Effects of structural rehabilitation on nutrient retention and uptake, community assemblages, and functional morphology of biotic communities in a small Midwestern stream

Biological Sciences

Graduate (M.S.)

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PI: Anabela Maia, PhD, Eastern Illinois University, amresendedamaia@eiu.edu, (217) 581-6360

Congressional District: IL-15

Background

Decades of anthropogenic pressure have devastated lotic ecosystems across the riverscapes of North America, resulting in the degradation of critical habitat and contributing to sharp declines in biotic integrity. For example, agricultural practices in the Midwest have led to increased levels of bank erosion, sedimentation, and nutrient loading, resulting in a loss of critical habitat for aquatic organisms (Berkman and Rabeni, 1987). Small streams are affected considerably by such pressures, with upwards of 85% of ecosystems displaying signs of degraded function (Dahl, 1990). In response, local stream restoration are increasingly frequent, yet comparably little effort has been allocated to monitoring (NRC, 1992; Moerke and Lamberti, 2003), leading to ambiguous results and limited project success. With habitat heterogeneity and biotic integrity being primary goals of rehabilitation (Gorman and Karr, 1978; O'Connor, 1991; Death and Winterbourn, 1995; Walser and Bart, 1999), it is imperative that projects are monitored with increasing frequency, and describe the factors affecting community structure and biotic integrity in impacted waterways to mitigate further loss. Lessons from the long-term rehabilitation and ecological monitoring of Kickapoo Creek in East-Central Illinois highlight some of the complex dynamics driving reach-scale restoration projects.

Kickapoo Creek is a unique system as it encompasses multiple anthropogenic pressures in a relatively small basin – sanitary treatment plant, golf course, and agricultural land. These pressures may stress the local aquatic ecosystem through habitat degradation and nutrient toxicity, and must be assessed. Nitrates from agricultural runoff and sanitary treatment effluent are harmful, and sometimes fatal to aquatic fauna, and must be closely monitored (Mueller et al., 1997; Royer et al., 2004; Camargo et al., 2005). Elevated estradiol (a form of estrogen) levels are also of concern due to the close proximity of the Mattoon sanitary treatment plant. This hormone has been associated with the production of intersex gonads in male fishes and cancer and reproductive abnormalities in humans (Singh et al., 2003; Braga et al., 2005; Shappell, 2006).

Our research looks at long-term effects of a previous instream rehabilitation on fish and macroinvertebrate assemblages in Kickapoo Creek, near Charleston, IL. In addition, this project will monitor a new restoration project, and will examine the effects of sanitary treatment plant effluent, golf course nutrient enrichment and bank erosion on nutrient uptake and fish and macroinvertebrate communities. During this research we will assess the effects of multiple rehabilitations on local biotic communities, and evaluate the overall success of each rehabilitation in regards to community assemblages, physical properties of the habitat and overall health of the system.

To compliment ecological data, laboratory experiments will examine the effects of changes in turbulence regime associated with restoration on selected fish species within Kickapoo Creek. Longear Sunfish (*Lepomis megalotis*) are both widely prevalent in Kickapoo Creek and fill vital roles in stream ecosystems, and thus will be ideal candidates for experimentation. Since fish live in a complex three-dimensional environment, and are affected by both biotic and abiotic variables (Liao, 2007), it is necessary to consider the organismal-level impacts resulting from habitat alterations. Proposed changes in instream structure and resulting changes in velocity could alter behavior and affect the way these fish use their habitat (Liao, 2007). Thus, it will be beneficial to examine the effects of the restoration to fish physiology as well as ecology. The last portion of our project will look at water chemistry, particularly estradiol levels to assess the anthropogenic impacts on the biotic communities. Fish estradiol levels will also be measured in the aforementioned species and correlated with oxygen consumption and metabolic scope.

If habitat diversity is maintained, we anticipate a continued increase in fish abundance, biomass, and diversity, as well as an increase in macroinvertebrate diversity at previous restored sites. If restoration increases habitat diversity, we expect to see similar post-restoration increases in fish and macroinvertebrate diversity at two new project sites. Each reach will be compared to itself over a period of time, while also being compared to longitudinal changes seen in reference, upstream, and downstream sites along the stream.

During laboratory testing, we expect to observe several physiological responses in wild caught fish. During respirometry testing, we expect to observe increased oxygen consumption under stressed conditions compared to resting metabolic consumption. We also expect increased turbulence to elicit the greatest change in metabolism. In terms of estradiol levels in the water and fish tissue, we expect to see elevated levels only downstream of the sanitary treatment plant (STP). We also expect elevated estradiol levels in the fish caught in reaches downstream of the STP. Lastly, we expect that fish exposed to estradiol will have elevated metabolism under stress conditions.

Methodology

Study Area

Kickapoo Creek (Latitude 39°27', Longitude 88°13') is a fourth-order, low gradient stream which originates south of Mattoon, Illinois and flows east for nearly 66 km until meeting its confluence with the Embarras River (Figure 1). Draining approximately 265 km^2, this human-impacted stream is subjected to multiple anthropogenic pressures within a relatively small basin, and land use within the Kickapoo Creek watershed consists primarily of agriculture, disconnected fragments of forest, grasslands, and urban stressors (e.g. road crossings, golf course, sewage treatment plant, and residential area). As part of the larger Embarras River watershed, a region which has been identified by the Illinois Environmental Protection Agency (IEPA) as a watershed of concern, this tributary has been a recent hotspot for rehabilitation and mitigation efforts. Prior to an instream rehabilitation project, all study reaches shared similar habitat characteristics, consisting of a shifting sand and gravel substrate regime accompanied by elevated levels of bank erosion and sedimentation (West 2013). Following a chemical-induced fish kill in 2001, mitigation from Illinois Department of Natural Resources (IDNR) enabled the structural rehabilitation of over 400 m of streambank and main channel habitat in September 2010. In an effort to improve habitat heterogeneity, and thus biotic integrity (Palmer et al., 1997) rehabilitation included construction of two artificial Newbury riffles (Newbury Hydraulics, Okanagan Centre British Colombia, Canada), which increased average water depths and simulated scour pool hydraulics within the rehabilitation reach (Pant, 2014). Rip-rap was employed along streambanks in the form of boulder cover and scouring keys to further facilitate geomorphic stabilization and improve hydrologic conditions (West, 2013; Pant 2014). Additionally, streambanks were revegetated with native grasses to further aid the recovery of riparian habitat and to reduce bank erosion and sedimentation.

Habitat Assessment

Stream habitat and integrity were monitored annually in the fall using the Qualitative Habitat Evaluation Index (QHEI; Rankin, 1989). Beginning immediately after the rehabilitation project in summer 2010, we examined habitat in three fixed 200 m sites — two located within the larger rehabilitation reach and associated with each artificial riffle, and one site approximately 1.8 km upstream which served as a reference, or control, for baseline comparisons. In 2012, an additional reach was added 1.8 km downstream of the rehabilitation reach to serve as an added reference. In teams of two researchers, each site was divided into ten equidistant transects where depth and substrate measures were taken at specified intervals along the width of the channel. Relative abundance of instream and riparian habitat was also estimated between each transect using a standard protocol. Water quality variables (dissolved oxygen, specific conductivity, water temperature, and pH) were collected instantaneously during each sampling event using a YSI multimeter probe (YSI Inc., Yellow Springs, OH). Additionally continuous in situ nitrate, temperature, and dissolved oxygen levels in Kickapoo Creek were monitoring by the US Geological Survey (USGS), and recorded using two USGS monitoring stations located within the rehabilitation reach and near the upstream reference. During the 2014 and 2015 sampling periods, ecological monitoring began at an additional three sites located within in the upstream reaches of Kickapoo Creek near Mattoon, Illinois (Figure 1). These areas were identified as impacted regions, characterized by decreased geomorphic stability with heavy amounts of bank erosion and siltation. Habitat assessments were conducted concurrently with our long-term monitoring project, and data were incorporated into ecological models.

Biotic Community Sampling

To ensure all fish were fully recruited to the gear, communities were sampled annually in the fall at baseflow water conditions, and concurrently with QHEI habitat monitoring. Blocking seines (mesh size, 5 mm) were employed during sampling at the upstream and downstream ends of seven 200 m sites, however, fish were only added to the sample from the downstream seine. Teams of six researchers conducted single-pass removal DC barge electrofishing surveys within each site using standardized protocols (Rabeni et al., 2009) where all available habitat within the stream channel was sampled, and fishing time was recorded as a measure of sampling effort. Whenever feasible, fishes were weighed (nearest gram), measured (nearest millimeter), identified to species and released unharmed near each site. Fishes which were unable to be identified in the field were euthanized using a lethal dose of MS-222, fixed in 10% formalin solution and later stored in 75% ethanol before further enumeration and identification using a taxonomic key (Pflieger 1997).

Changes in macroinvertebrate populations were measured using the IEPA’s (2007) multihabitat 20-jab method, with jabs allocated using the QHEI as a measure of available habitat. Macroinvertebrate collections were taken in the sediment using an 18 inch rectangular dip net, and an 18x18 inch area was thoroughly aggravated to ensure all insects were suspended and collected within the net. All samples were stored in 90% ethanol until identification and enumeration in the lab. A standard procedure was used to subsample macroinvertebrates within each site, using randomly selected grids to identify approximately 300 ± 40 macroinvertebrates per site. All macroinvertebrates were identified down to family, or lowest taxonomic resolution possible.

Metabolic Scope

To analyze the metabolic changes under stressed (i.e. turbulent) conditions we used standard respirometry techniques developed for aquatic fauna (Svendsen et al. 2014). Longear Sunfish collected from Kickapoo Creek were examined due to their broad distribution in warmwater streams and importance to local ecosystems. After fish were adjusted to being housed in the lab at Eastern Illinois University, they were tested in our sealed flow tank (Loligo® Systems 2016). Fish were first acclimated to the chamber or flume with the lid open for a minimum of two hours prior to testing. The fish were also not fed for a minimum period of 24 hours before testing to ensure oxygen measurements reflected locomotor effort and not digestive processes. Once fish were acclimated, the lid to the flume was closed and sealed, and an oxygen probe inserted into the chamber. During experiments we recorded oxygen levels within the intermittent flow chamber for a minimum of two hours due to the large size of the flume; this interval was chosen to maximize the resolution necessary to assess changes in metabolic oxygen condition. During trials, saturation of dissolved oxygen was not allowed to decrease below 85% due to physiological limitations of fish. Variables being tested in the flume were no turbulence (quasi-laminar), and turbulence (simulated). Turbulence was simulated using three equally spaced vertical cylinders, which produced horizontal streets of vortices similar to fish body depth. Oxygen concentration was plotted against time and the slope used to determine metabolic rates for each organism at varying stress levels (Svendsen et al. 2014). A mixed effects analysis of variance (ANOVA) model was used to test for significant differences between treatment types.

Estradiol analysis

Analysis of estradiol concentrations will be carried out using an Enzyme-Linked Immunosorbent Assay system (ELISA). The prefabricated ELISA system is designed to detect specific chemicals within samples, and the protocol is approved by the United States Environmental Protection Agency for sampling drinking water for a variety of pathogens and chemicals. Dr. Karen Gaines and the Ecotoxicology Laboratory at EIU house all necessary equipment, and has used this technique with success in other studies. She will be a collaborator during the estradiol analysis portion of this project. Estradiol concentration data will later be correlated with physiological and metabolic scope data.

Data Analysis

To analyze community data, fish assemblages were aggregated based upon taxonomic families, while macroinvertebrates were classified based on taxonomic order. As the most robust measure of distance in community ecology (Faith et al., 1987), we employed nonmetric multidimensional scaling (NMDS; Minchin, 1978) based on a Bray-Curtis dissimilarity matrix of scaled assemblage data across two dimensions to examine temporal and spatial trends in biotic communities within rehabilitated and reference sites. Community response to the structural rehabilitation was examined using post-restoration data from 2010 to 2015, and was tested using permutational multivariate analysis of variance (perMANOVA; Anderson, 2001), examining community structure as a factor of both time and treatment type (i.e. rehabilitated vs. reference). Additionally, we calculated a fish Index of Biotic Integrity (fIBI; Karr 1986) along with 95% confidence intervals to estimate changes in health of communities both before, and in years following instream rehabilitation. Health of macroinvertebrate assemblages were monitored using the macroinvertebrate Index of Biotic Integrity (mIBI; Tetra Tech, 2004).

Additionally, we examined linkages between habitat and distribution of fauna using general linear models (GLM) and multiple linear regression (MLR) analyses. Using stepwise model selection based on Akaike's (1973) Information Criterion (AIC), we assessed relationships between relative abundance of taxonomic families to the QHEI parameters. All modeling was completed in R (R Core Team, 2015), and unless otherwise denoted, results were deemed statistically significant at α=0.05.

Principle Findings

Fish Communities

During the seven-year study period, we sampled a total of 79,013 fishes comprising 46 species from nine taxonomic families. Species from families Cyprinidae (85.38%), Centrarchidae (5.6%), Percidae (3.98%), Catostomidae (2.29%), and Ictaluridae (1.46%) accounted for >98% of the total catch, with nominal contributions from Clupeidae, Poeciliidae, Fundulidae, and Atherinidae. Following implementation of artificial riffles, scouring keys, and riparian revegetation, we observed distinct temporal and spatial shifts in community structure in the six years following rehabilitation (Figure 2). Initially, assemblages in all sites were largely comprised of tolerant Cyprinid fishes, however, three years post-rehabilitation there was an apparent shift in community structure characterized by decreased abundance of Cyprinids and increased abundance of Centrarchidae, Catostomidae, Ictaluridae, and Percidae species. This was supported by a perMANOVA (Table 1), which indicated community structure was significantly influenced by the habitat rehabilitation ($F_{1,21}$=5.9304, R^2=0.1692, p=0.012), and varied over a temporal scale ($F_{5,21}$=2.6471, R^2=0.3777, p=0.045). We found a similar delayed response in biotic integrity, with fishes responding more than two years post-rehabilitation (Figure 3). While biotic integrity remained moderately low throughout the study in reference reaches, fishes in restored reaches experienced a steady increase in assemblage health, with recent samples reaching the moderate level of IBI classification, and possibly indicating the return of sensitive benthic invertivore species. Further evidence of this fundamental shift in community structure was observed when examining relationships between fish taxa and habitat drivers within the system. We found significant linkages between boulder substrate and mean depth driving relative abundance of Cyprinidae and Centrarchidae taxa in Kickapoo Creek (Figure 4). The implementation of artificial riffles, coarse boulder substrate and rip-rap keys allowed for the formation of deep scour pools which provided necessary refuge to facilitate recovery of degraded fish communities.

Macroinvertebrate Communities

During this study period we also collected and identified 9,310 macroinvertebrates from 20 orders comprising 66 taxonomic families. Seven orders accounted for >98% of the total catch, including Diptera flies (30.24%), Ephemeroptera mayflies (29.29%), Trichoptera caddisflies (13.32%), Oligochaeta worms (10.06%), Odonata dragonflies (9.45%), Basommatophora snails (3.68%), and Coleoptera beetles (1.9%). Other taxa were collected infrequently, and occurred in less than 1% of samples. When examining changes in macroinvertebrate community structure we did not find any distinct trends resulting from the rehabilitation. Results of a perMANOVA (Table 1) indicated significant effects of habitat rehabilitation ($F_{1,21}$=3.7999, R^2=0.0887, p=0.027) on assemblages in Kickapoo Creek, although the variance attributed to the rehabilitation and explained within the data was nominal (approximately 8% explained). Temporal variation accounted for nearly 55% of the variation within the data, and was found to be significantly driving macroinvertebrate communities ($F_{1,21}$=4.7071, R^2=0.5491, p=0.001). Our NMDS model displayed abundant overlap between communities across treatment types, and no clear temporal trends could be assessed (Figure 5), with communities within a given year appearing more similar than between treatment types. We observed similar trends when examining biotic integrity using the mIBI (Figure 6). Short-term response to the rehabilitation was positive, with community integrity steadily increasing in restored sites as reference assemblages fluctuated. However, recent samples indicate that interannual variation remains a driving force, and similar trends in biotic integrity were visible in both restored and reference communities. Although we did not find substantial long-term response to the rehabilitation project, the macroinvertebrate communities within the study reaches remain in stable fair to good integrity and are capable of supporting a robust and diverse community of fishes.

Metabolic Scope

We successfully observed Longear Sunfish swimming in the lab under a variety of stress conditions. Fish were exposed to quasi-laminar and turbulent flow regimes, and displayed dissimilar swimming abilities within the two regimes. In quasi-laminar flow, Longear Sunfish were able to station hold and maintain position with relative ease. In contrast, swimming in turbulent flow was noticeably disturbed and fish were frequently exposed to forced yaw maneuvering and spills, or loss of heading. Metabolic oxygen consumption was measured in three fish exposed to each flow regime repeated over three trials, for a total of six trials per fish. Mass-corrected oxygen consumption values ($\dot{M}_{O2}$) were obtained for each fish and were corrected for unstressed (i.e. quasi-laminar) condition. We obtained average $\dot{M}_{O2}$ values for Longear Sunfish swimming in quasi-laminar and turbulent flow regimes, and found significant increased cost of transport when navigating complex flow (Figure 7). On average, Longear Sunfish consumed 23% more oxygen when exposed to unsteady flows in the lab. In the field, this sunfish shows high fidelity to deep channels with multiple instream cover types and abundant deep silt-bottom pool habitat; areas characterized by low flow and turbulence. We found significant relationships between Longear Sunfish abundance in Kickapoo Creek and four driving habitat parameters — mean depth, submerged terrestrial vegetation, silt substrate, and boulders (Figure 8). Based on current ecomorphological models, the Longear Sunfish can be described as a habitat specialist, finding refuge in areas of low flow with abundant instream cover structures and deep pools. Given the high energetic costs incurred navigating complex flows, we demonstrate a physiological mechanism driving habitat use and behavior which helps explain shifts in ecology following instream habitat rehabilitation.

Estradiol

The estradiol exposure has not yet started. However, we have successfully established baseline data for geometric morphometrics of body shape and metabolic data. Mass corrected mean oxygen consumption during basal metabolism and maximum metabolism were 207.7

$mgO_2Kg^{-1}.h^{-1}$ and 243.1 $mgO_2Kg^{-1}.h^{-1}$, respectively in fish prior to exposure to estradiol. We show that this species has an aerobic capacity of approximately 35$mgO_2.kg^{-1}.h^{-1}$. We are expecting that fish exposed to estradiol will have this scope reduce, which would impair their ability to optimize energy consumption in their daily behaviors and compromise growth.

PCA analysis of geometric morphometric landmarks showed that four principal components explain 74% (PC1 28.9%, PC2 18.5%, PC3 16.7% and PC4 9.5%) of the variation in the body shape of male longear sunfish. PC1 can be explained mostly by a dorsoventral compression, while PC2 is mostly explained by a change in height of the caudal fin. PC3 is related to changes in the caudal peduncle while PC4 highlights shape changes in the ventral region. From the geometric morphometrics data we are expecting that the body shape will show less male related characters (e.g. higher head) in fish exposed to estradiol. Oxygen consumption and geometric morphometric measurements in the E2 exposed fish will be conducted and compared with the baseline values calculated at the beginning of the mesocosm experiment.

Training potential

This project has served as a unique learning tool for students at Eastern Illinois University. This project provided a graduate assistantship and served as a Master's Thesis for Carl Favata. Dr. Anabela Maia has also been able to advise another graduate student, Neeta Parajulee Karki, who, along with Camden Nix (Dr. Gaines advisee) have begun research on the energetic costs of estradiol exposure in stream fish. Dr. Robert Colombo's (co-PI on the IEPA grant), and Dr. Karen Gaines' (collaborator, estradiol) labs at EIU also gained a tremendous amount of field and laboratory experience stemming from this research. Graduate students Alex Sotola, Hanna Kruckman, Zachary Mitchell, Evan Boone, Clint Morgeson, David Petry, Shannon Smith, Dan Roth, Jordan Pesik, Bethany Hoster, and Camden Nix were instrumental in assisting with stream electrofishing, fish identification, habitat surveys, macroinvertebrate collections and identification, and estradiol analysis. Undergraduate students Missy Eaton, Kailee Schulz, Krista Zerrusen, Courtney Deters, Katherine Bottom, Alicia Kellup, Vantasia Joe, Georgina Govostis and Kelly Forbus were trained in and assisted with field work, data collection, lab identifications, and energetics experiments.

Publications

Below are listed the relevant publications and conference papers which were presented at local, state, national, and international scientific meetings during the term of this agreement:

Favata CA, Colombo RE, and Maia A. 2016. Managing structural rehabilitation: Ecological monitoring and factors driving community structure in a restored stream. Symposium presentation, American Fisheries Society Annual Meeting, Kansas City, MO.

Favata, C.A. (in preparation). Effects of habitat alteration on biotic assemblages and ecomorphology of fish in a restored stream. MS Thesis. Eastern Illinois University. Charleston, IL.

Favata CA, Colombo RE, Roseboom DR, Straub TD, and Maia A. (manuscript in preparation). Habitat factors driving fish assemblages in a restored stream.

Favata CA, Colombo RE, Roseboom DR, Straub TD, and Maia A. (manuscript in preparation). Habitat and macroinvertebrate response to stream restoration.

Favata CA and Maia A. (manuscript in preparation). Ecomorphology and energetics of Longear Sunfish (*Lepomis megalotis*) steady swimming in turbulent flow.

Favata CA, Colombo RE, and Maia A. (manuscript in preparation). Sampling selectivity and fish community comparisons for two common stream electrofishing gears.

Karki, NP, Colombo RE, Gaines K, and Maia A. 2016. Effects of 17β estradiol in the metabolism of Sunfish species. Poster presentation, Illinois Chapter of American Fisheries Society Annual Meeting, Springfield, IL.

Favata CA, Colombo RE, Roseboom DR, Straub TD, and Maia A. 2016. Community structure in a restored stream: what is driving dissimilarity? Oral presentation, Illinois Chapter of American Fisheries Society Annual Meeting, Springfield, IL.

Favata CA, Colombo RE, Roseboom DR, Straub TD, and Maia A. 2016. Factors driving fish assemblages in a restored stream. Oral presentation, Midwest Fish and Wildlife Annual Conference, Grand Rapids, MI.

Favata CA, Colombo RE, Roseboom DR, Straub TD, and Maia A. 2016. Ecomorphology and swimming energetics of longear sunfish Lepomis megalotis in turbulent flow. Oral presentation, Society of Integrative and Comparative Biology Annual Meeting, Portland, OR.

Favata CA, Colombo RE, Roseboom DR, Straub TD, and Maia A. 2015. Ecomorphology of fish assemblages in an East-Central Illinois stream. Oral presentation, American Fisheries Society Annual Meeting, Portland, OR.

Favata CA, Colombo RE, Roseboom DR, Straub TD, and Maia A. 2015. Ecomorphology of fish assemblages in an East-Central Illinois stream. Poster presentation, College of Sciences Sciencefest, Eastern Illinois University, Charleston IL.

Favata CA, Colombo RE, Roseboom DR, Straub TD, and Maia A. 2015. Ecomorphology of fish assemblages in an East-Central Illinois stream. Poster presentation, 18th Annual Sigma Xi Banquet, Charleston IL.

Favata CA, Colombo RE, Roseboom DR, Straub TD, and Maia A. 2015. Ecomorphology of fish assemblages in an East-Central Illinois stream. Poster presentation, 53rd Annual Illinois American Fisheries Society Chapter Meeting, Pere Marquette, IL.

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Table 1—Results of permutational ANOVA testing differences in community structure as a factor of habitat rehabilitation, and accounting for temporal (annual) assemblage variation. Community dissimilarity was monitoring in restored and reference (control) sites in Kickapoo Creek from 2010-2015.

	Term	DF	MS	F	R^2	p-value
Fish	treatment	1	0.0603	5.9304	0.1692	0.012
	year	5	0.0269	2.6471	0.3777	0.045
	interaction	5	0.0120	1.1761	0.1678	0.356
	residuals	10	0.0102			
	total	21				
Macroinvertebrates	treatment	1	0.1975	3.7999	0.0887	0.027
	year	5	0.2446	4.7071	0.5491	0.001
	interaction	5	0.0575	1.1060	0.1290	0.358
	residuals	10	0.0520			
	total	21				

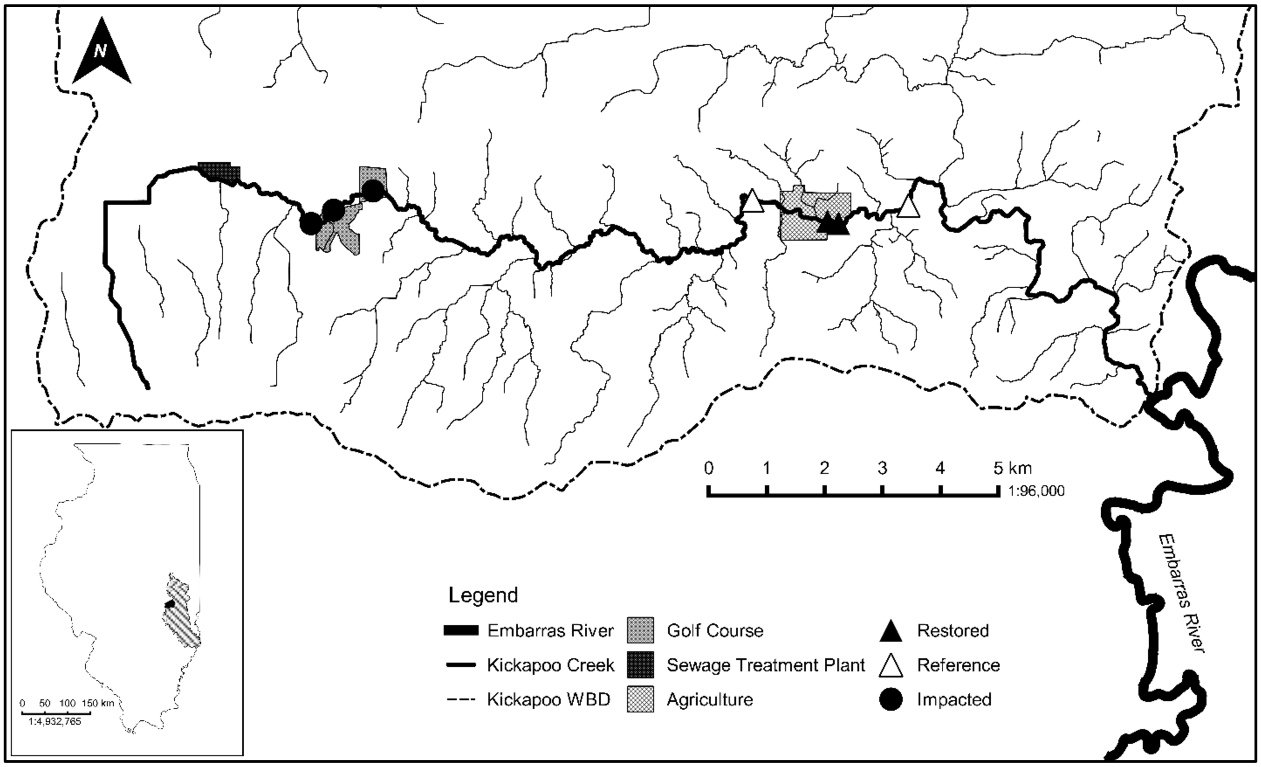

Figure 1—Locations of restoration, reference, and impacted sites monitored within the Kickapoo Creek watershed boundary (WBD) in East-Central Illinois from 2009 to 2015.

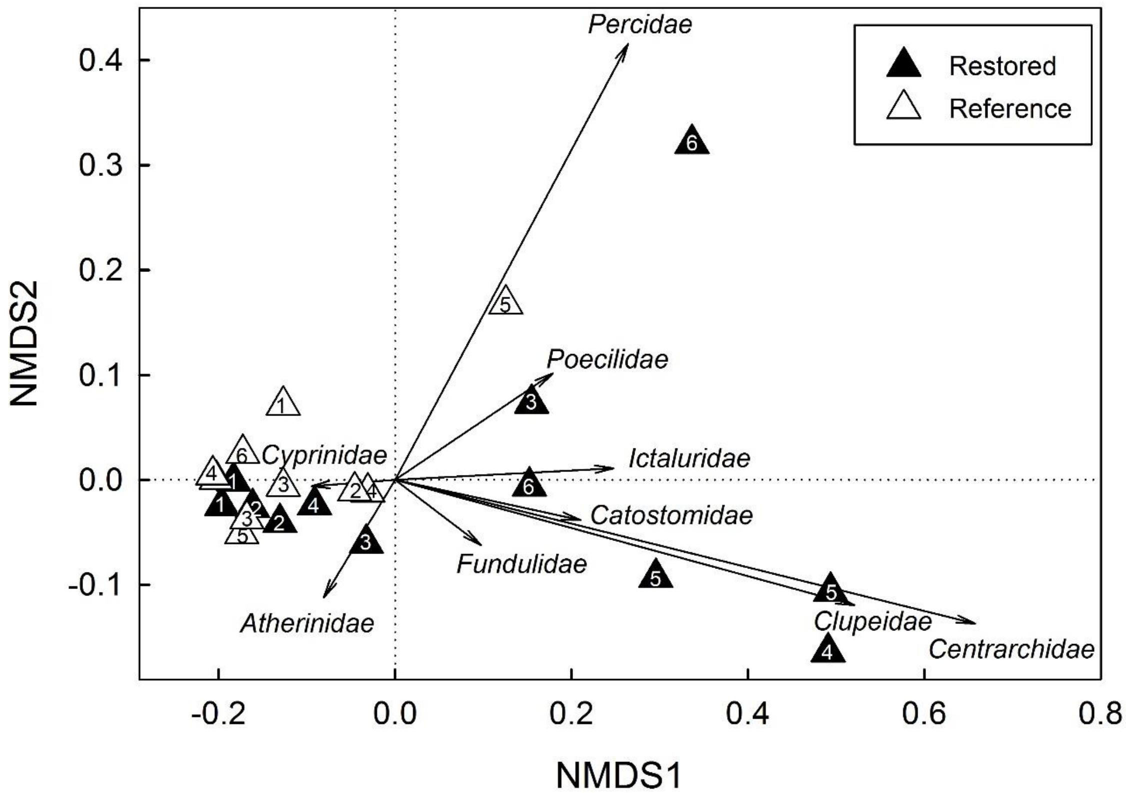

Figure 2—Nonmetric multidimensional scaling (NMDS) plot computed with a Bray-Curtis dissimilarity matrix examining temporal and spatial changes in fish community structure following an instream rehabilitation project in Kickapoo Creek. Assemblages were sampled in restored and reference sites from 2010 to 2015, and numbers within the plot correspond to years post rehabilitation (1-6). Relative loadings of taxonomic groups are represented by solid vectors, with direction and magnitude relating to respective correlations with the community matrix.

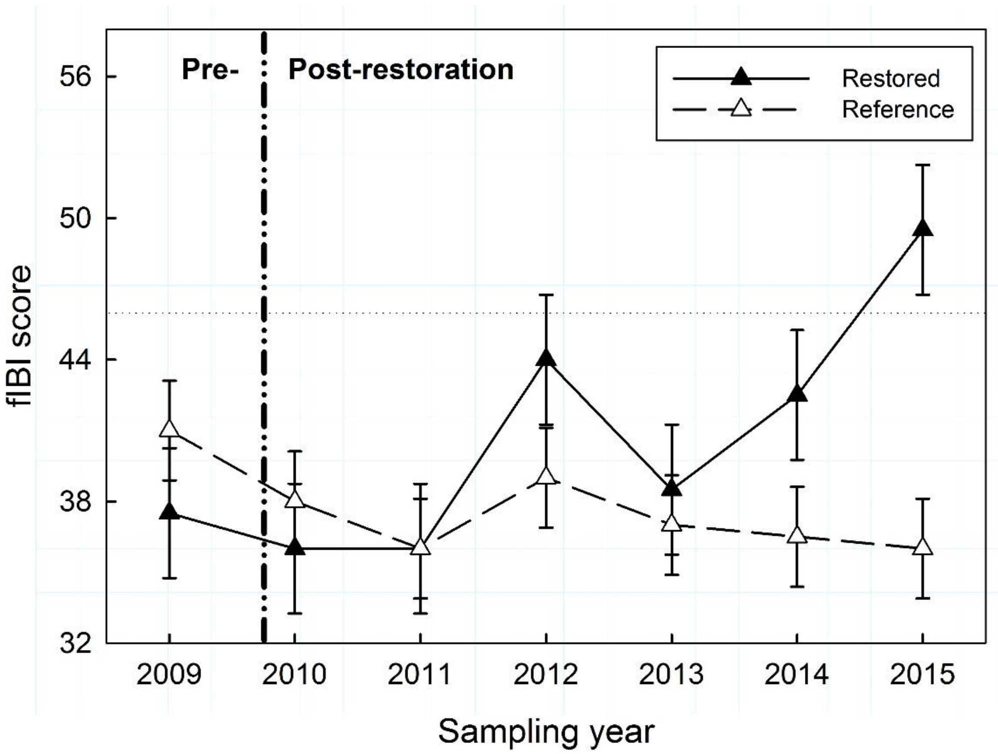

Figure 3—Fish Index of Biotic Integrity (fIBI) scores for fish assemblages in restored and reference sites sampled from 2009 (pre-restoration) to 2015. The vertical dashed line indicates the approximate completion of the rehabilitation project, while the horizontal dotted line separates the 'moderately low' integrity classification from 'moderate' biotic integrity. Error bars represent 95%-confidence intervals computed for assemblages within each of the treatment types.

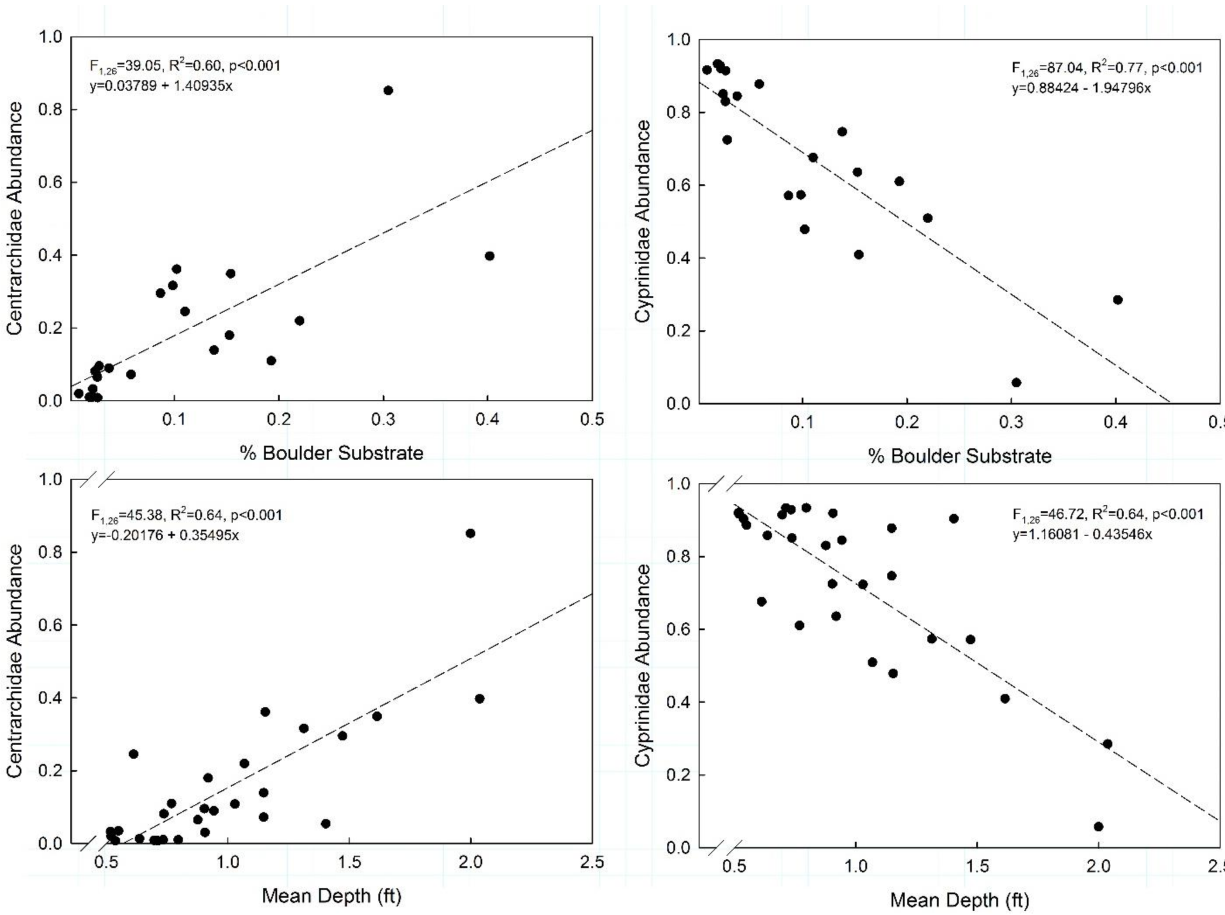

Figure 4—Relationships between relative abundance of Cyprinidae and Centrarchidae taxa with percentage of boulder substrate and mean depth within seven study sites along Kickapoo Creek, sampled from 2009 to 2015. Dashed lines represent best-fit linear regression models. Results of linear regression analysis and equations appear within each respective plot.

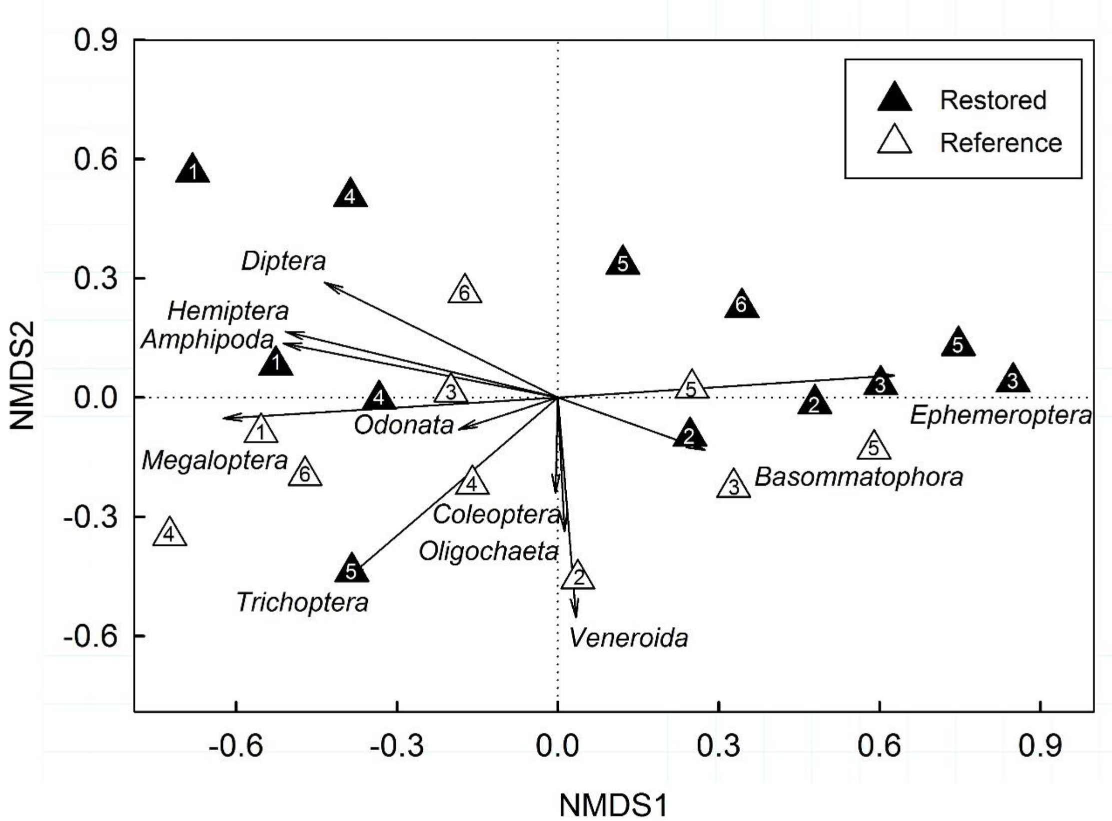

Figure 5— Nonmetric multidimensional scaling (NMDS) plot computed with a Bray-Curtis dissimilarity matrix examining temporal and spatial changes in macroinvertebrate community structure following an instream rehabilitation project in Kickapoo Creek. Assemblages were sampled in restored and reference sites from 2010 to 2015, and numbers within the plot correspond to years post rehabilitation (1-6). Relative loadings of taxonomic groups are represented by solid vectors, with direction and magnitude relating to respective correlations with the community matrix.

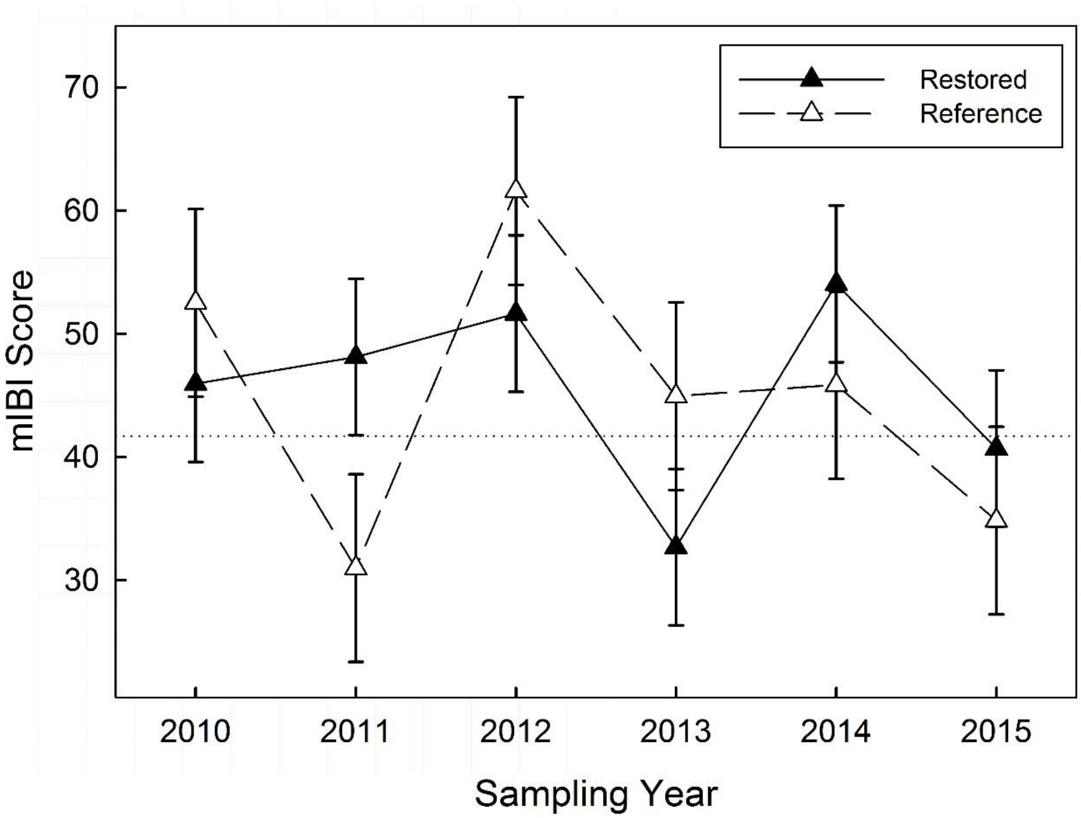

Figure 6—Macroinvertebrate Index of Biotic Integrity (mIBI) scores for fish assemblages in restored and reference sites sampled post-rehabilitation from 2010 to 2015. The horizontal dotted line separates the 'fair' integrity classification from 'good' biotic integrity. Error bars represent 95%-confidence intervals computed for assemblages within each of the treatment types.

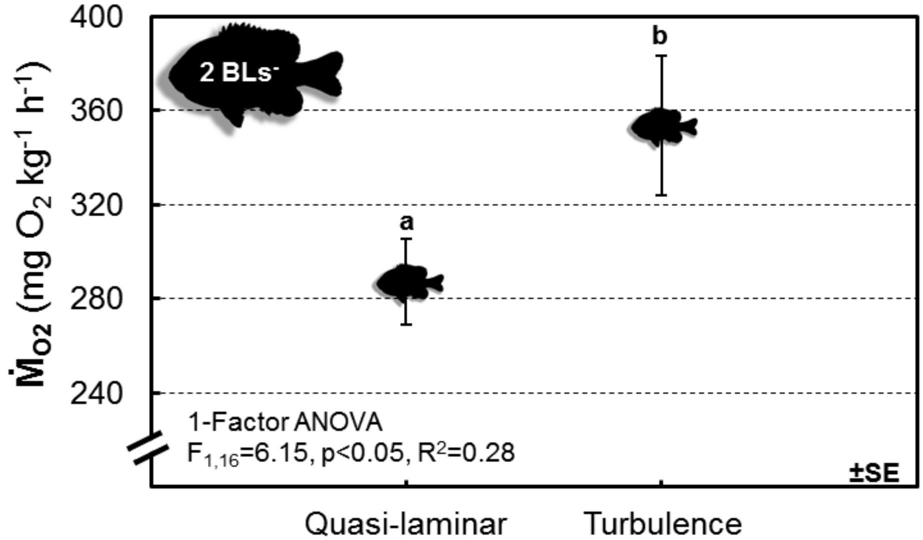

Figure 7—Mass-corrected rates of oxygen consumption ($\dot{M}_{O2}$) for Longear Sunfish swimming in quasi-laminar and turbulent flow. Results of the ANOVA are presented in the figure. Error bars represent ± standard error (SE)

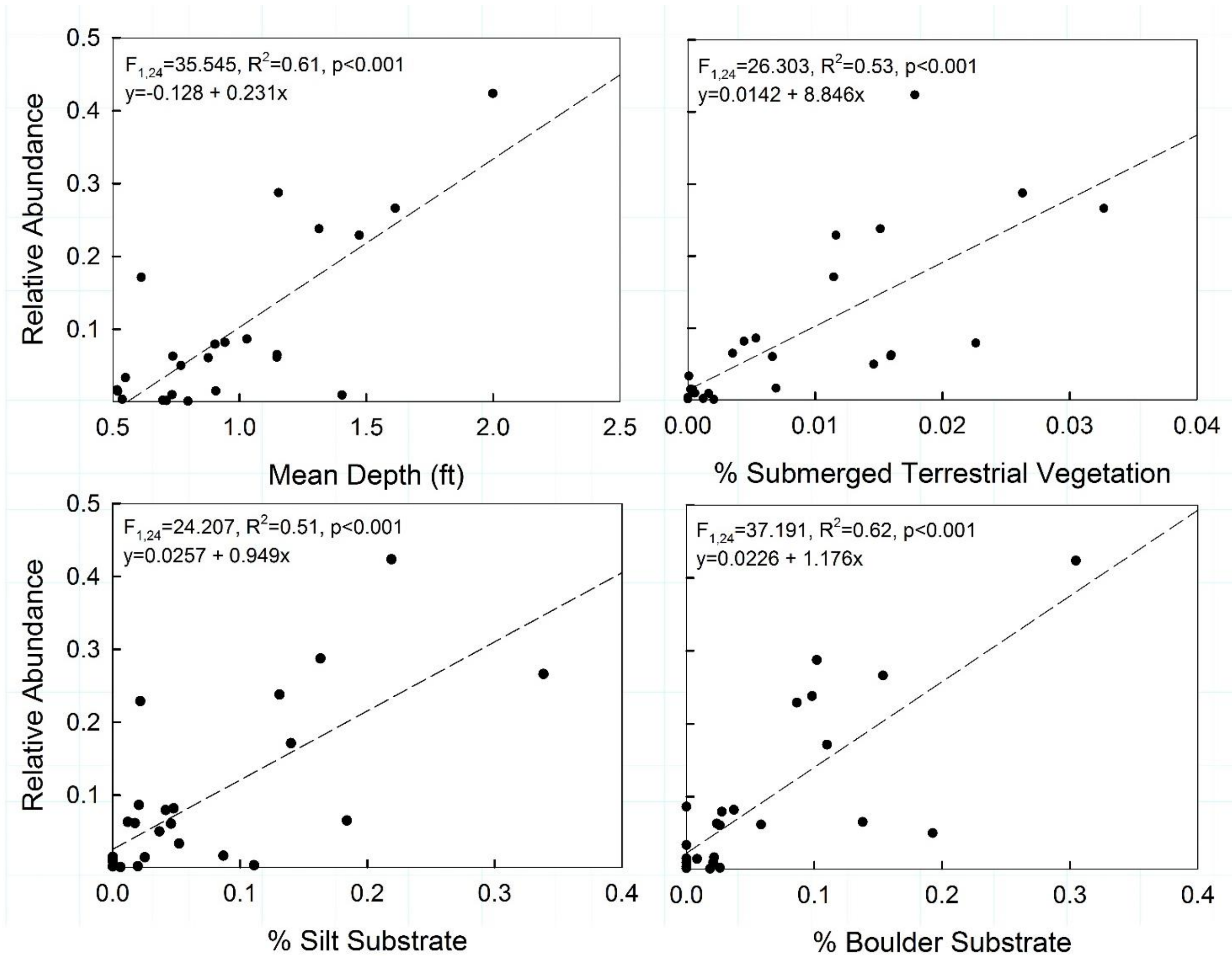

Figure 8—Relationships between relative abundance of Longear Sunfish and driving habitat factors in Kickapoo Creek. Results of linear regressions appear within each respective plot along with an equation for each best-fit dashed line.