THE MYSTERY OF THE BANDED KILLIFISH *FUNDULUS DIAPHANUS* IN THE MIDWEST, PART DEUX

Jeremy S. Tiemann, Jordan Hartman,

Illinois Natural History Survey University of Illinois

Eric R. Larson, Philip W. Willink

University of Illinois La Grange Park, IL

The recent inexplicable explosion of Banded Killifish *Fundulus diaphanus* populations across the Midwest was nicely summarized by Willink et al. (2019). To quickly recap these findings, the Banded Killifish was always considered imperiled in Illinois and found only in the kettle lake region of the five northeastern-most counties in the state, but it underwent a HUGE population expansion from 2000–2015 (Smith 1979; Rivera et al. 2013; Tiemann et al. 2015; Willink et al. 2018; 2019; Metzke et al. 2022). Its range in Illinois grew so quickly that the Illinois Endangered Species Protection Board (IESPB) considered de-listing the species. However, biologists from across the state sat down, looked at the data (and voucher specimens) and realized something was amiss (Willink et al. 2018, 2019). They recognized that this expansion was not a natural phenomenon of an imperiled species but was instead an invasion of a non-native taxon that was quickly colonizing new areas like so many invasive species do. The biologists then petitioned to the IESPB to recognize both subspecies and preserve the state-threatened status of the native Western Banded

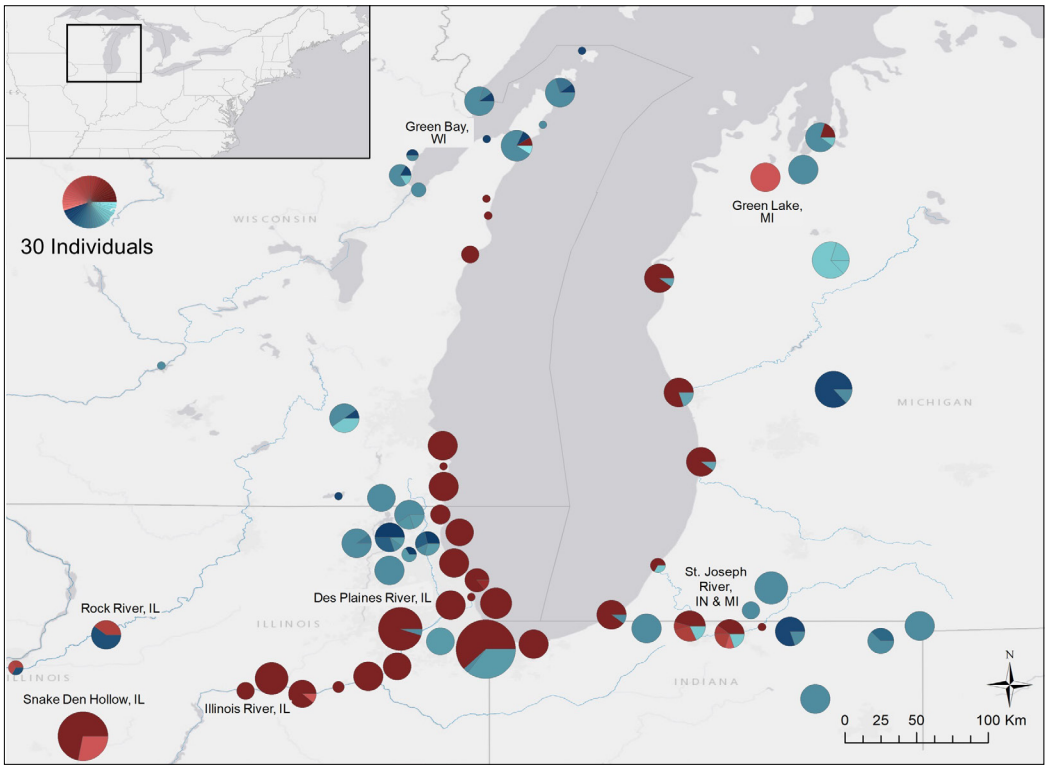

Figure 1. The distribution of mitochondrial DNA haplotypes for Western Banded Killifish (blue), Eastern Banded Killifish (red), and sites where individuals of both subspecies were found (both blue and red) across populations within our study (taken from Hartman et al. 2023).

Photos by the authors.

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.

Jordan Hartman is a PhD Candidate at the University of Illinois, Urbana-Champaign studying aquatic invasive species with a particular interest in genomics and landscape ecology. She grew up dreaming of being a marine biologist. Being from the Midwest, her appreciation of freshwater systems grew during her BS in Fisheries and Wildlife (University of Missouri, Columbia) and MS in Biology (Tennessee Technological University). She loves using genomic techniques to study freshwater fishes and mussels and hopes to incorporate landscape genomic research.

Figure 2. Green Lake, near Grawn, MI, where Western Banded Killifish were collected in 2020.

Figure 4. A backwater bay of Lake Michigan near Traverse City, MI, where Eastern Banded Killifish were collected in 2020.

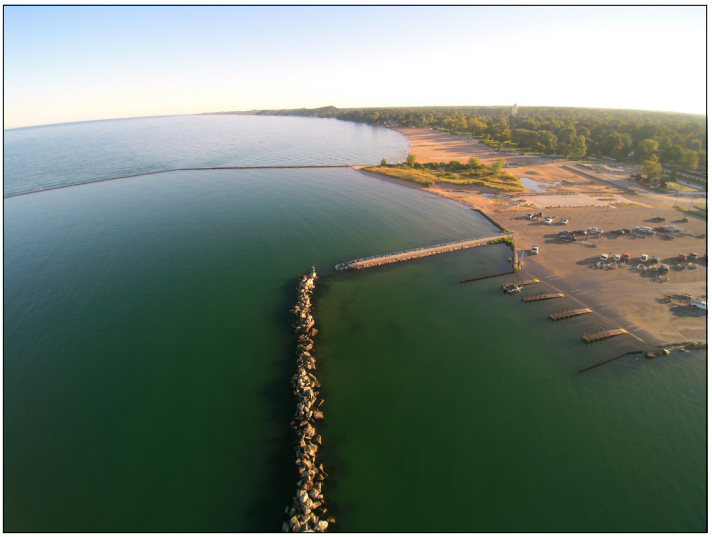

Figure 3. Lake Michigan, Ludington, MI, where Eastern Banded Killifish were collected in 2020.

Figure 5. A backwater bay of Lake Michigan near Racine, WI, where Eastern Banded Killifish were collected in 2020.

Killifish *F. d. menona* and recognize the non-native Eastern Banded Killifish *F. d. diaphanus*.

Phase 2 of our project was to determine which subspecies is where and how this invasion might have happened. We brought on new colleagues, including University of Illinois PhD student Jordan Hartman, and incorporated additional conservation techniques (e.g., genetics) to help us answer these questions. Like Phase 1, we reached out to several NANFA members to help us obtain specimens to build our dataset. We acquired 720 banded killifish from 104 sampling sites across nine states in the US and one province in Canada. These individuals were collected between 2004 and 2021 from our focal region in Illinois and the Lake Michigan drainage (Figure 1), localities elsewhere in the native range of both the Western and Eastern subspecies, and from populations of non-native Eastern Banded Killifish in the Ohio River watershed. The fish were collected using standard fisheries techniques, such as seining, electrofishing, trapping via mini-fyke nets, and

Eric Larson has been a professor at the University of Illinois' Natural Resources & Environmental Sciences since 2015. His main research interests are freshwater ecology and conservation science, with an emphasis in environmental DNA and invasive species. He received his BS in Fishery Resources at the University of Idaho in 2004, his MS in Biology at the University of Arkansas in 2007, and his PhD in Aquatic and Fishery Sciences at the University of Washington in 2011.

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.

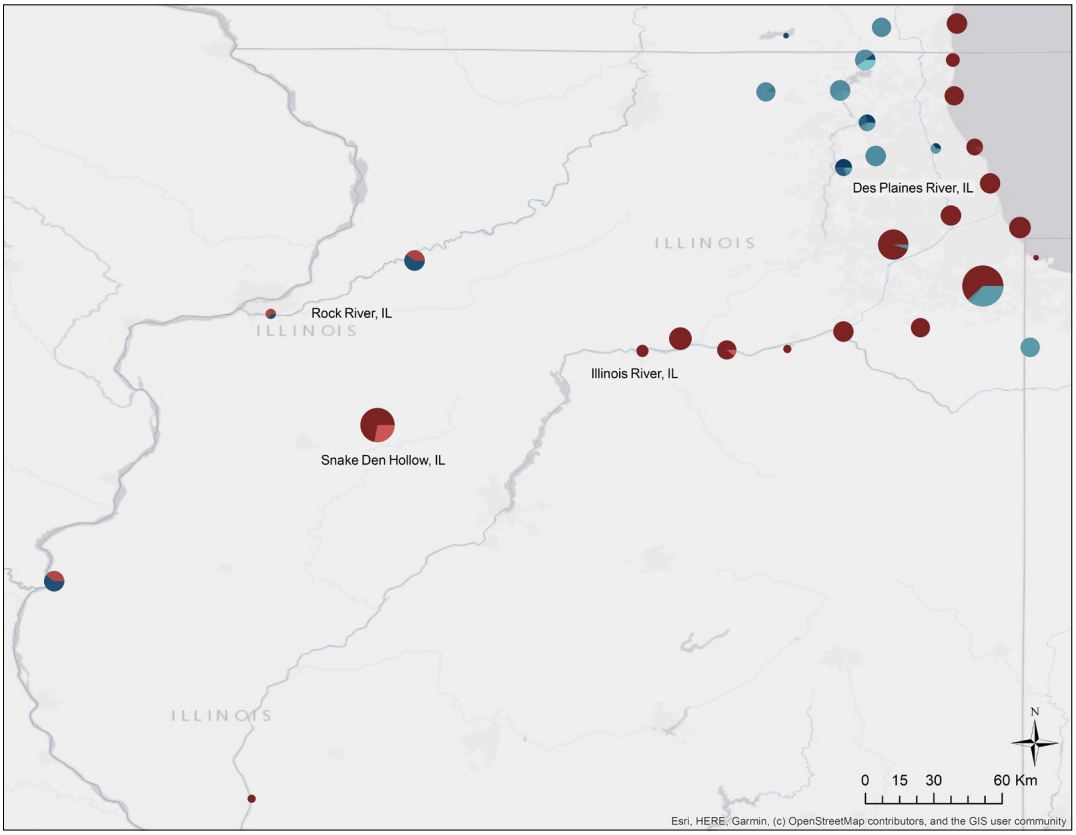

Figure 6. The distribution of mitochondrial DNA haplotypes for Western Banded Killifish (blue), Eastern Banded Killifish (red), and sites where individuals of both subspecies were found (both blue and red) across populations in Illinois.

dip-netting. Specimens were preserved in 95% ethanol and deposited in the Illinois Natural History Survey Fish Collection, Champaign.

This is where Jordan and I (Jeremy) must stop and step aside from our co-authors. We took a collecting trip up and around the Lake Michigan drainage during the summer of 2020, during COVID. Simply put, the trip was *EPIC* (Figures 2–5). We collected along the shoreline of Lake Michigan and in various kettle lakes throughout the basin. In 25+ years of collecting fishes professionally, this is one of my (Jeremy) favorite trips because I collected with an aspiring PhD student in one of America's most beautiful areas. At one site we saw Mother Nature at her most cruel when a Bald Eagle plucked a loon chick off the surface of a kettle lake while the mother loon protected the rest of her brood, and yet at another site we were able to showcase our native freshwater fishes by having a small group of kids help us sort through our seine.

This trip was the first sampling trip of my (Jordan) career that I was in charge of organizing and planning where we would sample. Up until the summer of 2020 during my PhD, I had spent most of my time in the genetics laboratory extracting and sequencing Banded Killifish DNA. I based our sampling locations on lakes where Banded Killifish had previously been caught and areas on Lake Michigan that seemed to have easy access and looked like good killifish habitat. During the beginning of our trip, I remember feeling incredibly defeated after the first few kettle lakes did not have any Banded Killifish, but I also remember how great it felt when we eventually found Western Banded Killifish! Overall, the trip turned out to be amazing. We collected Banded Killifish from numerous sites and it was an incredible experience for me to get to work with such a seasoned and supportive biologist.

AND NOW, THE REST OF THE STORY…

After we had the specimens in hand, Jordan extracted tissue samples and conducted a genetic analysis. If you are a scientist/geneticist, all the methods are outlined in Hartman et al. (2023). For the rest of us, simply put, Jordan led the way using cutting edge genetic methods, and the results supported what we thought: the genetic data showed that the kettle lakes are comprised of the Western Band Killifish and this influx of new locations (e.g., Lake Michigan and the Illinois River) is due to the non-native Eastern Banded Killifish colonizing new areas (Figure 1). As natural resource scientists with precious limited resources, we know now where to focus our conservation efforts (that is the kettle lakes), and we know what is artificial/non-native (e.g., those populations in Lake Michigan) when attempting to conserve the Banded Killifish. Jordan's next two chapters of her PhD dissertation will 1) use yet additional analyses to dive deeper into the genetics and explore the origin of the Easterns that invaded Lake Michigan and whether the two subspecies are hybridizing, and 2) use stable isotopes and diet metabarcoding to determine what, if any, ecological overlap there might be in how these two distinct subspecies feed. For example, does one feed at the surface while the other is more opportunistic, or are they competing for food resources? An interesting caveat to all of this comes from the observation of Smith and Harris (2020) that Eastern Banded Killifish aren't always *top*minnows, as they can go undetected by swimming along the lakebed of the Laurentian Great Lakes. Regardless, there will more to the story in future years.

From the Illinois perspective, the Banded Killifish in Lake Michigan, through the Chicago Area Waterway System (CAWS), and down the Illinois River are the non-native subspecies (Figure 6). They look different as they are more vibrant (and bigger) than what we'd find in the kettle lakes (Figure 7). For our counterparts elsewhere in the basin, let's talk. If it doesn't seem right, it might not be right. We feel the Eastern Banded Killifish could be elsewhere in the Great Lakes basin. We are here to help identify what you might have as times are weird with climate change, budget cuts, and, let's be honest,

Figure 7. An Eastern Banded Killifish collected from the North Branch Chicago River, Chicago, IL, in 2018 (top), and a Western Banded Killifish collected from Green Lake near Grawn, MI, in 2020 (bottom).

"dinky fishes" aren't in the limelight of most natural resource agencies. Regardless, please recognize that citizen scientists—like NANFA and other hobbyists—can help natural resource agencies advance the conservation of our precious natural resource, especially when documenting under-sampled species (e.g., Tiemann et al 2014; Jones et al. 2022; Lyons and Schmidt 2022). They are an extra set of boots on the ground (or in the water) collecting data and making observations. Let's work together and help conserve our fishes for the next generation.

ACKNOWLEDGEMENTS

We could not have done this research without the financial support provided by the International Association of Great Lakes Research Norman S. Baldwin Fishery Science Scholarship, the Illinois Department of Natural Resources Division of Natural Heritage (#375 IDNR 00501571) and US Department of Agriculture Hatch project ILLU-875-976, with additional support provided by the Illinois Department of Transportation and the Illinois State Toll Highway Authority. We are forever indebted to the following colleagues: D. Deegan – City of Elkhart, Indiana; K. Swagel – Field Museum; D. Kroeker, D. Watkinson – Fisheries and Oceans Canada; S. Silic, J. Phillips, and M. Feldman – Forest Preserve District of Cook County; J. DeBoer, A. Porecca, J. Sherwood, L. Solomon, A. Sites – Illinois Natural History Survey (INHS); R. Hilsabeck, D. Makauskas, S. Robillard, V. Santucci, T. Widloe, Z. Zuckerman – Illinois Department of Natural Resources; J. Adams, B. Fisher – Indiana Department of Natural Resources; M. Hawkins, J. Olson – Iowa Department of Natural Resources; S. Day, S. Lake, K. Skubik – Little Traverse Bay Band of Indians; M. Ashton – Maine Department of Natural Resources; A. Bowen, A. Briggs – Michigan Department of Natural Resources; B. Hrabik – Missouri Department of Conservation; S. Pothoven – National Oceanic and Atmospheric Administration; K. Schmidt, J. Kruckenberg, A. Otten – North American Native Fishes Association; K. Dockendorf – North Carolina Wildlife Resources Commission; M. Kirby, B. Zimmerman – Ohio State University; B. Falsih, B. Harris, J. Kingsbury, A. Mathis, B. Smith – United States Fish and Wildlife Service; D. Morrill – University of Illinois; M. Hove – University of Minnesota; O. Morissette – Université du Québec à Chicoutimi; J. Lamer – Western Illinois University (now with INHS), and D. Bloom – Western Michigan University.

Literature Cited

Hartman, J.H., J.S. Tiemann, J.L. Sherwood, P.W. Willink, K.T. Ash, M.A. Davis, and E.R. Larson. 2023. Eastern Banded Killifish (*Fundulus diaphanus diaphanus*) in Lake Michigan and connected watersheds: the invasion of a non-native subspecies. Journal of Great Lake Research 49(4):876–887.

Jones, A., J.S. Tiemann, B.E. Fisher, T. Thomas, J. Adams, C. Moody-Carpenter, and R.E. Colombo. 2022. First occurrence of the Streamline Chub *Erimystax dissimilis* and Tippecanoe Darter *Etheostoma tippecanoe* discovered in Illinois. American Currents 47(2):18–21.

Lyons, J., and K. Schmidt. 2022. An updated annotated list of Wisconsin's fishes. American Currents 47(4)1–56.

Metzke, B.A., B.M. Burr, L.C. Hinz Jr., L.M. Page, and C.A. Taylor. 2022. An atlas of Illinois fishes: 150 years of change. University of Illinois Press, Urbana. 424 pp.

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.

Smith, B.J., and B.S. Harris. 2020. Expansion of a banded killifish *Fundulus diaphanus* population into benthic nearshore waters of Lake Michigan around Calumet Harbor. American Midland Naturalist 183 (2):260–267.

Smith, P.W. 1979. Fishes of Illinois. University of Illinois Press, Urbana, Illinois. 314 pp.

Tiemann, J., T. Thomas, W. Schelsky, J. Epifanio, and U. Thomas. 2014. The Illinois Redspotted Sunfish project: not possible without NANFA. American Currents 30(4):6–8.

Tiemann, J.S., C.A. Taylor, D. Wylie, J. Lamer, P.W. Willink, F.M. Veraldi, S. 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.W., J.S. Tiemann, J.L. Sherwood, E.R. Larson, A. Otten, and B. Zimmerman. 2019. The mystery of the Banded Killifish *Fundulus diaphanus* population explosion: where did they all come from? American Currents 44(4):3–6.

Willink, P.W., T.A. Widloe, V.J. Santucci, Jr., D. Makauskas, J.S. Tiemann, S.M. Hertel, D.P. Haerther, 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(2):179–190.