Assessing and Optimizing a Rapid Road-Crossing Protocol for Aquatic Organismal Passage

Running Head: Assessing and Optimizing a Rapid Road-Crossing Protocol

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Abstract

For decades managers have sought to mitigate the effects of fragmentation on wildlife. In aquatic ecosystems, fragmentation strongly affects headwater streams due to the architecture of riverine networks and the abundance of road crossing culverts. Standardized road crossing assessments offer an alternative to historical methods in facilitating the identification and prioritization of barriers for restoration. However, the ecological relevance of these assessments are seldom empirically investigated, and most assessments assume homogenous environmental and biotic conditions observed during snapshot surveys. Our goal was to assess both the efficacy and assumptions of the widely adopted Southeastern Resource Aquatic Partnership’s (SARP AOP) Road Crossing Assessment for predicting fish passage. We used model selection of generalized linear mixed models to compare SARP AOP scores to observed movement calculated through mark-recapture. We also compared the SARP AOP score with a modified version of the score that included alterations to better reflect local environmental conditions. Although limited in scope, our results suggest an overall lack of support for the efficacy of the SARP AOP score in predicting fish passage and only marginal improvements under the modified score. This study is an important first step in our ability to modify standardized score calculations to increase efficacy without additional surveys. Regardless of the scoring framework efficacy, standardized road crossing surveys remain highly useful in collecting information related to potentially harmful structures (i.e., failing infrastructure). Future studies should further explore how to improve the efficacy of these assessments, which represent a promising tool to facilitate efficient and effective restoration.

**Key Words**: Connectivity, Culvert, Fragmentation, Standardized Survey, Movement

Introduction

Restoring the ability for organisms to freely move within and between populations (connectivity) is a common goal for conservationists aiming to preserve genetic diversity, access to habitat, and healthy ecosystem function. Connectivity is only possible when organisms can freely move; however, anthropogenic factors are increasingly interfering with connectivity through fragmentation and artificial barriers (Chase et al., 2020; Le Pichon et al., 2020). Contemporary dendritic ecological network theory predicts that organisms confined to movement within river networks (such as fish and bivalves) will be the most affected by barriers to movement, relative to organisms that can move over land (Grant et al., 2007). Unsurprisingly fragmentation of habitat is one of the leading factors rendering freshwater fish and mussels as two of the most imperiled faunal groups in North America (Jelks et al., 2008; Lopes-Lima et al., 2014). Thus, restoring connectivity within riverscapes by identifying and removing barriers is one of the most effective and cost-efficient ways to restore ecosystem functions and services (Roni et al., 2002).

Within river networks, headwater streams are more vulnerable to fragmentation partly because their small size leads to the installation of culverts, which are the most common barrier to aquatic organismal passage (AOP; Gido et al. 2016; Shiau, Jenny et al. 2020). However, culverts are not always barriers to AOP, so to restore connectivity managers are tasked with identifying problematic structures before applying restoration management options. Historical methods of barrier identification involved directly observing movement using inefficient methods limited in scope (e.g., mark-recapture Warren and Pardew, 1998). These methods are effective but have left millions of culverts unstudied (Perkin et al., 2020). The resulting knowledge gap led to the development of standardized barrier assessments, which seek to quantify AOP of a given structure without having to directly observe the movement of organisms. Although efficient, these assessments are seldom empirically tested (Nathan et al., 2018). For example, Nathan et al. (2018) found that a standardized protocol designed by the North Atlantic Aquatic Connectivity Collaborative (NAACC) was a poor predictor of movement inferred from genetic differentiation (Nathan et al. 2018). More recently, managers in the southeastern U.S. adopted the Southeast Aquatic Recourses Partnership (SARP) Stream Crossing Protocol, itself a modification of the NAACC protocol (S. Jackson et al., 2016; Southeast Aquatic Resources Partnership, 2017). Despite broad application, the efficacy of the SARP protocol for predicting aquatic organism passage is untested empirically.

The SARP protocol generates an AOP score aimed at estimating passage through a structure. Our primary objective was to empirically test the hypothesis that the SARP AOP score can predict directly measured movement at a given culvert. Further, one of the primary assumptions of standardized barrier protocols is that all species respond similarly to the same structure (no interspecific differences). This is despite a range of research documenting interspecific differences in movement ability and behavior among fishes in a given assemblage (Comte & Olden, 2018; Freeman, 1995; Roberts & Angermeier, 2007). Our second objective was to test the hypothesis that the inclusion of interspecific differences among fishes will significantly improve the predictive ability of the SARP AOP score.

The SARP protocol was developed based on the NAACC with the latter primarily applied to high gradient streams with coarse sediments and high discharge (S. Jackson et al., 2016). One of the primary factors these protocols attempt to account for is the velocity of streamflow in the structure. Additionally, relative to other variables, the protocol does not weigh the effects of scour pools highly and only includes them as an indicator of streamflow (S. Jackson et al., 2016; Southeast Aquatic Resources Partnership, 2017). Scour pools are large pools formed due to constriction of flow through the culvert (S. D. Jackson, 2003; Wellman et al., 2000). These pools are characterized by increased stream depth and width compared to the natural stream and are more likely to form in areas with finer and more mobile sediments at the downstream end of structures which restrict stream flow (Schmidt & Wilcock, 2008). Our third objective was to test the hypothesis that modifying the SARP AOP score to increase the weight of scour pool presence and decrease that of velocity will increase predictive power in lower gradient streams with relatively slow streamflow and finer sediments.

Methods

Study Area and Background

Our study area featured four focal Hydrologic Unit Code (HUC) 12 sub-watersheds (Black Creek, Grey Creek, Hudson Creek, and Bayou Rigollette) in central Louisiana (Fig. 1). This specific location was selected due to ongoing conservation efforts and generalizability to many headwater streams within the biologically diverse Gulf Coastal Plain. Each of the four watersheds are either adjacent to or within the Kisatchie National Forest and contain primarily low gradient headwater streams surrounded by upland pine forest. The study area is within the range of the federally threatened Louisiana Pearlshell Mussel (*Margaritifera hembeli*). Native freshwater mussels rely on fish for dispersal of their larvae (glochidia) and therefore their conservation is intimately connected to their host (Modesto et al., 2017). Several hosts have been suggested for Louisiana Pearlshell (Hill 1986; Johnson and Brown 1998), with multiple species yielding viable recruits in controlled settings (U.S. Fish and WIldlife Service, 2019); however, the suitability of other co-occurring species in the wild remains unclear. Therefore, this study will inform the management of this species by testing the efficacy of the SARP barrier protocol, which will aid managers in estimating the effects of fragmentation on species that co-occur with the Louisiana Pearlshell Mussel.

Site Selection

Site selection began after SARP protocol surveys at every road-stream crossing (Fig. 1). Aquatic Organismal Passage scores range from 0-1, with scores of 0.00-0.20 suggesting a severe barrier, 0.21-0.40 a significant barrier, 0.41-0.60 a moderate barrier, 0.61-0.80 a minor barrier, and 0.81-1.00 an insignificant barrier. We selected six sites across the gradient of AOP scores, while controlling for potential biases associated with stream size and presence of tributaries within the study reach (Fig. 1, Table 1). The presence of Louisiana Pearlshell was also considered to potentially inform future management decisions and restoration opportunities. Of the 241 road stream crossings surveyed, we identified 91 culverts. Based on the SARP AOP score, 20 (23%) culverts were identified as moderate, 13 (15%) were identified as significant or severe, and the remaining 58 (65%) were identified as insignificant or minor barriers (Fig. 1). Further information on the survey results or standardized protocol can be accessed from SARP’s Aquatic Barrier Inventory (Southeast Aquatic Resources Partnership SARP, 2024).

Because the SARP AOP protocol does not include quantitative velocity measurements and to ensure that velocity was not restricting fish passage, we collected velocity data using a Hach velocity sensor (Hach FH950) at each of the culverts we surveyed. Within each culvert, four transects were equally dispersed. Each transect was divided into points one meter apart (with minimum of 2 points) and velocity was measured at each of the points. Because culverts were surveyed during base flow conditions, we also repeated velocity measurements at the six selected sites during a range of hydrological conditions throughout the study period. We then conducted a literature review to identify the critical swimming speed of species similar to those in the current study. The mean minimum culvert velocity measurement recorded at any transect (mean = 4.2cm/s; Range = -6.0 – 48.2cm/s) was less than any of the critical swimming speeds identified from the literature (Table A.1). Further, none of the transects from the six selected mark-recapture sites had velocities greater than the minimum critical swimming speed identified in the literature (Scott & Magoulick, 2008).

Marking Surveys

Mark-recapture methods were used to detect movement of fish at the six sites. To evaluate movement across culverts, 150-m reaches were delineated directly upstream and downstream of each road crossing (Fig. 2). A control reach was delineated downstream of the downstream reach and was separated by a length of stream equal in distance to the length of the culvert. This design enables the direct comparison of movement rates in contiguous, natural habitats to movement rates across the culvert to assess the impact of each crossing. Because increased scouring directly upstream and downstream of culverts can result in disparate habitat features (S. D. Jackson, 2003; Wellman et al., 2000), these scour pools were delineated using the SARP stream crossing protocol and treated each scour pool as a separate reach. Additionally, any perennial tributaries that entered the study reach were delineated as an additional 150-m reach.

A field crew of four individuals used double-pass backpack electrofishing followed by seining in each reach to collect fish. Once fish were collected, they were placed in mesh holding containers in their reach of capture. Before marking, fish were placed in a temporary holding container and anesthetized using MS-222. With the exception of lampreys (*Petromyzontidae*), all individuals >26 mm (TL, mm) total length (Olsen & Vøllestad, 2001) were marked with a uniquely colored visible implant elastomer (VIE; Northwest Marine Technology) based on the reach, identified to species, and measured before being returned to a mesh holding container to recover. Once the fish were able to maintain equilibrium they were determined to be fully recovered and were returned to the stream in their reach of capture. Mortality during handling was recorded and resulted in a mortality rate of < 0.45%. Marking surveys were conducted during the spring of 2021 and repeated during the spring of 2022.

Recapture Surveys

Each site was resampled at 3-months after marking during the summer of 2021 and at one-month after during the summer 2022. Several fish marked in 2021 were recaptured in the spring 2022 marking survey. These fish were not included in analyses because interspecific tag retention rates were unknown and likely variable over longer periods (Goldsmith et al., 2003; J. R. Skalski et al., 2009). During the recapture surveys, fish were collected using the same procedure as described above except single-pass sampling was used instead of double-pass. Once collected, all individuals were placed in a mesh holding container, measured, identified to species, checked for a VIE tag, placed in a recovery container, and released into their reach of capture. The results are based on data across all samples (recapture attempt in an individual reach) except for the second recapture sample from Swafford Creek and Chandler Creek (1), which were excluded due to unanticipated complications during the second marking event. The final dataset included 29 samples from 6 sites across 2 years.

Movement Analyses

To control for potential biases associated with differences in recapture rates, only species with an average recapture rate >10% and >100 marked individuals per site were included in analyses. The recapture rate was determined by dividing the total number of recaptured individuals by the total number of marked individuals. Proportional movement was used to conduct analyses to control for the recapture rate of a given sample. This was calculated by dividing the number of movers across all focal species by the number of recaptured individuals (potential movers) in the sample. The Culvert Crossing Rate (CCR), the ability for each species to traverse the culvert, was calculated as the ratio of proportional movement across the control to proportional movement across the culvert. The CCR was calculated using the formula:

where *Mcul* and *Mcon* are the number of individuals that moved across the culvert and control, respectively, during a given sample, and *R* is the number of recaptures during that sample. One was added to the numerator and denominator to account for cases where the number of recaptures (*R*) was zero. To test the hypotheses generalized linear mixed models were first fitted using the lme4 package (Bates et al., 2015) in R (R Core Team 2023). A gamma distribution of errors with an inverse link function was used because the data adjusted best to this distribution. Despite the corrections from the gamma distribution with an inverse link, a slightly non-linear relationship existed between the predicted AOP values and the residuals. However, the model comparison was carried out for several reasons. First, the departure is relatively minor as the residuals were only larger than expected between predicted AOP values of 0.6-0.7. This minor departure is likely a reflection of one sample with a large CCR value. Second, the addition of a smoothing term to the AOP variable could better fit the data, but due to the small size of the dataset including a smoothing term would increase the model complexity and likely result in additional uncertainty and overfitting (Wood, 2017). Given the risk of overfitting, the minimal nature of the departure, and the fact that all other assumptions in the model were met, the Likelihood Ratio Tests (LRTs) and model selection were carried out using the same model structure.

Each of the models used CCR as the response variable and included a random intercept for site to account for unexplained differences among sites. Although sampling occurred on multiple occasions, sample was not included as a random effect because it had less than five levels (Harrison et al., 2018). To determine if the inclusion of sample as a fixed effect was necessary to avoid violating the assumption of independence used an LRT (P > 0.05; Harrison et al. 2018) and model selection with Akaike Information Criterion adjusted for small sample sizes (AICc). We used both methods as this has been recommended to avoid overlooking meaningful variables with p-values above the 0.05 alpha value (Sutherland et al., 2023).

An LRT was also used to test the second hypothesis that interspecific differences contributed significantly (*p* ≤ 0.05) to variation in CCR. To test the hypothesis that the AOP score explained variation in CCR*,* model selection was performed using Akaike Information Criterion corrected for small sample sizes (AICc) with a delta AICc cutoff of 6 (Harrison et al., 2018). The addition of species as a fixed effect or site as a random effect in candidate models were only included if LRTs indicated a significantly improved model. Three candidate models were fit to test the hypotheses (Table 2). Two of the models included AOP as a fixed effect (either SARP or Modified). The modified AOP score was calculated by altering the weights of variables included in the SARP AOP score. Specifically, the variable related to velocity was eliminated, and its associated weight value (0.08) was added to the scour pool variable (Table 3). The third model was a null model with only random effects. The assumptions for each of the models were checked by visually assessing diagnostic plots and calculating the Variance Inflation Factor (VIF; if more than one fixed effect was used) with a conservative threshold of 2 due to the small size of the dataset (Zuur et al. 2010; Fox and Weisberg 2019).

Based on our hypotheses we expected the candidate models including the AOP variables to rank above the null in the AICc with the modified AOP candidate model ranking the highest. We used the bootstrapping method of the “sjPlot” package to extract confidence intervals as well as the marginal and conditional R2 values to assess the strength of the model(s) (Ludecke 2023). The marginal R2 represents the variance explained by the fixed effects in the model, and the conditional R2 is the total variation explained by the model. Therefore, a larger ratio of marginal to conditional R2 indicates an increased amount of variation explained by the fixed effects in the model relative to the random effects. Regardless of ranking in the AICc, we hypothesized that the Modified AOP model would have an improved ratio (larger) of marginal to conditional R2 and a smaller confidence interval indicating improved explanatory power relative to the SARP AOP model.

Results

Marking Surveys

Marking surveys resulted in the capture and marking of 5,636 individuals representing 29 species (Table 4). The samples were dominated by the Redfin Shiner (*Lythrurus umbratilis*), which represented 34% of the total fish marked (39% in 2021 and 26% in 2022). Three other species represented 41% of marked fish: Blackspotted Topminnow (*Fundulus olivaceus;* 15% in 2021 and 26% in 2022), Striped Shiner (*Luxilus chrysocephalus*; 11% in 2021 and 17% in 2022), and Longear Sunfish (*Lepomis megalotis*; 9% in 2021 and 2022).

Recapture Surveys

During the recapture surveys across all sites 690 individuals were recaptured resulting in recapture of 11.4% across marked species. Redfin Shiner ( = 10.7, range = 0.5% – 47.0%), Striped Shiner ( = 10.8, range = 0.0% – 32.3%), and Blackspotted Topminnow ( = 13.1, range = 2.2% – 31.1%) had mean recapture rates >10% and met our criteria for focal species; other species are non-focal (Table 4). The most abundant species in the marking surveys were also the most abundant during the recapture surveys.

Movement Analyses

The CCR had a mean of 1.21 (SD = 0.83, Range = 0.25 – 4.00). Both the LRT and model selection indicated that the inclusion of sample (P = 0.13, dAICc = 1.3) and species (P = 0.07, dAICc = 0.9) as a fixed effects did not significantly improve the model and were not included in the candidate models. AICc indicated that none of the candidate models outperformed the null model (dAICc < 6; Table 2). However, the marginal and conditional R2 values of the Modified AOP model (0.122 / 0.156) were larger than that of the SARP AOP model (0.088 / 0.147; Table 2). The confidence interval of the AOP fixed effect was also smaller in the Modified AOP model (0.13 – 1.18) than the SARP AOP model (0.16 – 1.44; Table 2). Despite not being distinguished from the null, the estimates of both candidate models were positive indicating that the AOP score was weakly, but positively related to the CCR.

Conclusions

The goal of the current study was to test hypotheses related to the ecological relevance of the SARP barrier assessment protocol that is used extensively in streams across the southeastern United States. Our results failed to reject (p=0.07) the null hypothesis that the inclusion of a species fixed effect would not improve the models. Similarly, we found a lack of support for the SARP AOP score because it was not a good predictor of the CCR. We also showed that modifications to the AOP score based on local environmental context (scour pools) can improve the scores efficacy, albeit, in a minor way. Future studies should explore this more on different scales and in different biogeographic areas.

Interspecific Differences in Movement

The dAICc and P-value of the species LRT indicate no improvement on the model, which supports our hypothesis that interspecific differences would not significantly influence CCR. Although this result aligns with standardized road crossing assessments such as the SARP stream crossing protocol, which assume equal ability of all species to traverse a road-crossing structure, interspecific differences in stream fish movement are well documented (Comte & Olden, 2018; Freeman, 1995; Lonzarich & Warren, 1998). The current study focused on three species that have similar body sizes and reside in the middle or upper water column. Although previous studies have not directly compared these species, studies on topminnows (Fundulidae) found them less mobile than minnows (Leuciscidae) in similar environments (Clark et al., 2019; G. T. Skalski & Gilliam, 2000; Walker et al., 2013; Warren & Pardew, 1998). For example, Warren and Pardew (1998) found that Striped Shiners had a higher proportional movement across various road-stream crossing structures than Blackspotted Topminnows.

Given that previous studies found significant differences in movement using similar small stream fish species and the relatively low p-value within the current study, the effect of interspecific differences on the model may have been stronger with a larger dataset or increased diversity of species with greater morphological and ecological differences. Alternatively, movement is in part driven by local conditions, and the environmental state of the current study could have possibly led to reduced interspecific differences in CCR(Chapman et al., 2012). We included the random site effect to account for differences between sites, but local conditions could have still confounded our results. In conclusion, our findings support the first hypothesis, but the marginal nature of the results and previous research suggest that managers should cautiously interpret standardized AOP scores as an indication of fragmentation effects across taxa.

Efficacy of the AOP Score

We hypothesized that the AOP score would be a good predictor of movement. However, our results did not support this hypothesis as the top ranked model (Modified AOP) nor the secondarily ranked model (SARP AOP) were significantly different from the Null model. Hence, the ability of focal species to traverse the culverts was not captured by the AOP scores. This is perhaps not surprising given that the only other study to our knowledge to investigate the efficacy of the SARP AOP protocol also found poor support in predicting observed movement (Nathan et al. 2018). As noted, the small size of the current dataset may have inhibited our ability to detect a strong relationship. However, even a weak relationship between either the modified or SARP AOP scores and CCR was not apparent. For example, the top ranked model (Modified AOP) only had a 0.05 (5%) increase in conditional R2 relative to the null model.

Comparing the Modified and SARP AOP Scores

Despite both metrics of the AOP score not being well supported through model selection, we still wanted to test the second hypothesis that the modified AOP score was a better predictor of CCR than the SARP AOP score. As a reminder, the SARP assessment method was developed based on high gradient streams but has been utilized across a wide range of biogeographic contexts (Southeast Aquatic Resources Partnership SARP, 2024). Our goal was to investigate if the efficacy of the SARP AOP score could be enhanced by modifying the AOP score to better reflect local conditions by replacing the velocity variable with the scour pool variable. Based on our velocity measurements and literature review the removal of velocity from the SARP AOP score was clearly justified in the current study. While the Modified AOP score was only separated by 1 AICc from the SARP score, the AOP score marginal R2 increased and the confidence interval decreased. These results suggest that the modification of the AOP score enhanced the model by accounting for more of the unexplained variance in *CCR*. However, this effect was not strong enough to significantly influence the results of the model selection process.

This study, along with Sliger et al. (2024) are the first to explore using direct observational data to enhance the efficacy of standardized road crossing assessments. Future studies should weigh the costs and benefits of potential methods with the local environmental context. For example, Sliger et al. (2024) used swimming performance measurements to modify the SARP AOP score and enhance its efficacy for specific species. While this methodology may be straightforward and effective for road-crossings highly influenced by streamflow velocity, culverts in lower gradient streams such as those in the current study have much slower streamflow velocity, and therefore utilizing swimming performance in this context would be much less effective and unlikely to result in improved AOP score efficacy. To our knowledge, no studies to date have specifically investigated the effects of scour pools on movement across road-stream crossings. The current study highlights the need to address this knowledge gap along with a continued effort to better understand the interactions between aquatic organisms and culverts in low gradient watersheds.

Future Directions

While this study has limitations in the relatively small size of the dataset and limited scope, it serves as an important precursor to future research that should continue to use traditional methods to empirically investigate the efficacy of standardized barrier assessments. Future studies should explore utilizing a variety of methods that have been shown to be effective across a range of spatiotemporal scales such as community sampling (Faucheux, 2022), population genomics (Palamara & Pe’er, 2013), swimming performance (Sliger et al., 2024), and mark-recapture (Warren & Pardew, 1998). It is vital that we fully understand and improve upon the efficacy of standardized barrier protocols that represent a fundamental advancement in our ability to understand, assess, and restore aquatic connectivity (Curtis, 2024).

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Data Availability Statement

All data required to replicate these analyses will be available on data Dryad once this manuscript is published.

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Conflict of Interest Disclosure

There is no conflict of interest declared in this article.

Ethics Approval Statement

All research was performed in accordance with the Animal Care and Use Committee protocol number 22033001 approved by the Animal Care and Use Committee at the University of Southern Mississippi.

**Tables**

Table 1. Selected sites and their characteristics.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Site | Lat | Lon | Order | SARP AOP | Louisiana Pearlshell |
| Swafford Creek | 31.586 | -92.590 | 4 | Moderate | Stable |
| Black Creek | 31.646 | -92.590 | 3 | Minor | Stable |
| Jordan Creek | 31.515 | -92.530 | 4 | Moderate | Declining |
| Cress Creek | 31.523 | -92.588 | 4 | Insignificant | Extirpated |
| Chandler Creek 1 | 31.524 | -92.554 | 4 | Severe | Declining |
| Chandler Creek 2 | 31.544 | -92.557 | 3 | Minor | Stable |

Louisiana Pearlshell (Margaritifera hembeli) status is from USFWS (2020).

Table 2. Final candidate models and results from model selection and comparison.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Model | Formula | dAICc | Df | Weight | Marginal / Conditional R2 | CI |
| Modified AOP | CCR ~ Mod AOP | 0.0 | 4 | 0.39 | 0.122 / 0.156 | 0.13 – 1.18 |
| Null | CCR ~ 1 | 0.1 | 3 | 0.37 | 0.000 / 0.106 | 1.74 – 3.19 |
| SARP AOP | CCR ~ SARP AOP | 1.0 | 4 | 0.24 | 0.088 / 0.147 | 0.16 – 1.44 |

All model formulas also included a random intercept for site.

Table 3. Variables and associated weights used to calculate the AOP scores.

|  |  |  |
| --- | --- | --- |
| Variable | SARP AOP | Mod AOP |
| Drop | 0.161 | 0.161 |
| Physical Barrier | 0.135 | 0.135 |
| Constriction | 0.090 | 0.09 |
| Inlet Grade | 0.088 | 0.088 |
| Depth | 0.082 | 0.080 |
| **Velocity** | **0.080** | **0.000** |
| **Scour** | **0.071** | **0.151** |
| Substrate Type | 0.070 | 0.070 |
| Substrate Coverage | 0.057 | 0.057 |
| Height | 0.045 | 0.045 |
| Outlet Armoring | 0.037 | 0.037 |
| Internal Structures | 0.032 | 0.032 |
| Openness | 0.052 | 0.052 |

Values changed for the Modified AOP score are in bold. More information regarding how each variable is measured can be found in the SARP Barrier Protocol Handbook (Southeast Aquatic Resources Partnership, 2017).

Table 4. Species and recapture statistics for each sample.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Sample 1 | | Sample 2 | | Total |  |
| Species | N | Recap % | N | Recap % | N | % of Total |
| *Lythrurus umbratilis* | 2082 | 11.85% | 763 | 5.38% | 2845 | 31.77% |
| *Fundulus olivaceus* | 917 | 7.98% | 1073 | 0.00% | 1990 | 22.22% |
| *Luxilus chrysocephalus* | 989 | 14.64% | 560 | 4.73% | 1549 | 17.30% |
| *Lepomis megalotis* | 425 | 1.90% | 296 | 3.05% | 721 | 8.05% |
| *Lepomis macrochirus* | 228 | 3.29% | 120 | 4.00% | 348 | 3.89% |
| *Etheostoma artesiae* | 190 | 5.56% | 128 | 6.45% | 318 | 3.55% |
| *Noturus phaeus* | 115 | 0.00% | 138 | 9.86% | 253 | 2.83% |
| *Gambusia affinis* | 137 | 2.30% | 104 | 3.45% | 241 | 2.69% |
| *Ichthyomyzon gagei* | 223 | NA | 15 | NA | 238 | 2.66% |
| *Micropterus punctulatus* | 27 | 0.00% | 47 | 7.41% | 74 | 0.83% |
| *Aphredoderus sayanus* | 23 | 0.00% | 41 | 11.76% | 64 | 0.71% |
| *Percina sciera* | 35 | 0.00% | 28 | 0.00% | 63 | 0.70% |
| *Lepomis cyanellus* | 26 | 6.67% | 11 | 20.00% | 37 | 0.41% |
| *Erimyzon claviformis* | 17 | 0.00% | 16 | 0.00% | 33 | 0.37% |
| *Semotilus atromaculatus* | 18 | 0.00% | 10 | 0.00% | 28 | 0.31% |
| *Lepomis gulosus* | 20 | 0.00% | 2 | NA | 22 | 0.25% |
| *Moxostoma poecilurum* | 6 | 0.00% | 13 | 0.00% | 19 | 0.21% |

Table 4 (continued).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Sample 1 | | Sample 2 | | Total |  |
| Species | N | Recap % | N | Recap % | N | % of Total |
| *Notropis boops* | 0 | NA | 18 | 0.00% | 18 | 0.20% |
| *Esox americanus* | 13 | 0.00% | 4 | 33.33% | 17 | 0.19% |
| *Lepomis sp.* | 8 | NA | 5 | 0.00% | 13 | 0.15% |
| *Lepomis miniatus* | 8 | 20.00% | 1 | NA | 9 | 0.10% |
| *Lepomis marginatus* | 7 | 0.00% | 2 | 100.00% | 9 | 0.10% |
| *Ameiurus natalis* | 1 | NA | 6 | 0.00% | 7 | 0.08% |
| *Micropterus salmoides* | 5 | 100.00% | 0 | NA | 5 | 0.06% |
| *Notemigonus crysoleucas* | 4 | 0.00% | 0 | NA | 4 | 0.04% |
| *Labidesthes sicculus* | 1 | NA | 3 | 0.00% | 4 | 0.04% |
| *Etheostoma parvipinne* | 3 | 0.00% | 0 | NA | 3 | 0.03% |
| *Elassoma zonatum* | 2 | 0.00% | 1 | 0.00% | 3 | 0.03% |
| *Centrarchus macropterus* | 1 | 0.00% | 0 | NA | 1 | 0.01% |
| *Lepomis megalotis/cyanellus* | 1 | 0.00% | 0 | NA | 1 | 0.01% |
| Total | 5549 | 6.70% | 3406 | 9.11% | 8955 | 100% |

Recapture percentage (Recap %), number of individuals (N), and percent of the total sample for each of the species captured during surveys. Species with NA values represent those that were not captured during marking surveys or not marked at all (Ichthyomyzon gagei).

**Figure Legends**

Figure 1. Map of surveyed culverts.

Surveys occurred within Black Creek (Black), Grey Creek (Gray border), Hudson Creek (Purple border), and Bayou Rigollette (Red border). Selected sites are labeled by creek name and colored by SARP AOP score.

Figure 2. Sample design for the mark-recapture surveys.

If the upstream scour pool was absent the upstream reach started directly adjacent to the culvert.