PROJECT NAME:
Effect of Dams on the Genetic Structure of Fish Assemblages in the Vermilion River

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## PROJECT SUMMARY

For the past century river hydrology has been altered by the addition of in-stream structures for industrial, agricultural and recreational purposes. These structures (usually dams) are a major source of anthropogenic disturbance and a decisive impediment to river restoration. The physical effects of dams on a river system are well documented and include converting lotic habitats to lentic habitats, changing flow regimes, and increasing siltation upstream from the dam (Pringle 2003, Bednarek 2001). These habitat alterations can impact aquatic insect and fish assemblages by reducing species richness and abundance and influencing dispersal. Furthermore, dams have been shown to isolate populations and have an impact on genetic structuring and differentiation (Meldgaard et al. 2003). Due to increasing awareness of dams' multifaceted ecosystem impacts, dam removal has been gaining traction in the United States in recent years. However, fewer than 5% of the 500 dams removed nationwide in 2005 underwent ecological studies (Thomson et. al 2005). Additionally, most of the dams monitored were large dams as opposed to low-head dams; the latter are more prevalent in the United States. Approximately 48% of all dams in the U.S. are lower than 25 feet and are often overlooked in terms of their potential ecological impacts due to their relatively small size (USACE 2013). Two of these small dams (classified as low-head dams) are the Danville and Ellsworth Park dams in Danville, IL. The Danville Dam is located on the Vermilion River and is a barrier between the lower 22 miles of Vermilion River mainstem and the 1,290 $mi^2$ drainage area upstream. The Ellsworth Park Dam is located on the North Fork Vermilion River, approximately 0.53 miles upstream from the confluence of the North Fork River and the Vermilion River. These dams were scheduled for removal between the spring of 2014 and the fall of 2015, but both still stand as a result of budget and funding issues. Since the removal of the dams is on hold indefinitely, the current principal investigator focused on the effects that these low-head dams have on fish population genetics (a topic poorly represented in scientific literature) in addition to the monitoring of habitat and biotic communities. The primary objectives of this study were to 1) assess habitat quality above and below these two low-head dams, 2) assess fish community assemblages above and below the dams, and finally, to evaluate the effects that the dams have on genetic differentiation and dispersal in two fish species: Longear Sunfish (*Lepomis megalotis*) and Bluntnose Minnow (*Pimephales notatus*). We found that the dams have clear impacts on physical river characteristics like habitat quality; upriver sites in both rivers had significantly higher habitat quality compared to pool sites directly above the dams. Fish assemblages reflected these habitat patterns, with riffle specialist species having a significantly higher abundance at the high quality habitat North Fork River upriver sites compared to other sites. However, our genetic data show that dams themselves are not preventing dispersal of the two fish species. There was weak genetic differentiation in Longear Sunfish, and no discernible patterns in $F_{ST}$ values that would indicate that the dams are impeding movement. Bluntnose Minnow had two genetically distinct populations in the study area, but pairwise $F_{ST}$ comparisons reveal that this is likely due to an isolation by distance effect instead of the dams blocking fish dispersal.

## METHODOLOGY SUMMARY

Six sites on the Vermilion River and six sites on the North Fork Vermilion River were sampled for habitat quality and fish assemblages twice annually in the fall and spring seasons. On each river two sites are located below the dam, two in the pool created above the dam, and two are located upriver of the pool extent. To analyze habitat quality at each site, basic habitat metrics were collected and Qualitative Habitat Evaluation Index (QHEI) scores determined for all reaches. Fish communities on each river were sampled using DC boat electrofishing gear with supplemental gears such as seine nets and mini-fyke nets. To investigate the effects of a physical barrier on the genetic composition of populations above and below the dams, targeted fish species for genetic analysis (Longear Sunfish and Bluntnose Minnow) were finclipped. These species are representative of different life histories and movement patterns in order to gain a comprehensive picture of how impoundments may affect genetic structure in fishes.

To determine genetic differentiation in these two species we used microsatellites: non-coding sections of the genome that are highly variable in populations and therefore a good detector of genetic differentiation. We isolated DNA from 426 Longear Sunfish and 374 Bluntnose Minnow collected in different years and sampling seasons in order to account for any seasonal or annual fluctuation in alleles. Novel and preexisting (Landis et. al 2009, Gotoh et. al 2013) microsatellite loci were amplified to examine levels of genetic differentiation among study sites. We tested over 25 loci for each species to ensure that they were suitable for inclusion in the study; testing involved linkage disequilibrium and Hardy-Weinberg equilibrium analyses. Ultimately we used 11 loci for Bluntnose Minnow and 10 loci for Longear Sunfish. Microsatellite amplifications used fluorescent-labeled DNA primers in multiplex polymerase chain reactions (PCR) and fish genotypes were determined on a Li-Cor 4300 DNA Analyzer.

All ecological data analyses were carried out in the statistical software R (version 3.2.1). To analyze ecological data, habitat quality scores for each site were calculated from the QHEI. Relative abundances of fish and macroinvertebrates were calculated for each site and these data were used in non-metric multidimensional scaling (NMDS) analyses in order to examine patterns in biotic assemblages. Environmental variables such as habitat quality scores and flow were correlated with fish assemblage data using permutational analyses and distance matrices. Analysis of variance (ANOVA) with Tukey Honest Significance Difference post-hoc tests were used to test for significant differences.

To analyze genetic data, we used the program FSTAT (version 2.9.3) to calculate $F_{ST}$ values (a measure of genetic differentiation) for each species among all sites (overall) and between sites (pairwise). For overall comparisons an alpha value of 0.05 was used; for pairwise comparisons the B-Y method False Discovery Rate (FDR) adjusted critical value was applied (Narum 2006). The program STRUCTURE (Pritchard et al. 2000) was used to infer the number of genetic populations among all sites and to infer genetic differentiation.

## RESULTS AND DISCUSSION

Habitat quality between below dam, above-dam (pool) and upriver sites showed significant patterns in both rivers. The highest habitat quality scores were in the upriver sites farthest from the dams, and upriver sites had significantly higher habitat quality than the pool sites (ANOVA, $P<0.05$). Fish communities showed groupings that reflected this pattern seen in habitat quality among sites. Fish communities aggregated at the family level in NMDS analysis showed a separation of the North Fork River and Vermilion River sites. Catostomids clustered with below dam sites, which makes sense for a group of fishes that favors areas with noticeable current. Families that prefer riffle habitat clustered with the with the high quality habitat in the North Fork River upriver sites, notably the families Percidae (darter species) and Ictaluridae (madtom species). When fish were grouped into habitat guilds, riffle specialist species had higher abundance in the North Fork upriver sites when compared to North Fork River pool, Vermilion River pool, and Vermilion River below-dam sites (ANOVA, $P<0.05$). Flow was determined to be a significant predictor of habitat guild assemblages (perMANOVA, $P<0.05$). Although QHEI and substrate type were not statistically significant predictors of guild assemblages, both of these physical parameters of river systems are ecologically important factors in fish dispersal.

Genetic data revealed different results for Longear Sunfish and Bluntnose Minnow. For Longear Sunfish, overall $F_{ST}$ was very low yet statistically significant ($F_{ST} = 0.001$, $P<0.05$), indicating very low genetic differentiation in this species among sites. STRUCTURE also indicated that the Longear Sunfish in both rivers are genetically homogenous; there is only one genetic population overall. When comparing Longear Sunfish between sites, pairwise $F_{ST}$ values showed that Longear Sunfish in the North Fork River upriver sites are genetically distinct from Longear Sunfish below the Danville Dam in the Vermilion River ($P<0.01656$), which is not surprising given the distance between these sites. Additionally, Longear Sunfish in the two Vermilion River pool sites were also genetically distinct ($P<0.01656$), which may be due to the small home range of the Longear Sunfish. Although the overall differentiation is very weak, these pairwise comparisons reveal significant differentiation between sites that is likely too weak for STRUCTURE or overall $F_{ST}$ to detect. In summary, Longear Sunfish show very weak genetic differentiation among the study sites, which is likely due to the fact that these low-head dams are completely submerged during periods of high spring flows. This occurs multiple times every spring and allows unimpeded movement of these fish across the dams.

Like Longear Sunfish, Bluntnose Minnow also had a very low $F_{ST}$ value ($F_{ST} = 0.007$, $P<0.01$) indicating weak but significant genetic differentiation overall. However, Bluntnose Minnow data showed a strong pattern when making pairwise site comparisons. Bluntnose Minnow from the North Fork River upriver sites were genetically distinct from every site in the Vermilion River ($P<0.01572$). STRUCTURE corroborated these $F_{ST}$ values and determined that there were two genetically distinct populations of Bluntnose Minnow within the study area. This is likely a genetic isolation-by-distance effect where we see one population in the North Fork River upriver sites and another in the Vermilion River.

In terms of ecological data and physical habitat characteristics, these dams are clearly impacting these two river systems. The dams' presence drives habitat type and habitat quality, which in turn influences the fish communities in that area. However, our genetic analyses show that the dams themselves do not obstruct fish dispersal and movement to the point of genetic isolation. Although Bluntnose Minnow showed strong genetic differences, there were no patterns to suggest that the dams were driving these differences. We conclude that in this system these two species are not reproductively hindered by the dams and that the dams are causing no harm at the population genetic level.

## STUDENT INFORMATION

A great many students from Eastern Illinois University and the Fisheries and Aquatic Research Team contributed their time and energy toward this project. M.S. degree candidates (graduate students) past and present include Clint Morgeson, Carl Favata, Zach Mitchell, Evan Boone, Daniel Roth, Hanna Kruckman, V. Alex Sotola, and Samuel Gradle. Undergraduate students who assisted in laboratory work include Kailee Schulz, Kaleb Wood, Pabitra Aryal and Melissa Eaton.

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