Synchrony between fish feeding guilds and habitat associations in a regulated Great Plains river

by

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Approved by:

Major Professor
Dr. Keith Gido

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Abstract

The effects of river regulation on fish populations and their habitats are complex and further comprehension is needed for effective management and conservation. Specifically, we sought to understand if habitat variability alters the distribution of fish species and their diets in a Great Plains riverscape. Fish, aquatic macroinvertebrates, and habitat measurements were collected at seven sites in the regulated Kansas and Big Blue rivers. We used multivariate analysis of fish diets and fish habitat associations to identify feeding and habitat guilds and tested their dependencies using a fourth corner approach. We found that although there was a large degree of diet overlap for most assemblage members, species diets could generally be used to distinguish four feeding guilds: herbivore, omnivore, benthic invertivore, and pelagic/terrestrial invertivore. We also ascertained that species were distributed along a habitat gradient with mid-channel species like emerald shiners *Notropis athernoides* and carmine shiners *N. percobromus* on one end and a near shore assemblage of western mosquitofish *Gambusia affinis,* bluntnose minnows *Pimephales notatus,* and sand shiners *N. stramineus* on the other. The fourth corner analysis provided some support (p-value = 0.08) for the synchrony between the diets of species and their habitats, suggesting a mechanism by which river regulation and associated changes in habitat might influence fish populations through the food web. A more comprehensive understanding of how flow regulation impacts the distribution of habitats in the riverscape is necessary to better implement this knowledge into management and conservation of regulated rivers.

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Acknowledgements

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Chapter 1 - Synchrony between fish feeding guilds and habitat associations in a regulated Great Plains river

Introduction

Regulation of flow regimes in systems impounded by dams is in constant conflict with the dynamic nature of large rivers (Kondolf et al. 2014, Reid et al. 2019). Construction of dams to store water and prevent flooding interrupts historic regimes of water, sediment, and nutrient cycling that maintain river ecosystem functions (Ward and Stanford 1983, Dudgeon et al. 2006, Kondolf et al. 2014). Local communities adapted to historic river dynamics are disadvantaged in these perturbed ecological conditions (Nilson et al. 2005, Dudgeon et al. 2006). The life history traits of fish that were shaped by historic flow regimes are potentially maladapted to flows regulated to promote human habitation on the landscape (Lytle and Poff 2004, Nilson et al. 2005, Mims and Olden 2012, Mims and Olden 2013). Regulated rivers are accompanied by additional stressors including dispersal barriers, invasive species, poor water quality, and climate change (Jelks et al. 2008). While these stressors can lead to decreasing population size, local extirpation, and even extinction for some species, it can also boost the abundance and distribution of others (Mims and Olden 2013). Investigating functional responses of fishes and their resilience to river regulations can inform ecosystem managers tasked with curbing the negative effects of dams (Poff et al. 2010).

Food webs serve as a guide to how fish interact with their environment. As diet powers growth and reproduction, traits associated with food acquisitions are strongly tied to their survival (Gerking 1994). To survive in fluctuating environmental conditions, many fish species have adopted a generalist strategy of feeding (Gerking 1994). Generalists prey on a wide range

of food items as shifting abiotic conditions change where and when prey can be found (Hartley 1948, Maitland 1965, Angermeier 1982). For example, seasonal flooding of an intermittent stream in Australia increased the abundance and diversity of prey while also increasing the range and habitats the fish assemblage could forage (Balcombe et al. 2005). These fish consumed the same amount of food during flooding as they did when they were restricted to pools during base flows, highlighting their adaptability to finding food no matter the conditions. While morphology and behaviors might appear specialized for consumption of specific prey items and foraging conditions, these structures are often adaptable to diet switching whenever it is advantageous (Grossman et al. 1982, Gerking 1994). In the Amazon River Basin, fish notable for their frugivorous diets and herbivorous morphology consume fruits during the wet season (when fruits are ripe and most accessible) but switch their diets to terrestrial invertebrates, benthic microorganisms, and other items when the fruits are out of season and competition for this prey is high (Correa and Winemiller 2014). Thus, generalist diets minimize competition within the fish assemblage while maximizing prey consumption under variable environmental conditions (Angermeier 1982, Dill 1983). Fish communities switching their diets in response to environmental variability is well documented, but the specific mechanisms for these changes are less understood (Angermeier 1982, Grossman et al. 1982, Gerking 1994). While the availability and distribution of prey is an important factor (Angermeier 1982, Graeb et al. 2004, Wang et al. 2019, Ouellet et al. In Press), the effects of abiotic changes on where a fish can access and detect prey might also influence how fish diets react to changing environments.

Varying environmental conditions directly change fish diets by restricting where fish can forage and might interfere with fish sensory abilities (Abrahams and Kattenfeld 1997, Utne-Palm 2002). Habitat changes such as drying, fast currents, or other environmental shifts influence prey

accessibility and determine where fish can forage, regardless of the prey's availability (Ouellet et al. In Press). Pothoven et al. (2009) found that rainbow smelt *Osmerus mordax* unable to access benthic prey due to hypoxic conditions in Lake Erie shifted their diet to pelagic prey. Other factors, including turbidity, also diminish the ability of many fish species to locate and acquire prey even when they are available (Bonner and Wilde 2002, Ward et al. 2016, Ward and Vaage 2019, Ouellet et al. In Press). Even subtle habitat features such as differing leaf and stem configuration of aquatic plants can affect bass *Micropterus* spp. predation on macroinvertebrates and fish (Dibble and Harrel 1997). Understanding how shifting environmental conditions might generate fluctuations in prey detectability or accessibility and corresponding fish feeding behavior is necessary to predict how fish diets respond to historic or human-induced, environmental variability.

Great Plains streams were historically quite variable; with intense flooding caused by heavy precipitation interspersed with prolonged droughts (Vaughn et al. 2004, Costigan and Daniels 2012). The historic regimes likely altered prey availability, accessibility, and detectability with shifts in lateral connectivity, turbidity, and other stream habitat features. This variable environment shaped an aquatic assemblage of generalist feeders and their accessibility to food. Since the 1960s, largescale stream fragmentation, groundwater depletion, and flow modifications across the Great Plains, have altered the fish assemblages (Cross and Moss 1987, Gido et al. 2010). The impact of the currently manipulated environmental variability on the feeding ecology of the contemporary assemblage is poorly understood. With increased focus on the restoration of the region's rivers, understanding how fish diets might respond to habitat changes under regulated flow regimes can be used to assess the effectiveness of ecosystem management.

In the Kansas River basin, river regulation is largely managed by dams operated by the United States Army Corps of Engineers (USACE) and focuses on flood prevention, meeting water quality standards, and providing flow adequate for barge traffic in larger rivers downstream. Recent management efforts have included the environmental restoration potential of impoundments through the Sustainable River Program, a collaboration between The Nature Conservancy and the USACE that develops watershed specific programs of environmental flows to restore ecological variation for ecosystem restoration and enhancement. Novel sediment management experiments are also underway by the USACE, testing if water-injection dredging in a major Kansas River tributary can replenish sediments and nutrients downstream. Understanding how fish diets respond to the environmental variability in this regulated river system will help managers understand responses of the fish communities to these restoration activities.

We tested if the foraging guilds of fish species were related to the different habitats and environmental conditions in the regulated Kansas River. Specifically, we asked if feeding habits of species within a fish assemblage are synchronous with habitat use across a gradient of environmental conditions. We expected that species could be classified into feeding (e.g., terrestrial invertivore, herbivore, detritivore) and habitat (e.g., depth, velocity) guilds, and that species with similar feeding habits would occupy similar habitats, or there would be asynchronous habitat use by species with similar diets due to shifting resource availability, detectability, and accessibility within habitats (Figure 1). For example, diet synchrony would be a species feeding on benthic invertebrates in fast and deep habitats while another species fed on algae in slow and shallow habitats, while diet asynchrony would reflect constantly shifting availability and accessibility of algae and benthic invertebrates requiring species eat them

wherever and whenever they could consume them. Understanding how river regulation impacts fish functional responses to environmental variation might allow managers to focus and refine the use of environmental flows through a better understanding of how habitat changes influence food web interaction.

Methods

Study area – To test if diets of fish species were characteristic of the habitat in which they were collected fish, macroinvertebrates, and habitat were sampled seasonally across seven sites in the Kansas and Big Blue rivers (Figure 2). The Kansas River is the largest tributary of the Missouri River and a sand-bed river. While the mainstem is unfragmented upstream of Lawrence, KS (not shown on Figure 2), its major tributaries in our study area, the Smoky Hill River, the Republican River, and the Big Blue River, are all regulated by large dams. The land use surrounding these four rivers is primarily agricultural and bank stabilization structures (e.g., rip-rap, dikes, and levees) are common. River impoundment and flow regulations have all led to stream channelization and other habitat modifications, such as a reduction in coarse substrate.

Fish assemblage – Fishes were sampled in six, 30 m shoreline habitats at each site using a straight seine (1.8 m x 4.6 m-0.32 cm mesh). We restricted sampling to wadable habitats free from obstructions (e.g., large wood) to have comparable sampling effort across samples. Total length of the first 25 individuals of each species per habitat was measured and the rest were counted. If there were >500 fishes in a haul, then the assemblage was subsampled with a 12.5 cm x 11 cm aquarium net and individuals counted in one “scoop” was extrapolated to the total number of scoops of the remaining sample.

Diet analysis – Ten individuals of common species were collected at each site when sampling allowed and preserved in 10% formalin for diet analysis. In the laboratory, weight and

length were measured before the contents from the stomach/anterior intestine to the first bend were extracted and viewed under a stereoscope. The contents were identified to lowest taxonomic family and photographed to archive diet information for quality assurance. As a surrogate for mass of diet items, contents were spread evenly on a 1x1 mm grid and the surface area of each item was estimated following Franssen and Gido (2006). This was repeated until a maximum of ten diets that were not empty were analyzed per species.

Habitat – To quantify physical characteristics of habitats seined, measurements were taken at three points along three transects (n = 9 points) from the start of the seine haul (0 m), the middle of the seine haul (15 m), and the end of the seine haul (30 m). Habitat points for each transect were taken at 0 m, 2.3 m and 4.6 m, perpendicular to the seine haul, representing the two edges and middle of the seine haul. Depth (m), velocity (m/s at 60% depth; FH950, Hach, Loveland, CO, USA), and dominant substrate (Wentworth 1922) were recorded at each point. Coordinates at the middle of the habitat were recorded.

Macroinvertebrates were collected with a D-frame net on a 30 m transect running through the middle of the same habitats sampled with the seine. Macroinvertebrates were preserved in the field and identified to the lowest taxonomic family (excluding chironomids which were separated between Tanypodinae and other genera). Macroinvertebrates <2 mm in length were counted and those >2 mm were counted and photographed for quality assurance and control. Macroinvertebrate biomass (g/m^2) was calculated using existing length to weight relationships per invertebrate taxa (Benke et al., 1999) for all individuals in a sample and dividing this sum by the area sampled during each seine haul.

Data analysis – Our first step was to characterize differences in diets among species, sample locations, and seasons. The percent occurrence of diet items was based on the presence or

absence (Amundsen and Sánchez-Hernández 2019) of six different prey groups: algae, benthic aquatic invertebrates (BAI), pelagic aquatic invertebrates (PAI), terrestrial invertebrates, and amorphous detritus. These groupings were identified from the cumulative taxa identified across all samples (Appendix Table A. 1, Table 1) and all unknown or unidentifiable items were omitted (Amundsen and Sánchez-Hernández 2019). To characterize differences in diets across species, we analyzed the presence of the prey groups per site, season, and year using a principal coordinates analysis (PCoA) ordination based on a Jaccard similarity coefficient to visualize variation in diets across species. This was followed by a multivariate analysis of variance (MANOVA) to test for significant differences in the occurrence of diet groups among species. If there was an overall significant species effect, we used Bonferroni adjusted p-values for *post hoc* comparisons to test for species contributing to this difference within diet groups.

Our second step was to test if variation in fish assemblage structure measured in sampled habitats was explained by measured habitat variables. The measurements of habitats and macroinvertebrate data were used to calculate average velocity, average depth, percent occurrence of silt, percent occurrence of coarse sediment (sediment with diameter greater than sand), and macroinvertebrate biomass (g/m^2). We used a canonical correspondence analysis (CCA) to compare these variables with the square root transformed abundance of species counts per seine haul. We used a stepwise selection for each variable using Akaike's information criterion (AIC) and then ran a permutational analysis of variance test to calculate the significance of each variable and axis (Oksanen et al. 2022). All analyses were performed using R version 4.3.0 (R Core Team, 2023).

Data were also analyzed using the fourth-corner approach to correlate environmental data (R matrix) with species traits (Q matrix) linked through species abundances (L matrix; Dray and Legendre 2008), or in other words, we used fish abundance data to connect species' diet (Q matrix) with where they were sampled. The Q matrix was composed of the percent occurrence of presence/absence of the five different prey groups per species. We used the same habitat measurements described above for the R matrix and species counts per seine haul of species that had a minimum of 30 diets analyzed for the L matrix. These three matrices were used for R-mode linked to Q-mode (RLQ) ordination (Dolédec et al. 1996) which visualizes the correlation between the environmental variables, species traits, and species abundances (Figure 3). The fourth corner analysis tested the significance of the relationship between the three matrices by creating a fourth matrix (the "fourth corner") and weighing the relationship between the environment and traits, in this case species diets, with the abundance of each species (Legendre et al. 1997). Permutations of this matrix were used to test if there were significant relationships between the environment and the species diet traits using a x^2 test. A significant association between environmental variables and species diets provides evidence supporting the hypothesis that species diets are synchronous with their habitat use. Alternatively, species feeding guilds might be independent of their habitat use.

Results

Of the 34 fish species collected, diets were quantified for 21, but using a threshold of 30 diets, only 9 species were included in analyses (Table 1). Among the diets analyzed (n=1555), 198 were empty and 64 unique prey items were found and assigned to broad prey groups (Appendix Table A. 1). The individual diets were typically dominated by specific prey groups depending on the site, season, and year, but the cumulative diets between species differed and

were dominated by one or two prey groups (Table 1). Amorphous detritus was the least often consumed group and did not contribute towards the identification of a feeding guild because of its similarly low occurrence across species. The diet analyses provided support for 4 feeding guilds: herbivores, omnivores, benthic invertivores, and pelagic/terrestrial invertivores (Table 1, Figure 4). The feeding guilds of the herbivore juvenile river carpsuckers *Carpiodes carpio*, herbivore bluntnose minnows *Pimephales notatus*, and benthic invertivore juvenile channel catfish *Ictalurus punctatus* were easily distinguished by high occurrences of specific prey groups (90-100%), but the diet overlap of invertebrate and algae prey made distinguishing the guild membership of the six other assemblage members difficult. Using the occurrence of the prey groups (Table 1) we were able to identify sand shiners *Notropis stramineus* and bullhead minnows *P. vigilax* as omnivores, western mosquitofish *Gambusia affinis* as an additional benthic invertivore, and red shiners *Cyprinella lutrensis*, carmine shiners *N. percobromus*, and emerald shiners *N. athernoides* as invertivores that targeted pelagic and terrestrial invertebrates.

During fish sampling, we encountered relatively low flow conditions (Figure 5). In 2023, there was a historic drought within the study reach and flows ranged from 14 m^3/s to 114 m^3/s with a median of 24 m^3/s (USGS gage 06887500). 2024 had similarly low median (25 m^3/s) but experienced a greater range of flows (7 m^3/s – 354 m^3/s). Despite the high summer flows seen in 2024, the median values of both years were 2.5 times lower than the 40-year median.

The CCA ordination indicated measured habitat variables accounted for 8% of the variation in the fish assemblage structure, and only the first axis was significant (p-value = 0.02; Figure 6). The stepwise AIC variable selection omitted average depth, but all other habitat variables significantly contributed to the model. The first axis showed that emerald shiners, juvenile river carpsucker, and carmine shiner were positively associated with the average

velocity, macroinvertebrate biomasses, and non-sand substrate. On the other end of this gradient, western mosquitofish and sand shiner were associated with slow velocities, low macroinvertebrate biomass, and sand substrate.

The fourth corner test provided some evidence that species diets synchronized with habitat (p-value = 0.08). The RLQ ordination showed similar trends for the diet tendencies of species compared with the prior PCOA and MANOVA analyses and hints that predation of pelagic and terrestrial invertebrates primarily occurs in environmental conditions that differ from benthic feeding on algae (diatoms) and aquatic macroinvertebrates (Figure 7). Emerald shiners, carmine shiners, and red shiners consumed the terrestrial invertebrate and PAI groups while occurring in habitats with high velocities, high macroinvertebrate biomasses, and large percentages of coarse substrate. Within the species feeding on benthic items (i.e., algae vs. BAI), there were also differences in species and habitat associations. Juvenile channel catfish and western mosquitofish were strongly associated with BAI in habitats with low average velocities, average depths, low aquatic macroinvertebrate biomasses, and sandy substrates. Algivorous juvenile river carpsuckers and bluntnose minnows were more associated with habitats with low average velocities, high average depths, low macroinvertebrate biomasses, and high percentages of fine substrate. Sand shiners and bullhead minnows consumed benthic prey items in silty-sandy substrates, deep-shallow depths, low velocities, and low macroinvertebrate biomasses.

Discussion

The effects of environmental change on fish ecology are complex and we provide evidence that species' diets in the regulated Kansas and Big Blue rivers are partially synchronous with habitat use (Figure 1a). Although these fishes' diets overlapped with one another, as seen with many other studies of fish feeding ecology (Angermeier 1982, Gerking 1994), we identified

four feeding guilds present in the fish assemblage. The adaptability of individual fish diets is likely tied to the availability and accessibility of prey items, but the delineation of species feeding guilds is synced to their habitat, suggesting that natural or human-induced variation in habitat availability might affect these species through food webs.

We found fish habitat associations could broadly be defined across a velocity gradient. This continuum also represented a gradient from channel margins and other habitats with slow velocity to mid-channel habitats where velocity is higher. The ends of this gradient are clearly illustrated by emerald shiner and western mosquitofish (Figure 6). Alternatively, some species, such as red shiner, have been reported to have little habitat specialization, especially in the Great Plains rivers (Matthews and Hill 1980). While this species occurred across the velocity gradients measured in our study the greatest numbers tended to occur in slower velocity habitats. A shift of fish assemblages across velocity gradients has been observed in other lotic systems (Gorman 1987, Grossman et al. 1998). Gorman (1987) attributed the occurrence of smaller species in low velocities versus high flow pelagic habitat with increased piscivory. However, in a North Carolina stream another fish-velocity gradient was observed and predation was determined to be negligible (Grossman et al. 1998). Instead, Grossman et al. (1998) hypothesized that species mostly occurred in optimal velocity habitats that balanced prey capture success with energy expended (Hill and Grossman 1993). In other words, they observed fish habitat distribution synchronous with where species are most adept at finding and consuming prey. We found a similar pattern in the regulated Kansas and Big Blue rivers.

Fishes feeding on benthic invertebrates, algae, and omnivorously were found near shore and in slower velocity habitats, whereas those feeding on terrestrial or pelagic invertebrates were in faster velocity habitats. The synchrony between diet and habitat demonstrates different ways

the observed environmental variation can control the accessibility and availability of the prey in this system. Benthic invertivores were found in shallow habitats with low average velocity, dominant sand substrates, and little aquatic macroinvertebrate biomass. Fish predation on benthic macroinvertebrate by fish species like bluegill *Lepomis macrochirus* and other Great Plains species, have been found to have negative effects on macroinvertebrate communities, especially in habitats without invertebrate refugia (Gilinsky 1984, Diehl 1992, Bonjour et al. 2020). In our system, invertebrates, with little to no refugia in the sand dominated habitats, were easy prey for the benthic invertivores and they were able to effectively lower the macroinvertebrate biomass in these habitats. Herbivores were synchronous with slow-velocities and high percentages of fine substrates. Both factors are correlated with algal growth in rivers, particularly diatoms, which were the most consumed prey type of algae (Appendix Table A. 1). Lamb and Lowe (1987) observed that in slow velocities diatoms increased their vertical growth and stabilized sediments that promoted the colonization of other diatoms compared to faster velocities. Also, in predominantly sand bed rivers, algae cannot effectively establish in the channel due to shifting substrate (W. Dodds, Personal Communication). Further, Richards et al. (2020) found fine substrates subsidized nutrients for diatoms and promoted their growth. The fourth corner analysis indicates omnivorous diets are situated in habitats that are a mix of the habitats that promote benthic invertivory and herbivory although this might be an artifact of them consuming both groups at about equal intervals. Evans-White et al. (2001) observed that although an omnivorous fish primarily consumed algae and amorphous detritus, their production was primarily boosted by macroinvertebrate consumption. With the opportunistic abilities of fish diets, the omnivores we observed might consume algae for energy when invertebrates are not available to fuel growth (Ahlgren 1990). The diets of species eating a large proportion of PAI and terrestrial invertebrates

are uniquely associated with fast velocities, coarse substrates, and greater benthic macroinvertebrate biomasses. High velocity habitats require high levels of energy expenditure for fish to maintain their position (Hill and Grossman 1993). Fish species that are adapted to high velocities to forage for invertebrates find prey drifting in the water column or near the water's surface (Brittain and Eikeland 1988). The high benthic macroinvertebrate biomasses associated with species commonly eating pelagic and terrestrial invertebrates is probably related to the high velocity depressing predation of benthic macroinvertebrate and elevated oxygen and nutrient levels promoting macroinvertebrate production, especially for filtering species, in this habitat (Buffagni and Comin 2000, Whiles and Dodds 2002). Further examination of how regulation has manipulated these relationships between diet and habitat would provide managers options when restoring regulated rivers.

Some of the variation in habitat and diets in our study might have been attributed to interannual variation in flows. In 2023, our study reaches experienced record low flows due to drought and pelagic species like emerald and carmine shiners were seldom sampled compared to years prior to 2023 (K. Gido, unpublished data) and in 2024 prior to increased flows in spring and summer (Figure 5). We observed an opposite trend for the juvenile river carpsuckers which were low in abundance in 2024. Freeman et al. (2022) highlights the importance of linking different flow events with demographic processes and our findings suggest feeding behaviors and habitat use might provide a mechanistic link between species population response to flows give that river carpsucker used slow velocity habitats and emerald and carmine shiner used fast velocity habitats. However, we also acknowledge that interannual variation in flows might partially account for the observed habitat associations, assuming that variables measured at the mesohabitat scale (i.e., seine hauls) reflected the river discharge. That is, species associations

with velocity might reflect variation across sample dates, rather than a velocity gradient within or across sites measures in a single season.

This study was able to link habitat affinities of 9 species and their diets, but we did not comprehensively sample all available habitat in the Kansas and Big Blue rivers. Using a straight seine restricted our sampling to depths <1.5 m habitats with moderate velocities and we did not sample complex habitats with large wood. This shortcoming could have led to us under-represent some species or diets of species occupying those habitats. Regardless, the vast majority of habitats available at our sample sites were within the bounds of where we could sample, and we feel our results adequately represent the small-bodied fish assemblage of the river system. It is also important to note the statistical power of our analysis was limited by the number of species with adequate characterization of diet (i.e., replication across species with each diet guild). It is not clear how adding species that were collected in lower abundance might alter our findings. This might be an avenue for future research.

While we have documented the feeding and habitat associations of a contemporary fish assemblage of the Kansas and Big Blue rivers, future management of reservoir discharge will likely include recommendations for environmental flows (e.g., through the Sustainable River Program) or sediment releases following the water injection dredging. These activities will likely alter the availability of habitats in the system and the response of the fish assemblage remains to be seen. Results of this study, however, might help generate prediction on how the fish community will respond. For example, during periods of high water release the availability/accessibility of terrestrial invertebrates and PAI will likely benefit the species and individuals that can compete in this guild. If the habitat remains slow and in near-drought conditions, then the benthic invertivores, herbivores, and omnivores are likely to proliferate.

Other environmental impacts from these restoration activities, like the expected increase of turbidity from the water injection dredging, might benefit or have a neutral effect on the species that forage in habitats dominated by fine sediments. Further investigations of how dams have, and continue to, regulate the habitat, diets, and ecology of fish will produce a future riverscape that promotes the habitation of both fish and humans.

Figures

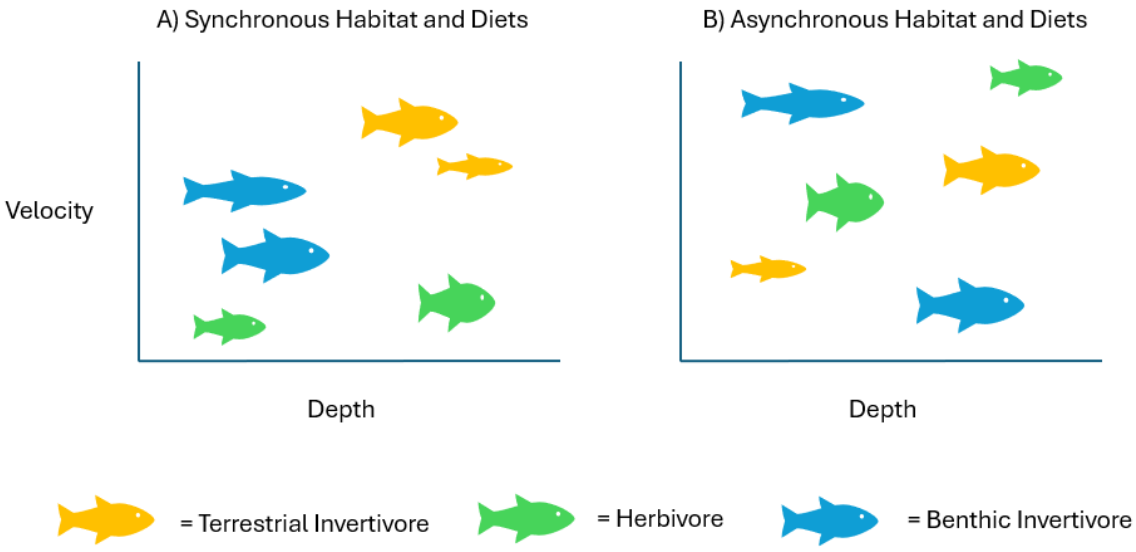

Figure 1. Conceptual figure of two hypotheses for how feeding guilds might be correlated with habitat associations of species. The figure depicts hypothetical fish assemblages with species differing in their habitat distributions relative to assigned foraging guilds.

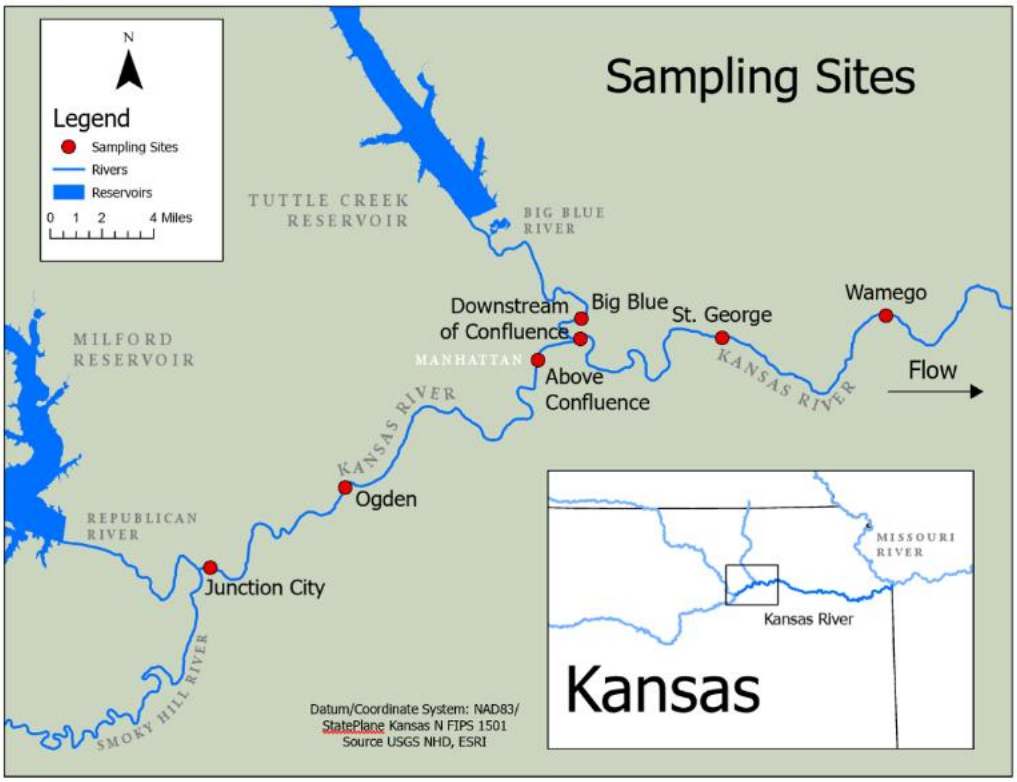

Figure 2. Map of fish, aquatic macroinvertebrates, and habitat sampling sites on the Kansas and Big Blue rivers in northeastern Kansas. Sites are denoted by red spots.

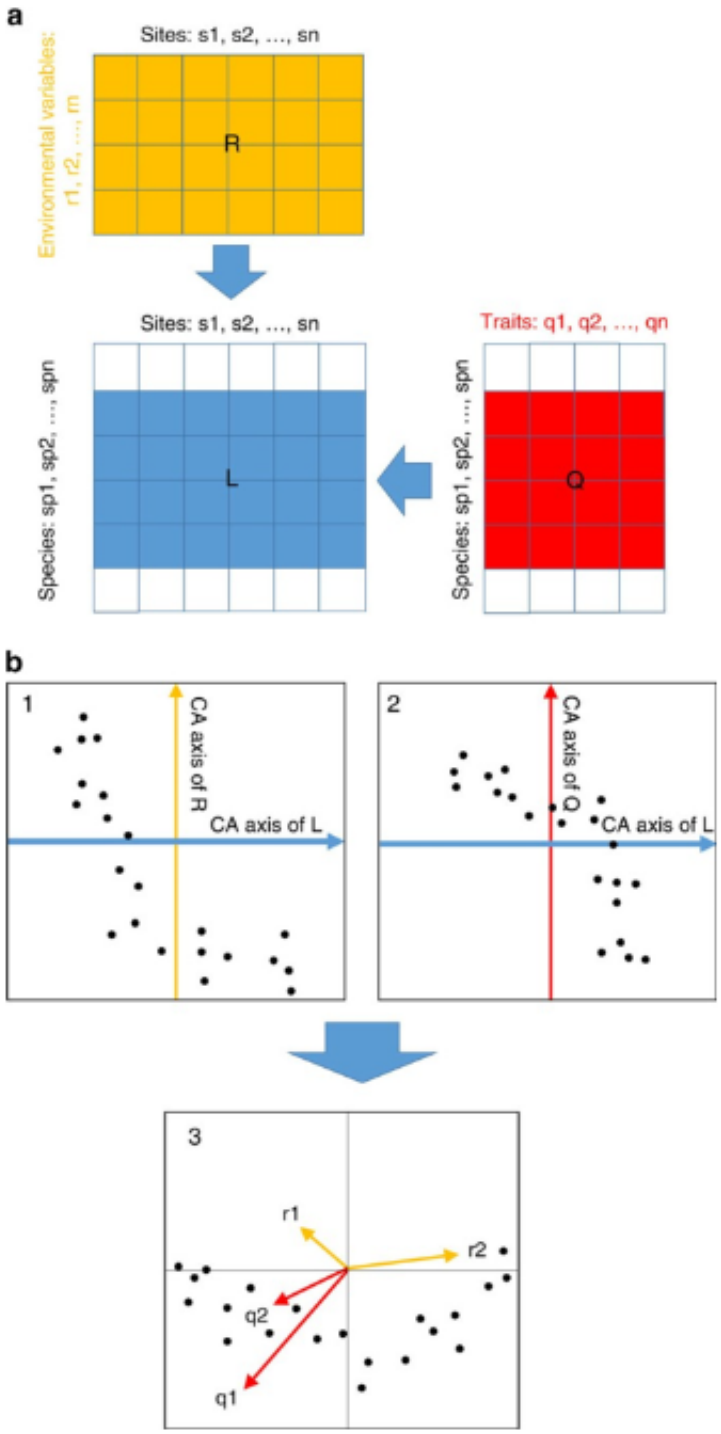

Figure 3. Conceptual diagram of RLQ ordination as seen in Gámez-virués et al. 2015. A) The structure of the R matrix (yellow), Q matrix (red), L matrix (blue), and how they are related to one another in the RLQ framework. B) Visualization of how the axis elucidated from correspondence analysis (CA) interact to display the interactions between the R and Q matrix in the ordination space.

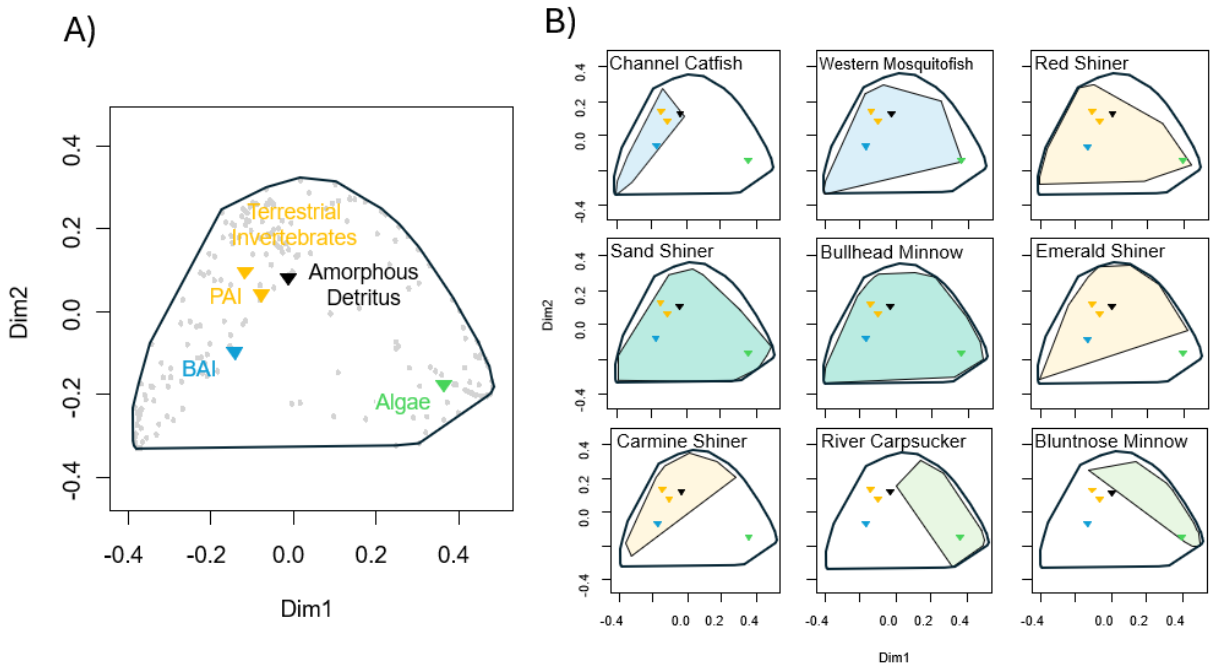

Figure 4. Principle Coordinates Analysis ordination illustrating variation in diet of nine species collected in the Kansas and Big Blue rivers. The ordination was based on a Jaccard distance matrix. Fish diets were collected in the spring, summer, and fall of 2023 and the spring and summer of 2024 using a seine. A) Diet items were simplified to functional prey groups and significant groups are represented in the ordination space by the color-coded triangles and text: Algae, benthic aquatic invertebrates (BAI), pelagic aquatic invertebrates (PAI), and terrestrial invertebrates (Appendix Table A. 1). Individual diets are represented by grey circles. B) The hulls of each species are displayed in the context of their combined diets (large hull) and the diet groups and color coded to guild where blue represents benthic invertivores, yellow represents terrestrial/pelagic invertivores, green represents herbivores, and turquoise represents omnivores.

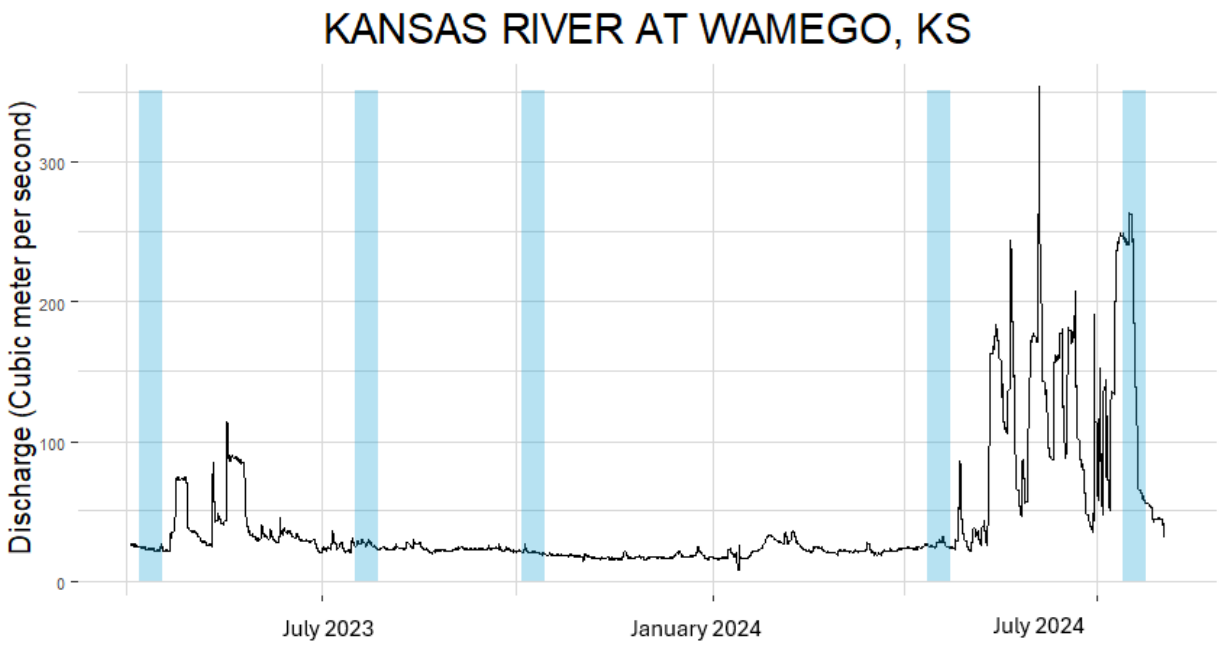

Figure 5. Hydrograph of the Kansas River at United State Geological Survey Wamego Stream Gauge (#06887500) from April 1st, 2023 to August 1st 2024 in cubic meters per second. Blue rectangles represent sampling periods starting in Spring 2023 until Summer 2024.

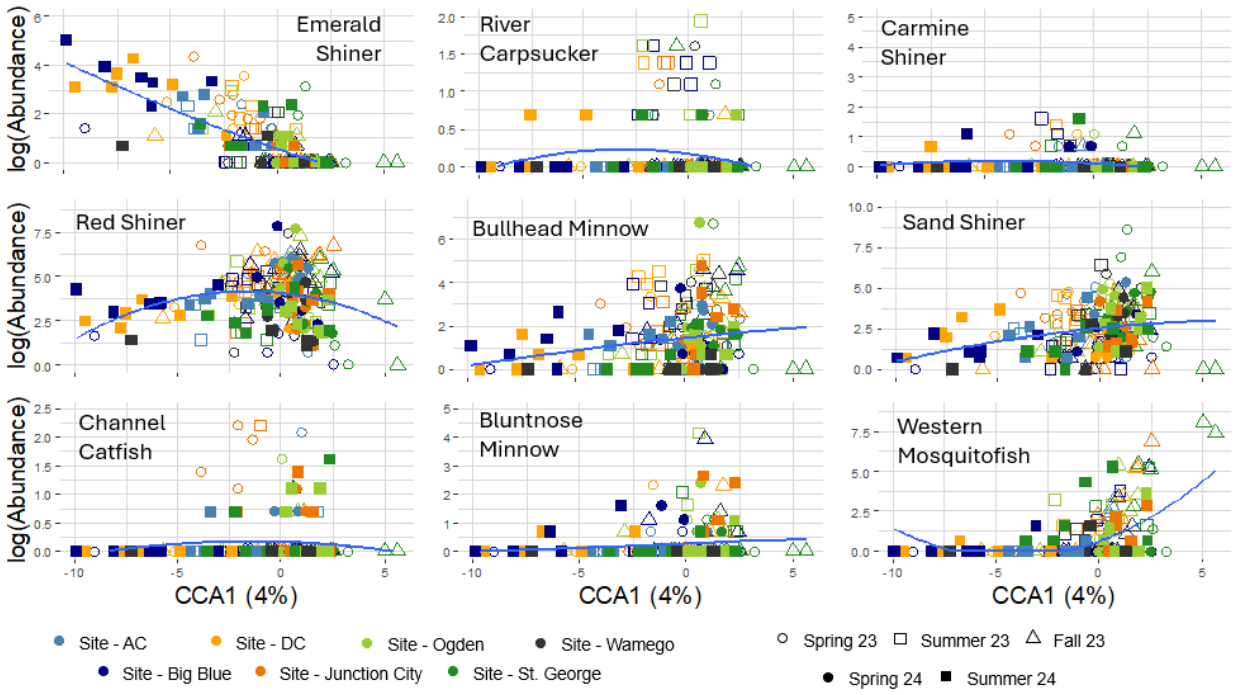

Figure 6. Graphs plotting the log-transformed abundance of 9 species of fish that were seined in the Big Blue and Kansas rivers during 2023-2024 with the significant CCA 1 axis. The samples are color coded to sampling site and shape coded to season and year. The general trend line is a second order polynomial in blue.

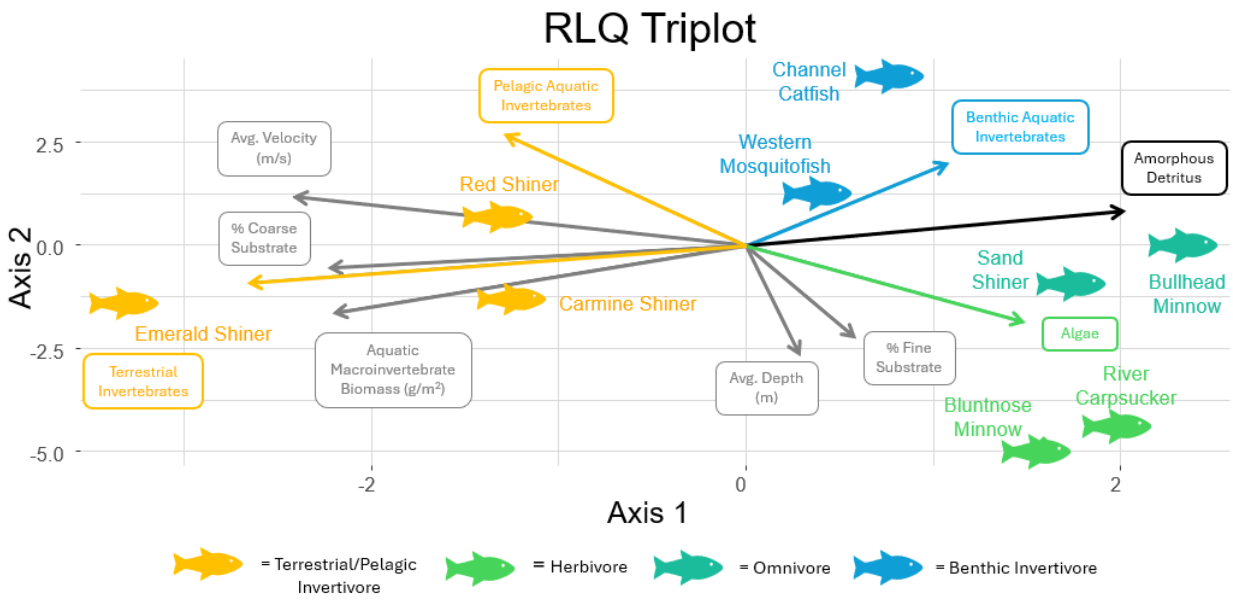

Figure 7. Triplot of RLQ ordination depicting the relationship between the square root transformed abundances of 9 fish species color coded by their feeding guild, the percent occurrence of prey groups in the species diets also color coded, and measurements of 5 variables from each habitat fish were sampled (grey). Yellow is associated with the pelagic aquatic invertebrates and terrestrial invertebrate prey groups and three study species, blue is associated with the benthic aquatic invertebrate group and two study species, green denotes algae and two species, turquoise is for the two omnivorous species, and black corresponds to the amorphous detritus prey which was not associated with any species.

Tables

Table 1: The percent occurrence of coarse prey groups in the five prey guilds for the nine fish species with 30+ diets analyzed. Asterisks (*) denote a significant difference across species (p-value <0.01) for diets from individuals grouped by year, season, and site and analyzed using MANOVA and the species had a significant adjusted p-value compared with the 8 other species in the post-hoc test. Functional groups were assigned for when individuals had >30% occurrence of any prey groups in their diet. Green represents herbivores, turquoise is for omnivores, yellow is for invertivores that consume pelagic and terrestrial prey, and blue is for invertivores that primarily consume benthic invertebrates. The post-hoc test. Functional groups were assigned for when individuals had >30% occurrence of any prey groups in their diet. Green represents herbivores, turquoise is for omnivores, yellow is for invertivores that consume pelagic and terrestrial prey, and blue is for invertivores that primarily consume benthic invertebrates.

Prey Groups	Bluntnose Minnow	Bullhead Minnow	Carmine Shiner	Channel Catfish	Emerald Shiner	Red Shiner	River Carpsucker	Sand Shiner	Western Mosquitofish
n	109	274	32	62	136	344	95	362	133
Algae*	90%	38%	20%	2%	15%	17%	97%*	50%	21%
Amorphous Detritus	5%	9%	5%	5%	1%	3%	3%	8%	8%
Benthic Aquatic Invertebrates*	5%	61%	50%	100%*	34%	51%	25%	48%	55%
Pelagic Aquatic Invertebrates*	4%	13%	10%	40%	26%	36%	0%	16%	35%
Terrestrial Invertebrates*	0%	3%	40%	10%	47%	22%	1%	6%	13%

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Appendix A

Appendix Table A. 1. Summary of fish diets from species collected in the Kansas and Big Blue river in 2023 and 2024. Specific prey items, which functional prey groups they have been placed in, and the count of each item in the diets of the 9 species with 30+ diets analyzed are shown. The grand total of each item is also provided. Some items are listed but are not found in any diet of the 9 fish species to provide context of other items being consumed in the community.

Prey Item	Species									Grand Total
	River Carpsucker	Red Shiner	Western Mosquitofish	Channel Catfish	Emerald Shiner	Carmine Shiner	Sand Shiner	Bluntnose Minnow	Bullhead Minnow	
Algae										
Filamentous Algae	1	37	9	2	11	1	33	1	15	110
Diatoms	94	14	17	0	7	6	110	82	53	383
Amorphous Detritus										
Amorphous Detritus	2	2	6	0	1	0	16	4	10	41
Fish Scale	1	3	9	2	1	2	0	0	0	18
Plant Matter	0	3	0	1	0	0	3	0	1	8
Benthic Aquatic Invertebrates										
Unidentifiable Aquatic Invertebrate	3	74	41	41	30	17	65	2	36	309
Baetidae	0	5	0	10	0	0	1	0	0	16
Caenidae	0	0	1	0	0	0	0	0	0	1
Ceratopogonidae	5	0	1	7	0	0	3	0	1	17
Chironomidae	64	99	50	846	13	5	138	3	161	1379
Coenagrionidae	0	0	0	0	0	0	0	0	0	0
Coleoptera	0	0	7	0	0	0	0	0	0	7
Collembola	0	0	0	0	1	0	0	0	0	1
Corbicula	0	0	0	0	0	0	0	0	0	0
Crayfish	0	0	0	0	0	0	0	0	0	0
Diptera	3	5	3	6	3	0	4	0	1	25
Dytiscidae	0	44	39	0	0	0	48	0	0	131
Elmidae	0	0	0	0	0	0	0	0	0	0
Ephemeridae	0	0	0	0	0	0	0	0	0	0

Ephemeroptera	0	0	0	2	0	0	0	0	0	2
Ephydridae	0	10	6	2	1	0	11	0	0	30
Gastropoda	0	0	2	0	0	0	0	0	0	2
Gomphidae	0	0	0	0	0	0	0	0	0	0
Gyrinidae	0	0	0	0	0	0	1	0	0	1
Hemiptera	0	0	0	1	0	0	0	0	0	1
Heptageniidae	0	1	0	0	0	0	0	0	0	1
Hirudinea	0	0	0	0	0	0	0	0	0	0
Hydrarachnidia	0	0	0	0	0	0	0	0	0	0
Hydrophilidae	0	0	0	0	0	0	0	0	0	0
Hydropsychidae	0	9	0	61	2	1	27	0	3	103
Isonychiidae	0	0	0	0	0	0	0	0	0	0
Isopoda	0	0	0	0	0	0	0	0	0	0
Leptoceridae	0	1	0	0	0	0	0	0	0	1
Leptohyphidae	0	0	0	0	0	0	0	0	0	0
Muscidae	0	0	0	0	0	0	0	0	0	0
Nematomorpha	0	5	0	2	0	0	0	0	0	7
Odonata	0	0	0	1	0	0	0	0	0	1
Oligochaeta	0	11	0	0	1	0	1	0	2	15
Plecoptera	0	0	1	0	0	0	0	0	0	1
Simuliidae	1	1	0	8	0	0	0	0	0	10
Stratiomyidae	0	0	0	0	0	0	0	0	0	0
Tabanidae	0	1	0	0	0	0	0	0	0	1
Tanypodinae	0	1	0	4	0	0	0	0	0	5
Tipulidae	2	5	0	0	0	0	1	0	2	10
Trichoptera	0	1	2	0	0	0	0	0	0	3
Unionidae	0	0	0	0	0	0	0	0	0	0
Zygoptera	0	0	0	0	0	0	0	0	0	0
Fish										
Fish	0	0	0	0	0	0	0	0	1	1
Gambusia affinis	0	0	0	0	0	0	0	0	0	0
Ictaluridae	0	0	0	0	0	0	0	0	0	0
Pelagic Aquatic Invertebrates										
Amphipod	0	0	0	0	0	0	0	0	0	0
Chaoboridae	0	268	3	0	31	0	15	1	19	337
Cladocera	0	62	0	0	81	0	6	7	64	220
Copepoda	0	171	4	0	0	0	55	0	5	235
Corixidae	0	23	34	1	9	0	9	0	0	76
Daphniidae	0	186	0	5	6	0	25	0	0	222
Diptera Pupae	1	45	65	48	16	2	31	0	21	229
Gerridae	0	0	0	0	0	0	0	0	0	0
Zooplankton	0	3	0	0	2	0	5	0	0	10

Terrestrial Invertebrates										
Terrestrial Invertebrate	1	166	17	8	115	12	19	0	4	342
Unidentifiable										
Unidentifiable	17	221	108	71	120	25	235	39	187	1023
Unknown	0	8	0	0	0	0	1	0	0	9