Comparison of fish community within the Blackwater River watershed before and after establishment of Northern Snakehead *Channa argus*

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Summary

Northern Snakehead is an invasive species initially discovered in the Potomac River in 2004, but has since spread to most major river systems of the Chesapeake Bay. In 2012, Northern Snakehead was first reported from the Blackwater River drainage on the eastern shore of Maryland. Fish community surveys were conducted in Blackwater River and Little Blackwater River in 2006 and 2007, before the establishment of Northern Snakehead there. Because of minimal habitat changes owed to protection by Blackwater National Wildlife Refuge, this dataset enabled us to document changes in the fish community that could be attributed to the establishment of Northern Snakehead. We replicated the 2006 and 2007 surveys (pre-Snakehead) over a year from 2018-2019 (post-Snakehead). Over all sampling periods we caught 35 species (32 fish species and 3 invertebrate species) totaling over 50,000 individuals. Of 21 species that were captured both pre- and post-Snakehead, 17 declined in relative abundance with percent reductions ranging from 30%-97%. We found that five of six sites had significantly different fish communities when comparing pre-Snakehead and post-Snakehead surveys. The main difference in fish communities was a reduction in overall biomass of most fish. Species dominance during the post-Snakehead period was significantly higher for both Blackwater and Little Blackwater River. Pre-Snakehead surveys were more evenly distributed and dominated by White Perch, Black Crappie, and Brown Bullhead, while post-Snakehead surveys were less even and dominated by Common Carp and Gizzard Shad. This study is the first to document major shifts in a fish community following establishment of Northern Snakehead. Further investigation into ongoing fish community changes and continued vigilance in minimizing spread and population growth of Northern Snakehead is warranted.

Background

Throughout the world, introduced fishes have been a significant source of concern where they become established (Cucherousset and Olden 2011). The focus of research for introduced species often includes investigation into control and management methods, determining current negative impacts to invaded ecosystems, and modeling potential future community and/or species changes due to the invader (Simberloff et al. 2013). Effects of introduced fishes can vary from minimal to extreme, such as causing extirpation or extinction of native species. Not all introductions lead to negative consequences (Cucherousset and Olden 2011; Martin and Valentine 2019), but generalized impacts can include: reductions in abundance of native fauna, loss of biodiversity (Gurevitch and Padilla 2004), or alteration of trophic dynamics (Britton et al. 2010, Gallardo et al. 2016, Kramer et al. 2019). As invasive species become abundant in an area, they could potentially use resources otherwise available to native fishes, which can reduce biomass and growth of native fish (Carey and Wahl 2010, Hughes and Herlihy 2012, Kramer et al. 2019). Experimentally, Carey and Wahl (2010) showed that increasing density of invasive carp reduced native fish growth. In addition to declines in biomass owed to reduced growth, declines in biomass can occur when the number of individuals declines via predation. Invasive lionfishes (*Pterois volitans* and *P. miles*) in the Western Atlantic have been shown to cause declines in prey fish biomass and/or their competitors for those prey (Green et al. 2012, Albins 2013). Such changes in biodiversity and biomass contribute to biotic homogenization, which could reduce resiliency of the ecosystem to natural or human disturbances and significantly alter the evolutionary trajectory of native species within a community (Olden et al. 2004; Petsch 2016). As introduced fishes expand their range and become more abundant, research investigating community level impacts have become of greater and broader need.

Northern Snakehead (*Channa argus*) is a freshwater fish native to parts of Asia and Russia (Courtenay and Williams 2004). It was first reported from the Potomac River in 2004 (Odenkirk and Owens 2005) and has since spread to nearly every major tributary of the Chesapeake Bay (Fuller et al. 2019). Its introduction and subsequent spread has caused concern among state and federal agencies about its impact to native ecosystems. To date, most studies have focused on potential competition with sportfish in Potomac River (Saylor et al. 2012, Love et al. 2015) and modeling potential ecological consequences (Love and Newhard 2012). No empirical research has addressed actual population or community level changes in response to Northern Snakehead establishment. In addition, the research that has been done has primarily focused on the population within the Potomac River watershed due to Northern Snakehead's initial establishment and population growth there (Odenkirk and Owens 2005, 2007, Odenkirk and Chapman 2019). Because climatic factors and natural ability have catalyzed the spread of Northern Snakehead beyond Potomac River and into the Chesapeake Bay watershed (Love and Newhard 2018), there is a need to continue research beyond Potomac River and into other ecosystems that differ in habitat type.

Northern Snakehead was first reported from the Blackwater River drainage (eastern Chesapeake Bay watershed) in 2012 (Fuller et al. 2019). Reports of Northern Snakehead in the area coincide with their expansion in the eastern Chesapeake Bay watershed from illegal introductions (King and Johnson 2011, Wegleitner et al. 2016) in Delaware, where the species was first observed in 2010 (unpublished observation, C. Martin, Delaware Department of Natural Resources and Environmental Control). Abundance of Northern Snakehead in Blackwater National Wildlife Refuge (BNWR) and Dorchester County has apparently increased dramatically since Northern Snakehead was first established and has led to a popular recreational fishery

(Ciekot 2019) that has been supported by state agencies (Love and Genovese 2019). The rapid increase in abundance of Northern Snakehead in eastern shore rivers somewhat mimics its increase in abundance following establishment in the Potomac River (Odenkirk and Owens 2007), where a recreational and commercial fishery also now exists (Newhard et al. 2019).

Prior to the introduction, establishment and rapid population growth of Northern Snakehead on the eastern shore of Maryland, fish community surveys in the Blackwater River drainage were conducted to inventory fish communities from various habitats that differed in salinity and position in the stream gradient (Love et al. 2008; Newhard et al. 2012). When long-term monitoring data is unavailable, comparison of current community data with historical data is a recommended strategy (Gallien and Carboni 2017). Because of minimal habitat changes owed to protection by BNWR, this dataset enabled us to document changes in the fish community that could be attributed primarily to the establishment of Northern Snakehead. Our objective for this study was to compare the fish community before and after Northern Snakehead introduction for sites in Blackwater River and Little Blackwater River.

Methods

Fish collection

We sampled three sites each within Blackwater River and Little Blackwater River (Fig. 1), areas that were inventoried before Northern Snakehead was introduced into the Blackwater River drainage (Love et al. 2008, Newhard et al. 2012). Similar to our reference study (Love et al. 2008), sites were sampled monthly from June 2018 to May 2019 (hereafter, post-Snakehead). Prior data were available for most sites for two years, in 2006 and 2007 (hereafter, pre-Snakehead), with the exception being Buttons Creek where data were only collected in 2007. Analyzing data for these two years afforded the opportunity to examine annual variability in community structure. Not all sites could be sampled during winter due to icing of the river, and no sites were sampled during January 2019 due to a federal government shutdown. At each site, fyke nets (1.25 cm mesh, 15.2 m lead line) were set for approximately 24 hours. Net set and pull times were recorded to standardize catch by hours fished (i.e., catch per unit effort or CPUE). Upon retrieving the nets, all fish were identified to species and individually counted. Any Northern Snakeheads captured were measured for total length and placed on ice for later dissection. Water quality was measured at each sampling event using a YSI Pro 2030. Water quality variables included: temperature (°C), dissolved oxygen (DO; mg/L), salinity (psu) and ambient conductivity (µs).

Analysis

We calculated CPUE for each species by dividing monthly abundances by number of net hours fished. We used monthly CPUE as a measure for species' relative abundances. Because biologists had wanted to identify specific times for habitat use by anadromous fish, surveys were conducted weekly during spring in 2006 and 2007. To help facilitate comparisons, we averaged CPUE across weeks for each month in spring 2006 and 2007 when multiple weeks within a month were sampled. In addition to relative abundance, we also analyzed the frequency of occurrence among months for captured species. The number of months when a species was present at a site was divided by the total number of months sampled (i.e., 12) to calculate the percentage of months the species was present both pre-Snakehead and post-Snakehead for all sites within the Blackwater River and Little Blackwater River.

To summarize and analyze data for relative abundances of all species caught within a site over time, we used non-metric multidimensional scaling (NMS). Each sample of species' relative abundances for a site was assigned a site score using NMS. Site scores were calculated using Sörenson (Bray-Curtis) distances that measured dissimilarity in ranked, relative abundances of species among monthly surveys. The NMS analysis is a multidimensional ordination analysis that ranks and places site scores on k-axes in order to reduce stress of the final k-dimensional configuration (McCune and Grace 2002). Final stress values ranging between 10-20 are common with community data and are generally considered acceptable (McCune and Grace 2002). We used 250 runs of our data and 250 Monte Carlo simulations to assess the significance of obtaining an equal or lesser stress value than our observed final stress value. The final k-dimensional configuration was used to determine whether site scores produced pre-Snakehead differed from those produced post-Snakehead.

We learned which species were responsible for changes in aquatic communities between periods by conducting a Spearman's rank correlation analysis on axis site scores and total monthly relative abundance of species at a site for: all fish; ubiquitous sunfish species [Black Crappie (*Pomoxis nigromaculatus*), Bluegill (*Lepomis macrochirus*), Pumpkinseed (*L. gibbosus*)]; White Perch (*Morone americana*); Northern Snakehead; Gizzard Shad (*Dorosoma cepedianum*); Common Carp (*Cyprinus carpio*); and Brown Bullhead (*Ameiurus nebulosus*). We also calculated Shannon-Weiner diversity indices for each site and monthly sample to determine if changes in NMS scores were correlated with changes in species diversity. Because water quality differences between periods may also influence community structure, environmental data (water temperature, dissolved oxygen, and salinity) were correlated with site scores for all fish using Spearman's rank correlations. Owed to relatively weak correlations (<0.50) of the variables and site scores within a year, environmental variables were not considered important predictors of changes in community structure. Using results from the reduced species list, we interpreted axes produced from each site-specific NMS.

We tested the hypothesis that aquatic communities significantly differed between pre-Snakehead and post-Snakehead periods using a multi-response permutation procedure (MRPP). The MRPP is a non-parametric analysis method designed to test the null hypothesis of no differences between communities of species (McCune and Grace 2002). The level of difference in between communities was computed using Sörenson distance. The analysis provided a test statistic (T), a measure of "effect size" (A), and a p-value. The T is the difference between observed and expected within-group distances among scores, divided by the square-root in variance of within-group distances. The A was measured by the chance-corrected within-group agreement, which describes within group homogeneity compared to a random expectation. The random expectation was determined by permuting the matrix of relative abundance and assigning each to either a pre-Snakehead or post-Snakehead group, then determining the mean distance in scores for each group. Species-specific monthly CPUE data were used to compare fish assemblages pre-Snakehead to post-Snakehead. All NMS and MRPP analyses were conducted using PC-ORD (Version 7.07, McCune and Mefford 2018). Spearman's Rank correlations were conducted using SYSTAT for Windows (Version 13.0, SYSTAT Software Inc.).

We used rarefaction to compare two measures of species diversity, species richness (i.e., the number of different species) and dominance (i.e., the fraction represented by the most common species), between pre-Snakehead and post-Snakehead periods for Blackwater River and Little Blackwater River. Because measurements of species diversity can increase with increasing abundance in the sample, we used rarefaction to compare species diversity at the lowest, common abundance (Sanders 1968; Hurlbert 1971). Individuals were drawn at random from each community of organisms until reaching a user-specified number of individuals (i.e., lowest, common abundance). Diversity measures were then computed for that level of abundance. This process was iterated 1000 times in order to generate a mean and variance for each diversity metric. When iterations were finished we compared metrics for the least abundant community to the 95% confidence interval generated for more abundant communities to determine if diversity significantly differed between the communities. Rarefaction was performed using EcoSim (Version 7.0; Gotelli and Entsminger 2001).

Results

Across all survey years we captured 35 species (32 fish species and 3 invertebrate species; Table 1) totaling 51,781 individuals. Prior to the introduction of Northern Snakehead, White Perch, Brown Bullhead, and Black Crappie were the most abundant species (in rank order). In fact, the species that were 90% of annual catch, were the same between 2006 and 2007 (White Perch, Brown Bullhead, Black Crappie, Pumpkinseed, and Bluegill). The rank order of dominant species also did not change between 2006 and 2007, indicating a fairly stable assemblage of aquatic organisms between years. Furthermore, site scores generated for those two years overlapped in distribution for most sites surveyed here (Figure 2), with a notable exception at LB1. Change in White Perch abundance is likely the driver of differences at LB1 between 2006 and 2007, where abundances dropped from 3,296 individuals in 2006 to 486 in 2007. After the establishment of Northern Snakehead, the three most abundant species (in rank order) were Common Carp, Gizzard Shad, and White Perch. Additionally, there were nine species captured in the pre-Snakehead period that were not captured in the post-Snakehead period, while there were 5 species captured post-Snakehead that were not captured pre-Snakehead (Table 1). Water quality conditions were generally similar between the two, though salinity was generally lower for 2018 and 2019 then it was for 2006 or 2007, especially for Blackwater River and Buttons Creek (Table 2).

During post-Snakehead surveys we captured 125 Northern Snakehead in fyke nets (Table 1). They were encountered at the same rate (45% of months sampled; Table 4) in both Blackwater River and Little Blackwater River, though they were more abundant in Little Blackwater River (N=110) than Blackwater River (N=15). Total length range of all Northern Snakehead captured was 121-680 mm (Mean=351, SD ±78). Of 31individuals that were dissected for gut contents, 77% had empty stomachs. Prey items from remaining stomachs included (in rank order): Gizzard Shad, Bluegill, American Eel (*Anguilla rostrata*), dragonfly larvae, and an unidentifiable fish species.

In general, relative abundances of most species were lower than they were pre-Snakehead (Table 3). Of all 35 species observed pre-Snakehead, 26 of them either had lower relative abundances or were not collected. The largest declines in relative abundance were observed for White Perch, Brown Bullhead, Atlantic Silverside (*Menidia menidia*), and Black Crappie. Of the 21 species that were captured in both pre-Snakehead and post-Snakehead periods, 17 species exhibited declines in average relative abundance. Percent declines in relative abundance of those species ranged from 30% to 97% (Average=58%, SD±20). There were 8 species that increased in relative abundance post-Snakehead, with only 3 of those being captured in both survey periods (Atlantic Menhaden (*Brevoortia tyrannus*), Gizzard Shad, and Common Carp). Common Carp had the largest increase in relative abundance (286%), followed by Atlantic Menhaden (78%), Bay Anchovy (*Anchoa mitchilli*) (72%), and Gizzard Shad (53%). These changes in relative abundance resulted in a modest change in the species that were 90% of the annual catch during 2018-2019. These species were Common Carp, Gizzard Shad, White Perch, Pumpkinseed, Black Crappie, and Bluegill.

Killifishes were less frequently collected post-Snakehead than pre-Snakehead (Table 4). During the pre-Snakehead period, three species of killifish had been collected: Banded Killifish (*Fundulus diaphanus*), Mummichog (*F. heteroclitus*), and Striped Killifish (*F. majalis*). Two of these species were not collected post-Snakehead and Mummichog was only collected in Little Blackwater River with a frequency that declined from 16.7% of surveys to 9.1% of surveys. The frequencies in collecting most other species were similar between pre-Snakehead and post-Snakehead periods. In some cases, the prevalence of species increased. In Blackwater River, for example, the prevalence of diadromous fish such as river herring (*Alosa* spp.), Yellow Perch (*Perca flavescens)*, and American Eel increased by at least 11%. However, this was not true for Little Blackwater River where prevalence for these species decreased by at least 22%.

Examination of assemblages at each site using NMS were generally summarized by two-dimensional solutions and had final stress values within acceptable range (12.0-16.6, Table 5). For Buttons Creek (site BC), a three dimensional solution was preferred, but we opted to only interpret axes 1 and 2 to ease interpretation. Final stress for the two-dimensional solution was still within acceptable range (Table 5). While there was some separation of monthly site scores along axes 1 and 2 for Buttons Creek, there was only a marginally significant difference in fish communities before and after Northern Snakehead introduction ($p = 0.06$; $A = 0.052$; Table 5). Buttons Creek scores for NMS axis 1 increased from pre-Snakehead to post-Snakehead and were positively correlated to sunfish and White Perch abundance (Table 6, Fig. 2). The relative abundance of sunfish and White Perch increased over time in Buttons Creek. While NMS axis 2 for Buttons Creek was most correlated to total fish abundance, no strong patterns in score values were evident for this axis.

Fish communities at all other sites significantly differed between pre-Snakehead and post-Snakehead periods (Table 5). Differences in fish communities were largely driven by differences in overall abundance and changes in abundance of dominant species. The NMS Axis 1 scores were strongly correlated to overall abundance (Table 6, Fig. 2), with the exception of BC. Sites sampled during the post-Snakehead period had species with lower overall abundances compared to the pre-Snakehead period. The NMS Axis 2 scores were differently correlated with species relative abundances among sites, but were generally associated with changes in abundance of dominant species. The NMS Axis 2 scores for BW1 and BW2 were most strongly correlated to monthly relative abundance of White Perch. At LB1, NMS Axis 2 correlated to relative abundances of White Perch and Gizzard Shad, while axis 2 scores for LB2 were correlated to relative abundance of White Perch and Brown Bullhead. The strongest correlation for axis 2 scores at LB3 was for Shannon-Weiner diversity index (Table 6). At BW1, BW2, LB1, and LB2, relative abundances of dominant species were significantly lower after establishment of Northern Snakehead. And at LB3, diversity increased after establishment of Northern Snakehead.

After standardizing sample size to a common abundance via rarefaction, we learned that species richness for Blackwater River was significantly higher before the introduction of Northern Snakehead (Fig. 3). Species richness pre-Snakehead averaged 26.7 (95% CI: 25 – 27), which decreased to 21 post-Snakehead (lowest common abundance = 4,602). However, there was no significant difference in species richness for Little Blackwater River following Northern Snakehead establishment (Fig. 3). The number of species post-Snakehead (23) at the lowest common abundance of 3,313, was not statistically different than the number of species estimated for the pre-Snakehead period (average = 22.0, 95% CI: 19 – 25). Species dominance was significantly higher for the post-snakehead period for both rivers, partially owed to dominance by Common Carp and Gizzard Shad. Blackwater River had the largest change in species dominance, which increased from 0.38 (95% CI: 0.38 – 0.39) during the pre-Snakehead period to 0.60 (lowest common abundance = 4,602) during the post-Snakehead period. A similar, but less dramatic increase in dominance was observed for the fish community in Little Blackwater River. Dominance increased from 0.28 (95% CI: 0.20 – 0.29) during the pre-Snakehead period to 0.32 (lowest common abundance = 3,313) during the post-Snakehead period.

Discussion

We found significant changes in aquatic community structure for fish and invertebrate fauna in the Blackwater River drainage since the introduction and establishment of Northern Snakehead. These changes were evidenced by both significant differences in ranked abundance and relative abundance for multiple species, with differences leading to measurable differences in fundamental attributes of species diversity. These differences can be explained by the introduction of a top predator (Northern Snakehead) and the installation of a water control structure and fresher water. Major reductions occurred for the overall abundance of several fish species after Northern Snakehead was introduced and became abundant. There was also a reduction in abundance of dominant species, and a shift in the dominant species that make up the fish community. Surveys completed in 2006 and 2007, before Northern Snakehead were known to inhabit waters on the eastern shore of Maryland, were dominated by an abundance of White Perch, Brown Bullhead, and a few sunfish species (F. Centrarchidae). Additional species, such as Banded Killifish, had been frequently caught before snakeheads were introduced, but were not observed in 2018 and 2019, suggesting a much reduced relative abundance and/or distribution. Banded Killifish, in addition to sunfish and perch, is a principal prey item of Northern

Snakeheads in Potomac River (Saylor et al. 2012, Isel and Odenkirk 2019, Lapointe et al. 2019). While not found in guts during our study, killifish species (*Fundulus*) were the numerically dominant prey item in Northern Snakehead guts sampled from Little Blackwater River and nearby rivers on the eastern shore of Maryland (J. Thompson, Maryland Department of Natural Resources, unpublished data). The loss of these prey species could be in part due to presence of an additional top predator like Northern Snakehead.

In general, results of our surveys are similar to other assessments of community level impacts of aquatic invasive species. One of the most pronounced differences we observed was in the reduction in abundance of many fish and invertebrate species. Invasive fishes can cause reductions in biomass where they become established (Albins 2013, Carey and Wahl 2010, Gallardo et al. 2016). The observed reductions in relative abundance of most species is likely why we observed a significant difference in species dominance for both Blackwater and Little Blackwater Rivers. Reductions in abundances and loss of native species can lead to biotic homogenization of fish communities (Rahel 2007). Introduced top-predators (such as Northern Snakehead) have been shown to be drivers of such homogenization and may facilitate successful future invasions of other species (Daga et al. 2015, Gallien and Carboni 2016). Bezerra et al. (2019) demonstrate that introduced Largemouth Bass (a top-predator) and African Cichlids (omnivore-detritivore) in neotropical lakes and reservoirs caused trophic downgrading and biotic homogenization of fish communities. While it is too early to determine if such effects are occurring in Blackwater River and Little Blackwater River, similarities exist with the establishment of Northern Snakehead alongside an apparently abundant population of Common Carp, an introduced detritivore. We observed fewer species post-Snakehead, along with significantly reduced biomass of most fish, at minimum suggesting homogenization may be occurring.

There was a significant difference in species richness for Blackwater River, but this may not indicate extirpation of the species from the drainage. One potential reason species richness was lower during the post-Snakehead period is the lower salinities observed in Blackwater River, likely a result of a water control structure. There was a loss of some euryhaline species (e.g., Spot (*Leiostomus xanthurus*); Atlantic Silverside (*Menidia menidia*)) in Blackwater River in 2018-2019. The highest recorded salinity in Blackwater River in 2006 or 2007 was 14.7 psu, while the maximum salinity observed was 6.2 psu during 2018-2019. The U.S. Fish and Wildlife Service completed a water control structure in upper portions of the Blackwater River in 2007 for the purpose of limiting saltwater intrusion into the watershed (Love et al. 2008). There was also significant rainfall during much of 2017 and 2018, which likely led to reduced salinities in Blackwater River. The fresher water may explain the increased prevalence of diadromous fishes in Blackwater River. Because similarly greater prevalence was not observed in Little Blackwater River, it is possible that the observed increase in Blackwater River is not due to increased precipitation but is instead the result of the water control structure.

In addition to a loss of euryhaline species, there was also a decline in freshwater-dependent species in Blackwater River, despite the greater availability of freshwater. Unlike during the pre-Snakehead period, we did not collect Largemouth Bass, Redfin and Chain Pickerel (*Esox americanus* and *E. niger*), or Channel Catfish (*Ictalurus punctatus*). These species were not numerically abundant in surveys completed prior to Northern Snakehead introduction, suggesting some gear avoidance or low catchability relative to other species. However, based on percent occurrence data, we determined that these species were encountered occasionally during the pre-Snakehead period and it is unknown why these species were not collected during the post-Snakehead period. Anglers reportedly caught Largemouth Bass and Channel Catfish in Blackwater River drainage in 2018-2019. It is possible that these species currently have a lower relative abundance or a different distribution than a decade ago.

Species dominance significantly differed between survey periods. Comparison of the fish community before Northern Snakehead were present showed high abundances (>1,000 individuals) of several species captured within a year, indicating a more even assemblage of fishes. However, surveys conducted after Northern Snakehead introduction showed that fewer species had high abundances (Common Carp, Gizzard Shad, and White Perch) along with much lower catches of some previously abundant species, such as Brown Bullhead, Black Crappie, and Bluegill. The reductions in abundance of these species likely explains lower evenness and greater dominance. Invasive fishes have been shown to reduce abundances of smaller bodied native fishes whether through competition, direct predation, or habitat alteration (Albins 2013, Pelicice et al. 2015, Kramer et al. 2019). The potential alteration of the fish community within the Blackwater River drainage warrants further investigation. If native fish diversity changes or is already low, an invasive fish may have greater success within an ecosystem (Carey and Wahl 2010).

This study compared two time periods that bracketed an intense community disturbance, the establishment of Northern Snakehead, in Blackwater River drainage. Currently Blackwater River drainage's fish community is dominated by Common Carp and Gizzard Shad. While White Perch and centrarchids remained relatively abundant in the post-Snakehead period, their biomass was significantly lower than during the pre-Snakehead period. Because there was no control or reference system for this analysis, we acknowledge that other factors may have caused changes in abundance and species diversity. Two common ecological factors that could affect aquatic communities include habitat changes and natural, interannual fluctuations in abundance of individual species. Our study periods were separated by over a decade, when habitat conditions and land use changes in the watershed could have altered the fish fauna. However, with the exception of the aforementioned water control structure, habitat conditions have remained largely unchanged during the period between surveys. Land use was relatively unchanged for Dorchester County between 2002 and 2010 (Dorchester County 2019). A significant portion of the Blackwater River watershed is owned and managed by the U.S. Government and no new land was developed. Additionally, the watershed of the Little Blackwater appears to be largely unchanged with no new significant changes over the last decade (M. Whitbeck, Supervisory Wildlife Biologist, BNWR, personal comment). Also, two sites on the upper Little Blackwater River (LB1, LB2) had significantly different fish communities over time, and water quality was not different in these mainly freshwater habitats. With the exception of salinity, habitat measurements (dissolved oxygen and temperature) recorded during the post-Snakehead period were generally not different from the pre-Snakehead period.

Changes in habitat conditions other than water quality and land use can affect abundance of species. de Mutsert et al. (2017) monitored the fish community in a Potomac tributary for over 30 years, which included several years with an established Northern Snakehead population. Banded Killifish increased in abundance over time and the increase was attributed to an increase of submerged aquatic vegetation (SAV) over time in their study area. Because SAV is not abundant in Blackwater and Little Blackwater River (J. Newhard pers. obs.; Orth et al. 2018), the growth of SAV is unlikely to influence the abundance of aquatic species in Blackwater River. Though their study was not designed to study the effects of Northern Snakehead, de Mutsert et al. (2017) also observed decreases over time for White Perch and Bluegill, as well as an increase in Gizzard Shad. Moreover, their results show an increase in species dominance over time, especially during the period with Northern Snakehead presence (Figure 6 in de Mutsert et al. (2017)). Differences in their study cannot necessarily be attributed Northern Snakehead alone, as habitat changed, and other introduced species such as Blue Catfish (*Ictalurus furcatus*) increased in abundance during that time. In another study, Isel and Odenkirk (2019) found that relative abundance of Bluegill (another common prey item of Northern Snakehead) was unchanged in one recently invaded Virginia lake with Northern Snakehead. Of critical importance when comparing results of studies is referencing density of Northern Snakehead and length of time since establishment of Northern Snakehead, as well as the resiliency of the aquatic community. Our study and the studies referenced here demonstrate that impacts to fish communities where Northern Snakehead are established may vary, especially as aquatic communities respond to biotic and anthropogenic factors alongside establishment of an invasive species.

Fish community changes observed in our study could be due to other processes not measured. For example, nutrient availability within an ecosystem can alter community dynamics separately from, or in conjunction with invasive species (Preston et al. 2018). There are also other introduced species present in Blackwater River drainage that can alter their environment. Common Carp are considered "ecosystem engineers" that alter native habitats through consumption of aquatic vegetation which can influence primary production, water clarity and nutrient cycling, among others (Carey and Wahl 2010, Cucherousset and Olden 2011). Common Carp were present in 2006 and 2007 surveys at consistent levels (7th most abundant in both years) and were the most abundant species in 2018-2019 surveys. While the reason for the increase in relative abundance of Common Carp is not known, it could be explained by a change in commercial fishing pressure in Blackwater River drainage whereby 5-times the number of carp were harvested between 2006 and 2008 than 2016 and 2018 (unpublished data, Maryland Department of Natural Resources). The addition of Northern Snakehead to the fish fauna as well as Common Carp could have additive effects that cause reductions in abundance and/or species diversity (Didham et al. 2007).

Continued monitoring of the fish community in Blackwater River and Little Blackwater River is warranted, especially if Northern Snakehead abundance continues to increase. Our results support well-established research that new and abundant aquatic predators can cause changes in aquatic communities, particularly via predation. They also further support risk assessments (Courtenay and Williams 2004) that Northern Snakehead is an invasive species that should be controlled. Management of Northern Snakehead should be a top priority to slow population growth in Blackwater and Little Blackwater Rivers, as they are important nursery habitats for resident and migratory fish species (Love et al. 2008, Newhard et al. 2012). Freshwater nursery habitats within the drainage have already been reduced in size due to sea level rise (Rogers and McCarty 2000) and may continue with climate change, which can also increase the spread of aquatic invasive species (Rahel and Olden 2008). Control options for Northern Snakehead are somewhat limited, especially in Blackwater and Little Blackwater River where electrofishing (one of the main control methods in many locations) is difficult, thus limiting managers to traditional fish collection methods such as nets and traps which are not as efficient at capturing Northern Snakehead. Additional control mechanisms could include recreational and commercial fishing operations, though these fishing sectors likely need to remove a significant portion of the population to cause noticeable declines (Newhard et al. 2019).

There are benefits to controlling the biomass and spread of invasive species. Reducing biomass of an invasive species can cause increases in biomass of native fish fauna (Abekura et al. 2004). Removal rates should be sufficiently high in order to see desirable outcomes (Kramer et al. 2019). While not measured here, the current fishing mortality in areas of the Potomac River did not cause significant declines in populations of Northern Snakehead (Newhard et al. 2019). However, modeling work has shown that reduction of Northern Snakehead adults can lead to reduced reproduction and potentially smaller populations (Hoff and Odenkirk 2019). Efforts to increase mortality of Northern Snakehead will likely need to be taken within Blackwater and Little Blackwater River, where the fishery is relatively new and fishing mortality is therefore assumed to be low. Such effort can be limited by public access and public apathy, thereby requiring effort from public agencies to achieve any established biomass reduction targets. Successful efforts may not only increase biomass of native fish fauna, but could also buffer against ecosystem changes and limit impacts of other invasive species (Carey and Wahl 2010).

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Table 1. List of all species captured at sites within Blackwater and Little Blackwater Rivers from 2006, 2007, and 2018-2019. Species with superscript “A” were captured during surveys before snakehead introduction (2006 & 2007) but not after (2018-2019). Species with superscript “B” were captured post-snakehead introduction but not before.

Common Name	Scientific Name	2006	2007	2018-2019
American Eel	*Anguilla rostrata*	88	41	33
Atlantic Menhaden	*Brevoortia tyrannus*	8	- -	31
Atlantic Silverside	*Menidia menidia*	115	66	2
Banded Killifish[A]	*Fundulus diaphanus*	169	3	- -
Bay Anchovy	*Anchoa mitchilli*	- -	1	2
Black Crappie	*Pomoxis nigromaculatus*	3,329	2,665	750
Blue Crab	*Callinectes sapidus*	370	404	105
Bluegill	*Lepomis macrochirus*	2,127	1,955	450
Bluespotted Sunfish[B]	*Enneacanthus gloriosus*	- -	- -	1
Brown Bullhead	*Ameiurus nebulosus*	4,365	3,776	282
Chain Pickerel[A]	*Esox niger*	- -	1	- -
Channel Catfish[A]	*Ictalurus punctatus*	55	1	- -
Common Carp	*Cyprinus carpio*	812	681	2,900
Crayfish spp.[A]		5	4	- -
Eastern Mudminnow	*Umbra pygmaea*	2	6	1
Gizzard Shad	*Dorosoma cepedianum*	891	978	1,171
Golden Shiner	*Notemigonus crysoleucas*	21	17	1
Hogchoker	*Trinectes maculatus*	5	53	3
Inland Silverside	*Menidia beryllina*	9	- -	1
Largemouth Bass[A]	*Micropterus salmoides*	14	19	- -
Longear Sunfish[A]	*Lepomis megalotis*	21	- -	- -
Mud Crab spp.[B]		- -	- -	1
Mummichog	*Fundulus heteroclitus*	2	38	1
Northern Snakehead[B]	*Channa argus*	- -	- -	125
Pumpkinseed	*Lepomis gibbosus*	3,227	1,310	774
Redbreast Sunfish[B]	*Lepomis auritus*	- -	- -	4
Redfin Pickerel[A]	*Esox americanus*	12	4	- -
River Herring	*Alosa aestivalis/pseudoharengus*	20	10	7
Silver Perch[B]	*Bairdiella chrysoura*	- -	- -	2
Spot[A]	*Leiostomus xanthurus*	2	6	- -
Striped Bass	*Morone saxatilis*	4	- -	1
Striped Killifish[A]	*Fundulus majalis*	10	65	- -
White Catfish	*Ameiurus catus*	26	139	6
White Perch	*Morone americana*	11,538	4,548	1,039
Yellow Perch	*Perca flavescens*	27	7	16

Table 2. Summary of yearly water quality measurements from all sites sampled within Blackwater River and Little Blackwater River. Measurements below indicate average (min-max) and include: water temperature (°C), dissolved oxygen (DO; mg/L), and salinity (psu).

Site	Year	Water Temp.	DO	Salinity
BC	2007	19.7 (4.5-30.1)	8.8 (4.5-13.8)	5.1 (0.1-11.3)
	2018-2019	17.2 (3.9-29.1)	8.0 (4.7-11.2)	0.84 (0.1-3.1)
BW1	2006	18.3 (5.7-25.0)	7.9 (4.0-13.7)	11.8 (7.8-14.3)
	2007	19.2 (7.1-28.5)	7.5 (4.1-12.5)	4.8 (2.6-12.9)
	2018-2019	18.2 (4.5-28.9)	7.4 (4.2-11.6)	2.9 (0.8-6.2)
BW2	2006	18.0 (3.7-31.2)	7.8 (2.6-13.5)	9.0 (3.4-14.7)
	2007	17.6 (4.0-29.3)	6.4 (3.4-10.5)	3.0 (0.8-9.2)
	2018-2019	17.0 (3.1-29.1)	6.6 (2.2-11.2)	1.5 (0.6-3.9)
LB1	2006	16.1 (5.2-29.3)	6.5 (0.4-11.8)	0.2 (0.04-0.7)
	2007	16.4 (1.9-28.7)	6.1 (1.6-9.5)	0.5 (0.0-3.5)
	2018-2019	16.8 (5.6-26.4)	5.4 (0.5-10.4)	0.1 (0.0-0.1)
LB2	2006	19.3 (6.6-29.2)	6.7 (1.8-11.0)	0.7 (0.1-1.5)
	2007	17.3 (3.4-29.3)	7.3 (3.4-13.8)	0.9 (0.0-5.2)
	2018-2019	18.5 (6.6-28.6)	5.7 (0.7-10.0)	0.1 (0.0-0.1)
LB3	2006	16.9 (7.2-27.4)	9.6 (5.7-13.4)	5.0 (0.4-9.5)
	2007	17.4 (2.3-30.2)	9.0 (6.1-13.0)	4.0 (0.1-13.2)
	2018-2019	18.3 (3.8-31.6)	9.1 (6.5-11.2)	1.0 (0.2-3.3)

Table 3. Monthly catch per-unit-effort (fish per hour) for all species captured at all sites sampled before Northern Snakehead introduction (2006 and 2007) compared to after Northern Snakehead introduction (2018-2019).

Species	Pre	Post	Difference
American Eel	0.15	0.08	-0.07
Atlantic Menhaden	0.18	0.32	0.14
Atlantic Silverside	1.41	0.04	-1.37
Banded Killifish	0.81		-0.81
Bay Anchovy	0.05	0.08	0.03
Black Crappie	1.68	0.63	-1.05
Blue Crab	0.43	0.24	-0.19
Bluegill	1.18	0.4	-0.78
Bluespotted Sunfish		0.05	0.05
Brown Bullhead	2.27	0.35	-1.92
Chain Pickerel	0.05		-0.05
Channel Catfish	0.15		-0.15
Common Carp	0.62	2.39	1.77
Crayfish	0.08		-0.08
Eastern Mudminnow	0.12	0.04	-0.08
Gizzard Shad	0.81	1.24	0.43
Golden Shiner	0.11	0.04	-0.07
Hogchoker	0.16	0.07	-0.09
Inland Silverside	0.1	0.04	-0.06
Largemouth Bass	0.05		-0.05
Longear Sunfish	0.15		-0.15
Mud Crab		0.04	0.04
Mummichog	0.32	0.04	-0.28
Northern Snakehead		0.24	0.24
Pumpkinseed	1.51	0.99	-0.52
Redbreast Sunfish		0.06	0.06
Redfin Pickerel	0.1		-0.1
River Herring	0.08	0.05	-0.03
Silver Perch		0.08	0.08
Spot	0.05		-0.05
Striped Bass	0.06	0.04	-0.02
Striped Killifish	1.02		-1.02
White Catfish	0.43	0.04	-0.39
White Perch	4	0.86	-3.14
Yellow Perch	0.09	0.06	-0.03

Table 4. Monthly percent frequency of occurrence of species captured in fyke nets from Blackwater and Little Blackwater Rivers before Northern Snakehead introduction (2006 and 2007) and after Northern Snakehead introduction (2018-2019).

	Blackwater		Little Blackwater	
Species	Before	After	Before	After
American Eel	0.500	0.636	0.833	0.455
Atlantic Menhaden	0.167	0.182	0.000	0.091
Atlantic Silverside	0.250	0.000	0.167	0.182
Banded Killifish	0.250	0.000	0.083	0.000
Bay Anchovy	--	--	0.083	0.091
Black Crappie	0.917	1.000	1.000	1.000
Blue Crab	0.750	0.818	0.750	0.545
Bluegill	0.917	0.909	1.000	1.000
Bluespotted Sunfish	0.000	0.091	--	--
Brown Bullhead	0.917	0.727	1.000	0.909
Chain Pickerel	0.083	0.000	--	--
Channel Catfish	0.083	0.000	0.667	0.000
Common Carp	0.667	1.000	1.000	1.000
Crayfish	--	--	0.333	0.000
Eastern Mudminnow	--	--	0.250	0.091
Gizzard Shad	0.583	0.818	1.000	1.000
Golden Shiner	0.250	0.091	0.583	0.000
Hogchoker	0.417	0.091	0.167	0.091
Inland Silverside	0.167	0.091	0.083	0.000
Largemouth Bass	0.500	0.000	0.750	0.000
Longear Sunfish	0.250	0.000	0.167	0.000
Mud Crab	--	--	0.000	0.091
Mummichog	0.250	0.000	0.167	0.091
Northern Snakehead	0.000	0.455	0.000	0.455
Pumpkinseed	0.917	1.000	1.000	0.727
Redbreast Sunfish	0.000	0.182	--	--
Redfin Pickerel	0.167	0.000	0.333	0.000
River Herring	0.167	0.273	0.500	0.273
Silver Perch	--	--	0.000	0.091
Spot	0.250	0.000	0.250	0.000
Striped Bass	0.083	0.000	0.167	0.091
Striped Killifish	0.167	0.000	0.083	0.000
White Catfish	0.167	0.182	0.583	0.364
White Perch	1.000	1.000	1.000	1.000
Yellow Perch	0.167	0.545	0.667	0.273

Table 5. Stress values from non-metric multidimensional scaling analysis (NMS Stress) and multi-response permutation procedure results (MRPP A) from comparison of fish communities before (2006 and 2007) and after (2018-2019) Northern Snakehead introduction at all sites in Blackwater River and Little Blackwater River.

Site	NMS Stress	MRPP A	p-value
BC	15.8	0.052	0.065
BW1	10.6	0.152	0.000
BW2	13.3	0.042	0.043
LB1	12.0	0.058	0.013
LB2	13.3	0.061	0.020
LB3	16.6	0.050	0.013

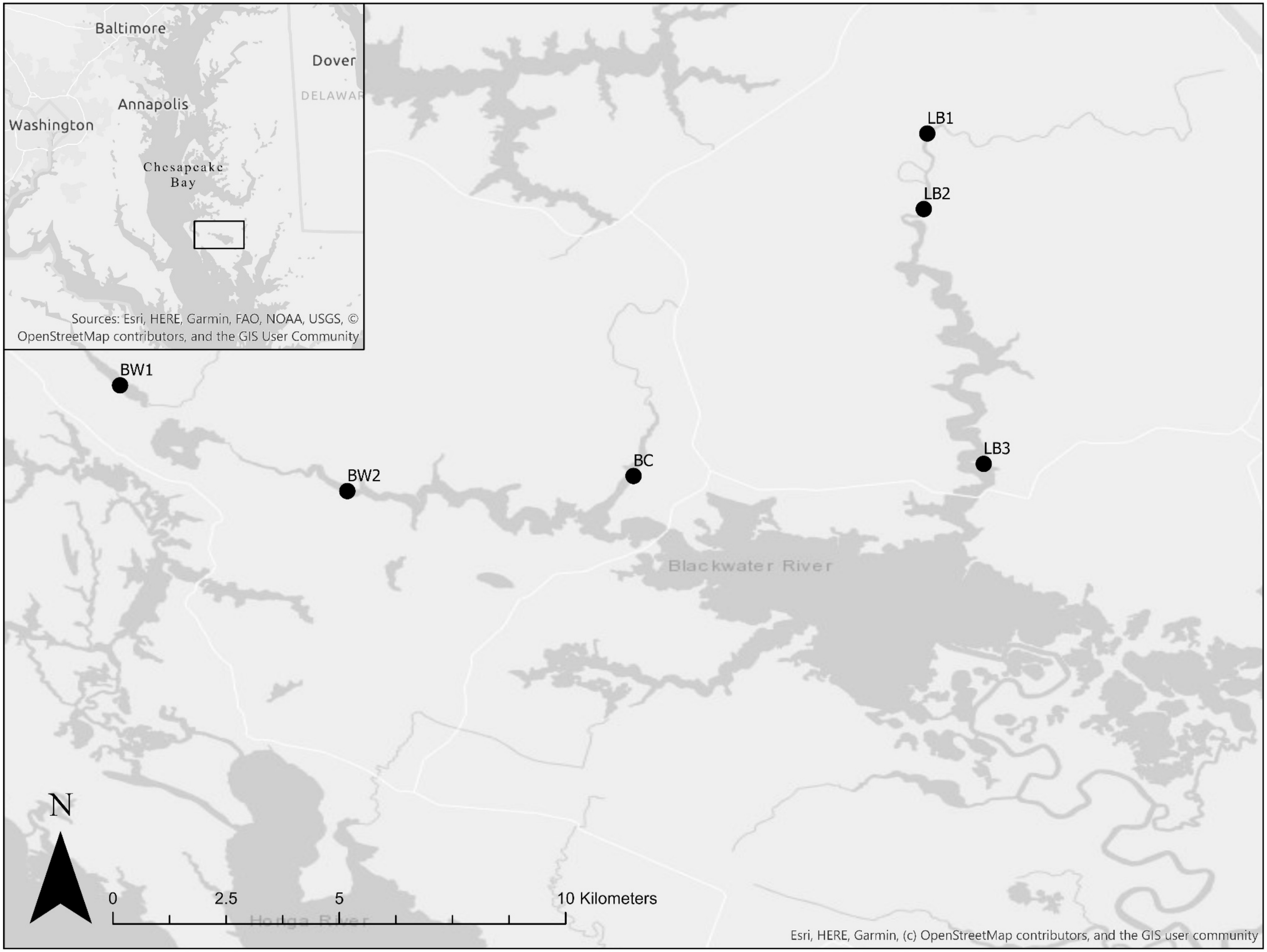
Table 6. Spearman's Rank correlations for non-metric multidimensional scaling (NMS) axis scores with total monthly fish abundance (ALL), Shannon-Weiner diversity index (H'), and monthly abundances for White Perch (WP), Northern Snakehead (NSH), Gizzard Shad (GS), Common Carp (CC), all Centrarchidae spp. (SUN), and Brown Bullhead (BB).

NMS Axis	Site	ALL	H'	WP	NSH	GS	CC	SUN	BB
1	BC	0.748	0.039	0.54	-0.022	-0.238	-0.454	0.925	0.34
	BW1	0.665	0.265	0.059	0.525	0.539	0.253	0.915	0.25
	BW2	-0.978	0.164	-0.822	0.151	-0.184	-0.041	-0.784	-0.432
	LB1	0.957	-0.25	0.416	-0.215	0.046	-0.43	0.918	0.704
	LB2	-0.938	0.41	-0.309	0.092	-0.005	-0.031	-0.927	-0.165
	LB3	-0.885	0.222	-0.93	0.15	0	0.177	-0.602	-0.469
2	BC	0.553	0.185	0.424	0.32	0.336	0.253	0.257	0.302
	BW1	-0.465	0.162	-0.888	0.391	0.055	0.205	0.248	-0.299
	BW2	0.018	0.037	0.479	-0.312	0.05	-0.34	-0.254	0.368
	LB1	-0.185	-0.259	-0.639	0.337	-0.736	-0.348	0.252	-0.415
	LB2	-0.27	0.154	-0.654	0.248	-0.456	-0.493	0.234	-0.824
	LB3	-0.038	0.538	-0.191	-0.132	-0.251	0.187	0.193	0.452

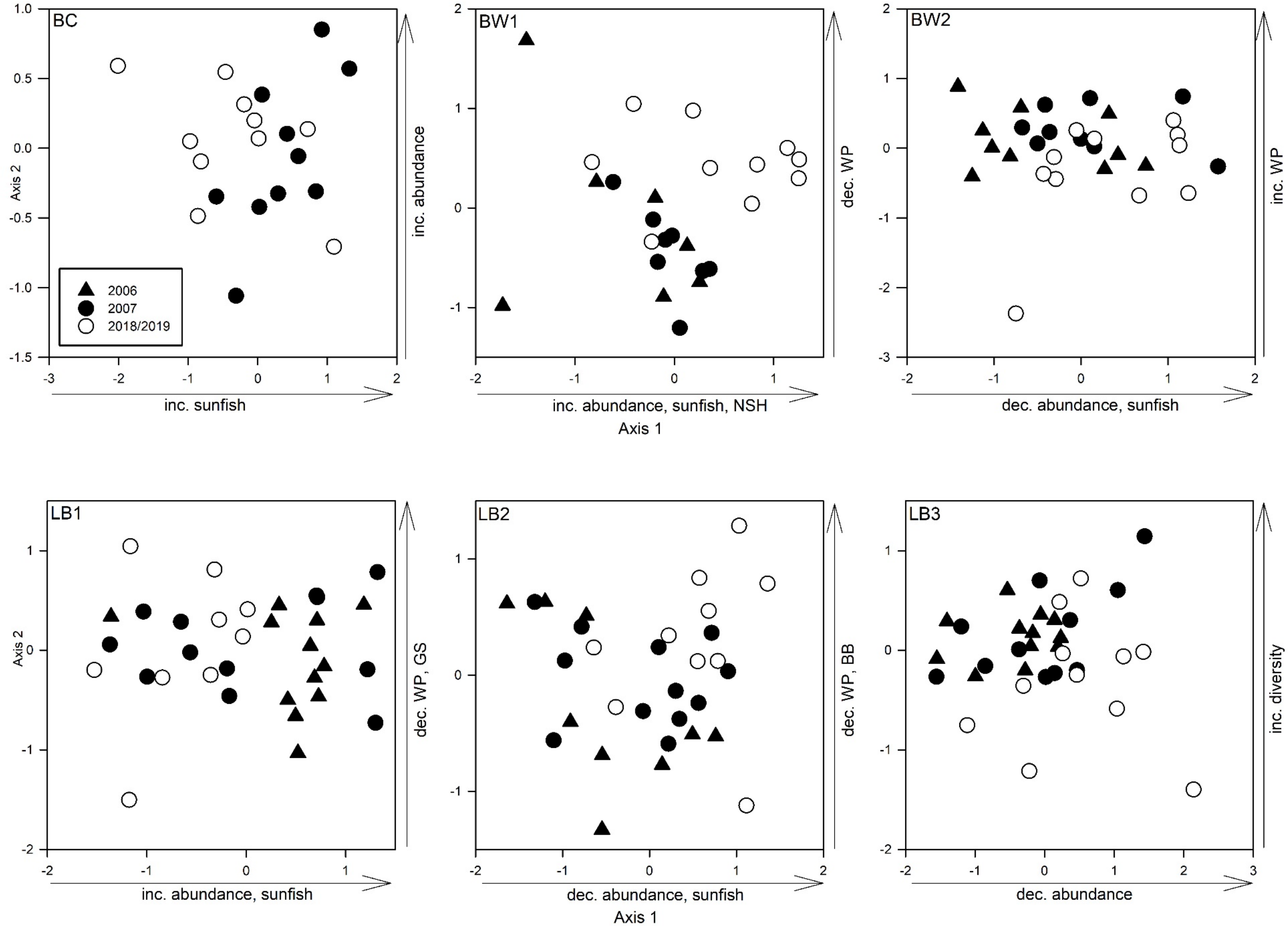
Figure 1. Map of sites for fish community surveys completed in 2006, 2007 and 2018-2019 in the Blackwater (sites BC, BW1, BW2) and Little Blackwater Rivers (LB1-3).

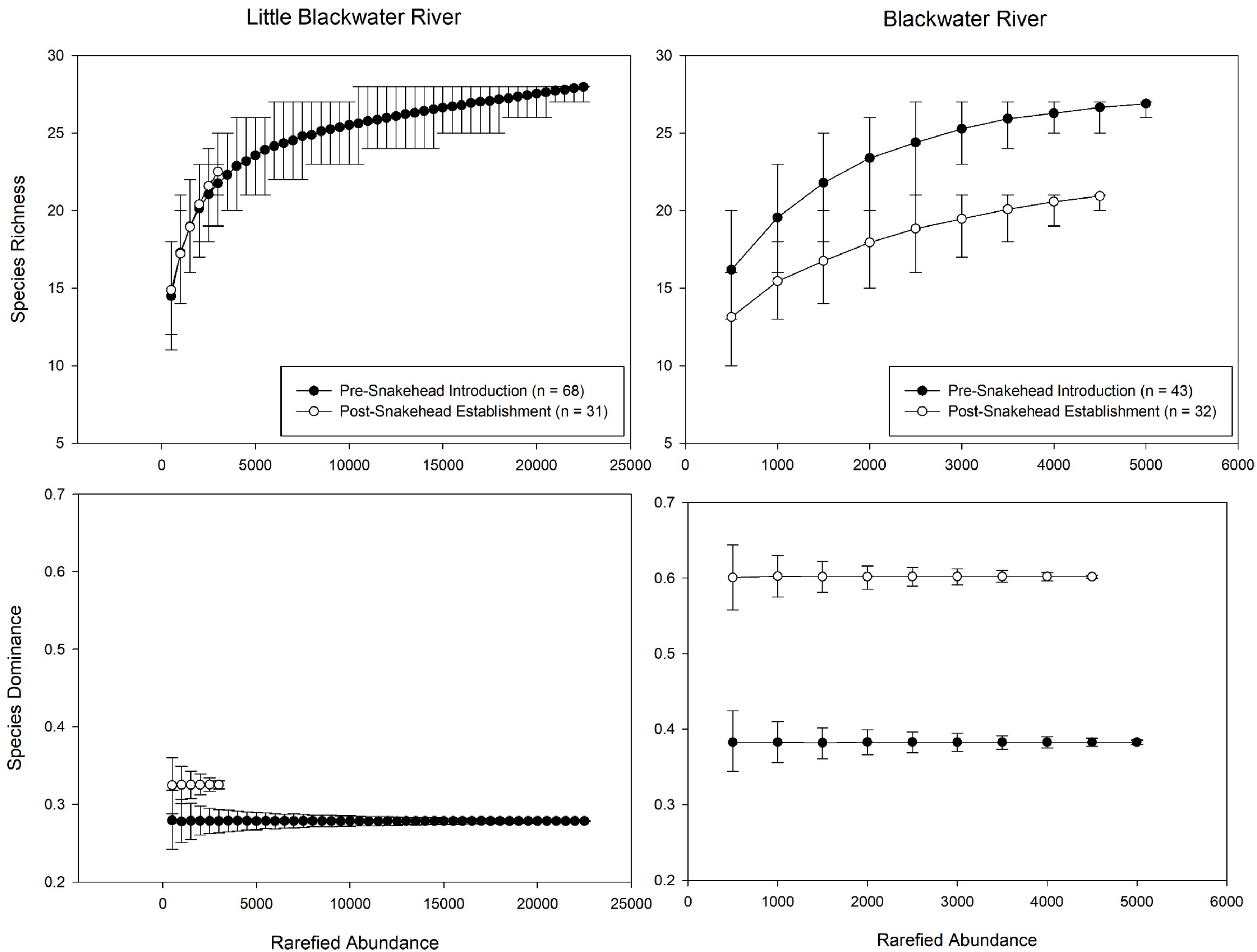
Figure 2. Non-metric multidimensional scaling analysis for fish community surveys conducted at sites in the Blackwater (BC, BW1, BW2) and Little Blackwater (LB1, LB2, LB3) Rivers before (2006, 2007) and after (2018-2019) establishment of Northern Snakehead (NSH). Axes were correlated to overall abundance of fish, as well as abundances of certain species, including: White Perch (WP), Gizzard Shad (GS), Brown Bullhead (BB), and sunfish spp. (Centrarchidae).

Figure 3. Comparison of diversity measures (species richness (upper panel) and species dominance (lower panel)) for Blackwater River (right) and Little Blackwater River (left) before and after establishment of Northern Snakehead.



Baltimore
Dover
DELAWAR
Annapolis
Washington
Chesapeake Bay
Sources: Esri, HERE, Garmin, FAO, NOAA, USGS, © OpenStreetMap contributors, and the GIS User Community
LB1
LB2
BW1
LB3
BC
BW2
Blackwater River
N
0
2.5
5
10 Kilometers
Honga River
Esri, HERE, Garmin, (c) OpenStreetMap contributors, and the GIS user community


BC
BW1
BW2
LB1
LB2
LB3
2006
2007
2018/2019
Axis 2
Axis 1
inc. sunfish
inc. abundance
inc. abundance, sunfish, NSH
dec. WP
dec. abundance, sunfish
inc. WP
inc. abundance, sunfish
dec. WP, GS
dec. abundance, sunfish
dec. WP, BB
dec. abundance
inc. diversity



Little Blackwater River
Blackwater River
Species Richness
Species Dominance
Pre-Snakehead Introduction (n = 68)
Post-Snakehead Establishment (n = 31)
Pre-Snakehead Introduction (n = 43)
Post-Snakehead Establishment (n = 32)
Rarefied Abundance
Rarefied Abundance