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Reproductive Health and Morphometric Comparison of Catostomid Species in Two Midwestern Rivers

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Eastern Illinois University

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Bethany Ellen Hoster

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REPRODUCTIVE HEALTH AND MORPHOMETRIC COMPARISON OF CATOSTOMID SPECIES IN TWO MIDWESTERN RIVERS

Bethany Ellen Hoster

B.A. Biology

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ABSTRACT

Wastewater treatment effluent, agricultural runoff, and impoundments can alter the water quality and flow regime of rivers. Fishes around the world are increasingly exposed to estrogenic compounds due to the discharge of wastewater into rivers. Estrogenic compounds, such as those found in birth control pills and soybean byproducts, are endocrine disruptors in vertebrates. The Sanitary District of Decatur serves the municipal and industrial entities of Macon County, IL, and discharges its effluent into the Sangamon River, which includes byproducts of industrial soybean processing. I assessed impacts of treated wastewater on the health of fishes in the Sangamon River. Abnormal caudal fins have been observed in several Catostomid species in the study reach of the Sangamon River. I assessed sex ratios, plasma vitellogenin concentrations, and the occurrence of gonadal intersex in three Catostomid species (River Carpsucker *Carpiodes carpio*, Smallmouth Buffalo *Ictiobus bubalus*, and Shorthead Redhorse *Moxostoma macrolepidotum*). I also assessed condition and morphometrics of these species to determine if relative weight is affected by fin morphology. Data were collected in spring of 2016 in the Sangamon and Embarras Rivers. All sexually mature fishes were sexed and blood was drawn from all adults identified as male and analyzed with ELISA test kits to determine vitellogenin concentrations. Fish testes were dissected, weighed, preserved for histology, and analyzed for the presence of testicular oocytes. No testicular oocytes were found in any of the species. Shorthead Redhorse from the Embarras River had a higher gonadosomatic index compared to fish from the Sangamon River, but no differences were found for River Carpsucker and Smallmouth Buffalo. Due to vitellogenin detection in a high percentage of males, populations were intersexed and female biased in both rivers for all species. Using traditional and geometric morphometrics, I used standard and total lengths to determine if River Carpsucker and Smallmouth Buffalo from the Sangamon River have significantly longer total lengths compared to standard lengths. River Carpsucker and Smallmouth Buffalo have longer caudal fins in the Sangamon River than the Embarras River. Longer caudal fins cause lower relative weight due to relative weight calculation incorporating total length. Geometric morphometrics revealed Sangamon River River Carpsucker and Smallmouth Buffalo have significantly different body shapes due to fin morphology than fishes in the Embarras River. The occurrence of feminization in both rivers, as well as the difference in body shapes between rivers, demonstrates these populations, especially in the Sangamon River, may be at risk for deterioration of health and further reproductive disruption due to contaminant exposure.

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ABSTRACT

Fishes around the world are increasingly exposed to estrogenic compounds due to the discharge of wastewater into rivers. Estrogenic compounds, such as those found in birth control pills and soybean byproducts, are endocrine disruptors in vertebrates. The Sanitary District of Decatur serves the municipal and industrial entities of Macon County, IL, and discharges its effluent into the Sangamon River, which includes byproducts of industrial soybean processing. This study aimed to assess impacts of treated wastewater on the reproductive health of male fishes in the Sangamon River. I assessed sex ratios, plasma vitellogenin concentrations, and the occurrence of gonadal intersex in River Carpsucker (*Carpiodes carpio*), Smallmouth Buffalo (*Ictiobus bubalus*), and Shorthead Redhorse (*Moxostoma macrolepidotum*). All sexually mature fishes were sexed and blood was drawn from all adults identified as male and analyzed with ELISA test kits to determine vitellogenin concentrations. Fish testes were dissected, weighed, preserved for histology, and analyzed for the presence of testicular oocytes. No testicular oocytes were found in any of the species. Shorthead Redhorse from the Embarras River had a higher gonadosomatic index compared to fish from the Sangamon River, but no differences were found for River Carpsucker and Smallmouth Buffalo. Due to vitellogenin detection in a high percentage of males, populations were intersexed and female biased in both rivers for all species. These results indicate that low amounts of feminization may be occurring in both of these rivers. These results indicate these populations may be at risk for reproductive disruption due to contaminants and highlight the need for continued monitoring of reproductive health.

INTRODUCTION

Lotic systems throughout the world are impacted by anthropogenic factors, such as impoundment, channelization, and the discharge of wastewater. Impounded rivers provide humans with a variety of resources, such as water supply, hydropower, and recreational access. Ultimately, these modifications can be harmful to riverine systems and the resident biota (Kondolf, 1997). River impoundment alters flow regimes and can cause poor water quality due to stagnation, as well as fragmentation of habitat when river discharge is low downstream (Abromovitz, 1996; Collier, Webb, & Schmidt, 1996; Ligon et al., 1995; Magnuson, Naiman, Firth, McKnight, & Stanford, 1995). Impoundments managed for urban and industrial water supplies exhibit an artificially controlled flow regime and often result in reduced river discharge below the dam, especially during periods of drought.

While stochastic hydrologic patterns from artificial impoundments lead to habitat loss and fragmentation, the additional impacts from both point and non-point sources of pollution affect the ecological integrity of many lotic systems and further exacerbate habitat degradation. Agricultural runoff is an example of non-point source pollution, while treated sanitary and industrial waste are examples of point sources of pollution. Although the Water Quality Act of 1972 led to upgraded wastewater treatment plants, effluents containing high amounts of nutrients and untreatable contaminants are released into waterways (Karr, Heidinger, & Helmer, 1985). Lotic systems affected by both agricultural runoff and wastewater discharge have higher concentrations of nutrients (Carpenter & Waite, 2000). During low water conditions, influx of treated wastewater can lead to higher concentrations of pollutants without enough flow for dilution. The release of both regulated and unregulated contaminants in treated wastewater can negatively impact river water quality and be detrimental to riverine organisms.

Point source pollution from discharged wastewater is responsible for the release of many harmful chemicals, including endocrine disrupting compounds (EDCs). Endocrine disruptors can mimic or interact with vertebrate hormones and become detrimental to vertebrate reproductive health (Liney et al., 2006; Shore & Shemesh, 2003). Endocrine disruptors that cause harm to aquatic vertebrates include estrogenic compounds that are introduced into systems through human activity. Many estrogenic compounds found in treated wastewater, such as estrone and 17β-estradiol, are naturally produced and excreted by vertebrates (Chimchirian and Rominder, 2007). Synthetic estrogenic compounds, such as those found in birth control pills, are excreted by humans and ultimately end up in wastewater. Agricultural runoff from livestock waste is another source of estrogenic compounds entering waterways (Shore & Shemesh, 2003). Exposure to endocrine disrupting compounds found in wastewater treatment effluent can cause reproductive disruption and feminization of male fishes (Vajda et al., 2008). The prevalent use of rivers for wastewater discharge introduces harmful contaminants to rivers and poses risks to aquatic life. Fishes, confined to these systems for the entirety of their life cycle, are especially at risk. Fishes are particularly at risk to endocrine disruption during the larval stage when sexual differentiation is occurring, potentially manifesting later in the life cycle, as well as during their reproductive period (Blazer et al., 2011). Such adverse effects experienced by fishes include premature sexual maturity, decreased testicular development, reduced embryo production, vitellogenin induction, loss of sexual dimorphism, increased embryonic mortality, and impaired immune function (Guillette, Crain, Rooney, & Pickford, 1995; Jobling et al., 2003; Liney et al., 2006). This exposure during sexual differentiation and the potential for feminized testicular tissue often leads to female or intersex biased populations (Leusch et al., 2006; Vajda et al., 2011; Vajda et al., 2008; Woodling, et al., 2006).

Although wastewater effluent is regulated by the Environmental Protection Agency, many estrogenic compounds are either not regulated or are difficult to remove through the water treatment process (Chimchirian and Rominder, 2007). Wastewater treatment facilities are required to meet standards for parameters such as total suspended solids, nitrogen, dissolved oxygen, and nickel (United States Environmental Protection Agency, 2004). Effluents can alter the hydrology of a river by comprising a large proportion of the total river discharge, especially during drought conditions, resulting in abnormally elevated concentrations of contaminants in the system. The presence of low water levels and high amounts of contaminants like estrogenic compounds may cause increased feminization of male fishes, early maturation of juvenile fishes, and disproportionate sex ratios compared to rivers without these pollutants. While removal of estrogenic compounds from wastewater is possible, it is heavily dependent on the efficiency of nutrient removal technology at wastewater treatment plants (Christiansen, Winther-Nielsen, and Helweg, 2002). In one study, plants with either activated sludge treatment followed by tertiary treatment stage in six lagoons or oxidation ditches and chlorination were more efficient at removing estrogenic compounds than plants with either three bioreactors followed by ultraviolet and chlorination disinfection or 10 treatment lagoons. Although two of these treatment plants were more efficient than the other two, none of these facilities were successful at removing all estrogens and xenoestrogens from their effluents (Ying, Kookana, & Kumar, 2008). Improvements in wastewater treatment technology are paramount to reducing the amount of harmful compounds being discharged into rivers. For example, upgraded technology in municipal wastewater treatment facilities to include full nitrification with nitrifying activated sludge for removal of ammonia decreased the severity of intersex condition occurring in male Rainbow Darters (*Etheostoma caeruleum*) previously exposed to estrogenic compounds in effluent (Hicks et al., 2016). In addition, Fathead Minnows (*Pimephales promelas*) exposed to endocrine disrupting compounds in municipal effluent experienced improvements in reproductive health following facility upgrades to activated sludge processes which increased removal efficiency of endocrine disrupting compounds (Barber, Vajda, Douville, Norris, & Writer, 2012).

Previous studies have documented increased rates of intersex occurring in fish species following exposure to endocrine disrupting compounds in rivers, lakes, and laboratory settings. This includes many studies involving lakes and rivers receiving discharged treated wastewater with estrogenic compounds. These studies found altered sex ratios in exposed wild populations, induction of vitellogenin in male fishes, reduced gonad size, and the presence of oocytes in testicular tissue of male fishes downstream of municipal wastewater treatment plants (Woodling et al., 2006; Vajda et al., 2008; Vajda et al., 2011). Species studied include White Sucker (*Catostomus commersonii*), Fathead Minnow, Rainbow Darter, and Shorthead Redhorse (Vajda et al., 2008; Vajda et al., 2011; Al-Ansari et al., 2010; Hicks et al., 2016(Al-Ansari et al., 2010)). Black basses (*Micropterus spp.)* are a commonly studied species in the southeastern United States for determining impacts of endocrine disruptors due to their recreational importance and the widespread incidence of intersex condition in male fishes (Hinck et al., 2009; Blazer et al., 2011). One such study found that Smallmouth Bass (*Micropterus dolomieu*) in a river receiving wastewater effluent and high amounts of agricultural runoff had a higher prevalence and severity of testicular oocytes compared to rates of intersex in this species that are considered a natural phenomenon (Blazer, Iwanowicz, and Iwanowicz, 2007). Additionally, Great Lakes male White Perch (*Morone americana*) in areas receiving effluents have been documented to have feminized gonads, high concentrations of vitellogenin, and large percentages of intersex individuals (Kavanagh et al., 2004). Vitellogenin induction has also been studied in male Zebrafish (*Danio rerio*), Rainbow Trout (*Oncorhynchus mykiss*), and Channel Catfish (*Ictalurus punctatus*) through estrogenic exposure in a laboratory setting (Bradley & Grizzle, 1988; Wedekind, 2014). Although these examples demonstrate the diversity of species impacted by this occurrence, there are still many species and family groups that are poorly studied in this regard. A limited amount of literature about potential impacts of wastewater effluent on the Catostomidae family calls for further examination these species.

The Sangamon River, the largest tributary to the Illinois River, flows for approximately 200 km with a 14,000 km^2 watershed extending through 18 counties in central Illinois. The Sangamon River receives multiple point and non-point sources of pollution throughout its course. Dominated by agriculture, the Sangamon River watershed has the highest percentage of surrounding land cover as cropland of all major watersheds in Illinois (IDNR, 2001). In addition to agriculture, the Sangamon watershed is the fourth most urbanized watershed in Illinois, which is home to more than 500,000 people (IDNR, 2001). The Sanitary District of Decatur (SDD), located in Decatur, IL, serves a population of 100,000 people in central Macon County, as well as 24 major industrial users. The effluent from the SDD enters the Sangamon River five kilometers downstream of the Lake Decatur Dam. Furthermore, the Sangamon River is affected by impoundment in Decatur, IL, which forms Lake Decatur and results in an altered flow regime. During periods of drought or low discharge from the dam, wastewater effluent accounts for the majority of water in the Sangamon River downstream of the Lake Decatur Dam.

Eastern Illinois University began a sampling program in 1998-1999 to annually monitor water quality and biotic communities to document spatial and temporal changes in an 8.5km reach of the Sangamon River in Decatur. This program is in collaboration with the SDD and will continue through the end of 2019. While this study was initially aimed at monitoring the health of the river over time by characterizing habitat quality and assessing biotic integrity, it became apparent that effluent dominates the river in this reach and the contaminants present could be detrimental to the organisms occupying this area.

The SDD, unlike many municipal wastewater treatment facilities, also processes industrial waste, of which a large component contains soybean processing byproducts. These soybean byproducts contain isoflavones, part of a chemical group known as phytoestrogens (Barrett, 2006). Phytoestrogens, compounds found within plants that have similar chemical structures to estrogens, mimic the estrogenic compounds that naturally occur within vertebrates (Barrett, 2006). The SDD uses preliminary, primary, and two-stage activated sludge treatment, followed by effluent disinfection. Estrogenic compounds, including phytoestrogens, are not efficiently removed by this current treatment and disinfection process and are ultimately discharged into the Sangamon River. Little is currently known about this source of estrogenic compounds in aquatic systems and their effects on wild fishes. As a result of this industrial component, a more intensive study began in spring 2016 to further assess the impacts of this effluent on three fish species in the Sangamon River

Using several methods, I assessed the reproductive health and intersex status of male fishes in the Sangamon River. I used the Embarras River, a 314 km tributary of the Wabash River located in East Central Illinois, as a reference river to compare fish populations to the Sangamon River. I chose River Carpsucker (*Carpiodes carpio*), Smallmouth Buffalo (*Ictiobus bubalus*), and Shorthead Redhorse (*Moxostoma macrolepidotum*) for a variety of reasons. First, they are abundant in both study rivers. Second, all three species are in the family Castostomidae and are widely distributed throughout the Midwestern United States. Third, although these fishes are abundant in many rivers, they have received relatively little attention in the scientific literature (Morris, 1965; Spiegel, 2010; Welker and Scamecchia, 2003). Their wide distribution and high abundance in lotic systems makes it important to study these species. Finally, these species are long-lived native fishes, feeding in the benthic zones of lotic systems, exposing them to many contaminants found in water and sediment (Edwards and Twomey, 1982; Spiegel, 2010). The effects of endocrine disrupting compounds in wastewater effluent on fishes are becoming increasingly well studied around the world. However, this study will be one of few to assess the impacts of wastewater treatment plant effluent containing both industrial and municipal waste on multiple fish species.

If increased feminization of fish is documented in the Sangamon River, this could have implications for other rivers impacted by both industrial and municipal wastewater, and emphasize the need for improved treatment technology at wastewater treatment plants. The goals of this study are 1) to determine the sex ratios of each species population, prevalence of feminized male gonads, and concentrations of vitellogenin in male fishes from the Sangamon River and 2) to determine if exposure to contaminants found in treated wastewater causes reproductive disruption in the Sangamon River. I expected altered sex ratios and a female biased population due to exposure to estrogenic wastewater contaminants prior to and during sexual differentiation. I also believe that if estrogenic wastewater contaminants are affecting fishes, there will be a high occurrence of intersex gonads. I expected to detect vitellogenin, an important biomarker for exposure to estrogenic compounds in male fishes, which should not occur in male fishes.

METHODS

Study Site

The Sangamon River, the largest tributary of the Illinois River, was chosen as the study site for this project due to the effluent discharged by the SDD. The SDD has a dry weather treatment capacity of 41 million gallons per day. The SDD serves more than 100,000 people and 24 major industrial users. The Lake Decatur Dam is located five kilometers upstream of the SDD effluent release point. The Embarras River, a tributary to the Wabash River, served as a reference site due to its similar fish community to the Sangamon River and the absence of similar wastewater treatment effluent in proximity to the study area. Due to limited boat access and difficulty sampling in these tributaries during periods of low river discharge, study sites were limited to stretches of river within reach of boat ramps. The study site in Decatur, IL included a stretch of river from the SDD sanitary effluent release point to approximately three and a half kilometers downstream. The Embarras River reference site included a stretch of river beginning in Charleston, IL, below the dam of Lake Charleston (Figure 1.1).

Study species

River Carpsucker, Smallmouth Buffalo, and Shorthead Redhorse were selected based on their high abundance in both study rivers, as well as the observation of abnormal fin morphologies in the Sangamon River. All three species belong to the family Castostomidae. These species are long-lived native fishes, feeding in the benthic zones of lotic systems (Edwards and Twomey, 1982; Spiegel, 2010). These species are easily sexed during their reproductive months, when tubercles develop on the faces and fins of the males and milt can be distinguished from eggs.

Field Protocol

I collected River Carpsucker, Smallmouth Buffalo, and Shorthead Redhorse from the Sangamon and Embarras Rivers using boat-mounted 60 Hz pulsed-DC electrofishing gear. All fishes of the study species were measured for total and standard length (mm), weighed (g), and sexed. All female and juvenile fishes were released following measurement and sexing. I collected blood samples from the caudal vein of male fishes using heparinized hematocrit vials. Following blood collection, fishes were humanely euthanized. I dissected, weighed, and preserved gonads in 10% neutral-buffered formalin for histological processing. Protocols to conduct this study were approved by the Institutional Animal Care and Use Committees (IACUC Protocol approval numbers 05-010 and 15-004)

Biological analysis

Saffron Scientific Histology Services prepared male gonads for histology using standard procedures. Tissue was embedded in paraffin, sectioned, and stained using hematoxylin and counter stained using eosin (H&E). Separate portions from throughout each gonad were embedded in order to evaluate the entire gonad. I examined the prepared slides to confirm sex and analyzed tissue for the presence and abundance of testicular oocytes. A minimum of six sections was analyzed for each fish for the presence of testicular oocytes.

Blood samples were centrifuged following field collection and blood plasma was frozen until assayed for vitellogenin. I measured plasma vitellogenin using enzyme-linked immunosorbent assay (ELISA) with an anticarp kit following the manufacturer's instructions in each male fish of all three species (Biosense Laboratories, Bergen, Norway & Abraxis, Warminster, PA). I ran all samples in duplicate as suggested by the manufacturer's instructions. I created a standard curve following the manufacturer's instructions by diluting the vitellogenin standard provided by the manufacturer and generated a 4-parameter standard curve.

Statistical Analysis

I calculated Gonadosomatic index (GSI) using the gonad weights recorded from dissection. I calculated GSI as:

$$\{[\text{gonad weight (g)}/\text{body weight (g)}] \times 100\}$$

I analyzed GSI, gonadal intersex, and vitellogenin concentrations for differences between rivers using one-way analysis of variance (ANOVA). Site differences in gonadal intersex prevalence were analyzed using one-way ANOVA. A natural log transformation was used for Shorthead Redhorse vitellogenin concentrations to normalize the data. Vitellogenin concentration data were non-normal and could not be transformed for River Carpsucker and Smallmouth Buffalo. Kruskal-Wallis one-way ANOVA on ranks was used to assess differences between rivers for River Carpsucker and Smallmouth Buffalo. Alpha levels of 0.05 were used to determine statistical significance. I calculated the sex ratio for each population as a frequency of males using the following equation:

$$[\text{males}/(\text{males} + \text{females} + \text{intersex}) \times 100].$$

To analyze sex ratio data, I used Fisher's exact test to compare each site and species to the predicted 1:1 female to male ratio, as well as compare ratios between rivers.

RESULTS

A total of 61 River Carpsucker, 42 Shorthead Redhorse, and 142 Smallmouth Buffalo was collected from the Sangamon River in the spring of 2016 using 60 Hz pulsed-DC electrofishing. Of these fishes, 18 River Carpsucker, 15 Shorthead Redhorse, and 42 Smallmouth Buffalo were identified as male. A total of 87 River Carpsucker, 73 Shorthead Redhorse, and 73 Smallmouth Buffalo was collected from the Embarras River in the spring of 2016. Of these fishes, 34 River Carpsucker, 38 Shorthead Redhorse, and 18 Smallmouth Buffalo were identified as male.

I found a statistical difference in GSI for Shorthead Redhorse between rivers. Shorthead Redhorse from the Embarras River had higher GSI than fish from the Sangamon River ($F=16.046$, $DF=1,50$, $P<0.001$). No differences were found between rivers for River Carpsucker or Smallmouth Buffalo (River Carpsucker: F= 0.000894, DF= 1,49, p=0.976; Smallmouth Buffalo: F=2.782, DF= 1,59, p= 0.101; Figure 1.2).

I calculated the sex ratio for each population as a frequency of males. I classified all males with vitellogenin detected as intersex, regardless of concentration, due to deviation from the accepted norm for males (Table 1.1, Figure 1.3). The male to female sex ratio for all species in both rivers were biased toward females, deviating from the standard 1:1 ratio (χ^2, $p < 0.05$). The frequency of males statistically differed between the Sangamon River and Embarras River for Shorthead Redhorse, with more males in the Embarras River (χ^2, $p < 0.05$).

No testicular oocytes were found in any of the gonadal histological sections analyzed. Embarras River Shorthead Redhorse had significantly higher vitellogenin concentrations than fish from the Sangamon River (F= 6.754, DF= 1, 48, p = 0.012; Table 1.2, Figure 1.4). No statistical differences in vitellogenin concentrations were found between rivers for both River Carpsucker and Smallmouth Buffalo (River Carpsucker: H= 1.523, DF= 1, p= 0.217; Smallmouth Buffalo: H= 0.598, DF= 1, p= 0.439; Figure 1.4). Vitellogenin concentrations were higher in Shorthead Redhorse than Smallmouth Buffalo and River Carpsucker (Figure 1.4).

DISCUSSION

Shorthead Redhorse had much higher vitellogenin concentrations in both rivers compared to River Carpsucker and Smallmouth Buffalo. I detected vitellogenin in the highest percentage of individuals for Shorthead Redhorse and also detected a difference in plasma vitellogenin concentrations between rivers. However, I did not observe any differences between rivers for River Carpsucker or Smallmouth Buffalo. Although only the Sangamon River is impacted by point source discharge of treated wastewater within the sampling reach, both the Sangamon River and Embarras River are heavily impacted by agriculture. High amounts of soy production and pesticide application result in large amounts of runoff entering the rivers, especially during heavy spring rainfalls when sampling occurred (Blazer et al., 2011). Examination of contaminants in each river is necessary to determine what is present and poses the largest threat to fishes in these rivers. Contaminants from runoff may be the reason why many individuals from both rivers exhibited vitellogenin, as a high prevalence of intersex fish has been correlated with the percent agriculture in the watershed (Blazer et al., 2011). Long-term exposure to these contaminants may result in more severe intersex condition and reproductive disruption in the future.

Although no gonadal intersex was detected in male fishes of any of the study species in either river, vitellogenin was present, which is the precursor protein to egg formation. Additionally, fish gonads from both study rivers appear reduced in size due to exposure compared to fishes in other rivers. For example, male Shorthead Redhorse in Canada were documented to have a mean GSI = 5.98 in November prior to spawning occurring the following spring (Al-Ansari et al., 2010). In my study, Shorthead Redhorse from the Embarras River had an average GSI = 4.8 ± 0.26, while the Sangamon River had an average GSI = 2.4 ± 0.7. Continued exposure to EDCs or any increase in vitellogenin concentrations in these fishes may impact reproductive health, further decrease gonad size, and eventually even lead to formation of testicular oocytes (Bahamonde, Munkittrick, & Martyniuk, 2013).

While vitellogenin was detected in a high percentage of fishes from each river, these concentrations are much lower than values reported in the literature for other species. The highest average vitellogenin concentration detected in this study, Embarras River Shorthead Redhorse, was <1% of the average concentrations detected in White Sucker downstream of a wastewater treatment facility in Colorado (Vajda et al., 2008) At this time, the vitellogenin concentrations I detected do not appear as severe as other studies. Nevertheless, vitellogenin should not be detected in any concentration in adult male fish, especially in a large portion of the population. Shorthead Redhorse had higher vitellogenin concentrations and a higher amount of individuals affected than the other two species; therefore, these populations should continue to be monitored to ensure their future health. It is important to note that this study used ELISA anti-carp kits, or antibodies from Common Carp, which have been used in previous studies examining intersex condition in other Catostomid species (Maltais, Roy, & Couillard, 2010; Vajda et al., 2008). While many studies have had success using these kits, other studies have found these commercially available anti-carp kits may underestimate vitellogenin concentrations for Shorthead Redhorse, suggesting that hybrid ELISAs should be developed and used instead to obtain more accurate results (Maltais et al., 2010). This suggests vitellogenin in the blood plasma of the three study species may not have bound well to the anti-carp kits used, resulting in apparently lower concentrations of vitellogenin. The development of antibodies that are specific to each of the study species is critical to better understand the differences in concentrations detected between species and obtain the most accurate results possible. This will allow us to determine if Shorthead Redhorse are more susceptible to feminization or if the difference observed between species can be attributed to properties of the species' blood plasma binding better with the antibody used in this project. Even though my results may underestimate the vitellogenin concentrations found in each of the study species, this highlights the seriousness of these findings and emphasizes the need for continued monitoring of these species' reproductive health in both rivers.

Presence and detection of testicular oocytes will help confirm the prevalence and severity of intersex condition. Although I did not detect testicular oocytes in any of the analyzed gonads, it is unclear if these fishes are simply not displaying gonadal intersex or if more sections of the gonads need to be processed and analyzed. The number of sections processed and analyzed was based on values presented in the literature for other species and financial limitations (Blazer, V.S., Iwanowicz, L.R., Iwanowicz, 2007; Vajda et al., 2008). The presence of testicular oocytes is often used to confirm the occurrence of intersex condition that was detected through vitellogenin analysis. It is imperative that a more extensive study of gonadal tissue be conducted because low levels of vitellogenin were detected in a high percentage of fishes. Since information on Catostomid intersex phenomena is limited, investigation into proper verification of intersex condition is necessary for these species. However, the detection of vitellogenin in these fishes is indicative of some degree of intersex condition occurring, leading to these study populations being female and intersex biased, rather than 1:1 of females and males. Skewed sex ratios can be the result of female biased offspring, as well as feminization of males occurring during development (Fentress et al., 2006). This, paired with the low number of unaffected males I sampled, could lead to continued and more severe reproductive disruption in the future.

Lower GSI in Shorthead Redhorse in the Sangamon River indicates lower reproductive condition. While I detected a difference in GSI between rivers for Shorthead Redhorse, this difference may be due to early spawning of this species and greater collection in the Sangamon River later in spring. However, GSI in both study rivers is lower than those documented in other systems. Smallmouth Buffalo and River Carpsucker, which spawn later in the spring and at higher water temperatures, were consistent in catch throughout sampling efforts in both rivers and no differences were found in GSI between rivers. Other Catostomid species show seasonal differences in GSI (Vajda et al., 2008).This suggests differences in GSI in Shorthead Redhorse could be an effect of timing, but are still lower than GSI documented in the literature (Al-Ansari et al., 2010).

. Although these three species are widespread and abundant, they are poorly represented in the literature. This study highlights the need for future studies on these species due to their abundance and range of sensitivity. Because these species rarely occur in pristine rivers, exposure experiments in a controlled laboratory setting would be best to determine the effects of exposure to EDCs. Additionally, continued monitoring of vitellogenin concentrations in these rivers is vital to mark detrimental physiological changes in these fishes to better inform fisheries managers Stricter regulations of wastewater and pesticides, additions of riparian buffer strips, and updated wastewater treatment facilities can limit harmful contaminants from entering waterways and lead to improved water quality. Continued monitoring and collaboration between fisheries managers, wastewater treatment facilities, and government entities managing water quality is necessary to assure the health of these rivers and fish communities.

TABLES

Table 1.1—Percent males for each river and study species. Male fish were considered as all individuals visually confirmed as male with no blood vitellogenin detected.

Percent Males		
	Embarras River	Sangamon River
River Carpsucker	25.7%	27.8%
Smallmouth Buffalo	20.9%	14.7%
Shorthead Redhorse	6.9%	0.0%

Table 1.2—Mean vitellogenin concentrations (ng/mL) for each study species and river with standard error in parentheses.

	Vitellogenin Concentration (ng/mL)	
	Embarras River	Sangamon River
River Carpsucker	145.41 (45.31)	70.75 (49.18)
Smallmouth Buffalo	227.19 (82.96)	259.025 (36.774)
Shorthead Redhorse	2339.59 (331.74)	1379.21 (536.23)

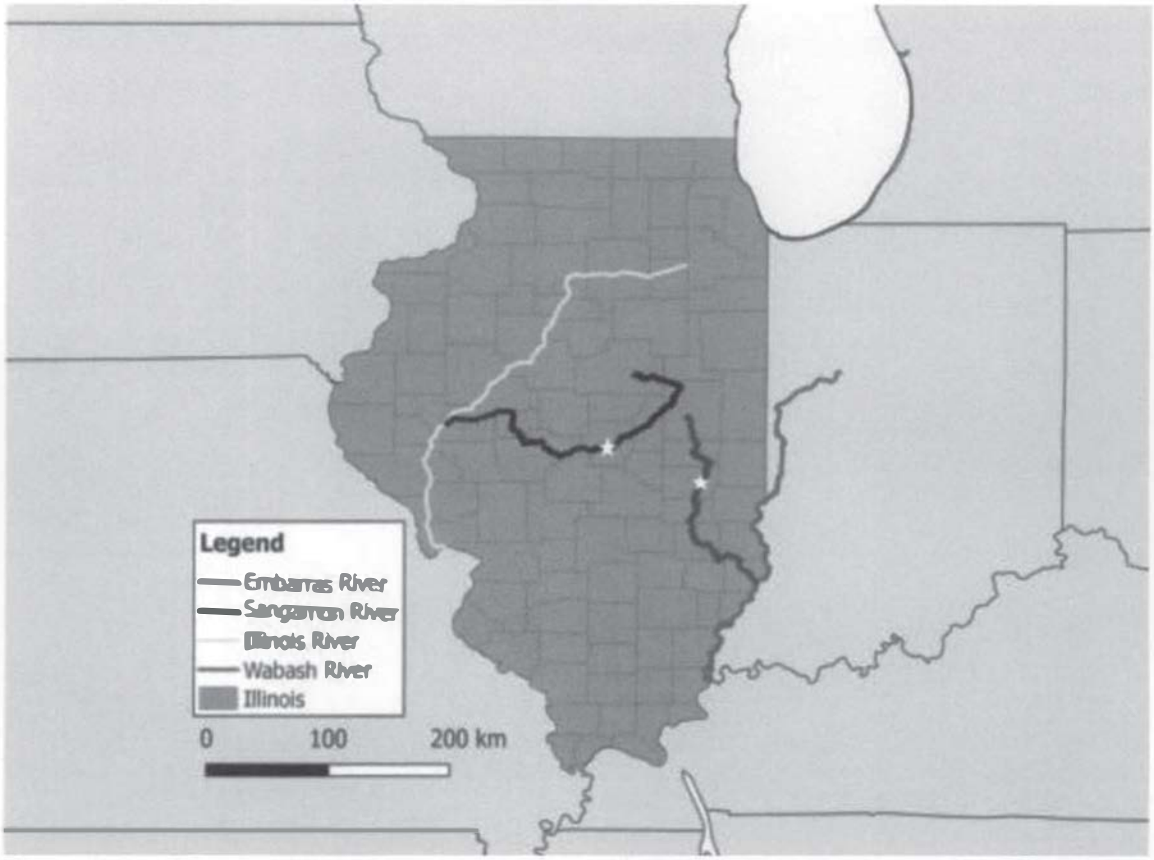

Figure 1.1—A map of the Sangamon River, located in central Illinois, and the Embarras River, located in east central Illinois. Study areas in Decatur and Charleston, IL, denoted with stars.

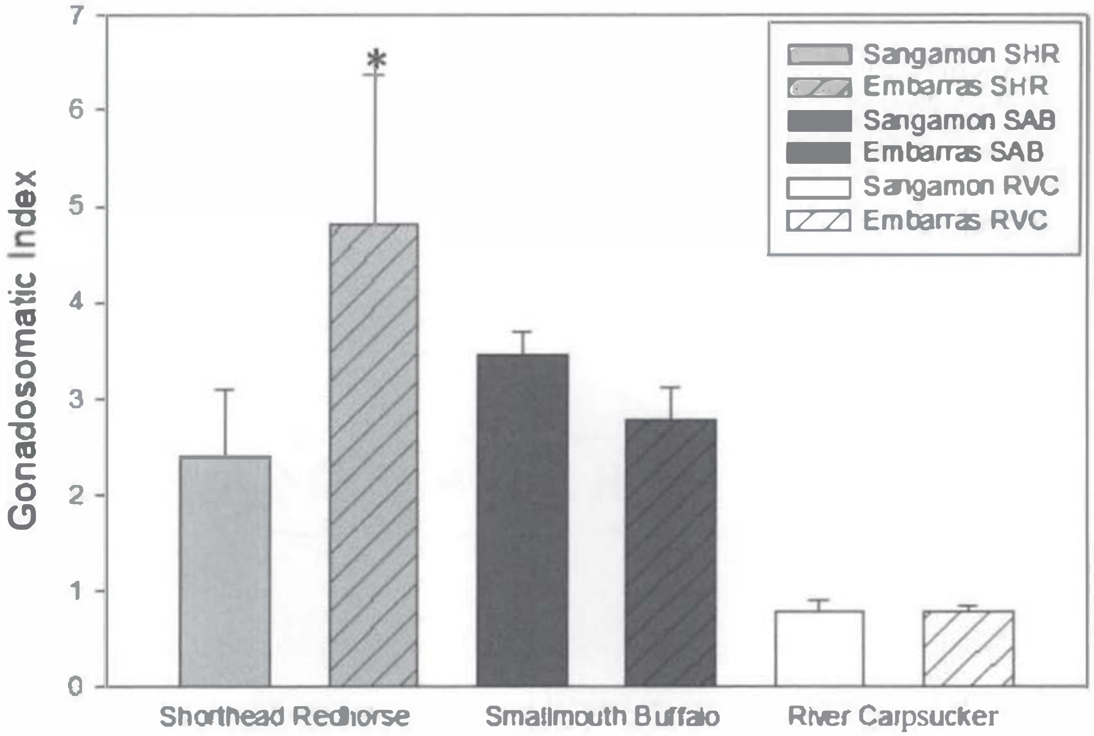

Figure 1.2—Gonadosomatic Index for each species from the Sangamon River and Embarras River sampled spring 2016 with standard error. Shorthead Redhorse (SHR) from the Embarras River had significantly higher GSI than the Sangamon River (F=16.046, DF=1,50, P<0.001). No differences were found between rivers for Smallmouth Buffalo (SAB) or River Carpsucker (RVC) (Smallmouth Buffalo: F=2.782, DF= 1,59, p= 0.101; River Carpsucker: F= 0.000894, DF= 1,49, p=0.976) .

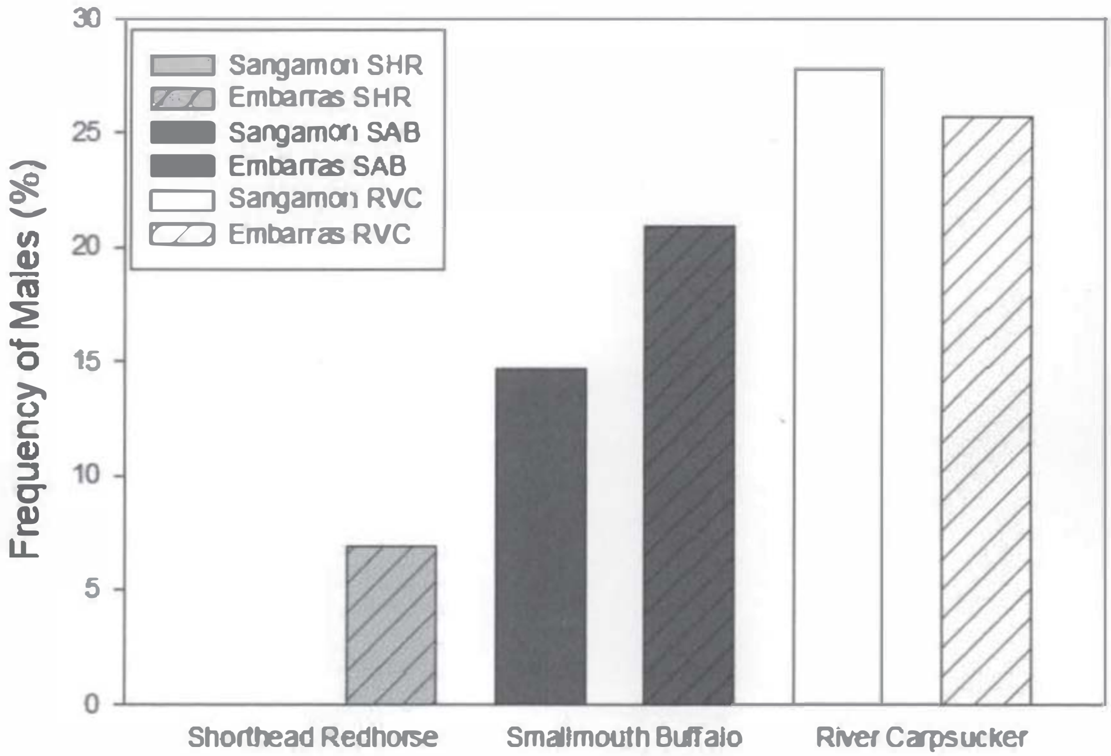

Figure 1.3—Frequency of males with no vitellogenin detected for each species from the Sangamon River and Embarras River sampled spring 2016, calculated as [males/(males + females +intersex) × 100]. Sangamon River Shorthead Redhorse, n = 36; Embarras River Shorthead Redhorse, n = 72; Sangamon River Smallmouth Buffalo, n = 116; Embarras River Smallmouth Buffalo, n = 43; Sangamon River River Carpsucker, n = 54; Embarras River River Carpsucker, n = 70.

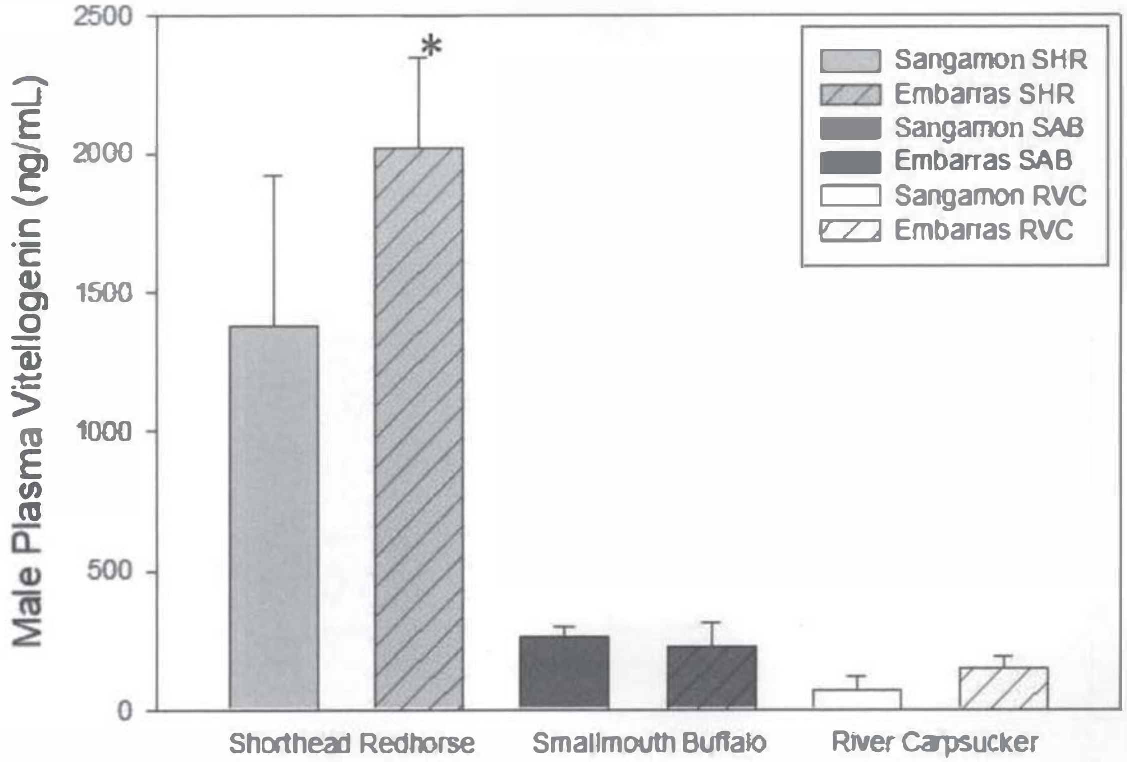

Figure 1.4—Plasma vitellogenin concentrations for each species from the Sangamon River and Embarras River sampled spring 2016 with standard error bars. Embarras River Shorthead Redhorse had significantly higher vitellogenin concentrations than the Sangamon River ($F = 6.754$, $DF = 1, 48$, $p = 0.012$). There were no statistical differences between rivers for both Smallmouth Buffalo and River Carpsucker (Smallmouth Buffalo: $H = 0.598$, $DF = 1$, $p = 0.439$; River Carpsucker: $H = 1.523$, $DF = 1$, $p = 0.217$).

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IMPACTS OF IMPOUNDMENT AND WASTEWATER ON CONDITION AND MORPHOMETRICS OF TWO CATOSTOMID SPECIES IN THE SANGAMON RIVER

ABSTRACT

Wastewater treatment effluent, agricultural runoff, and impoundments can alter the water quality and flow regime of rivers. The Sangamon River and Embarras River, located in Central Illinois, are both surrounded by agricultural lands. The Sangamon River is further impacted by the discharge of treated municipal and industrial wastewater, as well as impoundment for industry. Abnormal caudal fins have been observed in several Catostomid species in the study reach of the Sangamon River. River Carpsucker (*Carpiodes carpio*), Shorthead Redhorse (*Moxostoma macrolepidotum*), and Smallmouth Buffalo (*Ictiobus bubalus*) were sampled in spring 2016 to assess their condition using relative weight. Shorthead Redhorse had the highest relative weight among species for both rivers. River Carpsucker and Smallmouth Buffalo from the Sangamon River had significantly lower relative weights than the Embarras River. Using traditional and geometric morphometrics, I assessed fin morphologies of these species to determine if fin morphology affected the relative weight calculated. I used standard and total lengths to determine River Carpsucker and Smallmouth Buffalo from the Sangamon River have significantly longer caudal fins than the Embarras River. Longer caudal fins cause relative weight to be lower due to relative weight calculation incorporating total length. Geometric morphometrics revealed Sangamon River Carpsucker and Smallmouth Buffalo have significantly different body shapes due to fin morphology than fishes in the Embarras River. This demonstrates the need for investigation into the cause and impacts of elongated fin morphologies.

INTRODUCTION

Impoundment of rivers serves a variety of purposes, such as providing hydropower and municipal supplies of drinking water, but also disrupts the natural landscape and flow of rivers and impacts the resident organisms (Kondolf, 1997). Impoundments and the resulting altered flow causes fragmentation, decreased water quality due to stagnation, and loss of habitat (Collier et al., 1996; Ligon et al., 1995; Magnuson et al., 1995). Impoundments present riverine fishes with unique challenges to adapt and respond to the loss of natural flow regimes (Haas, Blum, & Heins, 2010). Dams controlled by industrial and municipal entities for water supplies lead to reduced river discharge below the dam, causing extreme daily and seasonal fluctuations (Finalyson, Gippel, & Brizga, 1994). The unpredictable magnitude, frequency, and duration of extreme flow changes caused by the nature of these impoundments can influence the success of species subjected to these events (Poff et al., 1997).

Fluctuations in flow affect riverine fishes in multiple ways, which includes causing body shape changes. Flow regime changes caused by dams led to morphological shifts in Blacktail Shiners (*Cyprinella venusta*) to suit the given flow conditions (Haas et al., 2010). Individuals found in reservoir environments had deeper bodies and shortened fins, compared to more elongated, streamlined morphologies seen in the rivers below (Haas et al., 2010). Similarly, Blackstripe Topminnows (*Fundulus notatus*) found in headwater streams exhibited deeper bodies than those found downstream, as a result of plasticity or local adaptations to flow (Schaefer, Duvernell, & Kreiser, 2011). These body shape shifts are the result of just one of the many abiotic factors impacting riverine systems as a result of human modification to rivers.

In addition to impoundment, many lotic systems are imperiled by both point and non-point source pollution, which includes discharge of treated wastewater and agricultural runoff. These sources of pollution contain multiple types of contaminants, such as endocrine disrupting compounds and heavy metals. Endocrine disrupting compounds interfere with hormonal receptors in vertebrates. These compounds include naturally occurring steroids, such as estrone, estradiol, and androgens excreted by humans, as well as synthetically produced steroids, such as those found in human birth control medications (Shore & Shemesh, 2003). This pollution source enters rivers through the release of municipally treated wastewater and runoff from agriculture, that consists of livestock waste, pesticides, and crop byproducts (Shore & Shemesh, 2003). These contaminants are capable of causing mutations and abnormal development in fishes' internal organs, such as the ovaries, testes, and liver (Liney et al., 2006; Moncaut, Nostro, & Maggese, 2003; Porter & Janz, 2003). Heavy metals are also harmful to fishes; adverse effects include alterations of gill structures, accumulation within tissue and internal organs, and DNA strand breakage (Eisler, 1998; Vinodhini & Narayanan, 2009). Wastewater treatment techniques are not always effective at removing these contaminants, especially in facilities not recently upgraded, allowing large percentages of contaminants to enter waterways (Ying et al., 2008).

Aside from internal maladies, exposure to contaminants can also lead to morphological changes. Multiple studies of Mummichogs (*Fundulus heteroclitus*) have given us insight into the morphological effects of exposure to endocrine disrupting compounds. 97.6% of larval and juvenile Mummichogs exposed for 60 days to 10,000 ng/L 17α-ethynylestradiol exhibited fin abnormalities (Boudreau et al., 2003). Further studies of Mummichogs revealed malformed fins and fin rays due to exposure to 17α-methyltestosterone, including elongated and squared caudal fins (Boudreau et al., 2005). Eastern Mosquitofish (*Gambusia holbrooki*), a sexually dimorphic species, exposed to androgenic compounds from treated sewage displayed elongation of the fourth anal fin ray, mimicking the male gonopodium (Leusch et al., 2006).

Water levels and the biotic integrity of the Sangamon River in Decatur, IL, are constantly fluctuating due to the type of impoundment, discharge of treated wastewater, urban impact, and agriculture. The Sanitary District of Decatur (SDD) releases high amounts of soluble nickel into the Sangamon River (Hoster, Gradle, Pederson, Laursen, & Colombo, 2017). These high concentrations of this contaminant are a result of processed industrial waste entering the SDD influent. It is also believed that high concentrations of endocrine disrupting compounds are released due to the discharge of municipal, hospital, and industrial waste, leading to vitellogenin production and feminization in male fishes (Hoster, unpublished). Urban activity and agriculture within the watershed lead to additional inputs of non-point source pollution. Eastern Illinois University conducts monthly and annual monitoring of water quality and fish communities in the Sangamon River to assess the impacts of the SDD effluent on the river's biotic integrity. Due to the presence of these contaminants, further assessment into the health of three abundantly found fish species in the Sangamon River began in 2016.

Although this study began by assessing condition to determine the health of fishes in the Sangamon River, the observation of abnormally elongated fins in two of three study species called for further investigation into the observed morphologies. While the cause or function of elongated fin morphologies remains unknown, I aimed to quantify these morphological changes in the Sangamon River. Using relative weight to assess condition, I compared fishes from the Sangamon River to fishes from the Embarras River, which served as a reference river. Through this study, I have determined that River Carpsucker and Smallmouth Buffalo from the Sangamon River have significantly lower relative weights than populations from the reference river. These differences called for additional examination into the observed fin elongations, especially caudal fins, since total length is incorporated into calculating relative weight. Increased caudal fin length in fishes from one river could cause skewed relative weights due to the increase it causes in total length. Therefore, the objectives of this study were to assess fin morphology using traditional and geometric morphometric techniques to evaluate if there were differences between rivers and if these differences impact relative weight.

Since River Carpsucker and Smallmouth Buffalo have significantly lower relative weights in the Sangamon River, I expected this lower condition to be due to longer caudal fins and not poorer body condition. I expect a difference in the relationship of standard length to total length between rivers for both River Carpsucker and Smallmouth Buffalo. Finally, I expect River Carpsucker and Smallmouth Buffalo from the Sangamon River to display different morphologies due to fin shape and elongation than fishes from the Embarras River.

METHODS

Study Site

The Sangamon River, the largest tributary of the Illinois River, was chosen as the study site for this project due to the effluent discharged by the SDD. The SDD has a dry weather treatment capacity of 41 million gallons per day and processes its wastewater with tertiary treatment. The SDD serves the municipal and industrial components of central Macon County, Illinois. The Lake Decatur Dam is located five kilometers upstream of the SDD effluent release point. This dam serves as a source for municipal and industrial water supplies, causing water to be held in Lake Decatur for extended periods of time. Flow from Lake Decatur to the Sangamon River only occurs during high precipitation events or when dam controllers release water. The Embarras River, a tributary to the Wabash River, served as a reference site due to its similar fish community to the Sangamon River and the absence of similar wastewater treatment effluent in proximity to the study area (Thomas, personal communication). Although the Embarras River also is impounded at Lake Charleston, this impoundment is a much smaller spillway, maintaining continuous water flow to the river below. Study sites were limited to stretches of river within reach of boat ramps due to limited boat access on both rivers. The Sangamon River study site, located in Decatur, IL, includes a stretch of river from the location of the SDD effluent input to approximately three and a half kilometers downstream. The Embarras River study site, located in Charleston, IL, includes a stretch of river beginning below the dam of Lake Charleston (Figure 2.1).

Study species

River Carpsucker (*Carpiodes carpio*), Smallmouth Buffalo (*Ictiobus bubalus*), and Shorthead Redhorse (*Moxostoma macrolepidotum*) were selected based on their high abundance in both study rivers, as well as the observation of abnormal fin morphologies in the Sangamon River. All three species belong to the family Castostomidae. These species are widely distributed throughout the Midwestern United States in medium-sized to large rivers (Pflieger, 1997). These species are long-lived native fishes, feeding in the benthic zones of lotic systems. While Shorthead Redhorse primarily feed on benthic invertebrates, River Carpsucker and Smallmouth Buffalo are more generalized feeders and will also consume large amounts of algae, detritus, and sediment (Gido, 2001; Spiegel, 2010). Although these three species commonly occur in the same river systems, they have different habitat preferences due to their feeding habits. Shorthead Redhorse primarily feed and spawn over riffles, whereas River Carpsucker and Smallmouth Buffalo are found in slower currents and wide varieties of substrates (Pflieger, 1997). These species are easily sexed during their reproductive months, when tubercles develop on the faces and fins of the males and milt can be distinguished from eggs.

Field Protocol

I collected River Carpsucker, Smallmouth Buffalo, and Shorthead Redhorse from the Sangamon and Embarras Rivers using boat-mounted 60 Hz pulsed-DC electrofishing gear with a 25% duty cycle. All fishes of the study species were weighed (g), measured for total and standard length (mm), and sexed. All individuals of the study species were sexed by visual identification of tubercles and milt in males or eggs in females. All female and juvenile fishes were released following measurement and sexing. Male fishes were humanely euthanized following collection for gonad dissection for other studies. Male fishes were photographed for morphometric analyses following euthanasia and prior to dissection.

Statistical and Morphometric Analyses

I chose Relative Weight as my condition metric due to its ability to compare condition across populations. Relative Weight allowed us to compare fish from the study population to a standard weight calculated for individuals of the same length for that species and to compare mean Relative Weight of each population to one another. I calculated Relative Weight as a measure of condition using total length and weight of each fish (Bister et al., 2000; Blackwell, Brown, & Willis, 2000; Gabelhouse, 1984). The following equation was used for calculating Relative Weight:

$$W_r = W/W_s \times 100$$

W_s is the standard weight for a fish of a specific length, predicted by a length-weight regression representative for an entire species. Although the accepted standard weights for the study species are standardized using total length and Relative Weight is calculated as such, I substituted standard length into the same equations used above to account for any differences caused by elongated caudal fins. While the number generated using this substitution is no longer centered on a standard of 100 for "good condition", it allows for the direct comparison between two separate populations. I compared Relative Weight and this modified version of Relative Weight for each species between rivers using t-tests to determine if condition varied between rivers

I measured total and standard lengths to calculate and compare caudal fin lengths between populations, as well as to compare the relationship of standard to total length between rivers. Standard length and total length were used to calculate caudal fin length for each fish and then taken as a percentage of the total body length. I used Pearson's Correlation to assess the relationship of standard length to total length for each species and river. Fisher's z transformation was used to convert Pearson correlation coefficients to z scores. I used t-tests on Fisher's z scores to determine if there were differences in the relationship of standard length to total length between rivers for each of the study species.

Photographs were taken of the left side of each male fish of the three study species. I used TPS Util to convert picture files and TPS Dig to assign landmarks to individuals of each species and from each river. The number of landmarks assigned and analyzed varied by species. Smallmouth Buffalo pelvic fin landmarks were not included in analyses due to positioning inconsistencies in images. Canonical variate analysis (CVA) and Discrimiant Function Analysis (DFA) were conducted in MorphoJ software to compare morphological differences of fish within and between rivers. CVA showed singular distinct groupings for each of the study species from the Embarras River, as well as Shorthead Redhorse from the Sangamon River. However, CVA showed two distinct groups within the Sangamon River for both River Carpsucker and Smallmouth Buffalo. These two distinct groups were separated for further analyses to account for outliers with more extreme changes in body shape. These Sangamon River groups, along with the group from the Embarras River, were then used for further analysis of differences between rivers. DFA was performed with 20,000 permutations and used two distances to differentiate between groupings: Mahalanobis distance measured how unusual an individual was relative to the mean and Procrustes distances measured the magnitude of shape deviation.

RESULTS

Traditional Morphometrics

Significant correlations of total length to standard length were found for all species in both rivers. Pearson correlation coefficients were transformed with Fisher's z transformation and then an upaired t-test was used to compare the relationship between rivers for each of the study species. No significant difference in the relationship was found between rivers for Shorthead Redhorse ($t=0.642$, $DF=67$, $p=0.523$) (Figure 2.5). However, the relationship of standard length to total length was significantly different for River Carpsucker and Smallmouth Buffalo between rivers (River Carpsucker: $t=4.146$, $DF=118$, $p<0.001$; Smallmouth Buffalo: $t=5.055$, $DF=134$, $p<0.001$) (Figures 2.6-2.7). River Carpsucker and Smallmouth Buffalo from the Sangamon River had much longer caudal fins than Embarras River fishes. Overall, Sangamon River fishes have longer caudal fins. River Carpsucker from the Sangamon River have caudal fins comprising 5.23% more of their total length than those from the Embarras River, while Sangamon River Smallmouth Buffalo have caudal fins comprising 5.58% more of their total length than those from the Embarras River. River Carpsucker and Smallmouth Buffalo from the Sangamon River were significantly larger in total length compared to fishes of the same standard length in the Embarras River (River Carpsucker total length: $t= -2.807$, $DF= 142$, $p= 0.006$; Smallmouth Buffalo total length: $t= -4.78$, $DF= 213$, $p < 0.001$).

Relative Weight

River Carpsucker and Smallmouth Buffalo from the Sangamon River had significantly lower Relative Weights than those in the Embarras River (River Carpsucker: t=11.145, DF= 151, p< 0.001; Smallmouth Buffalo: t=10.374, DF= 203, p< 0.001). No difference was detected between rivers for Shorthead Redhorse (t= -0.327, DF= 100, p= 0.745) (Table 2.1). Smallmouth Buffalo had the lowest Relative Weights for all species in both rivers.

Adjusted Relative Weight

Since River Carpsucker and Smallmouth Buffalo from the Sangamon River have longer total lengths compared to their standard lengths and weights, I substituted standard length into the Relative Weight equation. No difference in adjusted relative weight was found between rivers for River Carpsucker (t=-1.395, DF= 115, p=0.166) (Table 2). However, Sangamon River Smallmouth Buffalo have a significantly higher adjusted relative weight than fish from the Embarras River (t=-2.811, DF= 130, p=0.006) (Table 2).

Geometric Morphometrics

Canonical variate analysis (CVA) and Discriminant Function Analysis (DFA) were conducted for each river and study species. CVA showed no difference in morphologies between rivers for Shorthead Redhorse. The CVA showed three distinct groupings for River Carpsucker and Smallmouth Buffalo. These groups were Embarras River fish, Sangamon River fish (Group 1), and Sangamon River outlier fish (Group 2). The first CV explained 75.3% of the variation for River Carpsucker, while the second explained 24.7%. Both Sangamon River River Carpsucker groups had similar scores on the first CV, but did not align on the second CV; the Embarras River group aligned intermediately on the second CV. The first CV for Smallmouth Buffalo explained 62.72% of the variation, while the second explained 37.28%. Both Sangamon River Smallmouth Buffalo Groups 1 and 2 have similar scores for the second CV, with lower scores than the Embarras River group. Embarras River fish aligned similarly to the Sangamon River Group 1 fish along the first CV, with Sangamon River Group 2 fish having much lower scores. Identifying the landmarks associated with scores for each CV allowed me to identify the driving factors of the differences observed. The traits causing the largest differences in morphological traits for River Carpsucker are as follows: length and size of caudal fin, size of dorsal fin, and length of pectoral fins (Figures 2.10) The traits causing the largest differences in morphological traits for Smallmouth Buffalo are as follows: length of anal fin, length of pectoral fin, length and size of caudal fin (Figures 2.11). Discriminant function analysis found all groups of both River Carpsucker and Smallmouth Buffalo significantly differed from one another within species using Mahalanobis distance ($p < 0.05$). Similarly, all groups significantly differed from one another using Procrustes distances, except for Sangamon River Group 2 and Embarras River fish for both River Carpsucker and Smallmouth Buffalo (River Carpsucker Sangamon River Group 2 vs. Embarras River: $p = 0.0819$; Smallmouth Buffalo Sangamon River Group 2 vs. Embarras River: $p = 0.2785$).

DISCUSSION

River Carpsucker and Smallmouth Buffalo from the Sangamon River had significantly lower Relative Weights compared to fishes from the Embarras River. These low Relative Weights might normally suggest that these fishes are in poor condition and significantly worse condition than fishes from the Embarras River. Aside from finding differences between rivers for two species and no difference for Shorthead Redhorse, the calculated condition for all species and rivers are much lower than what is considered ideal (Bister et al., 2000; Blackwell et al., 2000). Low Relative Weights in River Carpsucker and Smallmouth Buffalo in the Sangamon River appear to be due to elongated caudal fins detected in these populations. Since calculating Relative Weight for these species takes into consideration the total length, a longer caudal fin causes individuals to have a longer total length compared to the standard length of a normal fish. This causes these fishes to have lower weights for their total length than the predicted Standard Weight. Therefore, standard length should be used instead of total length when examining condition in fishes displaying irregularly elongated caudal fins. Standard lengths and weights should be collected from multiple populations of these species in order to derive a Relative Weight equation based on standard length, rather than total length. This will allow for a more accurate direct comparison of condition between populations, as well as better assessment of abiotic and biotic factors influencing each population's health, thus leading to improved management (Blackwell et al., 2000).

River Carpsucker and Smallmouth Buffalo from the Sangamon River have significantly longer caudal fins than individuals from the Embarras River. This is further supported by the statistical difference between rivers for the relationship of total length to standard length for both River Carpsucker and Smallmouth Buffalo. The inaccuracy of calculating the standard weight, and thus Relative Weight, due to the caudal fin length of these populations in the Sangamon River demonstrates the need for further assessment of this morphology and adjustment of this metric. While it is apparent that longer caudal fins cause lower relative weights, cause of this fin morphology is still unknown and is reason for concern. Although studies have documented the need for adjustment of condition metrics due to seasonal and geographical differences, there are few, if any, studies on adjustments needed for differences in fin morphology between populations (Blackwell et al., 2000; Hansen & Nate, 2005).

The results of the geometric morphometric analyses show that River Carpsucker and Smallmouth Buffalo from the Sangamon River do exhibit different body shapes than fishes from the Embarras River. Nevertheless, there are fishes in the Sangamon River that have some similar characteristics to those in the Embarras River. These results support the traditional morphometrics results; however, geometric morphometric analyses provide a more complete examination of morphology, as it takes into consideration both size and shape, rather than just size (Zelditch, Swiderski, Sheets, & Fink, 2004). The separation of two distinct River Carpsucker and Smallmouth Buffalo groups within the Sangamon River demonstrates that although some fishes are more similar to those in the Embarras River, they still do not exhibit all of the same morphological traits. The primary source of these differences between rivers is the size and shape of the caudal fin, as well as the pectoral and anal fins. Multiple studies have demonstrated a connection between altered hydrology and body shape changes. (Haas et al., 2010; Schaefer et al., 2011). Furthermore, many studies have connected body shape changes and fin elongation with exposure to contaminants, including endocrine disrupting compounds (Boudreau et al., 2003, 2005; Leusch et al., 2006; Shinn, 2010). Additional investigation, using a more robust sample size, will provide insight into the cause and impacts of these body shapes, as well as determine the appropriate next steps to assure the future health of these populations.

Although we cannot say at this time what is driving the fin morphologies seen in the Sangamon River, there appears to be some stressor on the system that warrants closer monitoring. Further studies should also seek to determine when this morphology occurs during development and if this abnormality is present in other species in the Sangamon River, as well as if this is occurring in any other nearby river systems. One manner of determining the onset of fin elongation is using age and growth data with both standard and total length to determine the point at which total length grows more rapidly than standard length. Although I determined that relative weight is influenced by elongated caudal fins and that these Sangamon River fishes are not in poor condition, per se, continued monitoring seems prudent.

While the Sangamon River is the largest tributary of the Illinois River and is a moderate size river, it becomes a wadeable stream for extended periods of time, especially when water is being held in Lake Decatur (Figure 2.12). When water is released from the dam or with excessive rains, the river can rise 5-10 feet in the matter of hours. Conversely, the Embarras River has more of a natural flow regime that is less controlled by human intervention (Figure 2.13). Flow regime changes have been documented to cause morphological shifts for enhanced swimming performance for the given flow conditions (Haas et al., 2010). The unpredictable nature of the Sangamon

River below Lake Decatur could be the cause of observed morphological differences. Further investigation into the swimming dynamics of these fishes is critical to understanding how these elongated fins impacts or aids swimming. *Cyprinella venusta* were documented to have deeper bodies and shortened fins in reservoir environments, compared to more elongated, streamlined morphologies seen in the rivers below for decreased hydrodynamic drag (Haas et al., 2010). Since water levels are so variable in the Sangamon River, understanding the relationship of elongated fins to flow events will allow us to better determine if these fin morphologies are an adaptation, rather than abnormality. Studying these species in several other rivers with similar hydrologic patterns is critical to understanding if the morphologies observed are a function of flow dynamics.

In addition to effects of flow, genetic composition and diversity of Sangamon River fish populations may play a role in the observed morphologies. There are three *Ictiobus* species and *Carpiodes* species found in both rivers, all known to be able to hybridize within genera (Dellinger et al., n.d.; Edwards & Twomey, 1982; Johnson & Minckley, 1969). Hybridization may result in abnormal and not typically seen morphologies of these species. Alternatively, with the Sangamon River being so low and shallow for extended periods of time every year, it could cause populations to be segregated due to fragmentation from the rest of the river downstream. Lack of genetic diversity within fragmented populations could eventually lead to local adaptations or plasticity. While population genetics will not deliver a cause for elongation or determine if the observed elongations are a genetic abnormality, it would help determine the extent of hybridization in both rivers and if this is occurring at a higher rate in the Sangamon

River than other rivers. Population genetics will also help determine if the populations sampled in the Decatur stretch of the Sangamon River are genetically dissimilar from the populations further downstream in the Sangamon River and in the Illinois River.

The presence of treated wastewater and known soluble mutagens in the Sangamon River could also be the cause of the elongated fins. This is supported by elongated fins only being present in two of three study species. Shorthead Redhorse consume a more selective prey base, and thus may not be exposed to as many contaminants, like heavy metals, located in the substrate of the river. River Carpsucker and Shorthead Redhorse have only moderate diet overlap, with Shorthead Redhorse specializing on a few prey items while River Carpsucker ingest high amounts of algae, detritus, and substrate (Spiegel, 2010). Smallmouth Buffalo are also opportunistic benthic feeders and will eat detritus, like River Carpsucker, when other food sources are scarce (Edwards & Twomey, 1982; Gido, 2001). These disparities in diet may place Smallmouth Buffalo and River Carpsucker in different substrate types and areas than Shorthead Redhorse, leaving them more exposed to existing harmful compounds.

Since I quantified elongated fins in two benthic feeders, I would also like to assess fin morphologies in other species that spend time feeding or swimming in the benthos of the Sangamon River. Anecdotal data from the Sangamon River monitoring program with the SDD suggests elongated fins in multiple small-bodied Cyprinids. In addition, Common Carp (*Cyprinus carpio*), a large bodied member of the family Cyprinidae, share a similar feeding ecology to Smallmouth Buffalo and River Carpsucker. Therefore, Common Carp would be another useful study species for future assessment. Bigmouth Buffalo (*Ictiobus cyprinellus*) are abundant in the Sangamon

River, especially between the Lake Decatur Dam and the SDD. Bigmouth Buffalo and Smallmouth Buffalo are members of the same genus but have very different feeding methods and prey items, allowing Bigmouth Buffalo to spend considerably less time in the benthos. Assessing fin morphologies of Bigmouth Buffalo will be useful in determining if diet and life history play a role in fin morphologies, due to exposure to contaminants. Additionally, sport fishing is very popular in the study reach of the Sangamon River. Studying common sportfishes, such as sunfishes, basses, and catfishes, may offer insight into how these fishes are impacted and what health risks they pose to humans from flesh consumption.

Sampling populations of these species upstream of the Lake Decatur Dam and downstream of Springfield, IL will help determine if these fin morphologies are persistent throughout the river, or if these are isolated populations below the Lake Decatur Dam. I would also like to examine fin morphology in additional watersheds in Illinois and other states in order to determine rate of occurrence. If other populations experience the same morphologies, it will allow us to better understand the causes and purpose of elongated fins. By having a more accurate relative weight equation, fisheries managers will be able to make better health assessments and management recommendations for specific populations.

Quantifying the specific contaminants in the water and sediments of the Sangamon River will allow a better integrated view of the system. Numerous other studies have cited androgenic contaminants as a cause of elongated and abnormal anal and caudal fins of several fish species (Boudreau et al., 2005; Leusch et al., 2006). However, the link to endocrine disruptors and fin abnormalities is much more prevalent for highly sexually dimorphic species, such as mosquitofish. Since none of my study species have been reported to have sexually dimorphic characters affecting the fins, I may not be seeing the same effect as the other studies have. The onset of fin elongation during a fish's development is still not understood, and further investigation is necessary to determine if elongation occurs in larval stages or develops later on as a result of exposure.

This study emphasizes the need for ecotoxicologists and fisheries managers to work together to better assess populations and understand the factors that may influence assessments. At first glance, Sangamon River populations of River Carpsucker and Smallmouth Buffalo appeared to be in poor condition. Upon further examination, this seemingly poor condition was being influenced by increased caudal fin length driving down relative weight values. Catostomid species are commercially or recreationally important as non-traditional sport fishes and are highly mobile. Understanding the dynamics influencing metrics and properly assessing populations using these practices can be useful in managing other species and populations that may experience similar problems. This understanding, as well as continued studies to determine the cause of the above documented fin morphologies, can lead to better managed fish populations in the future and better control of the factors causing these morphologies.

TABLES

Table 2.1—Mean Relative Weight for each study species and river, with standard error in parentheses.

	Relative Weight	
	Embarras River	Sangamon River
River Carpsucker	89.33 (0.72)	74.58 (1.22)
Smallmouth Buffalo	77.41 (0.84)	65.92 (0.66)
Shorthead Redhorse	85.23 (0.94)	85.79 (1.50)

Table 2.2—Mean Adjusted Relative Weight for River Carpsucker and Smallmouth Buffalo and each river, with standard error in parentheses.

	Adjusted Relative Weight	
	Embarras River	Sangmaon River
River Carpsucker	163.35 (1.60)	167.53 (2.51)
Smallmouth Buffalo	145.84 (1.94)	162.89 (4.79)

FIGURES

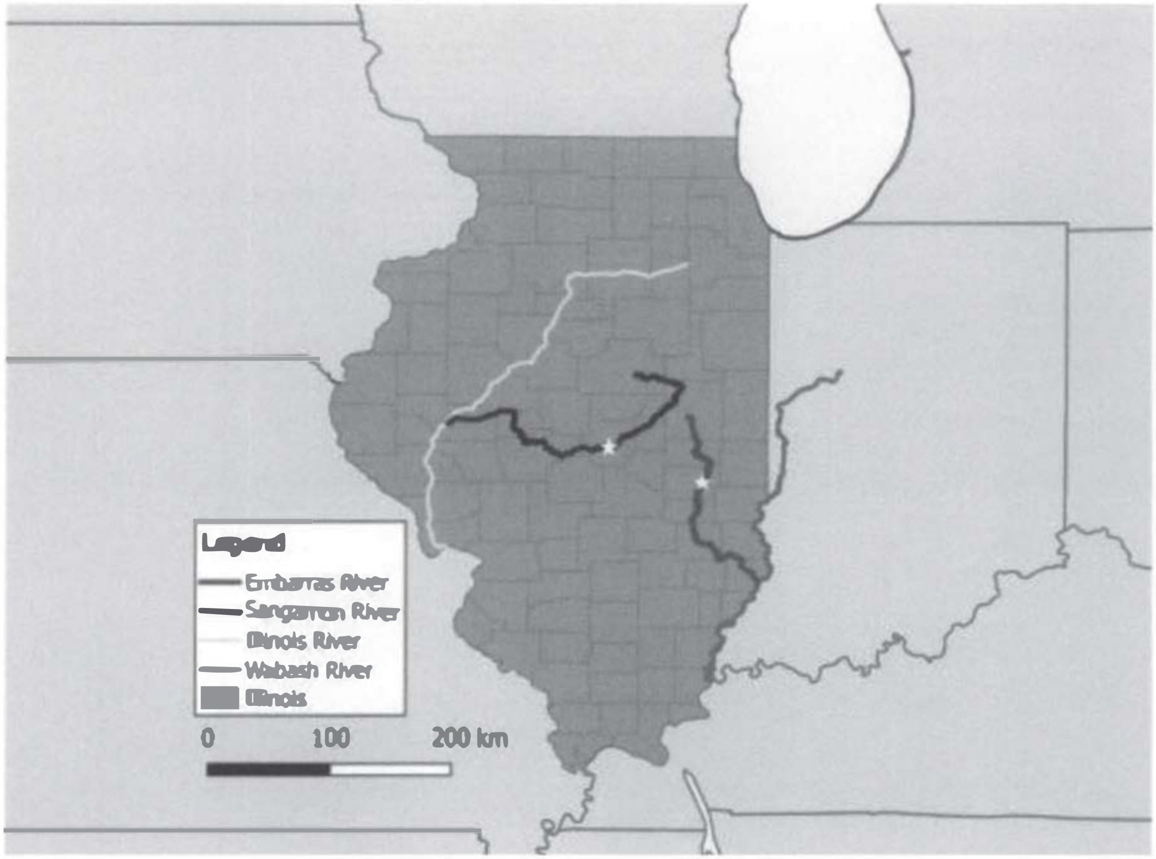


Figure 2.5— A map of the Sangamon River, located in Central Illinois, and the Embarras River, located in East-Central Illinois. Study areas in Decatur and Charleston, IL, denoted with stars.

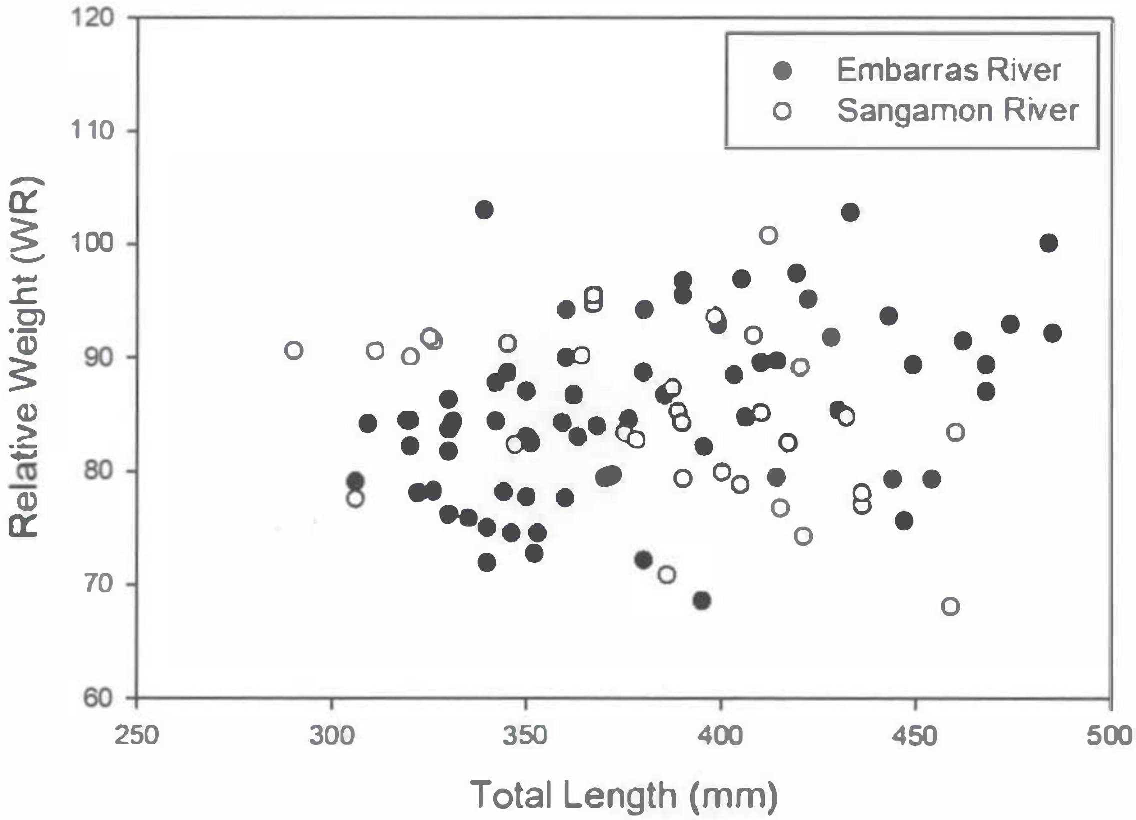


Figure 2.6—Total length (mm) versus Relative Weight (WR) for Shorthead Redhorse from the Sangamon River and Embarras River. There was no statistically significant difference between rivers.

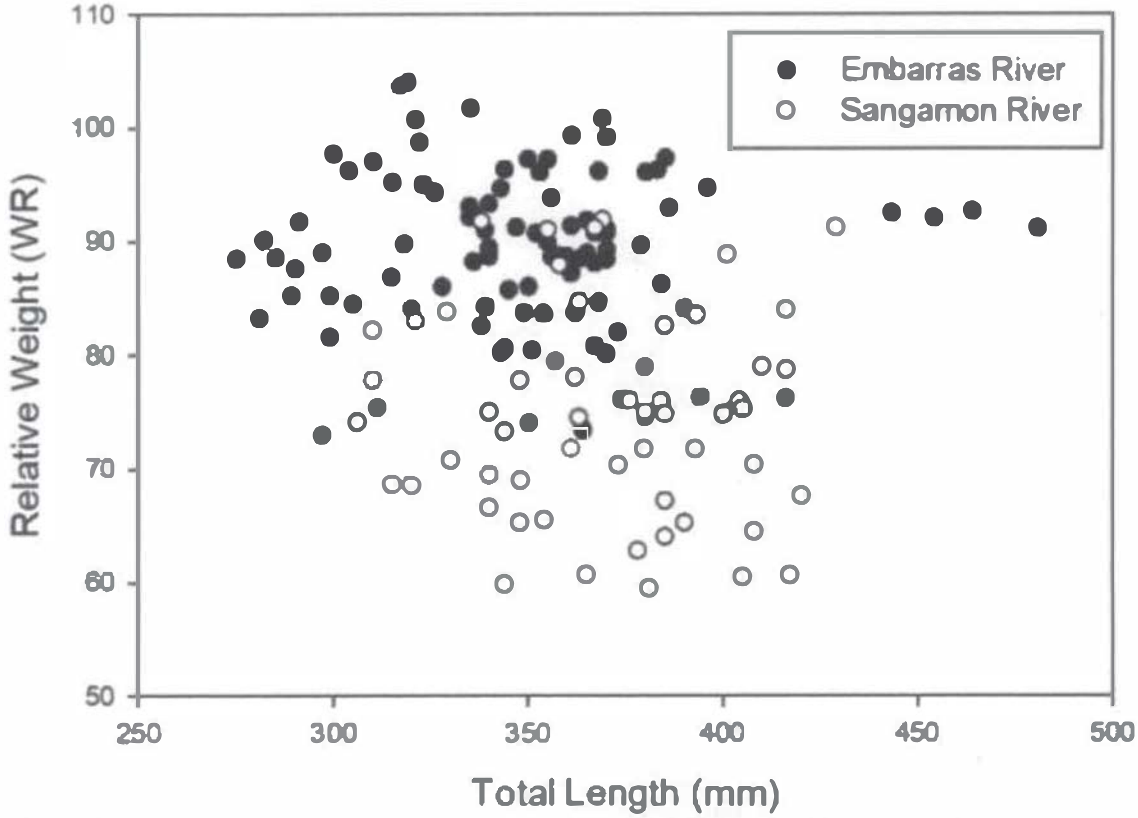


Figure 2.7—Total length (mm) versus Relative Weight (WR) for River Carpsucker from the Sngamon River and Embarras River. There was a statistically significant difference between rivers ($F = 124.206$, $DF = 1, 151$, $p < 0.001$).

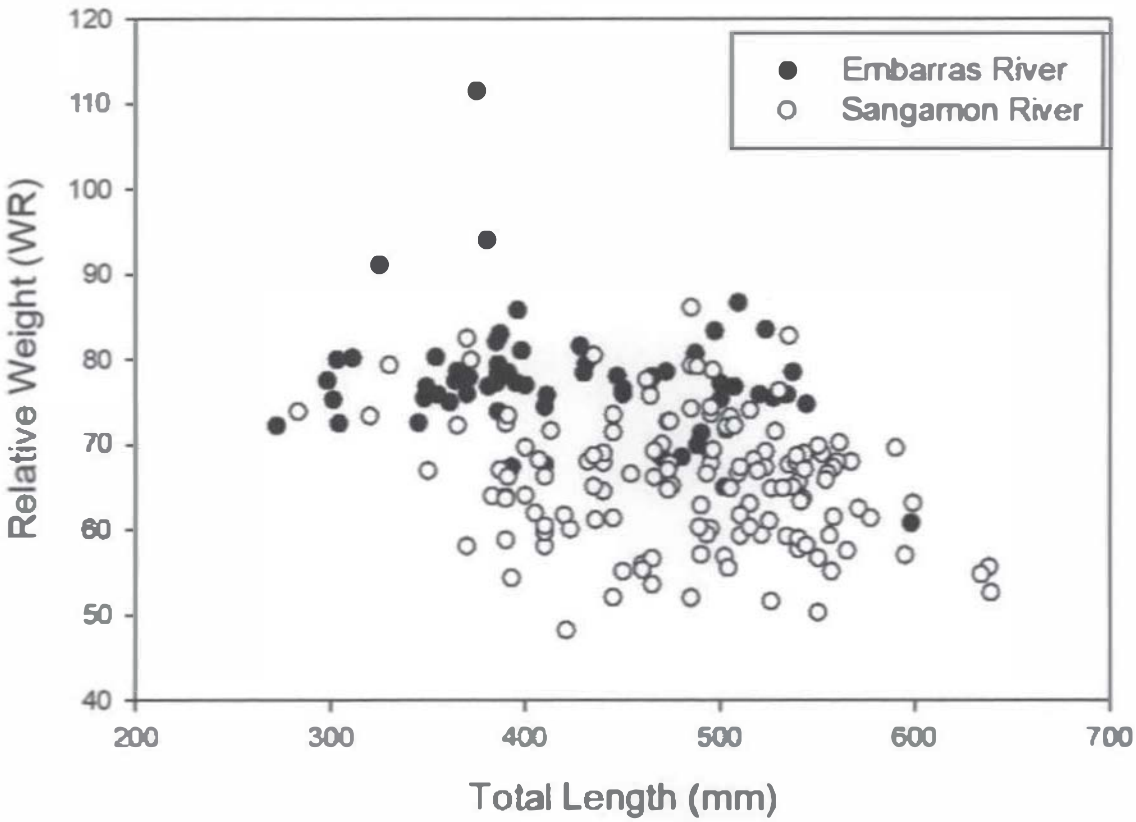


Figure 2.8—Total length (mm) versus Relative Weight (WR) for Smallmouth Buffalo from the Sangamon River and Embarras River. There was a statistically significant difference between rivers ($F = 107.619$, $DF = 1, 203$, $p < 0.001$).

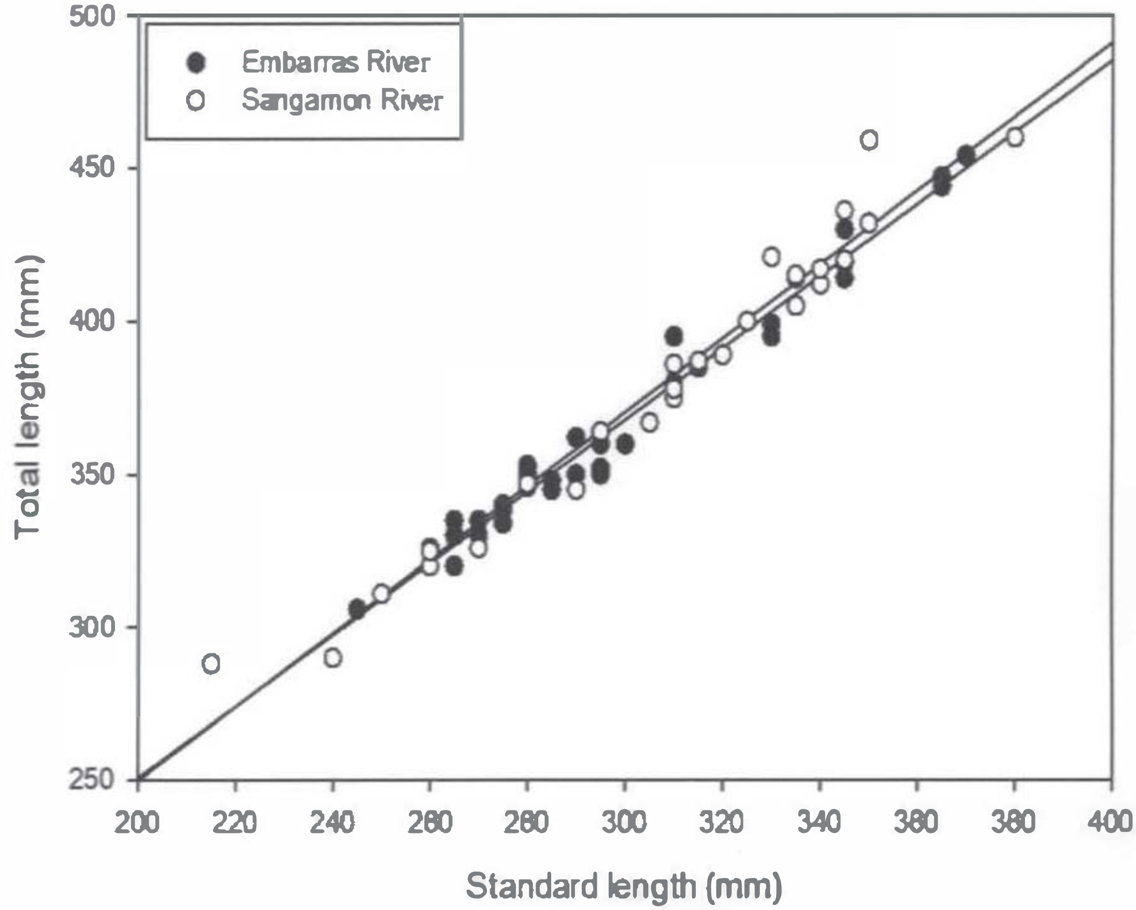


Figure 2.9—Standard length (mm) versus Total Length (mm) for Shorthead Redhorse from the Sangamon River and Embarras River. Pearson Correlation and Fisher's z transformation found no difference in the relationship between rivers. (t=0.642, DF=67, p=0.523)

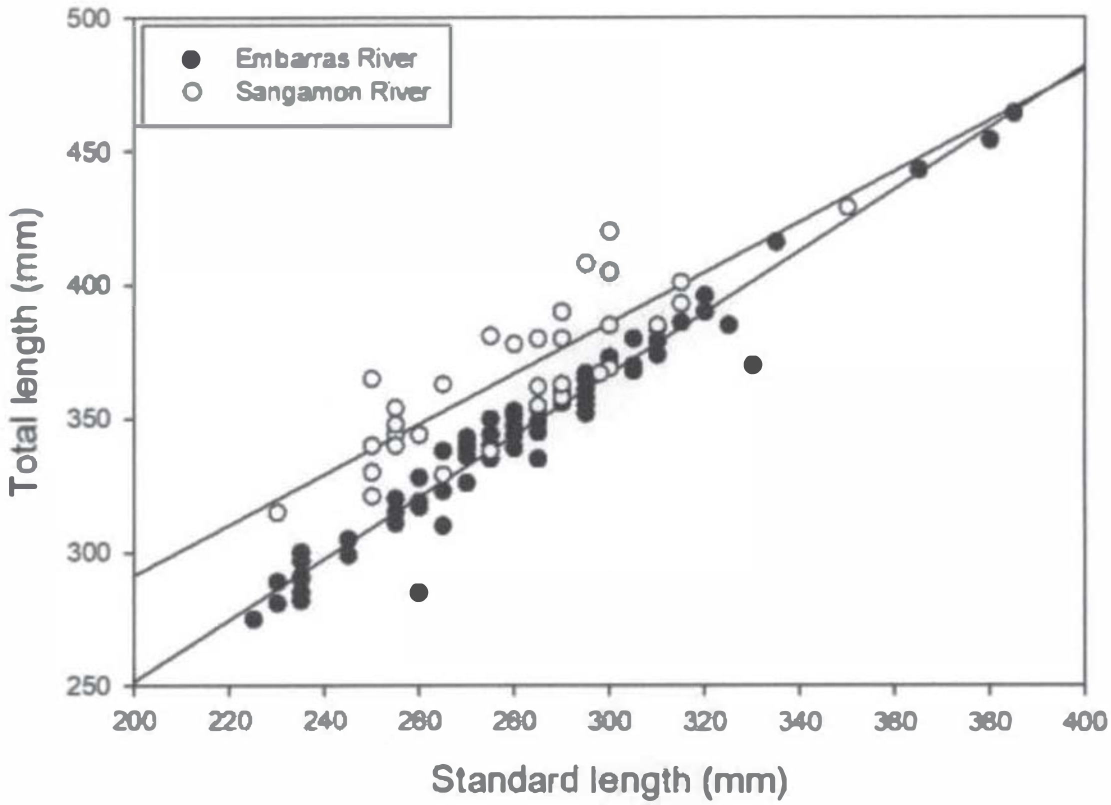


Figure 2.10—Standard Length (mm) versus Total length (mm) for River Carpsucker from the Sangamon River and Embarras River. Pearson Correlation and Fisher's z transformation found a significant difference in the relationship between rivers (t=4.146, DF=118, p<0.001).

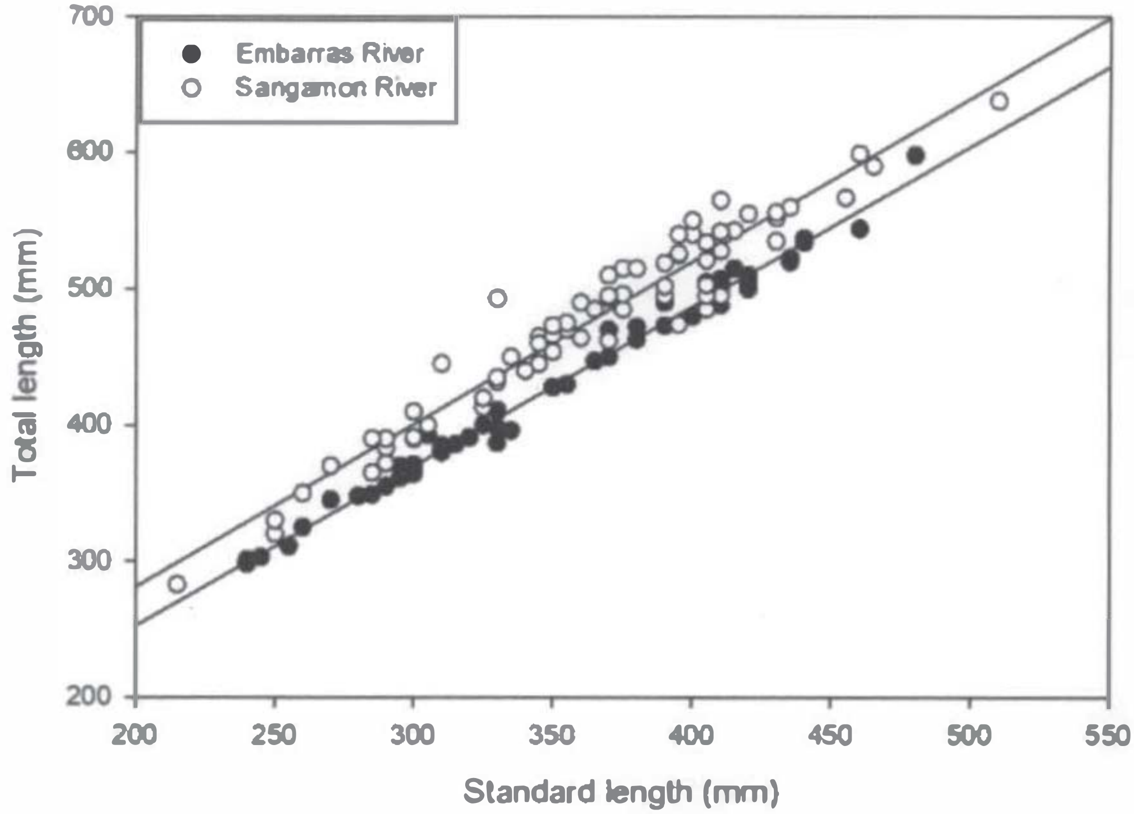


Figure 2.11—Standard length (mm) versus Total Length (mm) for Smallmouth Buffalo from the Sangamon River and Embarras River. Pearson Correlation and Fisher's z transformation found a significant difference in the relationship between rivers ($t=5.055$, $DF=134$, $p<0.001$).

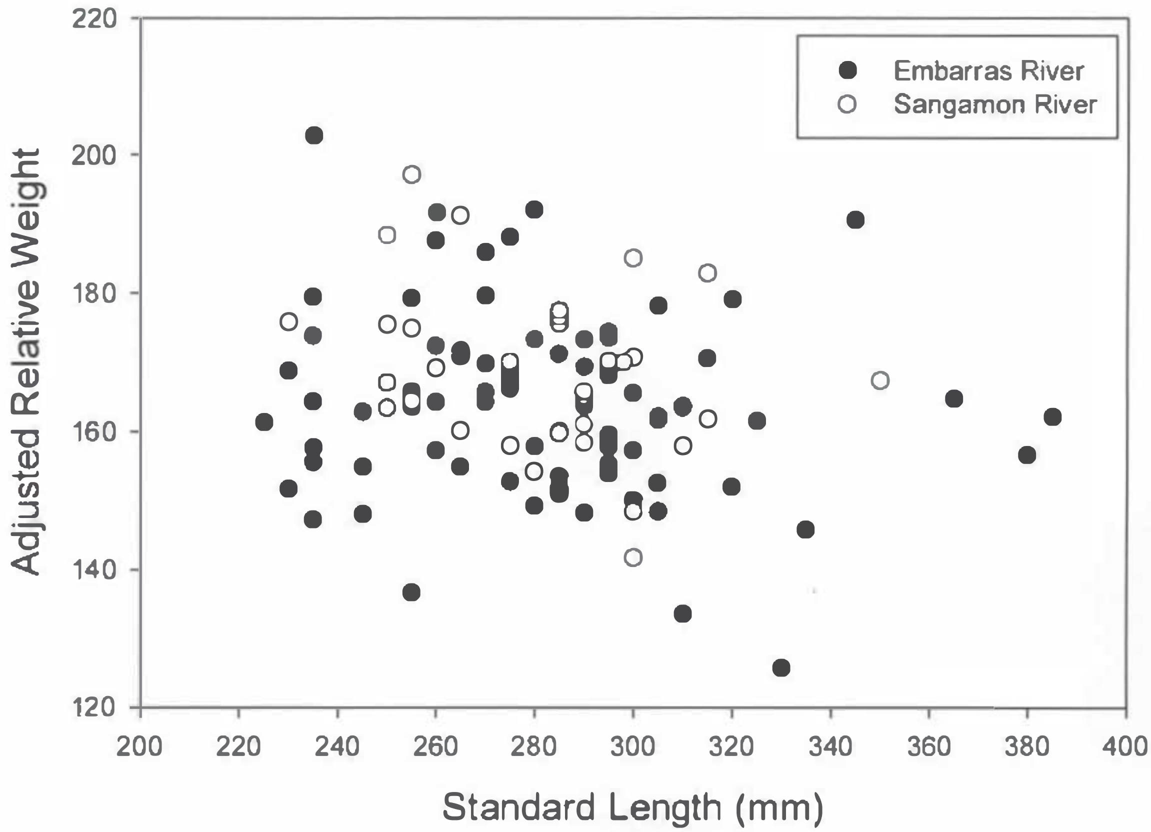


Figure 2.12—Standard Length (mm) versus adjusted Relative Weight for River Carpsucker from the Sangamon River and Embarras River. Adjusted Relative Weight did not differ between rivers ($t=-1.395$, $DF= 115$, $p=0.166$).

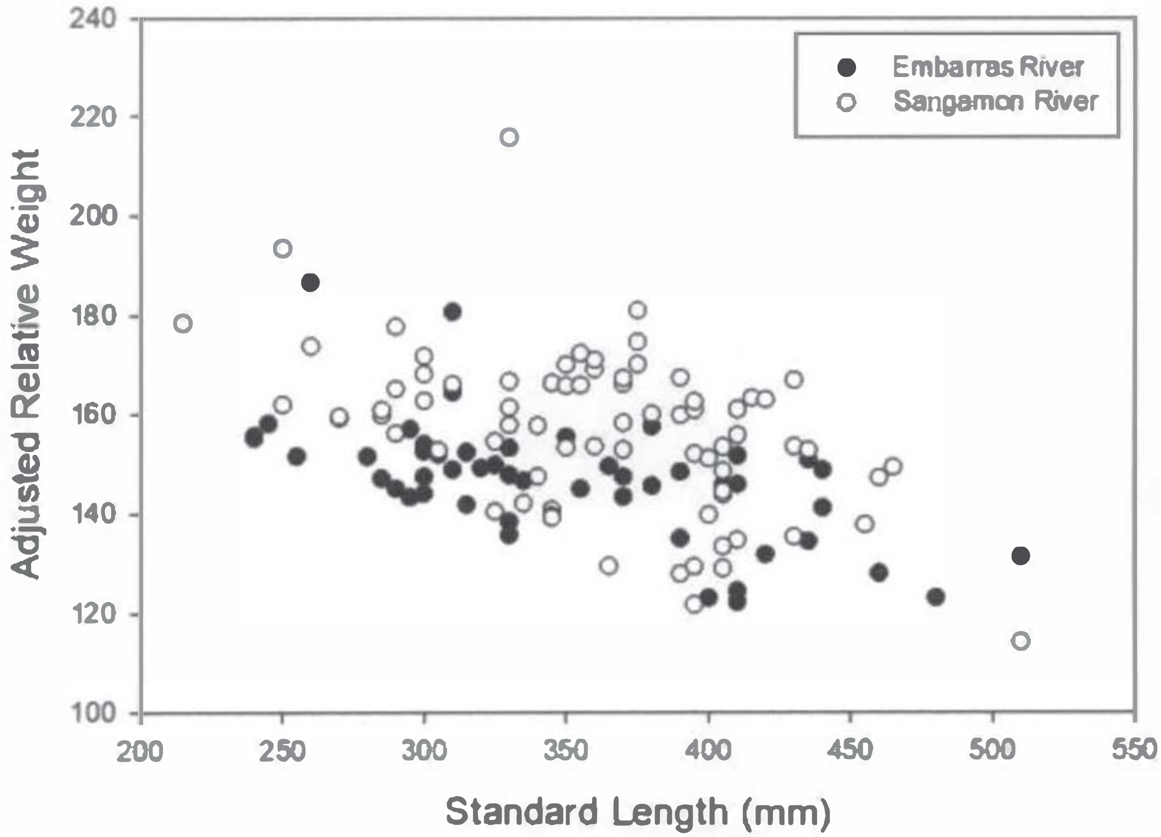


Figure 2.13—Standard Length (mm) versus Relative Weight for Smallmouth Buffalo from the Sangamon River and Embarras River. Adjusted Relative Weight was significantly greater in the Sangamon River (t=-2.811, DF= 130, p=0.006).

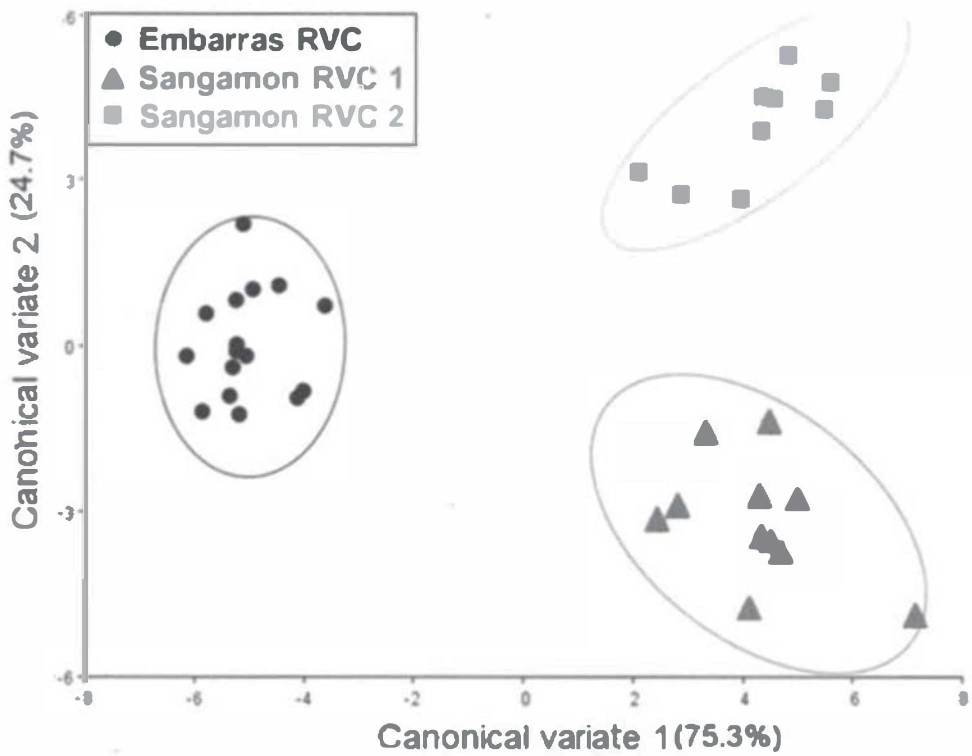


Figure 2.14—Relationship between canonical variates for three groups of River Carpsucker (fish from the Embarras River and fish from the Sangamon River in two separate groupings).

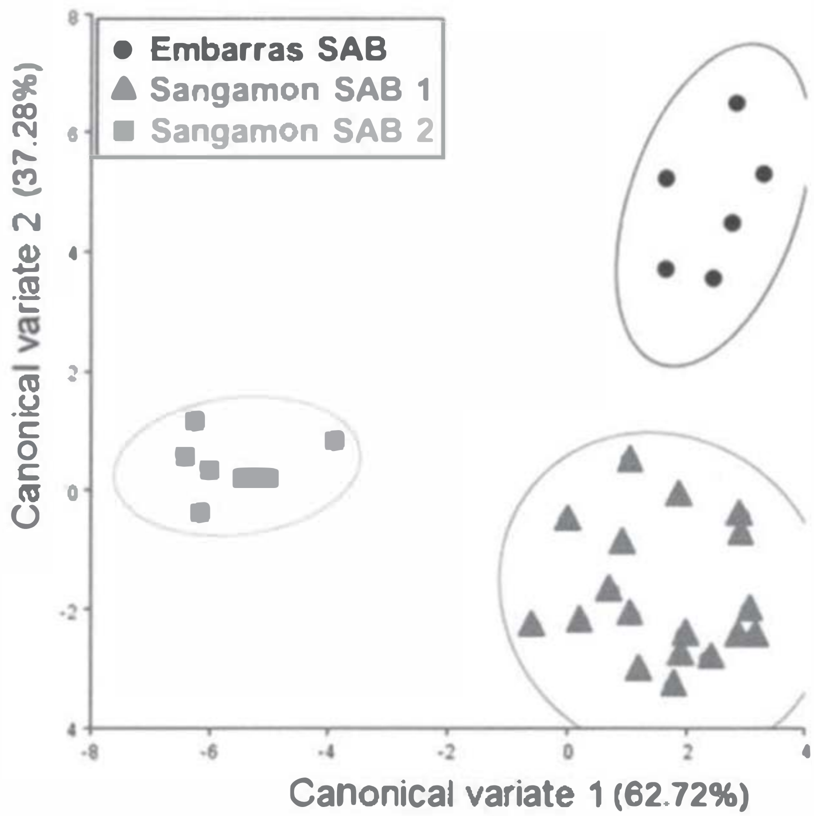
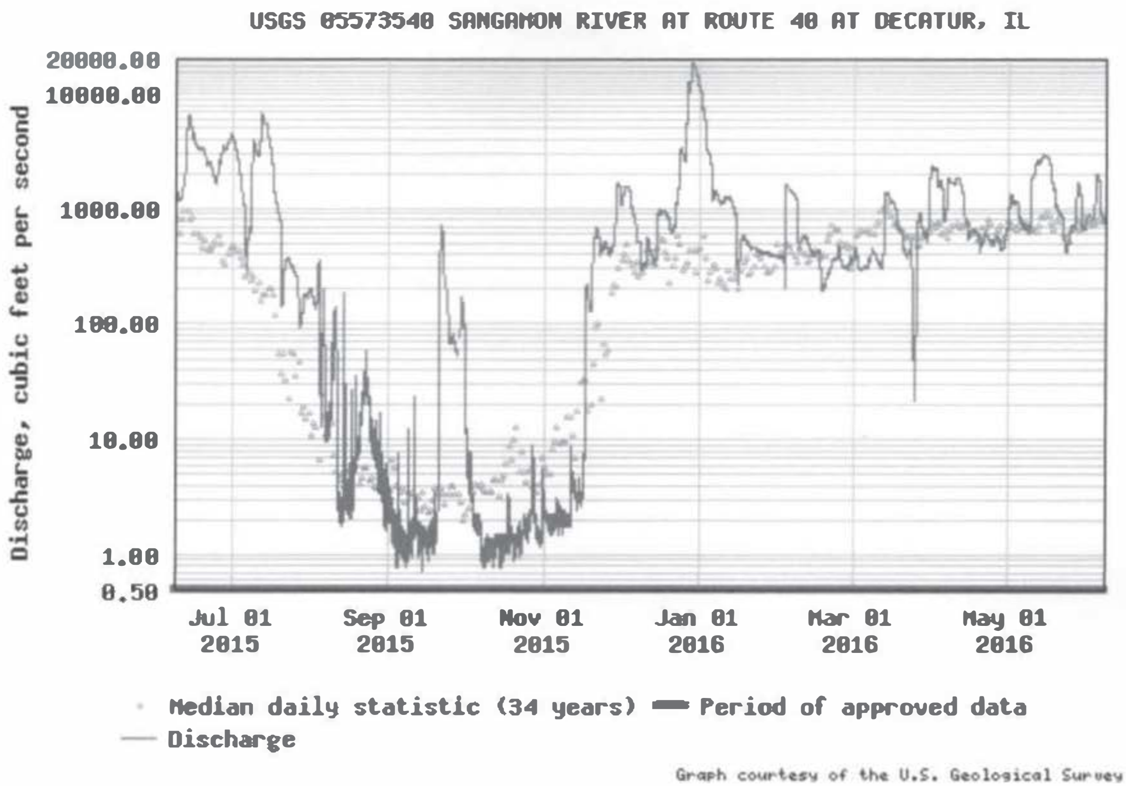


Figure 2.15—Relationship between canonical variates for three groups of Smallmouth Buffalo (fish from the Embarras River and fish from the Sangamon River in two separate groupings).



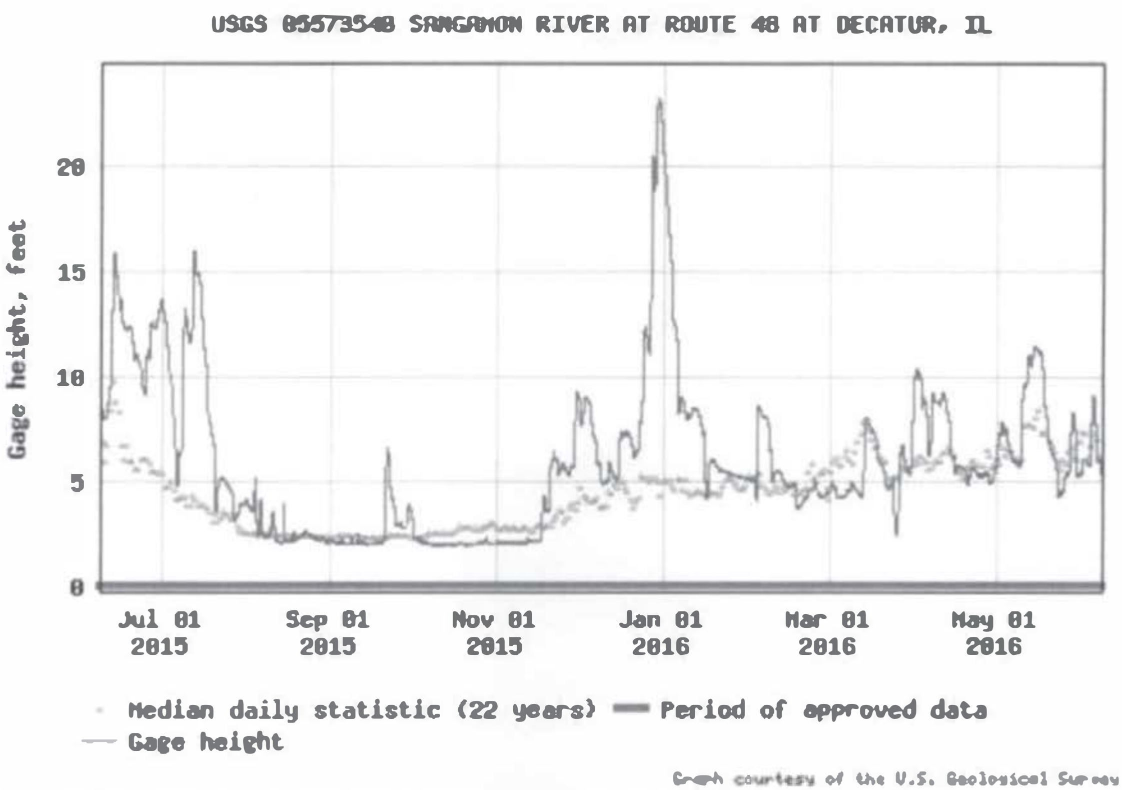


Figure 2.16—Discharge (cubic feet per second) and gage height (feet) for the Sangamon River from June 2015-June 2016.

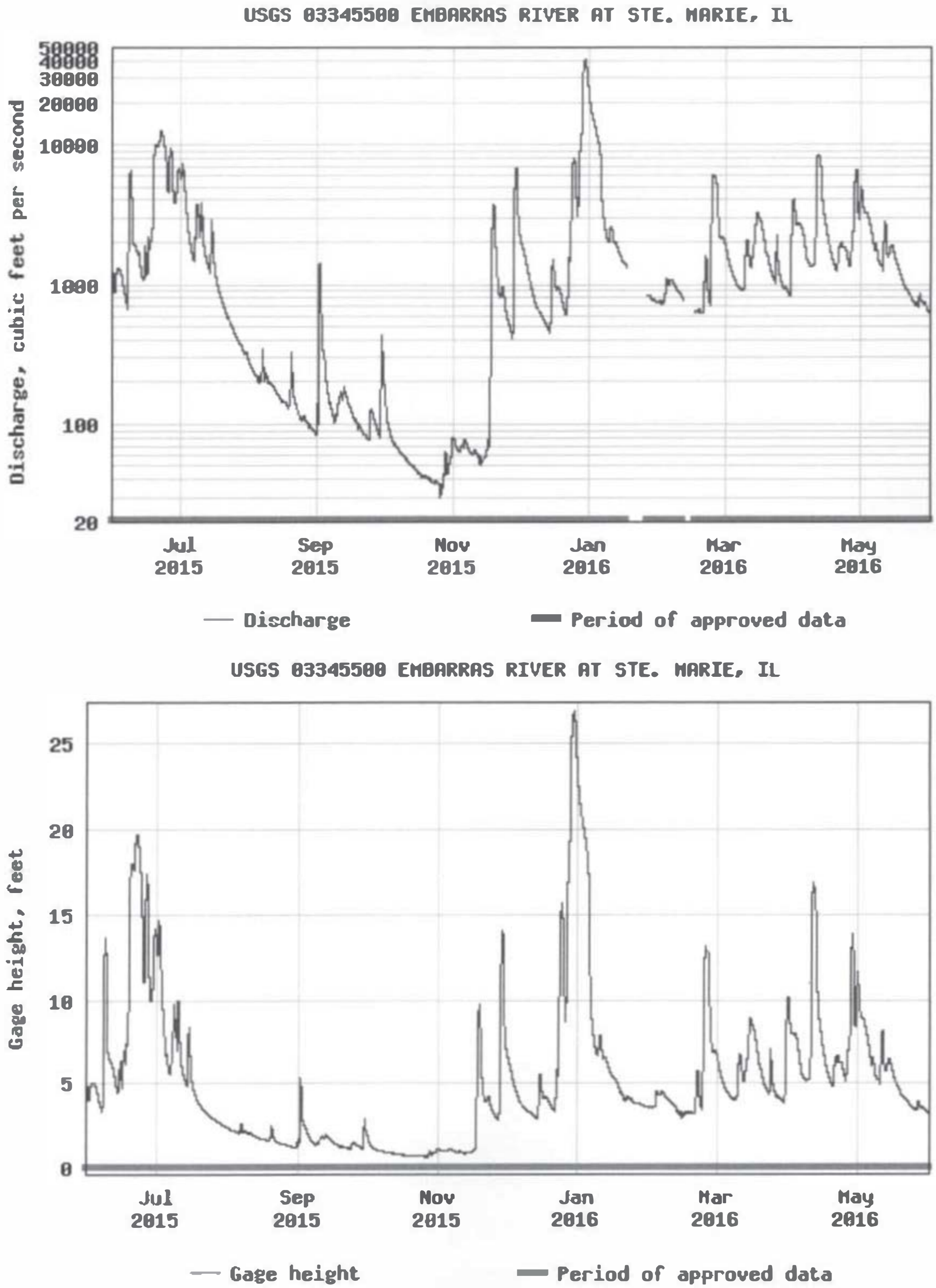


Figure 2.17—Discharge (cubic feet per second) and gage height (feet) for the Embarras River from June 2015-June 2016.

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