Expanded Distributions of Three *Etheostoma* Darters (Subgenus *Nothonotus*) within the Upper Ohio River Watershed

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Abstract - Within the upper Ohio River watershed, 3 *Etheostoma* darter species in the subgenus *Nothonotus* have been documented in disjunct populations and were listed as threatened or endangered in Ohio and Pennsylvania. Tailwater habitat below navigation lock and dam (L/D) installations has been shown to contain diverse darter assemblages. *Etheostoma camurum* (Bluebreast Darter), *E. maculatum* (Spotted Darter), and *E. tippecanoe* (Tippecanoe Darter) often live in similar habitats; thus, we hypothesized that all 3 were occupying tailwater habitat below navigational L/Ds. Electrified benthic trawling verified Bluebreast Darter and Tippecanoe Darter below 8 L/D installations and at water depths varying from 1.4 m to 4.5 m and 1.4 m to 5.9 m, respectively. Spotted Darter was only found below 1 L/D. In the Ohio River, benthic trawling documented Bluebreast Darter and Tippecanoe Darter utilizing habitat located within deposition zones and areas above and below islands. Analysis of contemporary and historic distribution data shows that Bluebreast Darter and Tippecanoe Darter now span large sections of the river, but the range of Spotted Darter is more limited and warrants close monitoring. Our study confirms the effectiveness of utilizing benthic trawling in non-wadeable rivers to survey for benthic species such as river-inhabiting darters.

Introduction

Etheostoma (*Nothonotus*) *camurum* (Cope) (Bluebreast Darter) was described from the headwaters of the Cumberland River in Tennessee (Cope 1870) and is known to have variable population sizes (Page 1983, Trautman 1981) and a disjunct distribution in the upper Allegheny drainage (PA, NY); Cheat, Little Kanawha, and Elk river drainages (WV); Walhonding and Scioto drainages (OH); Wabash drainage (IN, IL); Cumberland drainage (KY, TN); Licking and upper Kentucky drainages (KY); and Duck, Elk, and upper Tennessee drainages (TN, AL, VA) (see Supplemental File 1 available online at http://www.eaglehill.us/NENAonline/suppl-files/n24-2-N1537-Honick-s1, and, for BioOne subscribers, at http://dx.doi.org/10.1656/N1537.s1). Bluebreast Darter habitat is reported to consist of moderate to swift riffles, raceways, and runs of moderate- to large-sized clear streams, and

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rivers running over silt-free boulders, cobble, and gravel at depths of 0.5 m to 1.5 m (Boschung et al. 2004, Etnier and Starnes 1993, Stauffer et al. 1995, Trautman 1981).

Etheostoma (*Nothonotus*) *tippecanoe* Jordan and Evermann (Tippecanoe Darter) was described from the Tippecanoe River at Marshland, IN (Jordan and Evermann 1890). This species is known to have dramatic year-to-year variation in population sizes (Trautman 1981), and Stauffer (2016) noted that populations in French Creek, PA, cycled every 3 years. Tippecanoe Darters have disjunct distributions in the upper Allegheny drainage (PA); Elk and Little Kanawha rivers (WV); lower Muskingum River, Walhonding River and the Scioto River drainage (OH); East Fork White River and upper Wabash River drainage (IN); Licking River, Kentucky River drainage and Green River (KY); and Big South Fork, Red Stones, and Harpeth rivers (TN) (see Supplemental File 1 available online at http://www.eaglehill.us/NENAonline/suppl-files/n24-2-N1537-Honick-s1, and, for BioOne subscribers, at http://dx.doi.org/10.1656/N1537.s1). Tippecanoe Darters inhabit riffles of medium to large rivers with slow to moderate currents and substrates of clean, fine gravel, sand, and cobble (Cooper 1983, Etnier and Starnes 1993, Trautman 1981).

Etheostoma (*Nothonotus*) *maculatum* Kirtland (Spotted Darter) was described from the Mahoning River near Youngstown, OH (Kirtland 1840), but that population was extirpated by pollution from a steel mill sometime in the mid-1850s (Trautman 1981). Historically, the Spotted Darter has been found in low population densities over a few disjunct localities in the Beaver River (now extirpated) and upper Allegheny drainage (PA and NY), Elk River (WV), Walhonding River and the Scioto River drainage (OH), Tippecanoe River (IN), and the Green River (KY) (see Supplemental File 1 available online at http://www.eaglehill.us/NENAonline/suppl-files/n24-2-N1537-Honick-s1, and, for BioOne subscribers, at http://dx.doi.org/10.1656/N1537.s1). Of these 3 species, the Spotted Darter is less broadly distributed (Kuehne and Barbour 1983, Page 1983); the largest number of remaining populations occur in the upper Allegheny River drainage of Pennsylvania and Big Darby Creek in Ohio (see Supplemental File 2 available online at http://www.eaglehill.us/NENAonline/suppl-files/n24-2-N1537-Honick-s2, and, for BioOne subscribers, at http://dx.doi.org/10.1656/N1537.s2). Spotted Darters inhabit swift riffles in medium to large streams associated with large cobble and boulder substrates (Zorach and Raney 1967).

Until recently, all 3 species were listed as either threatened or endangered by the Ohio Division of Wildlife (ODNR 2015, Ohio Revised Code 2015) and the Pennsylvania Fish and Boat Commission (Pennsylvania Bulletin 1999). In the most recent compilation of imperiled North American freshwater fishes by the American Fisheries Society, Jelks et al. (2008) listed the Tippecanoe Darter as vulnerable and the Spotted Darter as threatened and declining.

Even though it is generally accepted that the nation's waterways have experienced improved water quality conditions since implementation of the Clean Water Act (1972), nationwide assessments by Brown and Froemke (2012) and the US Environmental Protection Agency (2009) indicate that the nation's water resources are experiencing increased stress from nonpoint-source pollution. Jelks et al. (2008) corroborated these claims and reported that imperilment of inland fishes

had substantially increased since the last assessment completed by the American Fisheries Society in 1989. In contrast, and on a regional scale, surveys since 2003 in Pennsylvania (Argent and Kimmel 2010; Freedman et al. 2009a; Howell 2007; Koryak et al. 2009, 2011) and assessments from Yoder et al. (2005) and the Ohio EPA (OEPA 2016) have found that the fish communities in non-wadeable rivers of the upper Ohio River watershed were recovering. Regardless, there remains a pressing need to accurately document the return of imperiled fishes from refugia (e.g., French Creek, PA, and Big Darby Creek, OH) and track their distributional changes for future assessment of imperilment. In this study, we documented the changes in the distribution of these 3 focal darter species that have been increasing in occurrence outside of their known refugia. Several factors have contributed to elucidating these changes: (1) in Ohio, extensive routine monitoring using rigorous boat-electrofishing protocols and trawling have documented changes throughout the mainstem Ohio River and (2) in Pennsylvania, historically there may have been less-rigorous routine sampling efforts, but there has been a recent switch in sampling protocols to include benthic and electrified benthic trawling in non-wadeable rivers and within lock and dam (L/D) tailwaters. Efforts in Pennsylvania and Ohio have demonstrated the extent to which these 3 darter species now occupy non-wadeable rivers in the upper Ohio River watershed. In addition, because previous work indicated the importance of L/D tailwater habitat to benthic riverine fish species (Argent and Kimmel 2014, Freedman et al. 2009a, Koryak et al. 2009), we hypothesized that these darters in Pennsylvania were occupying tailwater habitat below L/D installations. In summary, we performed electrified benthic trawling surveys and compiled contemporary and historic data from multiple sources in Ohio and Pennsylvania to re-assess the darters' current distributions. These data have increased our understanding of the focal species' distributions within non-wadeable rivers and provided a summary of regional distribution changes that are imperative to documenting recovery since the delisting of Bluebreast Darter (in Ohio and Pennsylvania) and Tippecanoe Darter and Spotted Darter (in Pennsylvania).

Study Area

We analyzed contemporary and historic fish-survey data collected from rivers and streams in the upper Ohio River watershed of Pennsylvania and Ohio. Target water bodies included the mainstem rivers and tributaries of the Ohio River from river kilometer (rkm) 790.0 (the Ohio/Indiana border) upstream to Pittsburgh, PA (rkm 0), the Allegheny River from Pittsburgh, PA, upstream to the Pennsylvania/New York border, and the Monongahela River from Pittsburgh, PA, to the Pennsylvania/West Virginia border (Fig. 1).

Methods

Sampling methods

In Pennsylvania, we sampled the tailwaters of 11 L/D installations on 4 river systems (Allegheny, Beaver, Monongahela, and Ohio rivers; Fig. 2) using a modified Missouri trawl (2.4 m x 1.2 m, 3.2-mm mesh) electrified with a Smith-Root VI-A

electrofisher (Smith-Root Vancouver, WA) and a 5000-W generator. The unit was powered with an output mode of 6.0 amps, 120 PPS DC, and 6.0 ms pulse width. We established a transect within the tailrace of the L/D (50 m to 150 m below the installation) as the starting point for 7 trawls and placed 1 trawl each within 10 m of the left and right descending bank, 1 trawl at center channel, and the 4 remaining trawls evenly spaced between center-channel and the descending bank. We manually deployed the trawl from the bow of a 6.1-m Sea Ark Jon-type boat with a 115-hp outboard motor moving backwards downstream at a speed slightly faster than river current. We aborted snagged trawls and started a new trawl adjacent to the original location. We used river depth to determine the length of rope deployed with each trawl with the following guidelines: 5.0 m of depth or less = 15.2 m of rope, 5.0 to 10.0 m of depth = 30.5 m of rope. Each trawl consisted of 2 minutes of sampling effort. We identified and enumerated all fish species. In addition to the electrified-benthic trawling, we electrofished 9 tributaries (Smith Root LR-24, backpack electrofisher; single pass) for 100 m starting at the first riffle upstream of the confluence with the main river. We sampled streams with moderate to high flow by electrofishing into a blocking seine (2.4 m x 1.8 m, 3.2-mm mesh). We identified and enumerated all fish species.

In Ohio from 2011 to 2014, we sampled the Ohio River from the Indiana/Ohio border to the Ohio/Pennsylvania border, the entire length of the Muskingum

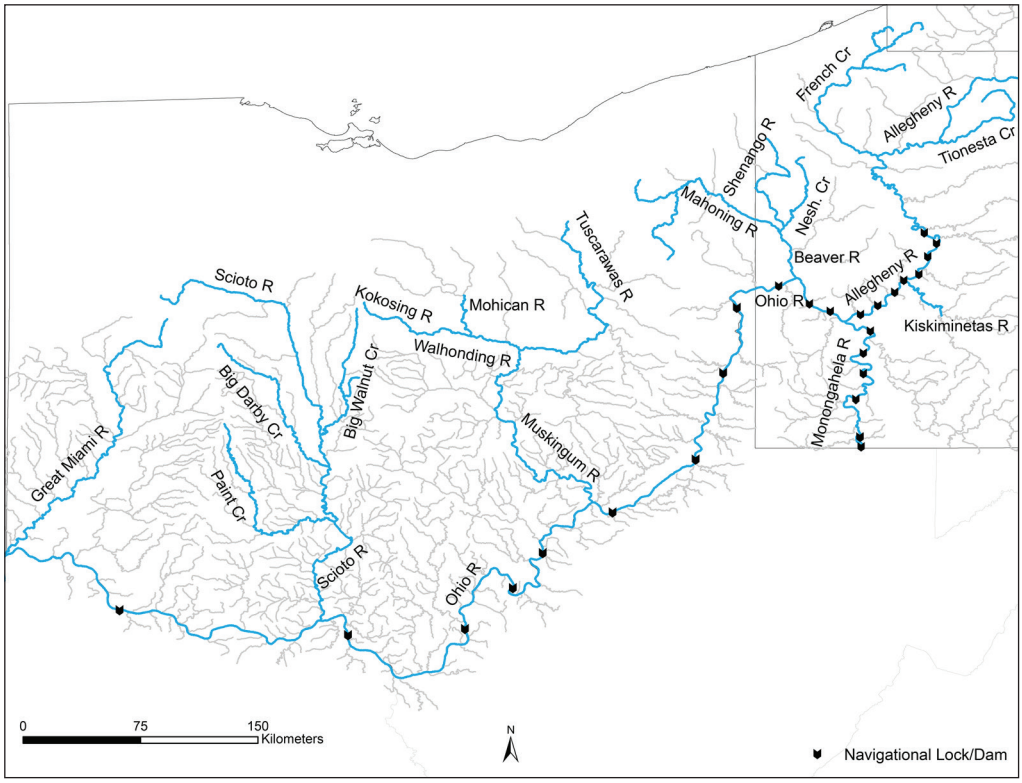

Figure 1. The major rivers and tributaries of the upper Ohio River watershed in Pennsylvania and Ohio.

River, and the Scioto River from Chillicothe to its confluence with the Ohio River at Portsmouth. We sampled all 3 systems approximately every 8 rkm (i.e., about every 5 river miles). The intent of the Ohio portion of this study was to provide presence/absence data for an improved understanding of the Ohio distribution of small benthic fishes in these large rivers. To conduct our samples, we employed a 2.4-m-wide mini-Missouri trawl (Innovative Net Systems, Milton, LA; Herzog et al. 2005) and a 1.4 m x 2.4 m (4.8-mm mesh) seine with a chain added to the lead line for better benthic contact. The trawl was manually deployed from the bow of a 4.8-m flat-bottomed boat (run in reverse) equipped with two 30-hp outboard motors at a target speed of 3.2–6.4 kmph. We classified as suitable habitat in non-wadeable areas all locations with unique features such as depositional zones at tributary mouths, current breaks at upstream and downstream ends of islands, tailwaters, and other areas of significant flow that might support darters and other benthic fishes. We conducted a minimum of 4 trawls varying from 30 s to 60 s in duration at each location. Large areas of suitable habitat were sampled more rigorously (e.g., at least 10 trawls). We sampled wadeable areas in the mainstem Ohio, Muskingum, and Scioto rivers with suitable habitat (as described above) using both kick seining and

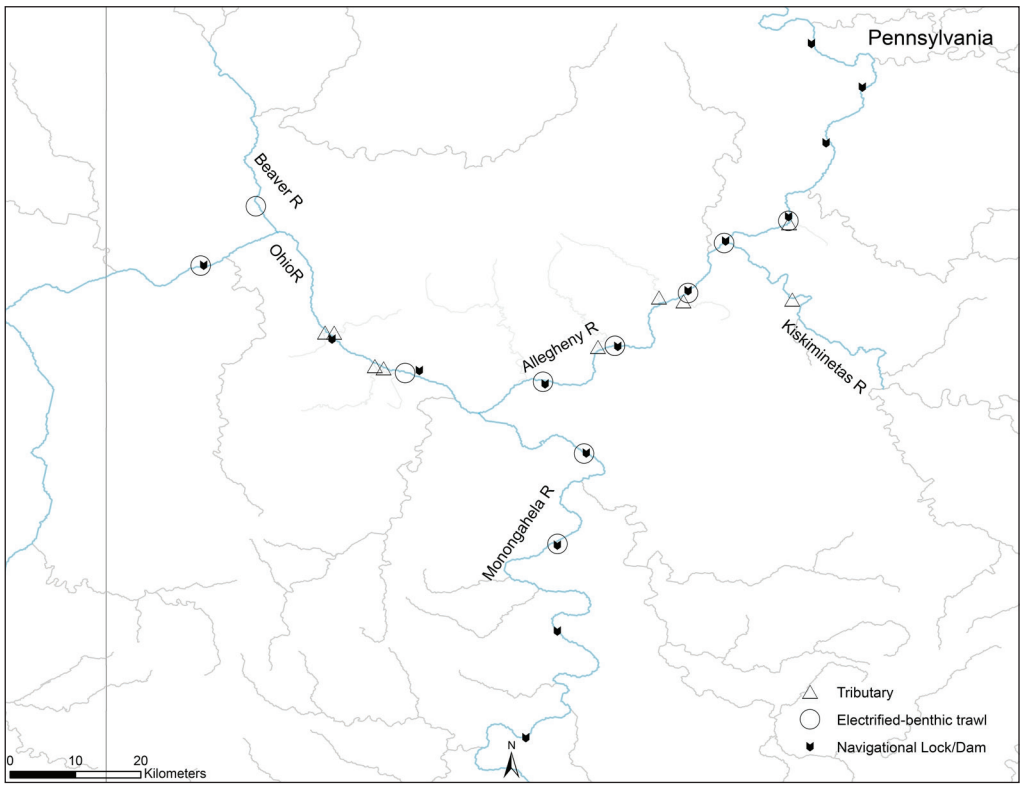

Figure 2. Sample locations of electrified-benthic trawls and the 9 tributaries sampled in this study. Note: Emsworth L/D consists of a main channel and a separate back-channel dam which we counted as 2 separate sample locations. All electrified trawls were conducted in dam tailwaters. The dam on the Beaver River is not a navigational lock and dam; therefore, the sample site is only designated with an open circle.

downstream hauls. When access was possible either by road or boating upstream from the mainstem river, we employed the seining methods described above to sample the first several riffles in the tributaries to these larger rivers. In this study, we use the term "traditional methods" to collectively refer to all fish-survey methods that were used prior to the development and implementation of the mini-Missouri trawl by Herzog et al. (2005). These protocols included backpack electrofishing, boat and tow-barge electrofishing, and all types of seining methods employed to survey for benthic fishes.

Historical data and map construction

We queried historical and contemporary survey data for Ohio from the Ohio State University Museum of Biological Diversity Fish Division database (OSU-MBD 2015; Table 1). OEPA is the largest contributor with over 400,000 records dating back to 1975. We compiled information on relative abundance, specific location, museum record/collection number, and gear type (see Supplemental File 2 available online at http://www.eaglehill.us/NENAonline/suppl-files/n24-2-N1537-Honick-s2, and, for BioOne subscribers, at http://dx.doi.org/10.1656/N1537.s2). Specific sampling protocols can be obtained from each respective agency.

We obtained historic and contemporary data from as many sources as possible for Pennsylvania records (Table 1). Raney (1938) compiled historic records for western Pennsylvania dating back to 1817. We collected information on relative abundance, specific location, museum record/collection number, and gear type (see Supplemental File 2 available online at http://www.eaglehill.us/NENAonline/suppl-files/n24-2-N1537-Honick-s2, and, for BioOne subscribers, at http://dx.doi.org/10.1656/N1537.s2). Specific sampling protocols can be obtained from each respective agency.

We quality-checked historic and contemporary records from Ohio and Pennsylvania for errors (e.g., duplicates, incorrect coordinates). When possible, we assigned coordinates based on site descriptions of the original survey record for historic records that did not have coordinate data and removed ambiguous

Table 1. Sources of historic and contemporary data.

State	Source
Ohio	
	Ohio State University Museum of Biological Diversity, Fish Division Database
	Ohio Environmental Protection Agency (OEPA)
	Ohio Department of Natural Resources - Division of Wildlife
	Ohio River Valley Water Sanitation Commission (ORSANCO) - http://www.orsanco.org/data/fish-population/
Pennsylvania	
	Pennsylvania Fish and Boat Commission (PAFBC)
	Pennsylvania Department of Environmental Protection
	US Army Corps of Engineers - Pittsburgh District
	The Pennsylvania State University Museum - Fish Collection
	California University of Pennsylvania
	Pennsylvania Natural Heritage Program

records. We constructed all distribution maps in ArcMap (v. 10.3.1; ESRI, Redlands, CA). In order to visualize distribution changes, we constructed maps for each species by grouping the data into 5 time-categories: pre-1981, 1981–1990, 1991–2000, 2001–2010, and 2011–2015. We plotted symbols denoting previous survey data (before 2011) on top of the most recent survey data to enhance visualization of distribution changes. For clarification, the terms "record" and "site record" both indicate that the respective species was positively identified during a sampling event at a specific location.

Results

Bluebreast Darter

Pre–1981. Survey records document Bluebreast Darter in a limited number of drainages across Ohio and Pennsylvania (Fig. 3). In Ohio, the Great Miami, Scioto, and Muskingum river watersheds contained extant populations of Bluebreast Darter. We found a total of 106 records, 60 of which were from sites located within Big Darby Creek (Fig. 3; Osburn 1901, OSU-MBD database 2015, Trautman 1981).

In Pennsylvania, populations of Bluebreast Darter were confined to the upper reaches of the Allegheny River, French Creek, and the tributaries that form the Beaver River (Fig. 3). Nine of the 19 records were documented in French Creek

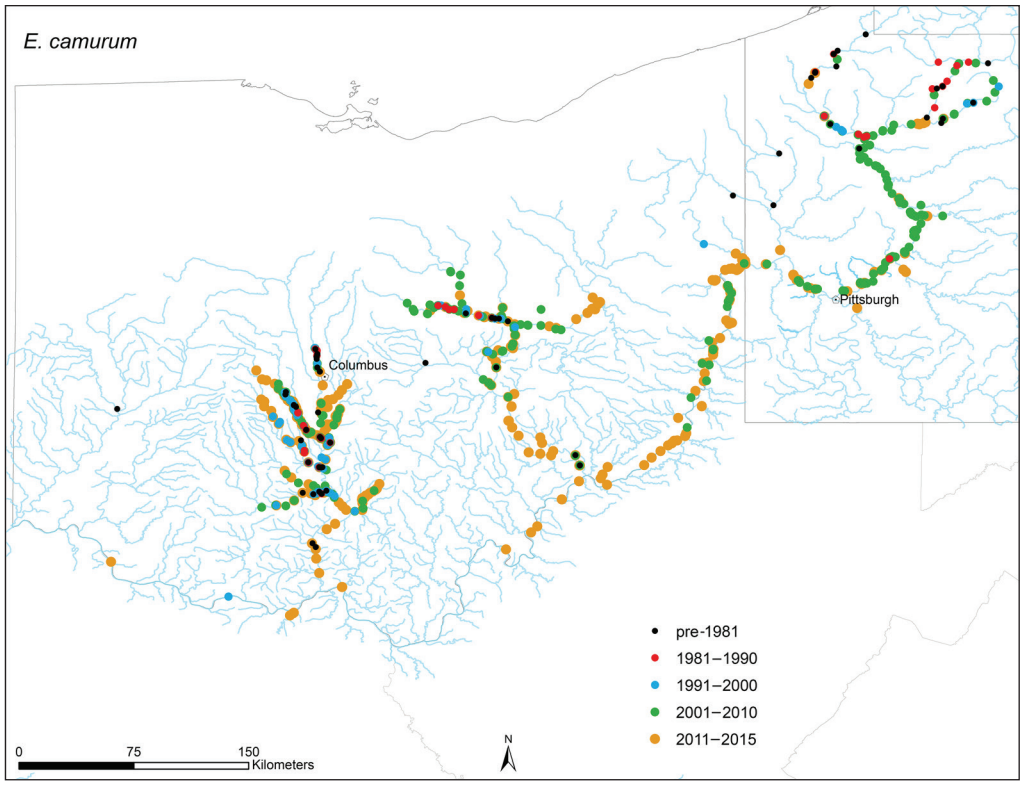

Figure 3. Distribution of Bluebreast Darter in the upper Ohio River system in Ohio and Pennsylvania showing all historic and contemporary data from pre-1981 to 2015.

(Pennsylvania Natural Heritage Program 2015, Raney 1938, Schwartz 1965); the remainder were located in the upper Allegheny River, Tionesta Creek, Little Coon Creek, and Sandy Creek (Pennsylvania Natural Heritage Program 2015, Raney 1938). Two additional locations in the upper Beaver River drainage were documented in the Shenango River and Neshannock Creek (Pennsylvania Natural Heritage Program 2015, Raney 1938).

1981–1990. Additional records for Bluebreast Darter between 1981 and 1990 showed minimal changes in distribution in Ohio and Pennsylvania. Within Ohio, 37 records showed an increased presence in Big Darby Creek, Deer Creek, Paint Creek, Kokosing, Walhonding, and Olentangy rivers (Fig. 3). During this time period, the OEPA greatly increased the amount of fish-sampling effort conducted across Ohio.

In Pennsylvania, records from the Pennsylvania Natural Heritage Program (2015) documented Bluebreast Darter at 14 sites—13 within French Creek and the upper Allegheny River above Tionesta (Fig. 3). Interestingly, 1 verified record in 1986 was collected in the Allegheny River below L/D 5, which was more than 144 rkm downstream from the nearest documented sites in the Allegheny River and French Creek at Franklin, PA (Fig. 3).

1991–2000. By 2000, a total of 93 additional records in Ohio began to show range expansion of Bluebreast Darter within 18 rivers and streams (Fig. 3). Substantial increases were documented within Big Darby Creek (16 sites), Deer Creek (18 sites), Paint Creek (11 sites), and the middle section of the Scioto River from the confluence of Big Darby Creek downstream to Indian Creek, where 26 records documented their presence (OSU-MBD database 2015). Furthermore, Bluebreast Darter was documented in 7 new Ohio tributary locations, including the Middle Fork Little Beaver Creek, Jelloway Creek, Mohican River, Salt Creek, Sugar Run, Tuscarawas River, Wakatamika Creek, and the first mainstem Ohio River site located near Manchester, OH (Fig. 3). In Pennsylvania, only 9 additional records were documented, all of them in the middle to upper reaches of Tionesta Creek, and the previously documented French Creek (Fig. 3).

2001–2010. By 2010, range expansion had become more apparent with 314 Bluebreast Darter records: 182 records in Ohio and 132 in Pennsylvania (Fig. 3).

The first upper Ohio River mainstem record along Ohio's border was documented in 2001 by the Ohio River Valley Water Sanitation Commission (ORSANCO) during boat electrofishing from the Hannibal Pool. Beginning in 2007, use of a modified mini-Missouri benthic trawl (Herzog et al. 2005) by ORSANCO documented 9 sites in the Ohio River from the Pike Island and Hannibal pools (OH) and 1 record in the Scioto River downstream in Chillicothe, OH (OSU-MBD 2015). Additional eastern Ohio range expansion was represented by collections in the Little Muskingum River; Short, Island, and Wheeling creeks; and the Ohio River at the Pike Island tailwater. Elsewhere in Ohio, despite little to no increase in sampling effort, continued surveys by OEPA documented range expansion within Salt Creek, Paint Creek, Walhonding River, and Muskingum River systems (Fig. 3).

Beginning in 2005, benthic trawls were also used to obtain Bluebreast Darter records in Pennsylvania. Of the 132 records, 76 were documented from benthic trawling, and of these, 69 sites were documented in the Allegheny River and 7 records were from the Ohio River below Pittsburgh (Freedman et al. 2009b, ORSANCO 2017). More-efficient sampling methods combined with traditional techniques revealed that Bluebreast Darter in Pennsylvania was present from the upper free-flowing sections of the Allegheny River to below the Montgomery L/D on the Ohio River (Fig. 3).

2011–present. A total of 451 Bluebreast Darter records have been documented in Ohio (367) and Pennsylvania (83) since 2011. In Ohio, 81 trawling records resulting from this study have expanded the known distribution of Bluebreast Darter in the Ohio River from the Pennsylvania state line, downstream to near Indian Creek just southeast of Cincinnati (Fig. 3). We also documented this species in multiple Ohio River tributaries upstream of Marietta, OH, including Yellow, Cross, McMahon, Wegee, Captina, and Sunfish creeks and Croxton Run. Further range documentation included Big Walnut Creek, Tuscarawas River, and progression down the Scioto and Muskingum rivers to near their confluences with the Ohio River mainstem (also documented by this study and continued efforts by OEPA).

Of the 83 records of Bluebreast Darter in Pennsylvania since 2011, 45 records were obtained by trawling with either a modified mini-Missouri trawl or an electrified Missouri trawl. Employing the electrified trawl in Pennsylvania during 2013–2014, we confirmed the presence of Bluebreast Darter in 2 new locations: (1) the lower Beaver River just below the first dam upstream from the confluence with the Ohio River, and (2) expansion of Bluebreast Darter into the lower Monongahela River just below Braddock L/D (Fig. 3). We documented a total of 4 new tributary site records—2 tributaries to the lower Allegheny River (Kiskiminetas River and Bull Creek) and 2 tributaries to the upper Ohio River (Moon Run and Montour Run) (Table 3, Fig. 3).

Tippecanoe Darter

Pre–1981. Prior to 1981, there were 27 records documenting the presence of Tippecanoe Darter in Ohio. All records were within the Scioto and Muskingum River drainages (Fig. 4), 20 of which were in Big Darby Creek (Osburn 1901, OSU-MBD 2015, Trautman 1981).

In Pennsylvania, 14 records for Tippecanoe Darter were documented from the upper reaches of the Allegheny River, including 12 records from French Creek and 2 records from the Allegheny River near Tidioute, PA (Cooper 1983, Pennsylvania Natural Heritage Program 2015, Raney 1938).

1981–1990. OEPA sampling effort increased dramatically in this time period, and by 1990, an additional 12 records of Tippecanoe Darter had been collected within the Scioto River drainage in Ohio. Big Darby Creek contained 10 of the 12 records (Fig. 4). Within Pennsylvania, 10 records for Tippecanoe Darter were recorded in the upper Allegheny River and French Creek (Fig. 4).

1991–2000. In Ohio, Tippecanoe Darter started to show signs of distribution changes towards the end of the decade, with a total of 50 site records. New records included Paint Creek (2 sites), and Little Darby Creek (4 sites) with expansion in

the Deer Creek system (6 sites) (Fig. 4). The middle reaches of the Scioto River from Walnut Creek downstream to Paint Creek contained 23 records, and sampling in Big Darby Creek documented the presence of Tippecanoe Darter with 15 records. French Creek was the only location within Pennsylvania, and an additional 9 records of Tippecanoe Darter had been documented by 2000 (Fig. 4).

2001–2010. A total of 100 Tippecanoe Darter records were documented in Ohio between 2001 and 2010. During this period, benthic trawling was added as a new sampling method for both Ohio and Pennsylvania. Of the 100 records for Ohio only 6 were obtained with trawling, but the trawling records documented Tippecanoe Darter in the Ohio River for the first time (Fig. 4, ORSANCO 2017). Other new locations included: Buckskin Creek, Little Beaver Creek (on the Ohio/Pennsylvania border), North Fork Paint Creek, Salt Creek, Walnut Creek, and Wheeling Creek (Table 2, Fig. 4; OEPA 2016, OSU-MBD 2015). Additional records showed an increased presence upstream in Paint Creek (8 records), and 5 records in the lower Muskingum River at the Lowell L/D tailwater (Fig. 4). Further known range expansion of Tippecanoe Darter was documented with 42 records on the Scioto River. A majority of the records from the Scioto River occurred in the reach from the Greenlawn Dam in Columbus, OH, downstream to Big Darby Creek, but 8 more records showed the movement of Tippecanoe Darter downstream to near Candy Run near Lucasville, OH, largely resulting from OEPA standard surveys (Fig. 4).

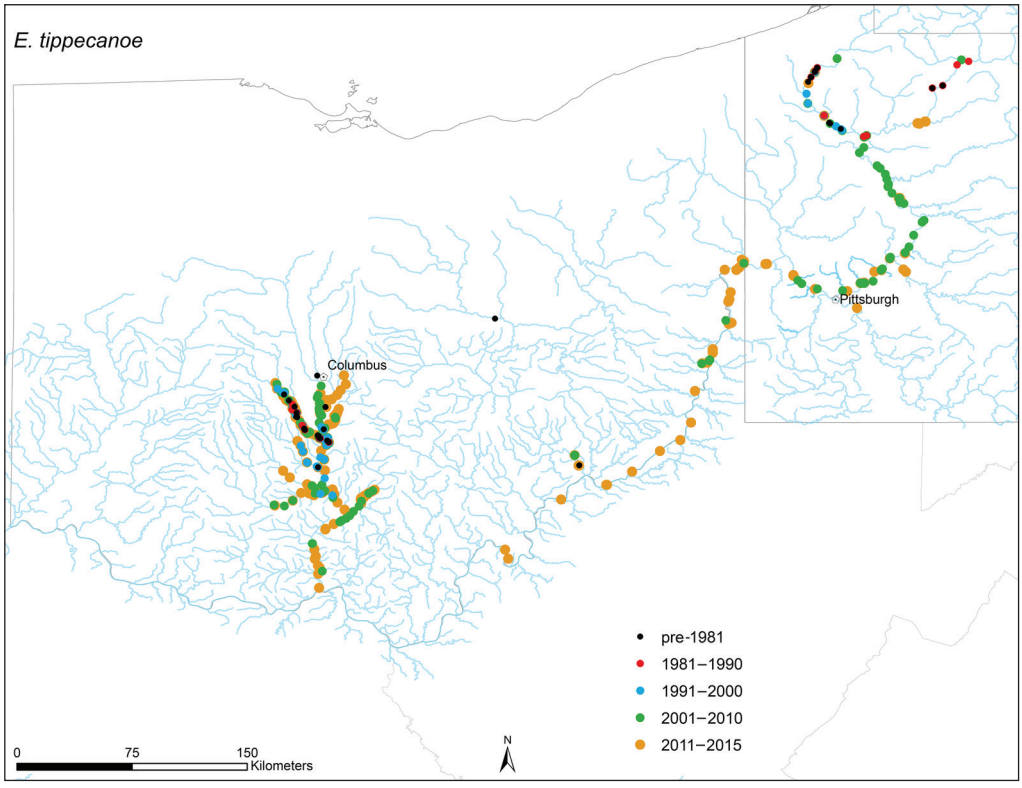

Figure 4. Distribution of Tippecanoe Darter in the upper Ohio River system in Ohio and Pennsylvania showing all historic and contemporary data from pre-1981 to 2015.

In comparison to Ohio, trawling in Pennsylvania accounted for 57% of the records of Tippecanoe Darter (40 out of 70). Trawling data combined with traditional sampling methods helped to document the Tippecanoe Darter's distribution from the free-flowing section of the Allegheny River downstream through the navigable reaches and into the Ohio River below the Dashields L/D. The Dashields record documented the Tippecanoe Darter ~224 rkm downstream from its previously recorded location near Franklin, PA (Fig. 4).

2011–present. Fish surveys in Ohio from 2011 to 2015 resulted in 189 records of Tippecanoe Darter (Fig. 4). Benthic trawling from this study resulted in 40 records, which documented Tippecanoe Darter in the Muskingum, Ohio, and Scioto rivers. Trawls also produced 32 records in the Ohio River, and extended the known range of the Tippecanoe Darter from the Pennsylvania/Ohio border downstream to the Racine L/D tailwater. Our sampling efforts also documented Tippecanoe Darter expansion into the lower portion of Cross Creek, a direct tributary to the Ohio River in eastern Ohio and in the North Fork of Paint Creek and the Scioto River to near its confluence with the Ohio River. Eight new records documented upstream movement past Osburn's 1897 site record on Big Walnut Creek to the confluence with the Rocky Fork (Fig. 4).

In Pennsylvania, there were 56 records for Tippecanoe Darter from 2011 to 2015. Benthic trawling accounted for 61% (34 out of 56) of the records and extended its known range in Pennsylvania with new site records at the tailwaters of the Montgomery L/D on the Ohio River, and up into the lower Monongahela River to the tailwaters of the Braddock L/D (Fig. 4). Additionally, benthic trawling confirmed the presence of Tippecanoe Darter in the tailwaters of the Allegheny River L/Ds 2, 3, 4, 5, and 6. Backpack electrofishing in the Kiskiminetas River and Bull Creek (Tarentum, PA.) produced new site records for Tippecanoe Darter within Pennsylvania (Fig. 4).

Spotted Darter

Pre–1981. Historic records of Spotted Darter in Ohio (total = 38) documented the species in 8 different waterbodies: Big Darby Creek, Big Walnut Creek, Deer Creek, Kokosing River, Mahoning River, Olentangy River, Walhonding River, and Yellow Creek (Mount 1959, Osburn 1901, OSU-MBD database 2015, Trautman 1981; Fig. 5). Twenty-three of these records were from Big Darby Creek.

Prior to 1981, there were 34 records for Spotted Darter in Pennsylvania from 5 streams or rivers: the upper Allegheny River, French Creek, Little Neshannock Creek, Otter Creek, and the Shenango River (Cooper 1983; PAFBC 2015; Pennsylvania Natural Heritage Program 2015; Raney 1938, Raney and Lachner 1939; Fig. 5).Twenty-five of the records were documented in French Creek.

1981–1990. In Ohio, 12 records were documented for Spotted Darter within previously identified locations. Big Darby Creek accounted for 11 of these records, 1 of which occurred upstream near the confluence of Little Darby Creek (Fig. 5). As previously mentioned, sampling effort greatly increased across Ohio in this time period. In Pennsylvania, 8 records for Spotted Darter represented 1 new site

in Sandy Creek (tributary to the upper Allegheny River), 5 in French Creek, and 2 in the Allegheny River near Tidioute, PA (Fig. 5).

1991–2000. There were 7 additional records for Spotted Darter in Ohio between 1991 and 2000. Two new sites were documented in the Scioto River just downstream of Big Darby Creek, and the others were in the Walhonding River and Big Darby Creek (Fig. 5). In Pennsylvania, French Creek contained all 6 Spotted Darter records. No new locations were documented.

2001–2010. Thirty Spotted Darter records were documented in Ohio. Several new site records were documented: near the mouth of Little Darby Creek and Paint Creek, and 4 records were from Walnut Creek just upstream of the confluence with Little Walnut Creek (Table 2, Fig. 5). Additional records documented Spotted Darter presence in the Kokosing River (9 records) and Big Darby Creek (15 records). Trawling did not produce any Spotted Darter records in Ohio.

Of the 42 Pennsylvania records, Spotted Darter was documented at 3 new sites: Woodcock Creek (tributary to French Creek), the mouth of Oil Creek (tributary to the Allegheny River), and the Ohio River just below Pittsburgh. The remainder of the records were within French Creek (11) and the Allegheny River (28). By 2007, the Spotted Darter was documented in the navigable reaches of the Allegheny River below L/D 3 (between the islands that make up Allegheny Islands State Park), and below the Dashields L/D, in the upper Ohio

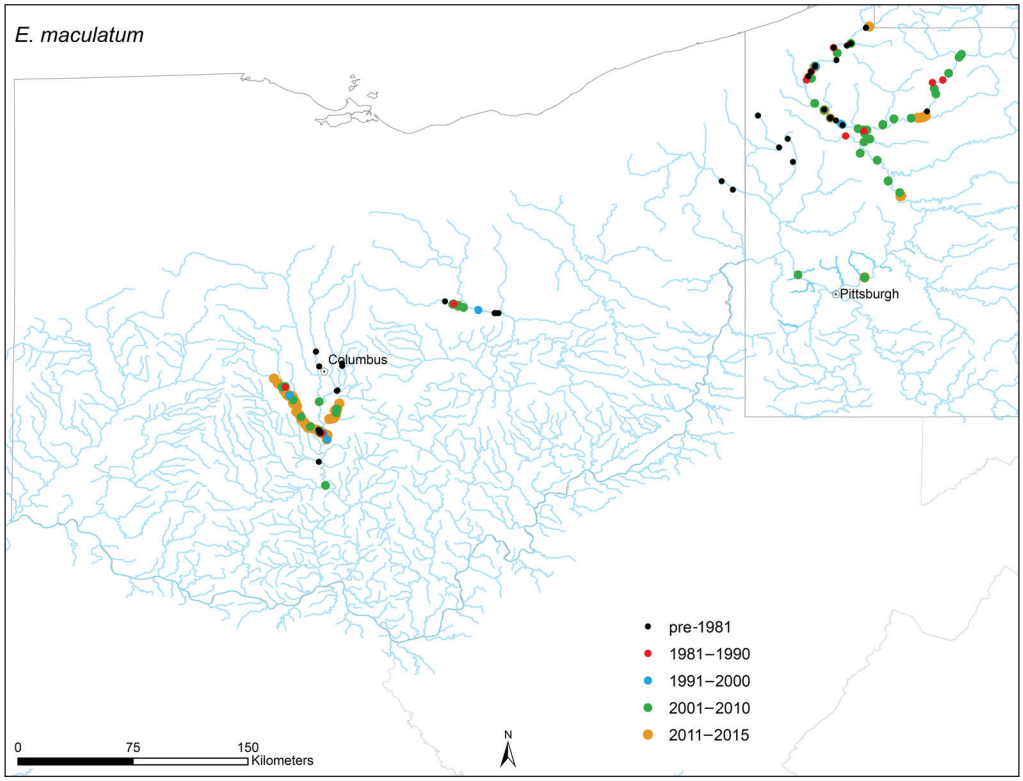

Figure 5. Distribution map for the Spotted Darter in the upper Ohio River system in Ohio and Pennsylvania showing all historic and contemporary data from pre-1981 to 2015.

River below Pittsburgh, PA (Fig. 5). Benthic trawling accounted for 19 of the records (18 in the Allegheny River and 1 in the Ohio River).

2011–present. Our seine sampling efforts helped to document 55 Spotted Darter records in Ohio. Range expansion, however, was minimal (Fig. 5). Additional sites showed slight movement up Little Darby Creek, but the majority of the records documented stable populations in the Kokosing River (3), a slight increase further upstream in Walnut Creek (12), and increased number of records in Big Darby Creek (34). The Spotted Darter has not been documented in the Ohio River within Ohio's borders and was never found during benthic trawling. To date, 21 additional records have documented the Spotted Darter within Pennsylvania. Fifteen of the records were within the Allegheny River and the 6 were in French Creek (Fig. 5). Benthic trawling documented 4 of the records within the Allegheny River.

Extirpations

Bluebreast Darter. There are 3 systems in Ohio where Bluebreast Darter appears to have been extirpated: (1) the Stillwater River (documented 1899), which is a tributary to the Great Miami River in western Ohio; (2) the North Fork of the Licking River (documented in 1899); and (3) Yellow Creek (documented in 1853), a tributary to the Mahoning River on the Ohio/Pennsylvania border (Fig. 3). In Pennsylvania, Bluebreast Darter appears to be extirpated from 2 upper tributaries of the Beaver River system in northwestern PA: (1) Neshannock Creek (documented in 1934), and (2) the Shenango River (documented in 1935) (Fig. 3).

Tippecanoe Darter. The current distribution of Tippecanoe Darter in Ohio illustrates 2 locations where they have been apparently unable to recolonize: (1) the Olentangy River (documented in 1896), which enters the Scioto River near Columbus; and (2) the Walhonding River (documented in 1962) which is in the upper Muskingum River system (Fig. 4). All historical locations for Tippecanoe Darter in Pennsylvania have extant populations.

Spotted Darter. Currently, the Spotted Darter appears to be extirpated from 3 systems in the Scioto River drainage including: (1) the Olentangy River (documented 1958, 1960, and 1963), (2) Big Walnut Creek (documented in 1897, 1959, and 1962), and (3) Deer Creek (documented in 1956) (Fig. 5). No recent surveys on the Ohio/Pennsylvania border have found the Spotted Darter in Yellow Creek (documented 1853) or the adjacent Mahoning River, which is the type locality (Kirtland 1840). The Spotted Darter has apparently not been able to reestablish populations in the Mahoning River since being presumed extirpated in the mid-1850s (Trautman 1981). In Pennsylvania, the Spotted Darter appears to be extirpated from the upper reaches of the Shenango River (documented 1905 and 1934) and Neshannock Creek (documented 1935) (Fig. 5).

Electrified benthic trawling

Electrified benthic trawling surveys below 11 L/D installations yielded varying results. We documented Bluebreast Darter below 8 installations: Allegheny River L/D 2, 3, 4, 5, and 6; Beaver River Dam 1; Monongahela L/D 2; and below the Emsworth back channel L/D on the Ohio River. We also found Tippecanoe Darter below

8 installations: Allegheny River L/D 2, 3, 4, 5, and 6; Monongahela River L/D 2; Ohio River Emsworth back channel; and the Montgomery L/D. We documented the Spotted Darter only below 1 installation: Allegheny River L/D 3.

Discussion

A large proportion of the contemporary survey records illustrate increases in the known ranges of darters of the subgenus *Nothonotus* into the non-wadeable riverine environments of the Allegheny, Ohio, and Monongahela rivers. Regional improvements in water quality that have resulted in improved fish assemblages (Yoder et al. 2005) may have influenced the distribution changes in these focal species. In addition, we suggest that recently developed and improved sampling techniques including the modified Missouri trawl (Herzog et al. 2005, 2009) and the PSU electrified benthic trawl (Freedman et al. 2009a) are responsible for elucidating these new records in the non-wadeable portions of the Allegheny, Monongahela, Muskingum, and upper Ohio rivers in depths >2.0 m where traditional methods can be less effective. In Ohio, the history of increased sampling that coincided with the inception of the OEPA surface-waters sampling program thoroughly documented an increase in distribution of *Nothonotus* darters and many other fish species as water quality improved (OEPA 2016, Yoder et al. 2005). Until recently, non-wadeable stream sampling in the basin was mainly limited to lock-chamber surveys (Thomas et al. 2005), boat electrofishing (Emery et al. 2003, Koryak et al. 2008), hoop/gill netting, beach seining, and various-sized mesh for trawling (Neebling and Quist 2011). Each method has valid applications, but they can also be biased towards certain species, body sizes (Neebling and Quist 2011), and aquatic habitats (e.g., pelagic fish vs. benthic fish). For example, Koryak et al. (2008) surveyed a navigable section of the Allegheny River with both night electrofishing and benthic trawling. Electrofishing resulted in 42 species (834 individuals), while benthic trawling documented 27 species (2903 individuals). Benthic trawling was more effective at collecting species in the family Percidae; electrofishing detected 4 species and trawling documented 12 (Koryak et al. 2008). The use of multiple sample gears to survey for large-river darters was also supported by Neebling and Quist (2011), who compared boat electrofishing, trawling, and shoreline bag-seining in non-wadeable rivers. Those authors surveyed 21 reaches from 3 to 5 km in length and found that 8 species were only detected by trawling and 4 of those species were darters. However, it is important to point out that, in Ohio, the OEPA has shown that boat electrofishing can be effective at detecting the presence of darter species by using an appropriate level of effort and detail within an electrofishing site (Yoder et al. 2005). It should be noted, though, that once depths are consistently > 2 m, effectiveness of this method is diminished. For all data from Ohio and Pennsylvania collected since 2005 and summarized in this study, trawling records accounted for 32% of all records of the 3 focal darter species. The number of trawling records since 2005 also varied by state—20% of the records in Ohio and 57% of the records in Pennsylvania were from trawling. Our surveys and analysis of historical records support previous assessments that concluded it is necessary to utilize benthic and/

or electrified benthic trawling to effectively survey non-wadeable riverine environments for benthic fishes (Freedman et al. 2009a, 2009b; Herzog et al. 2005, 2009; Koryak et al. 2008, 2011). We propose that benthic trawls are an effective

Table 2. Streams in Ohio that were sampled in the same location and the year *Nothonotus* appeared. Full references presented in Table 4. OEPA = Data queried from Ohio State University Museum of Biological Diversity, Fish Division database, analyzed by B.J. Zimmerman.

		Abundance			
Stream/site	Year	*E. camurum*	*E. maculatum*	*E. tippecanoe*	Reference
Middle Fork Salt Creek					
Site 1	1988	-	-	-	OEPA
	1997	-	-	-	OEPA
	2005	1	-	-	OEPA
Salt Creek					
Site 2	1992	-	-	-	OEPA
	2005	10	-	4	OEPA
Site 3	1984	-	-	-	OEPA
	1992	-	-	-	OEPA
	2005	15	-	27	OEPA
Site 4	1992	-	-	-	OEPA
	2005	-	-	3	OEPA
Scioto River					
Site 5	1997	-	-	-	OEPA
	2011	2	-	2	OEPA
Site 12	1979	-	-	-	OEPA
	1988	-	-	-	OEPA
	1992	-	-	-	OEPA
	2002	-	1	-	OEPA
Paint Creek					
Site 6	1992	-	-	-	OEPA
	1997	3	-	-	OEPA
	2006	6	-	2	OEPA
Site 7	1997	-	-	-	OEPA
	2006	6	-	1	OEPA
Site 8	1997	-	-	-	OEPA
	2006	3	-	1	OEPA
North Fork Paint Creek					
Site 9	1985	-	-	-	OEPA
	1997	-	-	-	OEPA
	2006	1	-	-	OEPA
Walnut Creek					
Site 10	1996	-	-	-	OEPA
	2010	72	3	14	OEPA
Site 11	1982	-	-	-	OEPA
	2005	21	2	1	OEPA
Killbuck Creek					
Site 13	1983	-	-	-	OEPA
	2009	1	-	-	OEPA

sampling method for small-bodied benthic species (e.g., darters) in conditions when depths are greater than >2.0 m and/or there is elevated turbidity.

Even though more-efficient sampling techniques may have elucidated the changes documented in the impounded reaches of the non-wadeable rivers, we also show evidence of range expansion of Bluebreast Darter, Tippecanoe Darter, and Spotted Darter into the unimpounded rivers and smaller tributaries. Surveys in multiple streams, with historic and contemporary samples using the same methodology, have recently documented new site records for these 3 darter species. In Ohio, there were at least 13 OEPA survey sites in 7 streams that have newly documented *Nothonotus* records (Table 2, Fig. 6). In Pennsylvania, 6 new records in 6 streams documented the recent expansion of *Nothonotus* species (Table 3, Fig. 7).

Since 2000, outside of the refugia areas of Big Darby Creek, OH, and French Creek, PA, the population sizes of Spotted Darter have been consistently lower than the other 2 focal species (see Supplemental File 2 available online at http://www.eaglehill.us/NENAonline/suppl-files/n24-2-N1537-Honick-s2, and, for BioOne subscribers, at http://dx.doi.org/10.1656/N1537.s2), which justifies continued monitoring. Previously, Lorson (2010) performed benthic trawling surveys of the Allegheny River from its headwaters to Pittsburgh, PA. Within the navigable section of the river, he only documented 1 Spotted Darter below 1 L/D

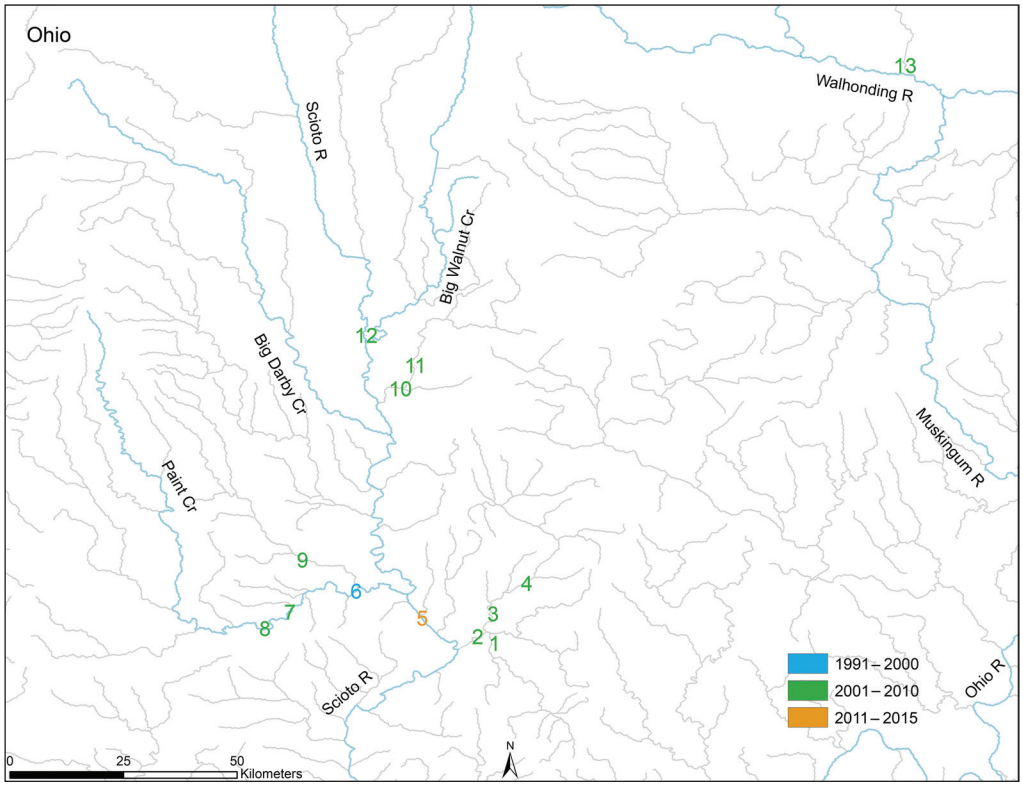

Figure 6. Sites in Ohio where *Nothonotus* species have only recently been documented after years of consistent sampling. Site numbers correspond to Table 2.

installation—the same pool where we documented them (pool 2 below L/D 3). Even within an 81-km reach of the free-flowing section of the upper Allegheny River, Argent and Kimmel (2014) only documented 4 Spotted Darters. In 2015, the Pennsylvania Fish and Boat Commission documented 11 individuals below L/D 3 on the Allegheny River, which suggests that there is a stable population at this location (Fig. 5). However, to our knowledge, documentation of the extension of Spotted Darters downstream into the Montgomery pool of the Ohio River (Freedman et al. 2009b) has not been duplicated, and additional surveys and cautious interpretation of the range extension of stable populations of the Spotted Darter within Pennsylvania are warranted.

Factors effecting Spotted Darter expansion

Habitat considerations and connectivity. The expansion of the known ranges of Bluebreast Darter and Tippecanoe Darter has been robust, but the Spotted Darter has been less successful at utilizing the navigable portions of the Allegheny, Monongahela, Muskingum, and Ohio rivers (Figs. 3, 4, and 5). Reasons for this lack

Table 3. Streams in PA that were sampled in the same location and the year *Nothonotus* appeared. ASH = data collected by A.S. Honick and B.A Porter. BAP = data collected by B.A Porter.

		Abundance			
Stream/site	Year	*E. camurum*	*E. maculatum*	*E. tippecanoe*	Reference
Little Sewickley Creek					
Site 14	2003	-	-	-	Koryak (2003)
	2006	-	-	-	MARIS (2016)
	2012	13	-	-	This study (BAP)
	2013	13	-	-	This study (ASH)
Montour Run					
Site 15	1982	-	-	-	USACE (1997)
	1991	-	-	-	USACE (1997)
	1996	-	-	-	USACE (1997)
	2003	-	-	-	Koryak (2003)
	2014	2	-	-	This study (ASH)
Moon Run					
Site 16	2003	-	-	-	Koryak (2003)
	2014	1	-	-	This study (ASH)
Pine Creek					
Site 17	2002	-	-	-	Hoskin et al. (2003)
	2005	1	-	1	Howell (2007)
Bull Creek					
Site 18	2006	-	-	-	MARIS (2016)
	2014	1	-	15	This study (ASH)
Kiskiminetas River					
Site 19	2009	-	-	-	This study (BAP)
	2010	-	-	-	This study (BAP)
	2011	-	-	-	This study (BAP)
	2013	-	-	4	This study (BAP)
	2013	10	-	25	This study (ASH)

of expansion may be directly related to the availability of optimal habitat. Historically, the Spotted Darter was considered an associate of the Bluebreast Darter and Tippecanoe Darter (Kuehne and Barbour 1983, Raney 1939), but was reported to occupy "deeper parts of riffles" that were often overlooked (Raney and Lachner 1939). In the Ohio River, along the border of Ohio, benthic trawling commonly documented Bluebreast Darters and Tippecanoe Darters in areas of moderate flow, including gravel outwashes near tributaries and the gravel/cobble habitat found up- and downstream of islands. However, benthic and electrified benthic trawling does not support the hypothesis that Spotted Darter is preferentially utilizing similar habitats in the navigable portions of the upper Ohio River watershed. Our electrified benthic trawling surveys below 11 L/D installations documented Bluebreast Darters and Tippecanoe Darters at 8 installations, and have revealed that these 2 species can occupy great depths; ranging from 1.4 m to 4.5 m and 1.4 m to 5.9 m, respectively. In contrast, the Spotted Darter was only found below 1 installation, within a wadeable riffle ~1.0 m deep. Raney and Lachner (1939) described Spotted Darters as occurring in deep, fast riffles and spawning at depths no greater than 0.6 m. Kessler and Thorp (1993) analyzed microhabitat use between the Spotted Darter and *Etheostoma bellum* Zorach (Orangefin Darter) in a tributary of the upper Green River, KY, and documented that Spotted Darters utilized deeper habitats (mean depth = 0.2 m) and were observed mostly under large rocks. Osier

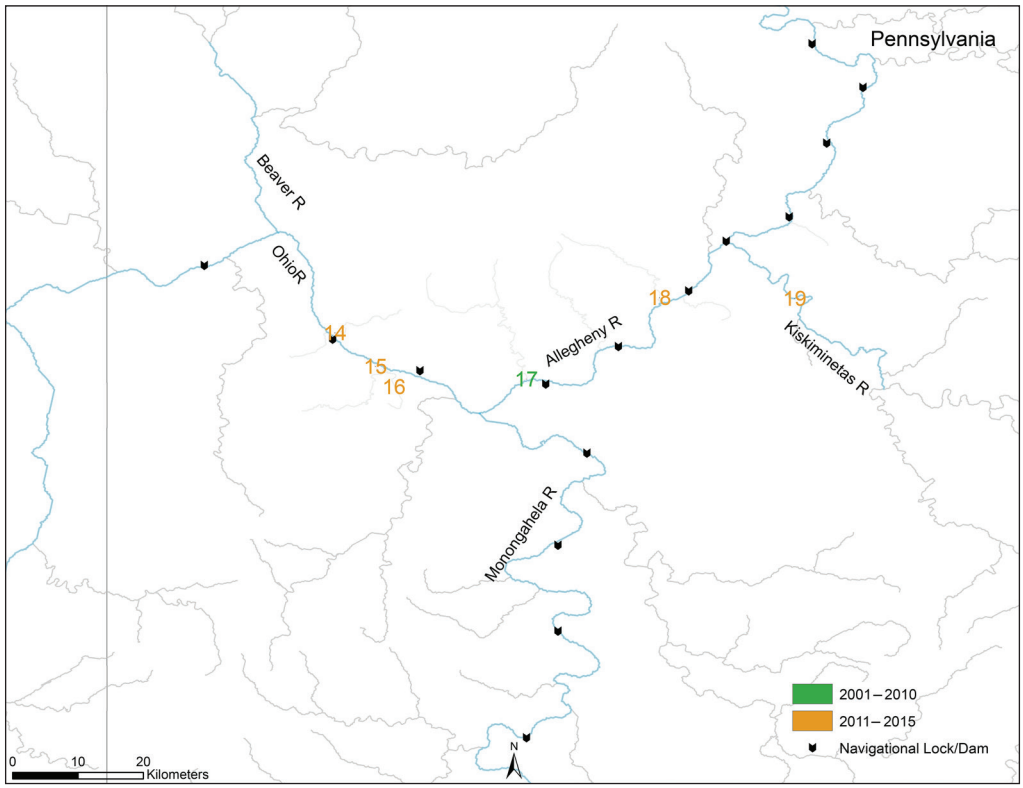

Figure 7. Sites in Pennsylvania where *Nothonotus* species have only recently been documented after years of consistent sampling. Site numbers correspond to Table 3.

and Welsh (2007) studied Spotted Darter habitat in the Elk River, WV, and found that they occurred with large rocks in the glide habitat between the riffles at depths ranging from 0.31 m to 0.49 m. These data suggest that the Spotted Darter is more of a habitat specialist and prefers deeper habitat. However, our data suggest that Spotted Darters may be restricted to shallower habitat within non-wadeable riverine environments, while Bluebreast Darter and Tippecanoe Darter may be benthic generalists that have the ability to utilize the more diverse and deeper habitat found within L/D tailwaters. The restricted expansion of Spotted Darters into the navigable portions of the Allegheny and Ohio rivers could be linked to current and historic dredging of the rivers for commercial aggregates and navigation requirements. Since 2004, the upper Ohio and lower Allegheny rivers have had over 13.6 million metric tons of substrate removed for commercial aggregates, and since the 1800s, it has been estimated that ~0.5 billion metric tons of substrate have been removed (R. Ventorini, Pennsylvania Fish and Boat Commission, Pittsburgh, PA, unpubl. data). Freedman et al. (2013) studied the navigable portion of the Allegheny River and found that dredging significantly changed the benthic fish community, reduced habitat heterogeneity, and shifted the fish assemblage towards habitat generalists. In summary, the Spotted Darter has a limited presence in the navigable portion of the Allegheny River and the upper Ohio River. We were only able to document this species below 1 L/D installation. Freedman et al. (2009b) documented 5 Spotted Darters below Dashields L/D, but that record hasn't been duplicated. Regardless of continued water quality improvements, the historic data compiled by Raney and Lachner (1939), Osburn (1901), and a report from the US Fish and Wildlife Service (2011) suggests that the Spotted Darter was likely never common throughout its range, which may be due to specific habitat requirements (Kessler and Thorp 1993, Osier and Welsh 2007, Raney and Lachner 1939). No surveys have documented Spotted Darters in the Ohio River downstream of the Pennsylvania state line; thus, we contend that the non-wadeable, impounded river environment may not have enough preferred habitat to support robust expansion of the Spotted Darter.

In addition to more-specific habitat requirements, Spotted Darter range extension may be negatively impacted by the Allegheny River's restricted connectivity between the upper free-flowing reaches and the now-lentic habitat of the navigable portion below L/D 9. Recently, Argent and Kimmel (2010) documented that fish community composition immediately above and below L/D installations were markedly different in both the Allegheny and Monongahela rivers. On the Monongahela River, the small-bodied fish assemblages consisted of 12 and 13 species above and below the installations with 2 darter species above and 5 species below. In contrast, in the Allegheny River, the small-bodied fish assemblages consisted of mostly darters, but only 2 darter species were documented above the installations, and 10 darter species were utilizing the tailrace habitat below the L/D installations. Regardless of the fact that the Monongahela River experiences higher lockage-frequency from more boat traffic, neither river indicated a correlation between small-bodied fish diversity and lockage frequency (Argent and Kimmel 2010). Therefore, Argent and Kimmel (2010) indicated that the physical restriction to fish movement posed by L/Ds may result in

isolated populations of darters within navigation pools. The navigational L/D system on the lower Allegheny River may be impeding the movement of Spotted Darters, which suggests that within the upper Ohio River watershed, the species needs to be closely monitored for proper conservation management.

Differences in reproductive strategies, spawning habitat requirements, and larval duration/transport. Field observations directly documenting fecundity and clutch sizes in Bluebreast, Tippecanoe, and Spotted Darters are sparse and are mostly from aquarium studies. Bluebreast Darter and Tippecanoe Darter have been documented as belonging to the egg-burying guild (Kelly et al. 2012, Stiles 1972). Field observations (Stiles 1972, Tiemann 2008) and aquarium experiments in the laboratory (Mount 1959, Page and Simon 1988, Warren et al. 1986) indicated that females of both species bury themselves into the gravel substrate while the males mount them and fertilize the eggs. Tiemann (2008) observed spawning behavior of Bluebreast Darter in the Vermilion River, IL, and documented that males stop defending their territories soon after spawning. Warren et al. (1986) collected Tippecanoe Darters from the Green River, KY, and in aquarium studies, showed that males established territories but quickly abandoned nests after spawning, just like Bluebreast Darters.

In contrast, under field and laboratory conditions, the Spotted Darter uses a different reproductive strategy and has different spawning habitat requirements. Raney and Lachner (1939), Winn (1958), and Stiles (1972) documented the Spotted Darter as belonging to the egg-clumper guild, in which females attach their eggs to the underside of large, flat rocks. They also observed that, in contrast to Bluebreast and Tippecanoe Darters, male Spotted Darters continue to defend their territory after spawning. Additionally, Raney and Lachner (1939) documented that regardless of the amount of suitable spawning habitat, Spotted Darter nests were spaced ≥1.2 m apart. Out of the 14 species of darters that Winn (1958) studied, the Spotted Darter was among the species laying the fewest eggs, and males provided substantial parental care. More recently, Ruble et al. (2016) studied reproductive behaviors of *Etheostoma wapiti* Etnier & J. D. Williams (Boulder Darter), *E. vulneratum* (Cope) (Wounded Darter), and Spotted Darter under laboratory conditions and found that Spotted Darter and Boulder Darter averaged fewer eggs per female and had lower egg-to-juvenile survival rates than Wounded Darter. Therefore, Spotted Darter exhibits characteristics of a K-selected species reproductive strategy, while Bluebreast and Tippecanoe Darters exhibit reproductive strategies more similar to r-selected species. This reproductive strategy and the lack of suitable spawning habitat featuring large unembedded cover stones or large boulders associated with swift currents are likely hindering population expansion by Spotted Darters. In contrast, the impounded portions of the Allegheny and Ohio rivers contain abundant gravel in areas with swift current to prevent siltation where Bluebreast and Tippecanoe Darters can bury their eggs.

Another potential reason for the differences in distribution changes among these 3 species may be linked to temporal variation in pelagic larval duration (PLD) and larval transport. Douglas et al. (2013) studied PLD in 23 darter species and Turner (2001) examined larval transport of 8 darters. Both reports found that PLD and

larval-transport times were highly variable across species of darters. Douglas et al. (2013) documented darter PLDs ranging from 0 to 60 days, with Spotted Darter exhibiting an average PLD of only 18 days. Of the 23 species studied, 12 were listed as imperiled (Douglas et al. 1013) and had PLD averages varying from 9 to 15 days. Short PLDs suggest that the species may have evolved that way to reduce downstream movement in attempts to stay within restricted habitats (Douglas et al. 2013), but reduced dispersal may essentially lead to isolated populations with small ranges (Sorte 2013), which is the pattern observed in Spotted Darter. The shorter PLDs of Spotted Darter relative to Bluebreast and Tippecanoe Darters may also have allowed the latter 2 species to re-establish in the larger rivers after water quality improvements in a shorter amount of time than Spotted Darter.

Summary/Conclusions

Populations of darters classified in the subgenus *Nothonotus* in the upper Ohio River system have historically been described as having disjunct distributions (Cooper 1983, Kuehne and Barbour 1983, Page 1983, Simon and Wallus 2006, Trautman 1981). Our surveys and analysis of ~1700 historic and contemporary survey records revealed major distribution changes for these darters in the upper Ohio River watershed. In Pennsylvania, all 3 species were listed as threatened in 1999 (Pennsylvania Bulletin 1999), and in Ohio, Bluebreast and Tippecanoe Darters were listed as threatened in 1990 and Spotted Darter was listed as endangered in 1974 (15 Ohio Rev. Code § 1531.25 - 2015). In Pennsylvania, assessment of recent survey data led the Pennsylvania Fish and Boat Commission to delist all 3 species in 2014 (Pennsylvania Bulletin 2014). Extensive surveys in Ohio from 2006 to 2012 led to the delisting of Bluebreast Darter in 2012 (ODNR 2012, OSU-MBD 2015) while the Tippecanoe and Spotted Darters maintained their threatened and endangered status, respectively. Our analysis showed that Spotted Darter was less common, had a smaller geographic range, and fewer individuals per sample site compared to Bluebreast and Tippecanoe Darters, which may be related to life-history characteristics, a lack of optimal habitat, and impaired connectivity throughout the navigable portions of the upper Ohio River watershed. Therefore, the stable Spotted Darter source populations should be closely monitored.

Based on previous observations, it is conceivable that the Spotted Darter is not expanding its distribution as effectively because (1) Bluebreast and Tippecanoe Darters employ an r-selected reproductive strategy, while the Spotted Darter displays a K-selected reproductive strategy (Ruble et al. 2016); (2) the Spotted Darter may require larger areas of suitable spawning habitat as a result of maintaining territoriality and nest defense, potentially producing fewer offspring per unit of available habitat; (3) Spotted Darter has been documented as having a short PLD that may be limiting their distance or rate of dispersal; and (4) the navigational L/D system may be restricting movement of Spotted Darters between the free-flowing sections of the upper Allegheny River and the navigable portions of the upper Ohio River watershed.

We were able to collect enough samples of Bluebreast Darter to investigate genetic structure of these populations. The results of our ongoing analysis will provide insight into metapopulation structure and dynamics and reveal if impaired river-connectivity has resulted in many genetically isolated populations within the navigable sections of the rivers. These data will facilitate development of management strategies that emphasize conservation efforts toward maintaining genetically diverse source populations compared to smaller, genetically depauperate, and ephemeral sink populations. In addition, efforts are underway in Ohio to reintroduce all 3 darter species back into historic locations where barriers have prohibited natural recolonization (B. Zimmerman, The Ohio State University, Columbus, OH, unpubl. data).

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