# THE MYSTERY OF THE BANDED KILLIFISH FUNDULUS DIAPHANUS POPULATION EXPLOSION: WHERE DID THEY ALL COME FROM?



# Philip W. Willink, Jeremy S. Tiemann, Joshua L. Sherwood,

La Grange Park, IL

Illinois Natural History Survey

Illinois Natural History Survey

## Eric R. Larson, Abe Otten, Brian Zimmerman

University of Illinois at Urbana-Champaign

Scott Community College The Ohio State University Stream and River Ecology Lab

Banded Killifish Fundulus diaphanus are no strangers to NAN-FAers. Over the past several years, there have been multiple articles in American Currents covering their distribution (Hatch 2015; Schmidt 2016a, 2018; Olson and Schmidt 2018; Li 2019), stocking to restore populations (Bland 2013; Schmidt 2014), and appearance in a hatchery (Schmidt 2016b). Their range extends from the Canadian Maritime provinces south along the Atlantic coast to the Carolinas, as well as westward through the Great Lakes region to the upper Mississippi watershed. Taxonomically they are split into an Eastern subspecies F. d. diaphanus and a Western subspecies F. d. menona. These subspecies are known to hybridize, and this has been studied from Lake Erie down through the St. Lawrence. The species has been known to hybridize with Mummichog F. heteroclitus along the Atlantic coast. There is also an interesting offshoot known as the Waccamaw Killifish F. waccamensis that is basically a slimmer version of the Eastern Banded Killifish that is only found in a few lakes in North Carolina (Page and Burr 2011; Stuber and Scanlan 2016).

Throughout much of their range, the status of both subspecies is considered secure. But there are some exceptions, such as the Western subspecies in Illinois and Ohio where they are listed as Threatened and Endangered, respectively. The reasons for their listings are because of their restricted ranges in these states and low population numbers. In Illinois, except for a couple localities in the center of the state where they were sampled in the late 1800s

After Philip Willink received his PhD from the University of Michigan, he worked at the Field Museum and then the Shedd Aquarium (both in Chicago). He now serves on several state, municipal, and non-governmental organizational boards. He has conducted fieldwork in a dozen countries around the world, described several new species of fishes, and appeared in numerous documentaries and media articles. Phil is now searching the depths of Lake Michigan for meteorites with the Adler Planetarium in Chicago.

and have not been seen since, they were only known from a handful of inland lakes in the far northeastern corner the state (Fig. 1). Even there, population numbers were low.

So it was with great excitement that in the early 2000s Illinois ichthyologists started to find more and more presumed Western Banded Killifish in Lake Michigan (Willink et al. 2018). They were even showing up in downtown Chicago (Willink 2011). It was hoped that this range expansion was evidence of an uncommon species making a comeback due to improvements in the environment. Perhaps it was time for a celebration!

Then the population spilled into the Chicago Area Waterway System, a name given to the Chicago River, Calumet River, and interconnecting canals. From there they eventually entered the Illinois River and spread like wildfire downstream (Fig. 1).

Meanwhile, Banded Killifish suddenly appeared in a Missis-sippi tributary near Davenport, Iowa, in 2009 (personal communication from NANFA member John Olson (retired), Ben Hucka, and Jerad Stricker, Iowa Department of Natural Resources). From there they moved up the nearby Rock River in Illinois and down the Mississippi between Missouri and Illinois as far as St. Louis. Prior to this, there were no historical records for Banded Killifish in this stretch of the Mississippi River (personal observation from NANFA member Abe Otten; personal communication from NANFA member Bob Hrabik; Rivera et al. 2013; Tiemann et al. 2015; Hrabik 2016; Lamer et al. 2016). A

Jeremy Tiemann has been a Field Biologist/Aquatic Zoologist specializing in stream ecology with the Illinois Natural History Survey since 2002. He grew up on a farm in northeastern Kansas and developed a keen interest in aquatic biology while traipsing through streams as a kid. This passion led to a BS in Biology from the University of Kansas in 1998 and a MS in Biological Sciences from Emporia State University in 2002. Professional research interests include stream ecology and life history of non-game fishes and freshwater mollusks.

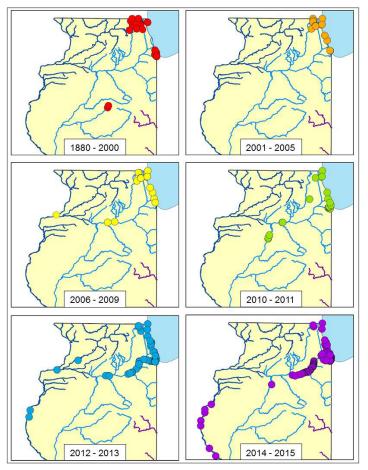


Figure 1. Illinois distribution of Banded Killifish divided into six time periods, starting in the late 1800s. (Map prepared by S. Hertel in Willink 2017)

similar expansion has been ongoing for many years in the upper Ohio River from around the Ohio/Pennsylvania line down to near the Indiana/Ohio line. Additional expansion appears to be happening in the waters of Lake Erie as well.

At this time, NANFA member Philip Willink received a State Wildlife Grant to assess the status of a dozen fishes across northern Illinois that were being considered for listing as Threatened (Willink 2017). One of those dozen fishes was the Banded Killifish. The situation also caught the attention of Tristan Widloe (Illinois Department of Natural Resources), who was part of the group monitoring Asian Carp but who were also catching lots of Banded Killifish during their surveys. NANFA member Josh Sherwood (Illinois Natural History Survey) also heard about the exploding population numbers, and considered it a potential dissertation topic.

By this point it was clear to a number of regional biologists that something was going on that was out of the ordinary. What ensued were a couple of very long and convoluted email chains involving fish experts, including several NANFA members, from Minnesota, Iowa, Missouri, Wisconsin, Illinois, Indiana, and Ohio. There was no shortage of opinions, but the only data were a few pictures of killifish taken in the field.

From there the project snowballed, incorporating NANFA member Jeremy Tiemann (Illinois Natural History Survey), Vic Santucci and Dan Makauskas (both of the Illinois Depart-

ment of Natural Resources Lake Michigan Program), Samantha Hertel (Loyola University, graduate student), and James Lamer (Western Illinois University, working extensively on the Mississippi River). Together, our group was able to generate the data to document the changing distribution of Banded Killifish across northern Illinois that has been described above (Fig. 1; Willink et al. 2018).

The dramatic and relatively rapid change in distributions is a compelling story but does not explain why it is happening. One of the suggestions from the email chains was the possibility that Eastern Banded Killifish may be involved. Historically, it was thought that states west of Ohio had only Western Banded Killifish, but perhaps something had changed. To figure this out, every preserved Banded Killifish in the collections of The Field Museum, Chicago, and Illinois Natural History Survey Fish Collection, Champaign, were examined. The specimens represented populations across almost the entire range of the species, and included individuals collected in the late 1800s to the present.

The first step was to figure out how to anatomically differentiate the Western subspecies from the Eastern subspecies. A number of characters have been proposed over the years, and these were explored. One character is pigmentation patterns, including the number of bars along the side and dark patches on the fins. Although there is something to this suite of characters, they were also problematic at times. Killifish are sexually dimorphic, so the males do not look exactly like females. Breeding condition (versus nonbreeding) is important. Also, pigmentation patterns appear to change during development. Add in the fact that some of the specimens from the 1800s were faded and pigment patterns were determined to be difficult to use.

The subspecies have also been documented to have differing numbers of dorsal and anal fin rays. This does appear to be the case, but the ranges of fin-ray values overlap significantly and only differ by a ray or two. So, although different populations could have different average values for these characters, if you only had one individual it would be very difficult to identify the subspecies.

Another potential character was lateral row scale count. It should be pointed out that it is *lateral row scale count* and not *lateral line scale count*. That is because Banded Killifish do not have a standard lateral line recognized by a distinct row of scales with pores. Instead, one has to count scales along a row where one would think a lateral line should be. This count is made a little more difficult because some individuals have irregular scales and hence do not always have distinct rows to follow. With practice and by developing a standardized method, it is possible to overcome these difficulties. But do not underestimate the importance of this character, because scale counts that differ by only one or two could make a significant difference in identification.

Having settled on lateral row scale counts as the character, we then needed to figure out what a Western Banded Killifish was and what an Eastern Banded Killifish was. This was made slightly more difficult because Easterns are known to hybridize with Mummichogs along the Atlantic Coast. So, these individuals had to be excluded from the analysis. But finally, after analyzing all the data from across the entire range of the species and comparing it to reported values in the literature, we came up with counts to differentiate the two subspecies.



Figure 2. Male and female Western Banded Killifish *Fundulus diaphanus menona*. (Photo by Philip Willink)

Based on lateral row scale counts, all Banded Killifish collected in Illinois prior to the year 2000 were Westerns (Fig. 2). This is what everyone previously thought. In parts of Lake Michigan, the Chicago Area Waterways System, and Illinois River that were colonized after 2000, the individuals had lateral row scale counts completely intermediate between Westerns and Easterns. This is a classic hybrid pattern that ichthyologists have been familiar with for decades (e.g., Hubbs 1955). Meanwhile, in the Mississippi, the majority of individuals were identified as Westerns, although one was identified as a hybrid. So, what we observed were hybrids of Western Banded Killifish and Eastern Banded Killifish (Fig. 3) that somehow invaded several new watersheds and expanded their range westward by several hundred miles!

Of course, this is the 21st Century, and counting scales is not really in vogue anymore. To validate the initial findings, we have begun examining the Banded Killifish genetics by collaborating with Eric Larson and Kurt Ash (both of the University of Illinois at Urbana-Champaign). Preliminary genetic results are congruent with morphology. Furthermore, we are increasing the scope of the project to encompass much of the Midwest. Thanks to NANFA member Brian Zimmerman, Brant Fisher (Indiana Department of Natural Resources), Jacob Adams (Indiana Department of Natural

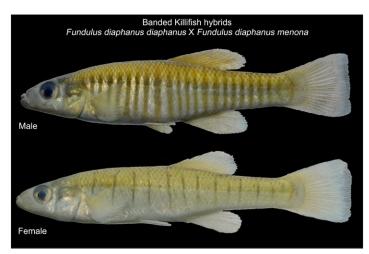


Figure 3. Male and female hybrids between Western Banded Killifish Fundulus diaphanus menona and Eastern Banded Killifish Fundulus diaphanus. (Photo by Philip Willink)

Resources), and Daragh Deegan (City of Elkhart, IN), we now have genetic samples from across Ohio, Indiana, and Illinois. We also have some additional material from Minnesota (thanks to NANFA members Konrad Schmidt and Mark Hove), Maryland (NANFA member Matt Ashton), Michigan (Lake St. Clair, Andrew Briggs), and Wisconsin (Brandon Harris and Bradley Smith).

Besides simply identifying individuals, another goal of the genetic project is to try and determine the pathway by which the invasion took place. If this can be figured out, then perhaps it will be possible to hinder future invasions. Another outcome of the analysis is conservation genetics. We are now in a position to recognize native populations of Western Banded Killifish, and various natural resource managers can use this information to protect the natural heritage of this species within their respective jurisdictions.

The project is already producing results. For example, in Illinois, Banded Killifish is listed as Threatened. But traditionally Illinois only recognized the taxonomic rank of species in its listing designations. As a consequence, both the native Westerns and invasive hybrids are currently protected. Banded Killifish as a species are now so abundant in Illinois that some believe they should be delisted. But much of this abundance is due to the invasive subspecies, and delisting would remove protection from the native Western subspecies. This conundrum forced the state to rethink its approach to listing species and subspecies as Threatened or Endangered, and it has recently decided to consider listing subspecies. Ohio, however, has recognized subspecies for some time now, and has already listed the Western Banded Killifish as Endangered and the Eastern Banded Killifish as an Injurious Aquatic Invasive Species.

The project is very much ongoing. We are now looking to increase our sample sizes from Michigan and Wisconsin. We have permits in hand and are now trying to secure funding and identify sampling locations. Any suggestions for these states would be greatly appreciated. We have already received feedback from NANFA member John Lyons (University of Wisconsin) about the status of Banded Killifish near Madison, which happens to be the type locality for the Western subspecies. But the northern and northwestern parts of Wisconsin are relatively unknown to us at this time. Also, our only sample from Michigan is from Lake St. Clair, so there is still much work to do there. We could even use more material of the Eastern Banded Killifish from its "native" range as well as the Waccamaw Killifish for comparative purposes.

This project started off as a simple attempt to map the distribution of Banded Killifish in Illinois and to describe how that distribution changed over time. It has exploded into a program that covers the Midwest with ramifications impacting taxonomy, species concepts, conservation, management, and legislation. And the scope continues to grow. We do not know how far these hybrids will spread. And who knows what type of interactions they may have with the recently introduced population of Mummichogs in Michigan (Muller 2014). Never underestimate the impact of seemingly inconsequential small brown fishes!

In case anyone is keeping track, at least eleven NANFA members have participated in this project to date, and we anticipate that number to grow. And eleven *American Current* articles published in the past few years were cited above. Stay tuned for future developments!

### **ADDENDUM**

NANFA members have continued to contribute specimens to this research. Konrad Schmidt and Jenny Kruckenberg collected Banded Killifish from six localities in Minnesota's Hudson Bay and Mississippi drainages. Bob Hrabik (Missouri) donated fin clips he took from Iowa's Lake West Okoboji (Missouri River drainage) specimens in 2011. However, there was concern whether theses samples were still usable so John Olson joined Mike Hawkins (Iowa Department of Natural Resources) for the annual survey of West Okoboji. Unfortunately, no Banded Killifish were found this year. Purely happenstance, Mike stumbled on to another population, and of all places, in a drainage ditch, and he sent specimens to Jeremy. Konrad mentioned the project to Doug Watkinson, who is a biologist with Fisheries and Oceans Canada. He has been very busy with research of his own, but had a free day. Despite his confidence in success being low, he and Derek Kroeker collected specimens from another locality in the Hudson Bay drainage. Doug added, "This increased the known collection of Banded Killifish in Manitoba from eight to more than 60 specimens."

Research funds for non-game fish projects are almost non-existent. It is admirable this NANFA network gladly heard the calling and took advantage of a very rare opportunity. Of course, the contributors also expressed interest in learning how these populations differ from one another across the species' range and within their respective state or province, and if possible, learning whether their populations of Banded Killifish are indigenous.

### Literature Cited

Bland, J. 2013. How do you spell success? The rare fish variety, that is. American Currents 38 (4):11–22.

Hatch, J. 2015. Minnesota fishes: just how many species are there anyway? American Currents 40 (2):10–21.

Hrabik, R.A. 2016. Fish species found along Missouri's border new to state. Missouri Conservationist 77(3):7.

Hubbs, C.L. 1955. Hybridization between fish species in nature. Systematic Zoology 4:1–20.

Lamer, J., E. Ratcliff, and K. Rivera. 2016. Banded Killifish in the Upper Mississippi River. The Upper Mississippi River Conservation Committee Newsletter 2016(summer):2.

Li, B. 2019. Exploration: a high school student's summer project in the Pine Barrens. American Currents 44 (2):10–14.

Muller, B. 2014. Mummichogs establish beachhead in Michigan. American Currents 39 (1):2–4.

Olson, J., and K. Schmidt. 2018. Iowa oxbow survey. American Currents 43 (4):1.

Page, L.M., and B.M. Burr. 2011. Peterson field guide to freshwater fishes of North America north of Mexico. Houghton Mifflin Harcourt, Boston. 663 pages.

Rivera, K.D., R.L. Haun, C.A. Anderson, and S.P. Romano. 2013. New distribution records for *Fundulus diaphanous* [sic] (LeSueur), family Fundulidae in Illinois. Transactions of the Illinois State Academy of Science 106:57.

Schmidt, K. 2014. Noah's fish ark. Stocking sensitive fishes in Twin Cities area lakes. American Currents 39 (1):8–12.

Schmidt, K. 2016a. In search of Minnesota's crossover connections. American Currents 41 (4):23–32.

Schmidt, K. 2016b. Iowa's Longear Sunfish mystery solved...maybe – Addendum. American Currents 41 (3):17–18.

Schmidt, K. 2018. In search of Minnesota's missing fishes. American Currents 43 (3):15–31.

Stuber, D., and J. Scanlan. 2016. Some observations on the Waccamaw Killifish (*Fundulus waccamensis*) with notes on spawning in captivity. American Currents 41(1):19–22, 29.

Tiemann, J.S., C.A. Taylor, D. Wylie, J. Lamer, P.W. Willink, F.M. Veraldi, S.M. Pescitelli, B. Lubinski, T. Thomas, R. Sauer, and B. Cantrell. 2015. Range expansions and new drainage records for select Illinois fishes. Transactions of the Illinois State Academy of Science 108:47–52.

Willink, P. 2011. Chicago lakefront fishes. American Currents 36(2):13, 15–18.

Willink, P.W. 2017. Assessing the status of potential Illinois endangered and threatened fish species. Report for United States Fish and Wildlife Service and Illinois Department of Natural Resources. 121 pp.

Willink, P.W., T.A. Widloe, V.J. Santucci Jr., D. Makauskas, J.S. Tiemann, S.D. Hertel, J.T. Lamer, and J.L. Sherwood. 2018. Rapid expansion of Banded Killifish *Fundulus diaphanus* across northern Illinois: dramatic recovery or invasive species? American Midland Naturalist 179:179–190.

