestern Iran. Keivany and Esmaeili suggested that the species should be in the IUCN's Red Data Book due to criteria such as restricted distribution, destruction of spawning grounds, environmental pollution and drought. There are several scattered patches of this fish species in spring systems around the Maharlu Lake basin isolated from each other by land or by a hyper saline lake. The authors investigated genetic structure from four different water bodies in the Maharlu Lake basin applying five microsatellite markers. The results are a high genetic diversity observed within the populations (99%) and low diversity (1%) among them indicating probably high level of gene flow among the studied populations at the present time or in the past. The reasons can be hypotheses of annual floods around the lake make a fresh water area in some parts of it, and fishes can be migrated from one spring to another. Secondly the currently isolated populations may be relicts of a recently widespread species around the Maharlu Lake and therefore, isolated populations did not have enough time for differentiation and form part of

a single genetic population.

[Stefano Valdesalici]

Intraspecific variation and plasticity in mitochondrial oxygen binding affinity as a response to environmental temperature. Chung DJ; Morrison P; Bryant H; Jung E; Brauner C; & Schulte P. *Scientific Reports*, 7:16238, 2017. DOI https://doi.org/10.1038/s41598-017-16598-6

Mitochondria supply the energy needs of cells. Most studies focus on the maximum capacity of mitochondria under certain environmental conditions and extrapolate those data to hypoxic and thermal conditions. In this paper Chung et al investigates mitochondrial oxygen kinetics at submaximum capacity and how it could contribute to local adaptations and plasticity in response to changes in temperature.

The authors used two subspecies of *Fundulus heteroclitus* subspecies for these experiments. They acclimated the fish to 15°C and studied how oxygen consumption rates at 50% of the mitochondrial capacity. They found that the southern subspecies is more tolerant of thermal stress and hypoxia and had higher oxygen affinity compared to the northern subspecies. Acclimation to temperature extremes (5 and 33°C) altered the oxygen affinities of the mitochondria for both subspecies. At 33° the southern and northern subspecies didn't show any difference in mitochondrial oxygen affinity. The northern fish had lower oxygen affinity in their mitochondria. In acute exposure to thermal stress the northern subspecies still had lower oxygen affinity. Blood oxygen affinity was also investigated but, interestingly, showed no difference under the temperate conditions.

The authors report that there were clear effects due to genetic background and environmental acclimation on mitochondrial oxygen affinity. The higher oxygen affinity observed in the southern subspecies are probably an adaptation given that they are from a warmer climate and more likely to experience oxygen deprivation due to thermal stress and this might be a molecular target for thermal adaptation in other species.

[Tyrone Genade]

Preliminary Insight into Winter Native Fish Assemblages in Guadiana Estuary Salt Marshes Coping with Environmental Variability and Non-Indigenous Fish Introduction. Gonçalves R; Cruz J; Ben-Hamadou R; Teodósio MA; Correia AD; & Chícharo L. *Fishes*, 2:19, 2017. DOI http://dx.doi.org/10.3390/fishes2040019.

While the Mummichog may be facing some pressure in Delaware Bay (see previous review) in the Guadiana estuary of South-East Portugal it is the Mummichog applying the pressure. The authors surveyed an extensive fish fauna across the estuary and found that in certain parts environmental degradation is leading to extreme conditions in certain areas that favour just two species, a native goby and the non-indigenous Mummichog (between them comprising over 99% of catches). *F. heteroclitus* and many other species have been introduced by humans and though many have fared poorly the Mummichog, with its high adaptability and tolerance, appears to be on the edge of gaining a foothold. Various sizes of individual were found, from 1.6 mm up to 52 mm, suggesting local breeding. The estuarine fish fauna may therefore be at risk and the authors urge further monitoring of the area.

[Andy Patel]

Ryanodine receptor and FK506 binding protein 1 in the Atlantic killifish (*Fundulus heteroclitus*): A phylogenetic and population-based comparison. Holland EB; Goldstone JV; Pessah IN; Whitehead A; Reid NM; Karchner SI; Hahn ME; Nacci DE; Clark BW; & Stegeman JJ. *Aquatic Toxicology*, 192:105 – 115, 2017. DOI https://doi.org/10.1016/j.aquatox.2017.09.002.

Mummichogs once again prove their worth as a model species for research into the effects of pollution on physio-

logical pathways. This study looks at the effect of widely encountered non-dioxin like polychlorinated biphenyls (NDL PCBs) and their neurotoxic effects. By studying two populations of Mummichog, one tolerant and one intolerant the authors identify six genes that activate Ryanodine receptors (intracellular Ca^{2+} channels). They point to the relevance of their work in establishing a framework against which to compare other teleosts and mammals and their responses to NDL PCBs. Perhaps of some interest to killifish researchers but of greater interest to toxicolgists.

[Andy Patel]

Growth and movements of Mummichogs (*Fundulus heteroclitus*) along armored and vegetated estuarine shorelines. Crum KP; Balouskus RG; & Targett TE. *Estuaries and Coasts*, Epub ahead of print, 2017. DOI https://doi.org/10.1007/s12237-017-0299-x.

A study to determine the impact of urbanization on the widely distributed Mummichog in the Delaware Coastal Bay Area. The authors use tagging and recapture to measure growth rates and movement of individuals along 4 differing types of shoreline, two armored by humans to stabilise the shoreline and two naturally vegetated. The armored comprise bulkheads and riprap (stone aggregate) and the vegetated are marsh areas, populated by *Spartina alterniflora* and *Phragmites australis*. Mummichog show strong site fidelity in natural environments and this behavior is replicated in all 4 shoreline types with most specimens recaptured within 5 metres of their original tagging site. Only a few specimens overcame their natural stay-put tendency and traveled to different area types, mostly from armored to vegetated. Perhaps unsurprisingly the armoring shows a negative impact on Mummichog numbers when compared to marsh areas. However growth of the reduced number of individuals increased in armored areas, but not sufficiently to overcome the negative overall effect on productivity. So it appears that the decrease in population is not attributable to reduced prey, rather to unknown other factors. This leads the authors to warn against traditional armoring methods, which can adversely affect native fauna, and urge use of alternative methods for shoreline protection.

[Andy Patel]

Zinc chloride rapidly stimulates efflux transporters in renal proximal tubules of killifish (*Fundulus heteroclitus*). Zaremba A; Miller DS; & Fricker G. *Toxicology and Applied Pharmacology*, 334:88 – 99, 2017. DOI https://doi.org/10.1016/j.taap.2017.09.001.

The authors set out to characterize the effect of $ZnCl_2$ on the activity of Multidrug resistance-related protein 2 (Mrp2). Zinc is an essential nutrient that is needed to fight infection, modulate inflammation, male fertility and maintain nervous system function (cognition, sensory systems and mood). Mrp2 is involved in the excretion of metabolic toxins and xenobiotics (eg. antibiotics) across the cell membranes of the renal tubules cells into the urine but is also present on the cells of lung, gut, liver and blood-brain barrier. Zaremba et al chose to experiment on *Fundulus heteroclitus* as it is a well established model organism for studying the effect of chemical toxicants and pollutants.

Zaremba et al dissected renal tubule tissue from freshly killed fish and then exposed them to $ZnCl_2$ or $CdCl_2$ to determine their effect on ion flux across the tubule cell membranes. The movement of ions accross the membrane was measured using a florescent dye, Texas Red. $ZnCl_2$ increased flourescent labeling in the kidney tubule lumen about 5 times while $CdCl_2$ had only a small (but significant) effect. The effect of the $ZnCl_2$ increased with $ZnCl_2$ dose. This effect was not affected by stopping protein synthesis with cycloheximide but was stopped by inhibition of the endothelin receptor type B/nitric oxide synthase/protein kinase C signaling pathway. Inhibitors of phosphatidylinositol 3-kinase activity, glucocorticoid-inducible kinase 1 and mTOR (e.g. rapamycin)

also abolished the effect of the $ZnCl_2$. $ZnCl_2$ also stimulated P-glycoprotein and Breast cancer resistance protein.

From a biochemical perspective I find this a very interesting paper. Why would $ZnCl_2$ increase the activity of Mrp2 to pump Texas Red out of the cells into the lumen? Is there an optimum level of Zn^{2+} needed to faciliate proper kidney function, to pump out xenobiotics and other metabolic toxins? In very soft aquarium water, would our fish benefit from some zinc in the water? Are their diets sufficient in zinc for fish health and fertility? Fish need 15–40 mg/kg feed (Watanabe et al. 1997. Aquaculture 151:187–207) and most feeds have zinc in excess. For example, black soldier fly larvae have 108 mg zinc per kg (dry mass) and earthworms have 198 mg/kg while shrimp has only 15 mg/kg. High levels of copper, phosphorus, calcium and phytates inhibit zinc uptake by the gut (http://www.fao.org/docrep/field/003/ab470e/ab470e06.htm) which could mean problems for our softwater fish kept in hard water or water full of phytates. Experiments on rainbow trout showed that dietary supplementation with zinc enriched yeast did improve immune function (Gharekhani et al, 2015. Iran J. Vet. Res. 16:278–282). It might be possible to enrich *Artemia* but feeding baby grindal worms (which are Annelids like earthworms) might be an easier option. Sadly no data is available on zinc content of *Artemia* or grindal worms but there is evidence of the latter accumulating zinc in its tissues from the environment—as much as 458 mg/kg in worms from polluted flood plains in the Netherlands (Van Vliet et al. 2006. Euroean Journal of Soil Biology, 42:S117–S126) suggesting that grindal and white worms could be enriched by feeding them foods rich in zinc or adding a small amount of zinc chloride directly to their growth medium.

[Tyrone Genade]

Age validation and seasonal growth patterns of a subtropical marsh fish: The Gulf Killifish, *Fundulus grandis*. Vastano AR; Able KW; Jensen OP; López-Duarte PC; Martin CW; & Roberts BJ. *Environmental Biology of Fishes*, 100:1315–1327, 2017. DOI https://doi.org/10.1007/s10641-017-0645-7.

The authors investigated populations of *Fundulus grandis* in marsh areas of the northern Gulf of Mexico. Initially fish were captured and tagged. They were bathed in oxytetracycline to leave discernible marks on their otoliths and released. When subsequently recaptured, either in April (to determine winter growth rate) or September (to determine summer growth rate) the otoliths were removed and examined microscopically. Results supported the validity of using otoliths to measure ageing, as winter growth produced a more opaque area than summer. This allowed them to identify individuals as under 1 year, more than 1 year and more than 2 years and then refine the age estimates by measuring the actual amount of growth of the otoliths between first and second capture. The oldest fish was estimated at 2.25 years (± 0.25).

Growth of the otoliths was fastest in summer, as might be expected. Growth of the actual fish was also recorded (body length) and this actually showed significantly faster somatic growth in winter. This is in contrast to previous findings in *F. grandis*. The authors comment on the fact that studies of reproductive activity in Gulf Killifish show reductions as temperature rises (stopping at 30°C), which may correlate with growth. Recorded temperature at the trial areas ranged between 1.7°C and 34.7°C with the summer mean close to that 30° cutoff. As they point out this is in marked contrast to the Atlantic Killifish (*F. heteroclitus*), a predominantly temperate species whose growth increases with temperature. Despite their similarity *F.heteroclitus* also live longer (upto 4 years).

With age and growth rates now discernible for *F. grandis* the authors promote its potential as an indicator species in the marsh areas of the Gulf.

[Andy Patel]

Salinity and temperature effects on element incorporation of Gulf Killifish *Fundulus grandis* otoliths. Nelson TR; DeVries DR; & Wright RA. *Estuaries and Coasts*, Epub ahead of print, 2017. DOI https://doi.org/10.1007/s12237-017-0341-z.

The chemical analysis of otoliths for various assays rely on the ratio of strontium to calcium and barium to calcium. The authors set out to determine how salinity and temperature affect these two chemical ratios.

They collected *Fundulus grandis* from various locations in the northern Gulf of Mexico. The ratio of strontium to calcium increased with temperature and salinity. Conversely, the ratio of barium to calcium decreased with salinity but increased with temperature.

The authors caution that temperature variability can confound extrapolation of past salinity from otolith analysis and recommend using both the strontium to calcium and barium to calcium ratios for reconstructing fish environmental history.

[Tyrone Genade]

Preservation of behavior after fifteen years of isolation: comparisons of wild and captive endangered pupfish in their natural habitat. Black A; Snekser JL; & Itzkowitz M. *Environmental Biology of Fishes*, Epub ahead of print, 2017. DOI https://doi.org/10.1007/s10641-017-0662-6.

The Leon Springs pupfish, *Cyprinodon bovinus* having been extirpated in the wild has been subject to conservation measures involving maintaining a relatively large captive population. Between 1998 and 2001 captive fish were reintroduced in an effort to repopulate its native environment. Most quickly died out but a small number (just 1 breeding male amongst them) were assisted by habitat regeneration and persist as a small population. Noting that captive populations can undergo genetic and morphological changes that can render them incapable of adapting to reintroduction the authors decided to compare behaviours between the captive and 'wild' populations. As of 2013 each population had passed through 15 generations and indeed there were clearly discernible differences between them. They introduced captive fish to a separate pool from the wild fish and made observations to see if there were any actual behavioral differences that might have implications for future repopulation efforts. To mitigate against habitat differences they also dug out similar area in each locale so that they could observe directly comparable foraging, mating and territorial behaviors albeit with a limited number of fish.

They found that despite genetic divergence none of the key behaviors were materially affected. They note that this may be a positive sign for ongoing and future efforts to preserve extirpated species by captive maintenance.

[Andy Patel]

Posters & Conference Abstracts

***Nothobranchius furzeri*: A new model organism of alpha-synucleinopathy**. Genade T. *J Alzheimers Dis Parkinsonism*, 7:5 (Suppl), 2017. DOI https://doi.org/10.4172/2161-0460-C1-029. Presentation at the 3rd International Conference on Parkinson's disease and Movement Disorders, Sept 25-26, 2017, Chicago, USA.

Annual killifish, *Nothobranchius furzeri*, discloses Parkinson's disease phenotypes. Matsui H. *Journal of the Neurological Sciences*, 381:580 (Suppl), 2017. DOI https://doi.org/10.1016/j.jns.2017.08.1634. Presented at the World Congress of Neurology (WCN 2017), September 16–21, 2017, Kyoto, Japan.

In brief, both Prof Matsui and I have been exploring the feasibility of *N. furzeri* as a model of Parkinson's Disease. In 2013 I discovered that the protein α-synuclein accumulates in the brains of *N. furzeri* Gonarezhou with age. This is the pathological protein in Parkinson's and several other neurological Diseases. Prof Matsui has confirmed this observation as well as the loss of dopaminergic neurons in the motor system of *N. furzeri* (among several other discoveries). In my presentation I present histological evidence for α-synucleinopathies in the brain of the fish as well as evidence for the dysfunction of DJ-1, the protein coded by the PARK7 gene which is implicated in human familial Parkinson's Disease. It is a remarkable coincidence that we both presented our work at almost the same time. We had no idea what each other were doing.

[Tyrone Genade]

Theses & Dissertations

Integrating Physiology and Genomics to Identify Mechanisms of Adaptation in Killifish. Brennan RS. , *University of California, Davis*, 2017. URL https://search.proquest.com/openview/5fdef32b308743b3efea0272551b7809/1?pq-origsite=gscholar&cbl=18750&diss=y

Fundulus heteroclitus has diverged from marine to fresh water several times and has produced genetically distinct, locally adapted populations. The authors set out to understand the mechanisms enabling the evolution of this fish into diverse habitats. The author integrated physiological, transcriptomic and genomic mechanisms to identify mechanisms contributing to evolutionary divergence. The author identified gene loci that are under divergent selection.

[Tyrone Genade]

Effects of embryonic arsenic exposure on killifish (*Fundulus heteroclitus*) growth, feeding behavior, and intestinal morphology and cell types. Sims KC. Masters thesis, *Clemson University*, 2017. URL https://tigerprints.clemson.edu/all_theses/2741

A thesis that makes use of the popular model fish *Fundulus heteroclitus* to examine whether arsenic exposure in human fetal development is likely to cause long term harm and determine ways that might occur. Mummichog are chosen for their prolific egg production (increasing number of test results) their similar reaction to arsenic exposure and of course their much shorter life cycle. A clear and logical literature review is presented establishing the basis for the study and reasoning for the subsequent experimental investigations.

Experimentation involved exposing embryos to different arsenic concentrations and then examining the persistence of three effects; alteration of feeding behaviour, disruption of nutrient uptake, reduction in insulin dependent growth factor (IGF). While all three are affected in the short term, it is

the latter that appears to have the most detrimental long-term effect in that it probably reduces condition and growth during the (pivotal) juvenile stage.

Of some interest to killifish enthusiasts the thesis is predominantly focused on examining the potential dangers to humans of low level arsenic exposure and concludes that current recommended levels may need to be lowered. Nevertheless the subject is fascinating and for those with an interest in the effects of low level exposure to toxic substances (regardless of animal group) a recommended read

[Andy Patel]

Ed's note: Arsenic is a common contaminant of ground water. The author reported affects from 10 μg/L. Aquarists drawing water from wells for their fish should keep this in mind. See https://water.usgs.gov/nawqa/trace/arsenic/ for USA data on arsenic concentrations in ground water. Similar results exist for the UK: http://www.bgs.ac.uk/research/highlights/2013/arsenicSW.html. If you are using well water you should have it tested.

[Tyrone Genade]

Multigeneration effects of chronic exposure to polycyclic aromatic hydrocarbons in Gulf Killifish (*Fundulus grandis*). Yammine A. Masters, *Louisiana State University at Baton Rouge*, 2017. URL https://digitalcommons.lsu.edu/gradschool_theses/4327/

The author set out to examine potential adaptation within two generation of gulf killifish exposure to polycyclic aromatic hydrocarbons. They also tried to determine whether mucus could be used to determine levels of 11-ketotestosterone and vitellogenin levels in gulf killifish.

Both mucus 11-ketotestosterone and vitellogenin levels correlated positively with blood levels of these two substances; and both were responsive to exposure to pollutants. Exposure to polycyclic aromatic hydrocarbons causes a drop in sperm motility and oocyte cell wall thickness. The author was able to show changes in polycyclic aromatic hydrocarbons metabolism and immunology within two generations.

This is a very rapid adaption to environmental changes! It seems that *Fundulus heteroclitus* isn't the only member of this genus that is able to rapidly evolve to adapt to cope with negative environmental changes. But perhaps this isn't unexpected. Molecular phylogenies place *F. grandis* as sister species to *F. heteroclitus*.

[Tyrone Genade]

The annual killifish *Nothobranchius furzeri* as a new model in long-term ecotoxicological research. Philippe C. , *Katholieke Universiteit Leuven*, 2017. URL https://lirias.kuleuven.be/handle/123456789/599381?mode=full&submit_simple=Show+full+item+record

This thesis isn't available for reading. The abstract indicates that much of it is based on Philippe et al's previous paper on the use of *Nothobranchius* ecotoicology (Philippeet al, Ecotoxicology and Environmental Safety, 144:26–35, 2017. DOI 10.1016/j.ecoenv.2017.05.047). The abstract indicates that additional experiments were done testing the interaction between toxicant and temperature as well as the effect of sublethal concentrations of chlorpyrifos. It would appear that several interesting papers are still to come from this research.

[Tyrone Genade]

Interesting research on other fish

Understanding the effects of temperature on sex ratio in a sexually dimorphic fish species. Chance DL. Senior thesis, *Universty of California, Santa Cruz*, 2017. URL https://norriscenter.ucsc.edu/natural-history-resources/student-created-resources/index.html

Chance explores the connection between ambient temperature and sex ratios. It is theorized that an increase in ambient temperature would result in more males to females because the increased temperature exacerbates the metabolic costs to females, which need to invest more energy into the production of eggs, over males.

Chance surveyed 20 geothermal sites in California and New Zealand. The author controlled for the fish's temperature preference in open habitats where a temperature gradient was present. The study showed that across the sites studied the ratio of males to females increased with an increase in temperature. Interestingly, within open systems the sex ratio is closer to 1:1 than in closed sites and more females were found in the warmer regions of the habitat. The author speculates that the gravid females favor the warmer sites in such open environments. The observation of spatial variation in sex ratio has consequences for future studies of how temperature effects sex ratios in the environment.

No controlled studies were conducted in aquaria. It would be interesting to know whether the effect is preserved under captive conditions, and whether all populations respond the same way to increased or decreased temperature.

[Tyrone Genade]

Mating and aggressive behaviour of *Brachyrhaphis olomina* (Cyprinodontiformes: Poeciliidae). Garita-Alvarado CA; Naranjo-Elizondo B; & Barrantes G. *Journal of Ethology*, Epub ahead of print, 2017. ISSN 1439-5444, DOI https://doi.org/10.1007/s10164-017-0523-y

An interesting paper, with video links embedded, looking at the degree of mating behaviour vs aggressive behaviour between males and females of a Costa Rican species. The authors provide commentary on the current state of research into mating and aggressive behaviours in livebearers and identify some previously undocumented behaviours in *B. olomina*. They also note that behaviours can vary widely between species and draw attention to the need to study multiple species in order to elucidate how such behaviours have developed. Their study does not include male-male interactions but in male-female interactions they do see evidence that the males can detect female breeding condition and from some distance. No female behaviour was noticed that might specifically account for this though they note the short trial duration may not have allowed time for the females to demonstrate it. Well referenced and raising many questions on male-female interactions that require further study.

[Andy Patel]

The protected areas system in Brazil as a baseline condition for wetlands management and fish conservancy: the example of the Pantanal National Park. Polaz CNM; Ferreira FC; & Jũnior MP. *Neotropical Ichthyology*, Epub ahead of print, 2017. DOI http://dx.doi.org/10.1590/1982-0224-20170041.

This paper is a report on the Pantanal National Park and how it is being used as a baseline by which to measure biotic integrity in other systems. The authors focused on the fish fauna of the park. The authors studied four categories of aquatic habitat: channels in the flood plains, permanent bays, temporary bays and rivers. 146 fish species were identified and fish sampling occurred during the dry season. There was no relationship between the habitat category and fish diversity. Only one killifish was identified: *Melanorivulus punctatus*[6].

[6] KDI regards *Melanorivulus* as a subgenus of *Rivulus*.

[Tyrone Genade]

Are fancy guppies (Poecilia reticulata Peters, 1859) invasive? Bandaranayake C & Chandrasekara W. *Sri Lanka Journal of Aquatic Sciences*, Epub ahead of print, 2017. DOI http://doi.org/10.4038/sljas.v22i2.7536.

The simple answer is no. Fancy guppies can't compete, at least not against the wild type forms they were compared to in experiments. The paper doesn't gage the ability of the fancy guppies to compete with native species. One thing not considered is that fancy strains spontaneously regress to the wild type form.

[Tyrone Genade]

Forebrain activation during social exposure in wild-type guppies. Cabrera-Álvarez MJ; Swaney WT; & Reader SM. *Physiology & Behavior*, 182:107–113, 2017. DOI https://doi.org/10.1016/j.physbeh.2017.10.012.

The authors undertake two experiments with wild type Trinidadian guppies to determine whether social interaction can be detected by changes in immediate early gene expression within four areas of the brain. By exposing individual guppies to the stimulus of no (zero), small (two) and large (ten) shoals and also by studying preferences for individuals with no, small and large shoals. Using gene assay techniques they looked for activity within regions of the brain known (or analagous to those known) to contribute to the social decision making network in other vertebrates and fish species such as *Fundulus diaphanus*; the pre-optic area, the dorsal section of the ventral telencephalon, the ventral section of the same telencephalon, the supracommissural part of the ventral pallium. Their findings confirmed previous studies in which guppies showed marked preferences for social interaction and a preference for larger groups. However the assay techniques only revealed changes within the pre-optic area. They speculate on potential reasons as to why this might be and argue for more research into the brain biology of the important model species—the guppy.

[Andy Patel]

Photo by Hristo Hristov©

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