

**The Effects of a Native Fish Reintroduction on Resident Fish Assemblages**

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## Abstract

Native fish reintroduction can be a valuable conservation tool used to curb declines in biodiversity. Previous native fish reintroduction projects have focused on monitoring population responses of the target species, yet potential changes in the resident fish assemblages have received less attention. The reintroduction of the Bluebreast Darter (*Etheostoma camurum*) to the Upper Licking River basin in Ohio was used as a model to understand how reintroduction may alter resident fish assemblages. This reintroduction began in 2016, with one additional year of stocking in 2017, and yearly follow-up surveys through 2019. Fish community, water-chemistry, and fluvial geomorphic measurements were also performed at the reintroduction sites. I found that the benthic fish assemblage diversity and evenness increased over time in response to the reintroduction, with coarser substrate emerging as an important mechanism of reintroduction success. Both diversity and evenness increased post reintroduction, peaking in 2018 and then dropping back down to similar levels at the beginning of the project. This research helps to better understand how reintroductions may impact aquatic assemblage architecture and inform future reintroduction efforts.

## Introduction

Many native freshwater fishes have experienced dramatic declines in their population numbers and reductions in their geographic ranges in the previous century due to anthropogenic effects on aquatic ecosystems such as pollution and habitat degradation (Jelks et al. 2008; Karr et al. 1985; Andreen, 2003). However, with the gradual improvement in water quality over the past 50 years stemming from stricter environmental regulations, aquatic habitat has been restored in many areas making fish reintroductions feasible (Shute et al. 2009). Native fish reintroduction has become an increasingly popular conservation tool used by organizations across the world to restore aquatic biodiversity in areas where native species have been extirpated or significantly reduced in their native ranges (Jelks et al. 2008). Over the past 40 years, many different reintroduction projects have taken place with varying degrees of success, which presents a broad set of evidence for how to effectively reintroduce species (Shute et al. 2009).

In particular, benthic riffle fishes of the eastern United States have been heavily studied for reintroduction projects due to their high diversity and sensitivity to water quality (Simon, 2006). Riffle habitats provide complex niches where adaptive radiation has led to diverse benthic fish assemblages (Near & Benard, 2004). Darters of the genera *Etheostoma* include over 150 recognized species, and many species can overlap even within a small stretch of riffle habitat (Van Snik Gray, Ellen, et al., 1997). Therefore, habitat niche partitioning is important for darters when interspecific competition is high (Van Snik Gray, Ellen, et al., 1997). Abiotic variables like water depth, water velocity, and substrate have been shown to exhibit a strong influence on this partitioning (Welsh and Perry, 1998).

The Bluebreast Darter (*Etheostoma camurum*) is a small benthic riffle fish found throughout moderate to large streams and rivers in the Ohio River basin from western New York

to eastern Illinois, and south to the Tennessee River in North Carolina and Tennessee in the USA (Rice & Zimmerman, 2019) (Figures 1, 2). Bluebreast Darters typically occupy cobble bed riffles with moderate to swift current, and forage on small stream invertebrates (Tiemann, 2008).

Darters have also been the subject of conservation concern in recent years, due to their declining populations throughout their range (Tiemann, 2008). Specifically, Bluebreast Darters have seen a precipitous drop in their range throughout Ohio, although they have begun to recover in recent years in some areas due to improved habitat and water quality (Honick et al., 2017).

The Upper Licking River basin is a 48-km long segment of the Licking River located in central Ohio, with the city of Newark situated at the confluence of the North and South Forks. The Bluebreast Darter is native to the basin but has been extirpated since the early 1900s due to poor water quality throughout the 20th century (Sullivan, Zimmerman, & Symonds, 2015). Over the past 40 years, drastic improvements in environmental regulation in the area has caused the waterway to improve enough to again support the Bluebreast Darter (EPA 2008). However, Dillon Reservoir Dam prevents movement between the upper and lower Licking River basins. Reintroduction efforts of the Bluebreast Darter (*Etheostoma camurum*) to the Upper Licking River basin in Ohio by Dr. Sullivan's Stream and River Ecology (STRIVE) Lab led to a total of 1,914 fish tagged and translocated between 2016 and 2017. Translocation occurred in various riffles throughout the basin, with stocking ending in 2018 due to evidence of population establishment (Sullivan, Zimmerman, & Symonds, 2015).

The resident benthic riffle fish assemblage of the Upper Licking basin includes many species that may have strong interactions with the Bluebreast Darter. The Rainbow (*E. caeruleum*), Greenside (*E. blennioides*), Fantail (*E. flabellare*), Banded (*E. zonale*), and Johnny (*E. nigrum*) darters are all part of the native *Etheostoma* assemblage (Trautman, 1981). The

Johnny Darter is least likely to overlap in habitat, with most of their habitat consisting of slack backwater pools: areas of slow moving water that do not coincide with riffle habitat (Rice and Zimmerman, 2019). However, Rainbow, Greenside, Banded, and Fantail Darters all inhabit moderate to swift currents in riffles with cobble and boulders, leading to the strong likelihood of sympatry and some degree of interspecific competition between these species (Rice and Zimmerman, 2019). Other potential interacting species include the Stonecat Madtom (*Noturus flavus*), and the Northern Mottled Sculpin (*Cottuse bairdii*), most likely through predation (Rice and Zimmerman, 2019). Although darters have been shown to occupy diverse niches that minimize overlap (Welsh and Perry, 1998), studies have also shown that similarity in diets cause overlaps regardless of how specialized the niche is (White and Aspinwall, 1984). One study found that when invertebrate taxa are limited, there is substantial overlap in taxa consumed between darter species (Van Snik Gray et al., 1997). In addition, competition has been shown to be more impactful in assemblage organization at local scales (i.e., riffle) due to these overlapping microhabitats (Jackson et al. 2001). Thus, it is plausible that Bluebreast reintroduction may influence assemblage structure across various spatial scales.

While the reintroduction of an extirpated stream fish is not expected to mimic the introduction of an invasive species, it may prove instructive to consider theoretical constructs of invasion ecology to better understand the potential impact a native reintroduction can have on the native fish assemblage. The basic steps of an invasion consist of transport, inoculation, establishment, spread, and integration (Moyle and Marchetti 2006), which also serves as a useful outline for reintroduction projects. For the sake of this study, I propose focusing on that last two phases: spread and the impact this spread has on integration. Although it is common to view invasion impacts through measures of decline in native diversity (Carey and Wahl, 2010), I plan

to focus on changes in assemblage composition of the resident fish assemblage that might be expected to change following the reintegration of species that have been absent for decades. The Shannon-Wiener diversity index (Qinghong, 1995), biomass, and population densities before and after introduction are all common metrics used to analyze effects of invaders on the native ecosystem (Carey and Wahl, 2010). Applying this invasion framework to reintroductions may help to better inform future reintroductions by raising new considerations for success, mainly more of a focus on the native fish assemblage structure and diversity.

The overall aim of this study is to assess the potential impacts of Bluebreast Darter reintroduction on native fish assemblages. I hypothesize that the reintroduction of the Bluebreast Darter will lead to significant alterations in diversity of the resident fish assemblage. Specifically, I predict that reintegrating Bluebreast Darter will increase competition among darters of the genus *Etheostoma* for food and niche habitat, resulting in changes to the relative abundance of species within the assemblage. I also predict that the heterogeneity of habitat at individual stream sites will create variability in Bluebreast abundance owing to the Bluebreast Darter's preference for large cobble.

## **Methods**

Reintroduction of the Bluebreast Darter to the Upper Licking River basin occurred in the summers of 2016 and 2017. Six reintroduction sites were established (LR1, LR2, NB1, NB2, SB1, SB2), along with three control sites (LRC, NBC, SBC), representing sites similar in physicochemical characteristics but where no darters were reintroduced (Figure 3). Two “reference” sites from the Kokosing basin where Bluebreast Darters were never extirpated were also included. These two sites were used to represent a benthic darter assemblage that is at a stable equilibrium to which the Licking sites may eventually shift. Within each site, three riffles were chosen based on visual assessments indicative of habitat suitable for Bluebreast Darter establishment (Honick et al., 2017) and assigned designations of “A”, “B”, or “C”.

### ***Fish Surveys***

Every autumn (and spring for 2016 and 2017), the fish community was surveyed using a Smith-Root LR-24 Backpack Electroshocker (Smith-Root, Inc., Vancouver, Washington, USA) and 3 m seine with a standardized unit effort (Shute et al., 2009). Riffles were electroshocked and kicked towards a seine in order to disturb the fish living in the riffles into the seine roughly every 7 m of riffle (Shute et al. 2009). This was done for a standardized time and attempts. Lengths and individual weights of Bluebreast Darters, as well as counts and batch weights of all other species, were recorded. Introduced Bluebreast Darters were tagged using Visible Implant Elastomer (Northwest Marine Technology, Inc., Anacortes, WA) to enable identification of when and where individual fish were reintroduced during future sampling and to differentiate fish that were born after the reintroduction.

Fish community surveys also occurred in the year prior to the reintroduction began (2015). However, these surveys were done without a Smith-Root LR-24 Backpack Electroshocker and instead were just conducted using standard kick-seining methods. Therefore, these samples are not used directly in the analysis, but are instead used as a descriptive reference for composition of the resident fish assemblages prior to reintroduction. All fish surveys were conducted under IACUC protocols 2010A00000172-R2 and 2010A00000172-R3 and Ohio Division of Wildlife Wild Animal Collection Permit #21-134 (Appendix 1).

### *Physicochemical Variables*

In addition to these community surveys, abiotic variables were also taken at each site. Pebble counts were conducted at each riffle by measuring 100 clasts at 3 transects (Wolman, 1954). These data were subsequently used to calculate the median substrate size ( $D_{50}$ ). Additional physicochemical data collected at each site before fish community sampling included water temperature, pH, conductivity, and dissolved oxygen (DO). Water samples were also collected at each individual riffle sampled for concentration of nutrients. Samples were refrigerated before submission to The Ohio State University's Service Testing and Research (STAR) Laboratory for analysis of total nitrogen (N;  $\text{mg L}^{-1}$ ) and total phosphorus (P;  $\text{mg L}^{-1}$ ). Total N and P were analyzed using flow injection analysis (Lachat Quick Chem 8500 Flow Injection Analyzer, Hach Company, Loveland, Colorado)

### *Statistical Analysis*

All statistical analyses and graphs were created using R Studio (Core team 2013). Diversity metrics were run by first creating a matrix of the entire fish assemblage sampled and



then calculating rarefied species richness, Fisher's alpha, and Hill number diversity for each sampling event (Chao et al., 2014). These same calculations were then run on a subset of the matrix to only include benthic riffle species (i.e., excluding species from the families *Centrarchidae* and *Cyprinidae*), as this was hypothesized to have more predictive power based on stronger competition between species within this subset.

Central Stoneroller (*Campostoma anomalum*) abundances were extremely high relative to all other species, in some cases 3x higher than the total abundances of all other species combined. To account for the possibility that Central Stoneroller abundances overwhelmed other important relationships, analyses were run on the full data set as well as without Central Stonerollers. Year, treatment, season sampled, and Bluebreast abundance (in the models not using Bluebreast abundance as the response variable) were used as predictors with the covariates of  $D_{50}$  and nutrient concentrations to create multiple linear mixed models (LMM) assessing the random effect of these variables on Hill number diversity, assemblage evenness, and Bluebreast Darter abundance. Post-hoc analyses of the LMM's were done using a pairwise Tukey method in an estimated marginal means package in R (Russel, 2021). Graphical assessments and post-hoc regressions of various predictor variables such as  $D_{50}$  and nutrient concentrations with response variables were also run to analyze some of the more direct relationships in the study.

## **Results**

The top four most abundant benthic species sampled throughout the entire project in descending order were Banded Darters, Rainbow Darters, Mottled Sculpins, and Greenside Darters (Figure 5). While specific abundances varied widely among sites, these four species represented roughly 90% of assemblage composition across the entire study (Figure 5). Response

variables also varied widely between sites. Bluebreast Darter abundance averaged around 5 individuals per reach, (SD = 10.2) (Figure 5). Diversity (as measured by Hill numbers) ranged from 1.285 to 5.866, and evenness ranged from 0.320 to 1 (a completely even assemblage) (Figure 6).

Although data from 2015 was not included in these aforementioned descriptive statistics, the following presence/absence data provides important baseline assemblage information. 2015 surveys caught Greenside, Fantail, Banded, Rainbow, Johnny, Orangethroat, and Logperch Darters, indicating a nearly identical darter assemblage composition as subsequent surveys (Blackside Darters were caught in very small abundances in 2018). Additional benthic assemblage species caught included Mottled Sculpin, Stonecat Madtom, White Sucker, Northern Hogsucker, and Channel Catfish.

After model comparison between different response variables, it was decided to use evenness and Hill number as the primary metrics of changes in assemblage structure. Shannon's, Simpson's, and Inverse Simpson's were all still calculated as lower level diversity statistics (Figure 4), but none of them were used in subsequent analyses, due to the predicative power of Hill numbers.

Bluebreast Darter abundance was different by treatment ( $F_{2, 26.4} = 30.11, p < 0.001$ ), year ( $F_{4, 144} = 2.67, p = 0.035$ ), and  $D_{50}$  ( $F_{1, 101.7} = 4.57, p = 0.035$ ). Treatment differences were driven by the reference sites in the Kokosing basin, and no relationship was found when those sites were excluded ( $F_{1, 7.67} = 3.15, p = 0.116$ ). Both Bluebreast abundance ( $F_{1, 10.2} = 9.16, p < 0.001$ ) and year ( $F_{4, 155.8} = 3.57, p = 0.008$ ) influenced diversity (as measured by Hill numbers). Evenness was different by treatment ( $F_{2, 9.79} = 3.14, p = 0.036$ ) and year ( $F_{4, 145.56} = 2.56, p = 0.041$ ), and also showed a trend with Bluebreast Darter abundance ( $F_{1, 127.1}, p = 0.078$ ). Season

emerged as a salient predictor for Bluebreast Darter abundance ( $F_{1, 142} = 21.7, p < 0.001$ ) and diversity ( $F_{1, 154} = 17.5, p < 0.001$ ), but not for evenness.

The relationship between Mottled Sculpin abundance and Bluebreast abundance was explored graphically (Figure 5), lending evidence to the hypothesis that Mottled Sculpin may be acting as an invasion barrier for Bluebreast Darter reintroduction. However, a Pearson's  $r$  of -0.11 indicated a weak overall relationship, due to skew in the graph.  $D_{50}$  was positively related to Bluebreast Darter abundance ( $r^2 = 0.113, F_{1, 176} = 23.8, p < 0.001$ ); i.e., larger substrate was associated greater Bluebreast Darter abundance.

Post-hoc analysis of pairwise differences revealed a consistent nonlinear change in all three response variables over time. Hill diversity peaked in 2018, and then trended back down in 2019 and 2020 (Figure 7a). Evenness showed a similar trend to Hill in absolute numbers, but there was no significant difference between years (Figure 7b). Bluebreast Darter abundance peaked in 2017 (the last year of reintroductions), then dropped back down in subsequent sampling years (Figure 7c).

Estimated marginal means were also used to explore the effect of treatment on response variables. Bluebreast Darter abundance (Figure 8a) and evenness (Figure 8c) showed no significant difference between control and introduction sites, but a difference between control and reintroduction sites and the Kokosing reference sites for Bluebreast Darter abundance amounting to 8x more abundance at the reference sites ( $p < 0.0001$ ). For Hill diversity, there was a difference between all three treatments, with introduction sites being between the higher values of control sites and lower values at reference sites ( $p = 0.0097, 0.0230$ ).

An ANOVA between the Hill number diversity model and the same model including Central Stonerollers in the diversity index yielded AIC's of 487.88 and 495.53 respectively,

indicating that excluding Central Stonerollers from the analyses improved model fit. This was expected, and therefore none of the previous models included Central Stonerollers in any of the indices. No relationship was found between phosphate or nitrate levels and any of the response variables ( $p > 0.05$ ).

## **Discussion**

Native fish reintroduction projects are an important tool for bolstering the ranges of imperiled species (Jelks et al. 2008; Karr et al. 1985; Andreen, 2003). However, these projects are often focused on the target species, leaving out crucial data on the resident fish assemblage. Through this study, I identified various ways in which the reintroduction of an extirpated benthic darter species can alter current benthic fish assemblages. Hill number diversity for the benthic fish assemblage changed over time and in relation to Bluebreast abundance, indicating a changing fish assemblage post-reintroduction. This is consistent with literature on reintroductions in terms of impact on native assemblages (Kiffney et al., 2009), but unique in how it shows specific abundances of species within the assemblage changing. Evenness had a similar trend in which the reintroduction of Bluebreast Darters altered the distribution of species, but differences were small enough that no significant relationship was found over time.

With the lack of comparable pre-reintroduction data, it is difficult to make assertions about how the fish assemblages changed in direct response to the reintroduction. However, the presence/absence data that is available provides a rough glance at the assemblage structure in 2015. Considering all of the species found before the reintroduction are still found in the follow-up surveys, it is possible to conclude that the reintroduction of Bluebreast Darters did not

fundamentally alter the benthic fish assemblage structure through competitive exclusion. However, little can be said about how the specific metrics such as diversity and evenness have changed since then.

Post-hoc analyses revealed a trend in metrics consistent with other reintroduction projects. With 2017 being the last year of physical reintroduction of Bluebreast Darters, it follows reason that that would also be the year of the highest Bluebreast Darter abundance throughout this short-term monitoring of populations. However, the trend in Hill diversity and evenness that illustrated peaking in 2018 and then declining in subsequent years was not fully expected. While it is difficult to point to specific mechanisms that may have caused this, one plausible explanation is that Bluebreast Darters originally acted as competitors (i.e., in the first few years) (Welsh and Perry, 1998), but then adapted to unfilled niche space once their numbers declined.

By looking at the reintroduction through the lens of invasion ecology, how Bluebreast Darters spread and the impact that this spread has on integration yields an interesting view of reintroductions. According to definitions outlined in invasive species literature (Moyle and Marchetti, 2006), Bluebreast Darters became fully established in the Upper Licking basin, but their impact on the resident fish assemblage was quite different than a traditional invader. The slower spread of Bluebreast Darters following their establishment is indicative of evolutionary co-adaptation with the resident fish assemblage worth further investigation.

The strong positive effect of  $D_{50}$  on Bluebreast Darter abundance was expected and speaks to the importance of habitat considerations when attempting to understand the community dynamics of a reintroduction project. While this preference for large cobble is known to literature (Tiemann, 2008), this analysis takes it a step further in establishing how this plays a

role in the distribution of benthic fish assemblages. Considering how *Etheostoma* tend to have very specific microhabitats (Welsh and Perry, 1998), this relationship lends additional evidence towards the mechanism of specific substrate acting as refugia for different species. This reintroduction may have had a stronger influence on the resident fish assemblage at first, then trended towards less impact as Bluebreast were able to adjust to their differing microhabitats and become established in the proper refugia/substrate for their life history.

As previously mentioned, the Bluebreast Darter abundance and diversity models showed high significance between sampling seasons. This was expected, as it most likely indicates that spring sampling at the beginning of the study may not have been fully indicative community surveys. High water levels and differing life history of benthic riffle dwelling species means that spring samples are typically not used for fish community surveys (Shute et al., 2009). Spring samples were originally done to attempt to have a more robust sampling of the fish assemblages, so including them in this analysis proved valuable in its ability to give weight to these assumptions on the poor quality of spring samples.

The weak correlation between Bluebreast Darter and Mottled Sculpin abundances can be attributed in part to the skew of the values. While there is a clear relationship of low Mottled Sculpin populations at sites with high Bluebreast Darter abundance and vice versa, the trend is lost at intermediate values and therefore is difficult to interpret. More complex statistical analyses of this relationship in the future may yield more informative results, but based on the existing analysis, little can be said. Ultimately, Mottled Sculpin abundance was removed from the mixed models for this reason.

This project analyzed the change in fish assemblages over time following the reintroduction of Bluebreast Darters. Despite promising results indicating a changing benthic fish

assemblage, the nuanced effects of interspecies competition between benthic darter species becomes difficult to quantify when control and introduction sites are connected within the same basin and there are many confounding stressors of a relatively urbanized watershed. Original site selection criteria were also heavily based on choosing areas where Bluebreast Darters would have the greatest success, and therefore were highly variable between sites. That being said, important potential mechanisms in the assemblage structure were identified, and insights about the establishment and spread of a previously extirpated species provided a new perspective on invasion theory. In conclusion, future studies with more controlled conditions between treatments are needed to further understand the effects of reintroducing a benthic fish species.

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**Tables:**

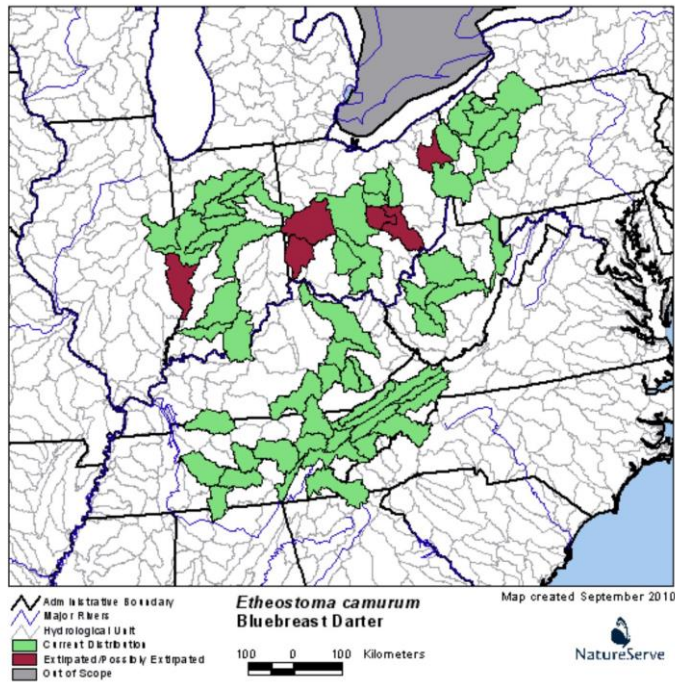
**Table 1.** Descriptive statistics for benthic riffle fishes considered in the fish assemblage metrics

	vars	n	mean	sd	min	max	range	se
White Sucker	1	186	0.280	1.447	0	16	16	0.106
Northern Hogsucker	2	186	5.446	9.455	0	48	48	0.693
Flathead Catfish	3	186	0.022	0.145	0	1	1	0.011
Channel Catfish	4	186	0.194	0.873	0	7	7	0.064
Yellow Bullhead	5	186	0.070	0.499	0	6	6	0.037
Brindled Madtom	6	186	0.177	0.898	0	9	9	0.066
Stonecat Madtom	7	186	4.188	8.430	0	54	54	0.618
Blackside Darter	8	186	0.323	4.326	0	59	59	0.317
Logperch Darter	9	186	3.435	7.784	0	64	64	0.571
Johnny Darter	10	186	1.398	4.263	0	32	32	0.313
Rainbow Darter	11	186	62.484	98.459	0	839	839	7.219
Orangethroat Darter	12	186	0.075	0.382	0	3	3	0.028
Banded Darter	13	186	91.769	84.468	0	467	467	6.193
Greenside Darter	14	186	25.333	30.421	0	195	195	2.231
Fantail Darter	15	186	3.710	5.290	0	28	28	0.388
Mottled Sculpin	16	186	48.156	88.718	0	587	587	6.505
Bluebreast Darter	17	186	5.145	10.191	0	75	75	0.747
Variagate Darter	18	186	0.263	1.657	0	15	15	0.122

**Table 2.** Descriptive statistics for each of the response variables (excluding Bluebreast Darter abundance, which is included in Table 1)

	vars	n	mean	sd	min	max	range	se
Pebble D50	1	186	53.502	32.735	11.000	180.000	169.000	2.400
phosphate	2	186	0.345	0.417	0.015	1.370	1.355	0.031
nitrate	3	186	1.468	0.532	0.312	2.238	1.926	0.039
hill_diversity	4	186	3.733	1.010	1.285	6.115	4.830	0.074
Evenness	5	186	0.689	0.124	0.327	1.000	0.673	0.009
Species_richness	6	186	6.651	1.671	2.000	10.000	8.000	0.122
Simpsons_diversity	7	186	0.635	0.121	0.128	0.813	0.684	0.009

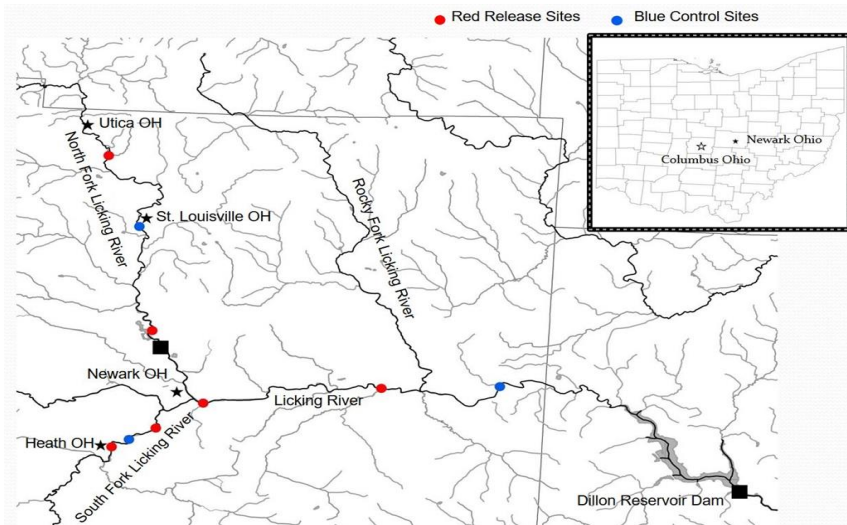
Figures:



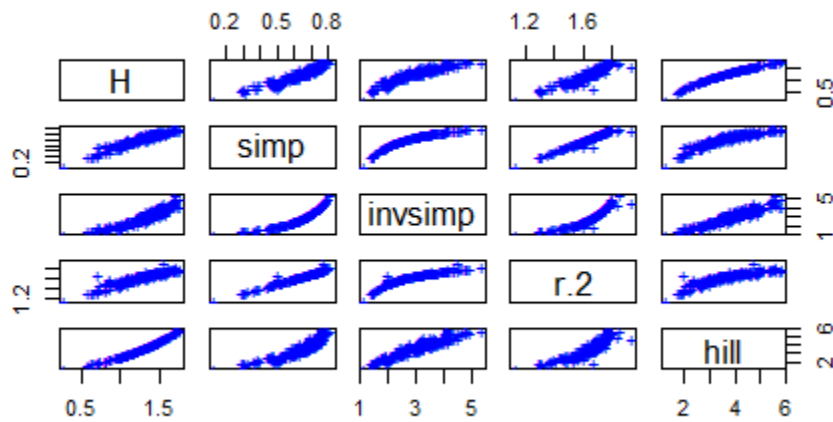
**Figure 1.** Distribution of the Bluebreast Darter throughout the Eastern United States (*NatureServe Explorer 2.0*).



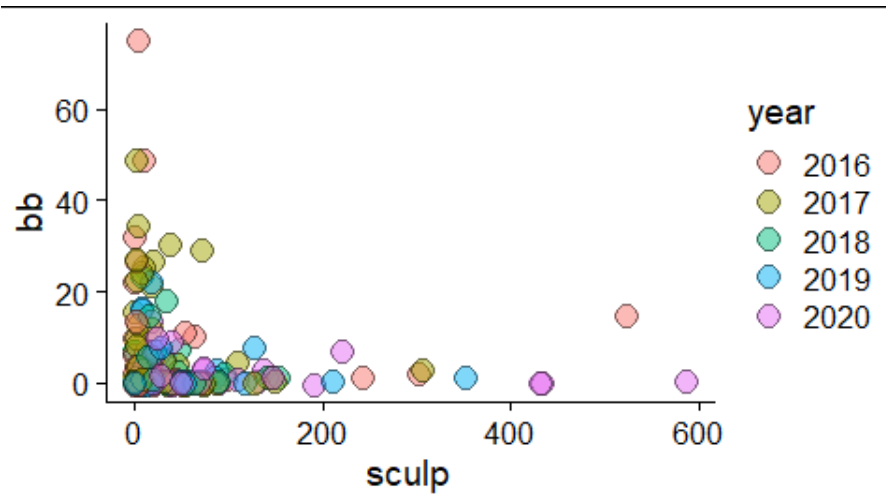
**Figure 2.** Female (top) and Male (bottom) Bluebreast Darters (*Etheostoma camurum*) with blue VIE directly below the front dorsal fin. Images courtesy of Brian Zimmerman.



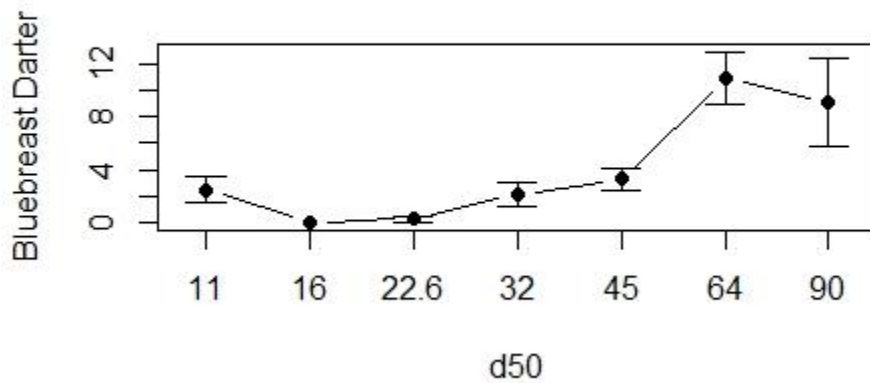
**Figure 3.** Map of sites for the reintroduction to the Upper Licking River. Red dots represent reintroduction sites, and blue dots represent control sites with similar physicochemical characteristics but where no fish were reintroduced. Black stars and squares represent urban areas.



**Figure 4.** H (Shannon's diversity), simp (Simpson's diversity), invsimp (Inverse Simpson's diversity), r.2 (Species richness), and hill (Hill number diversity) plotted against each other to show the relationship among diversity indices.

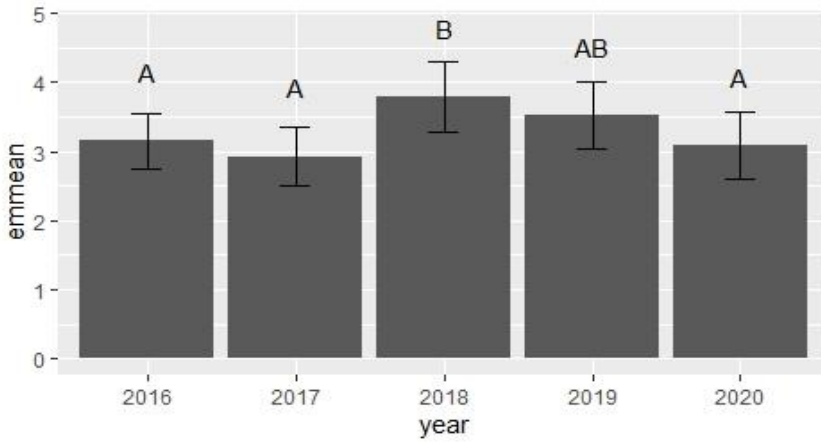


**Figure 5.** Bluebreast Darter abundance (bb) vs. Mottled Sculpin abundance (sculp), with different years represented by different color points.

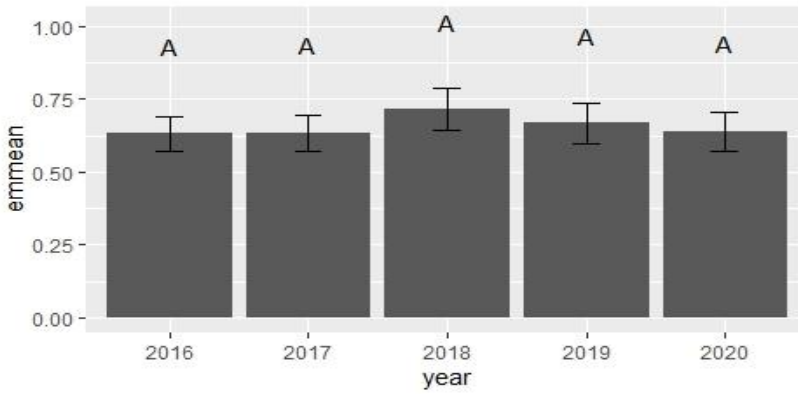


**Figure 6.** Substrate  $D_{50}$  vs. Bluebreast Darter abundance. Sites with  $D_{50} > 90$ mm were excluded, as they were indicative of bedrock and therefore the relationship became reversed due to lack of cover.

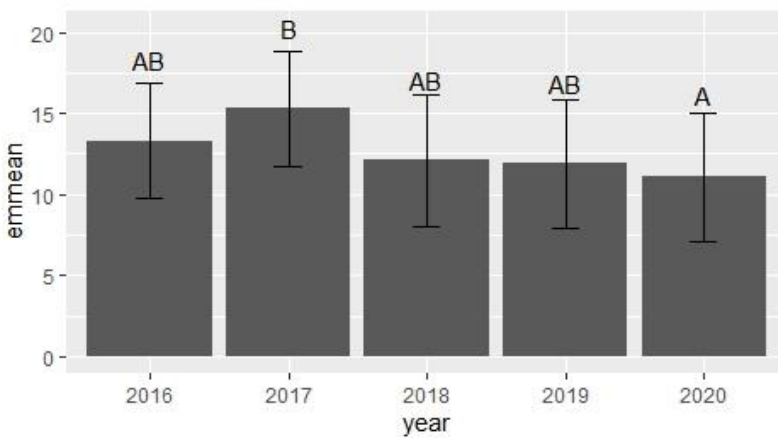
(a)



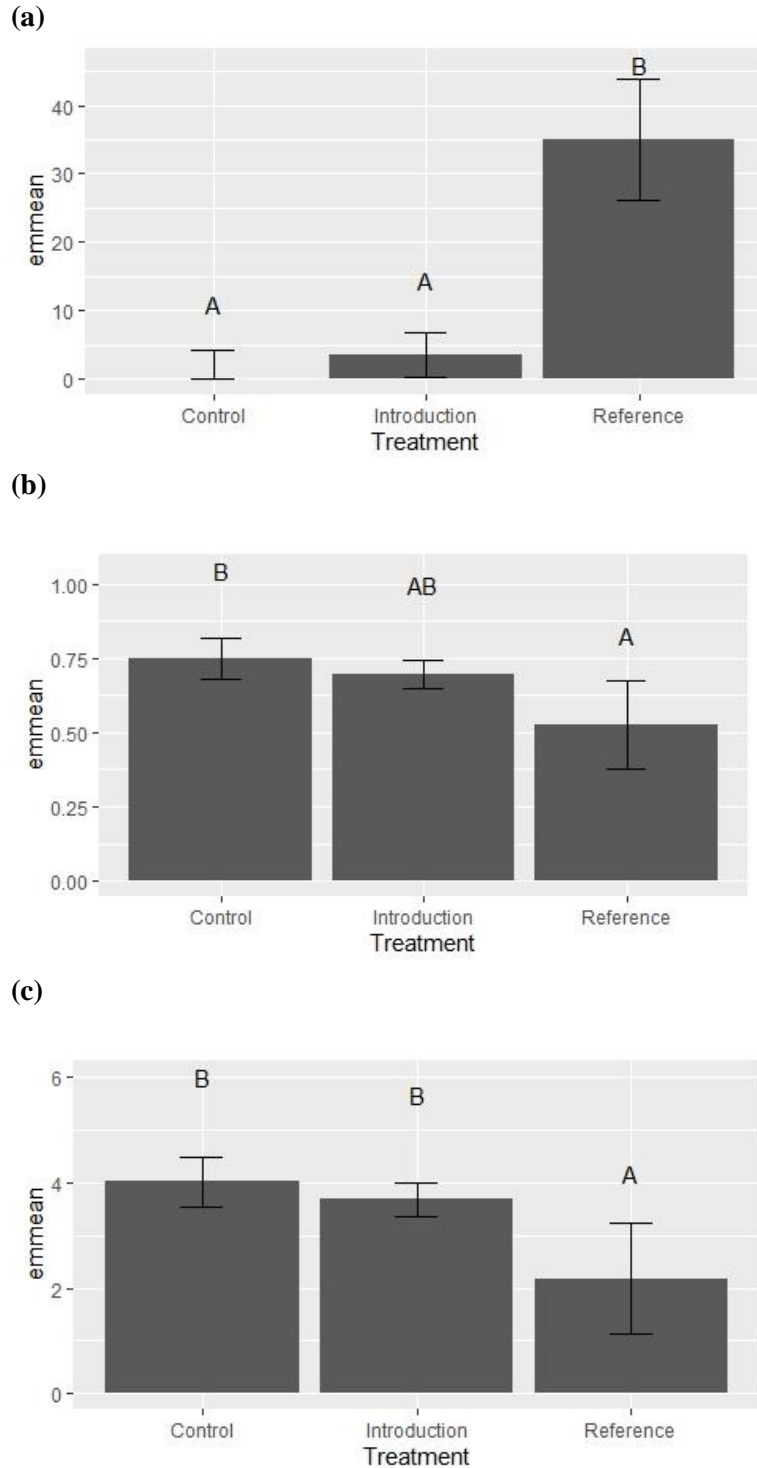
(b)



(c)




**Figure 7.** Differences in estimated marginal means (emmean) of (a) Hill number diversity, (b) evenness, and (c) abundance by year. Different letters (A, B) indicate significant differences.



**Figure 8.** Differences in estimated marginal means (emmean) of (a) Bluebreast Darter abundance, (b) evenness, and (c) Hill number diversity by treatment. Different letters (A, B) indicate significant differences.



Appendix:

	<b>DIVISION OF WILDLIFE</b> Ohio Department of Natural Resources	Division of Wildlife Headquarters 2045 Morse Road, Bldg. G Columbus, Ohio 43229-6693 1-800-WILDLIFE	
		Chief, Division of Wildlife: <b>Michael R. Miller</b>	
<b>WILD ANIMAL PERMIT: 21-134</b>		<b>DATE ISSUED</b>	
SCIENTIFIC COLLECTION		<u>6/14/2018</u>	
MAZEIKA SULLIVAN THE OHIO STATE UNIVERSITY 352 WEST DODRIDGE ST COLUMBUS, OH 43202		Others authorized on permit	
		YES	(SEE ATTACHMENT)

is hereby granted permission to take, possess, and transport at any time and in any manner specimens of wild animals, subject to the conditions and restrictions listed below or any documents accompanying this permit. This permit, unless revoked earlier by the Chief, Division of Wildlife, is effective from:

6/14/2018 to: 3/15/2021

The Chief of the Division of Wildlife will not issue permits for Dangerous Wild Animal (DWA) species (ORC 935.01 except native DWA, required for specific projects. The permit issued by the Chief does not relieve the permittee of any responsibility to obtain a permit pursuant to R.C. Chapter 935 except as specified for the animals and purposes permitted herein. The permittee must adhere to all additional requirements under R.C. Chapter 935.

**THIS PERMIT IS RESTRICTED AS FOLLOWS:**

1. Permittee must notify the Division of Wildlife of each study site location for mussel surveys and receive prior approval before conducting collection activities. Contact: John Navarro at john.navarro@dnr.state.oh.us.
2. Permittee may collect fish, birds, reptiles, amphibians, mussels and macroinvertebrates for survey and inventory. Raccoons may also be live trapped for research. No sport fish over six inches may be retained. Live mussels may not be removed from the site and must be immediately released to the collection location upon identification. Dead shells only may be removed and added to the mussel reference collection held at Schiemenier Olentangy River Wetland Research Park.
3. Areas where the northern riffleshell and cutshell have been reintroduced must be avoided.
4. Permittees may collect blood and feather samples from birds. A USFWS permit is required for migratory species.
5. Any specimen held longer than 30 days must be humanely euthanized or retained for further research/education.
6. Please notify John Navarro by email or phone at 614-265-8715 if a new location for a state-listed species is found. Contact Mr. Navarro within 24 hrs if an undocumented invasive species is found.
7. Twenty-four hours prior to collecting activities, permittee must contact the local wildlife officer to advise locations and duration of sampling.
8. All traps must be clearly labeled with the contact information of the responsible person. Traps must be checked each calendar day.
9. All voucher specimens are to be deposited at The OSU, Museum of Biological Diversity Schiemenier Olentangy River Wetland Research Park or the CMNH.
10. Collection is prohibited in the Killbuck, tributaries to and east branch of the Chagrin River above I-80, Fish Creek (Williams County) and Division of Wildlife property without explicit written permission from the Division of Wildlife. Sampling is further restricted in streams that may have federally listed mussels. See Appendix A of the Ohio Mussel Survey Protocol (April 2014 @ <http://dnr.wildlife.ohio.gov/licensing-and-permits/specialty-licenses-permits>) for locations of federally listed mussels.
11. Permittee must provide an annual electronic report of collecting activities in the Diversity Database Excel spreadsheet to the Division of Wildlife. A copy of any published materials that result from this project should also be forwarded to the division.

**Locations of Collecting:**  
STATEWIDE WITH NOTED EXCEPTIONS

**Equipment and method used in collection:**  
ELECTROSHOCKER, SEINES, BLOCK NETS, DIP NETS, HOOP NETS, MINNOW TRAPS, SURBER SAMPLES, MIST NETS, HAND NETS, BOX TRAPS AND ROCKET NETS

**Name and number of each species to be collected:**  
FISH, BIRDS, MACROINVERTEBRATES, MUSSELS, REPTILES, AND AMPHIBIANS. RACCOONS MAY BE LIVE TRAPPED FOR OBTAINING TISSUE SAMPLES. SAMPLING BY ELECTROSHOCKING ONLY ARE PERMITTED IN THE BIG DARBY.

**RESTRICTIVE DOCUMENTS ACCOMPANYING THIS PERMIT? YES**

**NO ENDANGERED SPECIES OR AQUATIC NUISANCE SPECIES MAY BE TAKEN WITHOUT WRITTEN PERMISSION FROM THE CHIEF**