

Trophic ecology of gars (*Lepisosteidae*) in a Mississippi River floodplain

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by  
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## Certificate

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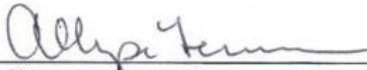
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## Abstract

River-floodplain ecosystems provide important spawning, feeding, and nursery habitat for large-river fishes. Anthropogenic modifications can reduce river-floodplain connectivity, limiting access for fishes, and influencing community structure and trophic ecology. The purpose of this study was to identify changes in the trophic ecology of gars (Lepisosteidae), primarily piscivorous fishes often dependent on floodplain habitats, during seasonal inundation. Shifts in trophic ecology were evaluated using stable isotope analysis of fin tissue. Carbon ( $\delta^{13}\text{C}$ ) was used to determine habitat use and nitrogen ( $\delta^{15}\text{N}$ ) to estimate trophic position. Fin tissue clips ( $n = 198$ ) were collected from four species of adult and young of the year (YOY) gars, Longnose Gar *Lepisosteus osseus* ( $n = 55$ ), Shortnose Gar *L. platostomus* ( $n = 87$ ), and Spotted Gar *L. oculatus* ( $n = 54$ ), and Alligator Gar *Atractosteus spatula* ( $n = 2$ ) in riverine and floodplain habitats of Loch Leven, a restored Mississippi River floodplain in Wilkinson County, Mississippi. Gars on the floodplain ( $16.25 \pm 0.09\text{‰}$ ) were significantly ( $F\text{-ratio} = 79.42$ ,  $df = 1$ ,  $P < 0.001$ ) depleted in  $\delta^{15}\text{N}$  relative to gars in riverine habitats ( $17.37 \pm 0.08\text{‰}$ ). Adult Longnose Gars exhibited the greatest  $\delta^{15}\text{N}$  enrichment ( $17.44 \pm 0.11\text{‰}$ ), followed by Shortnose ( $16.60 \pm 0.08\text{‰}$ ) and Spotted Gars ( $15.97 \pm 0.15\text{‰}$ ). The  $\delta^{15}\text{N}$  value of YOY ( $16.41 \pm 0.08\text{‰}$ ) was similar to adults, but  $\delta^{13}\text{C}$  ( $-28.47 \pm 0.15\text{‰}$ ) varied significantly ( $F\text{-ratio} = 157.70$ ,  $df = 1$ ,  $P < 0.001$ ), reflecting that of floodplain baseline organisms ( $-27.86 \pm 0.33\text{‰}$ ). These results indicate that floodplain access influences gar trophic ecology. Low  $\delta^{15}\text{N}$  of gars on the floodplain suggests a lower trophic position relative to gars in riverine habitats. Similarities in  $\delta^{13}\text{C}$  between YOY and baseline organisms indicate YOY originated on the floodplain and capitalized on abundant floodplain resources. Understanding the influence of river-floodplain connectivity on

trophic ecology of aquatic organisms can better inform restoration, conservation, and land management practices.

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## **Introduction**

River-floodplain ecosystems serve as important habitats for a diversity of organisms (Junk et al. 1989; Pander et al. 2019). In these ecosystems, the flood pulse facilitates hydrologic connectivity between the mainstem river and associated floodplain, allowing for lateral exchange of nutrients, sediments, and aquatic organisms (Junk et al. 1989; Zeug et al. 2005). As a result, main-channel productivity and biota are directly impacted by river-floodplain interactions (Junk et al. 1989). During the flood pulse, many aquatic organisms, including large-river fishes, move from the river to the floodplain to exploit abundant wetland resources and habitats (Robertson et al. 2008; Rypel et al. 2012; Kluender et al. 2016). Due to high primary productivity and increased vegetative cover relative to the main channel, many large-river fish species depend on floodplain inundation for spawning, feeding, and nursery habitat (Allen et al. 2014; Dembkowski and Miranda 2014; van der Most and Hudson 2017). Use of floodplain habitat can also increase fitness, resulting in larger body sizes and faster growth rates (Gutreuter et al. 1999; Rypel et al. 2012; Phelps et al. 2015).

Leveeing, damming, and channelization of large rivers has significantly altered floodplain ecosystems worldwide (Bayley 1995; Pander et al. 2019). Extensive levee construction along the Lower Mississippi River has disconnected approximately 90% of the historic floodplain from the main channel (Baker et al. 1991). Such anthropogenic modifications to large rivers can alter the timing, intensity, and duration of floodplain inundation (Baker et al. 1991; Galat et al. 1998; and Pander et al. 2019), potentially disrupting the lateral exchange of resources and aquatic organisms between river and floodplain habitats (Chen et al. 2020).

Shifts in river-floodplain connectivity can significantly influence fish assemblages within floodplain habitats (Benke et al. 2000; Lemke et al. 2017; Meng et al. 2020). The seasonal

migration of some fish species on to the floodplain for spawning can be dependent on a simultaneous increase in water temperature and rising water levels (Snedden et al. 1999; Kluender et al. 2016). When spawning coincides with floodplain inundation, young-of-year (YOY) can move into slack-water nursery habitats and exploit abundant floodplain resources (Zeug and Winemiller 2007). If anthropogenic modifications to the river-floodplain system disrupt the natural seasonality of the flood pulse, the flood pulse may occur before suitable spawning or nursery habitat has established, leading to a potential decrease in fish reproduction (Lemke et al. 2017). Reduced river-floodplain connectivity may also physically limit the lateral movement of fishes on to the floodplain, thereby reducing access to valuable resources (Ickes et al. 2005; Miranda 2005). As a result, populations of species that evolved life histories that depend on floodplains for spawning, feeding, and nursery habitat may face an increased risk of extirpation (Kluender et al. 2016; van der Most and Hudson 2017).

Trophic ecology of fishes can also change in response to river-floodplain connectivity (Fry 2002; Roach et al. 2009). As the floodplain becomes inundated and habitat connectivity increases, food resources that were not otherwise available become accessible to river fishes (Pereira et al. 2017). Prolonged inundation can be associated with some fishes consuming a greater diversity of prey items, resulting in a broader niche breadth (Luz-Agostinho et al. 2008; Pereira et al. 2017; Azevedo et al. 2021). Following seasonal inundation and access to alternative food sources, fishes in the planktivore, omnivore, insectivore, and piscivore guilds may feed at lower relative trophic positions (Roach et al. 2009). According to Azevedo et al. (2021), the trophic niche breadth of several Amazonian fish species was influenced by the annual flood and dry cycle, with broader niche widths observed after floodplain inundation. Similarly, Fisher et al. (2001) found omnivory and diet variability in fishes from a Missouri River backwater peaked in

mid-summer, when the floodplain was fully inundated. These findings suggest that many fish species capitalize on seasonally available floodplain resources.

Stable isotopes of nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ) can be used to understand the trophic ecology and energy flow through aquatic food webs, respectively, including river-floodplain ecosystems (Hamilton et al. 1992; Herwig et al. 2007; Azevedo et al. 2021). The abundance of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  present in the tissues of consumers reflect assimilated food and provide useful information regarding diet, such as level of trophic diversity and primary energy sources (Vander Zanden et al. 1999; Post 2002; Herwig et al. 2007). However, a natural delay, known as tissue turnover rate, prevents the isotopic signature of food sources from being incorporated into consumer tissue immediately (Fry 2006; Vander Zanden et al. 2015). In adult fishes,  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in tissue reflect food consumed two to four months prior (Vander Zanden et al. 2015).

As  $\delta^{15}\text{N}$  passes from prey to predator, it becomes enriched by a mean of 3.4‰, making it useful in determining the relative trophic position of an organism (Minagawa and Wada 1984; Vander Zanden and Rasmussen 2001; Post 2002). As the composition of  $\delta^{15}\text{N}$  changes in a predictable manner (Peterson and Fry 1987), it can be used to compare the relative trophic position of fishes within a given waterbody (Gu et al. 1996; Herwig et al. 2007; Roach et al. 2009) or across multiple watersheds (Fredrickson 2020). Previous studies have demonstrated that fishes exhibiting greater  $\delta^{15}\text{N}$  enrichment feed at higher a trophic level relative to fishes depleted in  $\delta^{15}\text{N}$  (Minagawa and Wada 1984; Zeug and Winemiller 2008).

Comparably, the abundance of  $\delta^{13}\text{C}$  in fish tissue can be indicative of habitat use (Peterson and Fry 1987; Fry 2002). Because  $\delta^{13}\text{C}$  exhibits little to no enrichment from primary producers to consumers (Vander Zanden and Rasmussen 2001; Post 2002), it reflects the ultimate source of dietary carbon (Post 2002, Zeug and Winemiller 2008). High levels of  $\delta^{13}\text{C}$

depletion ( $< -29.00\text{‰}$ ) are characteristic of fishes in unconfined rivers and floodplains (Hamilton et al. 1992; Fry 2002), while higher carbon isotope values ( $-28.00$  to  $-26.00\text{‰}$ ) are associated with main-channel fishes (Fry 2002; Zeug and Winemiller 2008).

Similar to other large-river fishes, gars (Lepisosteidae) benefit from periodically connected floodplains and capitalize on available spawning and nursery habitat (Robertson et al. 2008; Buckmeier et al. 2013). Gar migration from the main channel onto the floodplain typically occurs in early spring and coincides with rising water levels and increasing water temperatures (Snedden et al. 1999; Buckmeier et al. 2017; Smith et al. 2019). Spotted Gars *Lepisosteus oculatus* have been observed spawning in quiet, weedy waters (Echelle and Riggs 1972), characteristic of floodplain habitats. For Alligator Gars *Atractosteus spatula*, access to floodplain habitat and long-duration flood pulses can significantly influence the reproductive success and result in stronger year classes (Buckmeier et al. 2017; Robertson et al. 2018).

As primarily piscivorous fishes and apex predators throughout their native range (Warren and Burr 2014), gars also feed in seasonally available floodplain habitats (Robertson et al. 2008; Walker et al. 2013). While gars are considered opportunistic feeders, typically consuming other fishes such as smaller centrarchids and cyprinids (Scarnecchia 1991; Walker et al. 2013; Smith et al. 2019), gars may also prey on crayfish, other terrestrial and aquatic invertebrates, and amphibians (Robertson et al. 2008; Walker et al. 2013). Prolonged floodplain inundation can result in piscivorous fishes consuming a greater diversity of prey items (Luz-Agostinho et al. 2008; Pereira et al. 2017). Following access to floodplain resources, some piscivores may feed at lower relative trophic positions (Roach et al. 2009). As piscivorous fishes feeding in floodplain habitats, gar trophic ecology may exhibit a similar shift in response to the diversity and abundance of prey on the floodplain.

As the ecological importance of river-floodplain connectivity becomes more broadly recognized, there is a growing interest in floodplain restoration (Miranda 2005; Pander 2019; Meng et al. 2020). The Nature Conservancy is currently restoring Loch Leven, a 2,470-hectacre floodplain adjacent to the lower Mississippi River. The primary goal of this restoration is to improve hydrologic connectivity with the main-channel river and allow the floodplain to receive a more natural flood pulse. Three gar species, Spotted Gar, Longnose Gar *L. osseus*, and Shortnose Gar *L. platostomus*, are common in the lower Mississippi River, and portions of Loch Leven have been identified as suitable spawning habitat for Alligator Gar, which are less abundant relative to other gar species (Baker et al. 1991; Allen et al. 2014). Analyzing the trophic ecology of gars, a group of fishes dependent on floodplains during several stages of life history (Snedden et al. 1999; Robertson et al. 2008; Buckmeier et al. 2017), may be useful in understanding the significance of river-floodplain connectivity and implications of large-scale restoration projects, such as Loch Leven.

Given the dependence of gars on floodplains for spawning, feeding, and nursery habitat (Snedden et al. 1999; Robertson et al. 2008; Buckmeier et al. 2013; Kluender et al. 2016), I hypothesized that Longnose, Shortnose, Spotted, and Alligator Gars would use the Loch Leven floodplain extensively. Because floodplains provide a greater diversity of forage habitats compared to the main-channel (Fry 2002), I also hypothesized that stable isotopes of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  would shift during high-water and low-water periods to reflect floodplain connectivity. Roach et al. (2009) demonstrated that piscivorous fishes, such as Largemouth Bass *Micropterus salmoides* and Smallmouth Bass *Micropterus dolomieu*, fed at significantly lower trophic levels in backwater habitats following floodplain inundation. Therefore, I predicted that the  $\delta^{15}\text{N}$  value of gars would decrease with floodplain access. Given that floodplain dependent fishes exhibit

$\delta^{13}\text{C}$  depletion (Hamilton 1992; Fry 2002), I also expected gars collected on the floodplain to be similarly depleted in  $\delta^{13}\text{C}$  relative to riverine habitats. However, because  $\delta^{13}\text{C}$  exhibits little to no change from prey to predator (Vander Zanden and Rasmussen 2001; Post 2002), I hypothesized that any change in  $\delta^{13}\text{C}$  would be minimal, yet detectable. This project included the following objectives:

1. Identify and establish a baseline fish assemblage for Loch Leven based on presence and absence prior to restoration activities
2. Identify and analyze patterns of resource use by focus species (Lepisosteidae) in floodplain and main-channel habitats during periods of high and low water levels
3. Use stable isotope analysis of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  to compare variation in resource use and trophic ecology of focus species in riverine versus floodplain habitats

## Methods

### Loch Leven

Loch Leven is a privately owned, 2,470-hectare floodplain located in Wilkinson County, Mississippi. The site is bordered by the Mississippi River to the west and surrounded by Lake Mary on the north, east, and south (Figure 1). While approximately 25% of Loch Leven is maintained for agriculture, the site also encompasses five floodplain lakes, a reservoir, numerous canals, and deciduous forest habitat.

Historically, the annual flood pulse from the Mississippi River has inundated adjacent floodplains (Baker et al. 1991), but a ring levee built around Loch Leven in the 1840s has disconnected the floodplain from the main-channel Mississippi River for approximately 180 years (S. Lemmons, The Nature Conservancy, personal communication). While the ring levee is still maintained for agricultural and recreational purposes, extensive flooding on the Mississippi River topped the levee in 8 of 10 years since 2011 and brought irregular flood pulses to Loch Leven. In the early 2000s, Loch Leven became part of the Wetland Reserve Program, a voluntary program with the goal of aiding landowners in wetland restoration and conservation. In 2020, The Nature Conservancy (TNC) installed a 2.5 m by 3.0 m water control structure (WCS) at Jackson Point Road (31°11'54.6"N, 91°34'55.1"W; Figure 1), successfully reconnecting the floodplain to the main-channel Mississippi River via a tributary known as the Narrows. Prior to restoration efforts and construction of the WCS, the only corridor funneling water and aquatic organisms between Loch Leven and the Mississippi River was a single pipe measuring 1.0 m in diameter (S. Lemmons, The Nature Conservancy, personal communication).



**Figure 1.** The Nature Conservancy's Loch Leven restoration site in Wilkinson County, Mississippi (yellow star). The site is bordered by the Mississippi River to the west and surrounded by Lake Mary to the north, east and south. The Narrows, a tributary connecting Lake Mary and the Mississippi River, is located to the northwest. The red square identifies the location of the water control structure (WCS) at Jackson Point Road.

Construction of the WCS was completed in November 2020, with floodwaters from the Mississippi River entering the structure for the first time in spring of 2021. Throughout the course of this study, Loch Leven was an active restoration site. All sampling occurred prior to completion of the WCS. The Narrows and the location of the WCS were grouped as “riverine habitat”. These locations were characterized by directional flow confined within a channel and had limited vegetation. Sampling locations within the ring levee, including floodplain lakes and the reservoir, were classified as “floodplain”. During inundation, these locations lacked directional flow, and many had vegetative cover.

### Sampling Events

To evaluate the floodplain fish assemblage and trophic ecology of focal species, six sampling events occurred at Loch Leven between 11 June and 13 November 2020. Sampling events were timed to coincide with the seasonal flood pulse but were strongly influenced by COVID-19 work and travel restrictions.

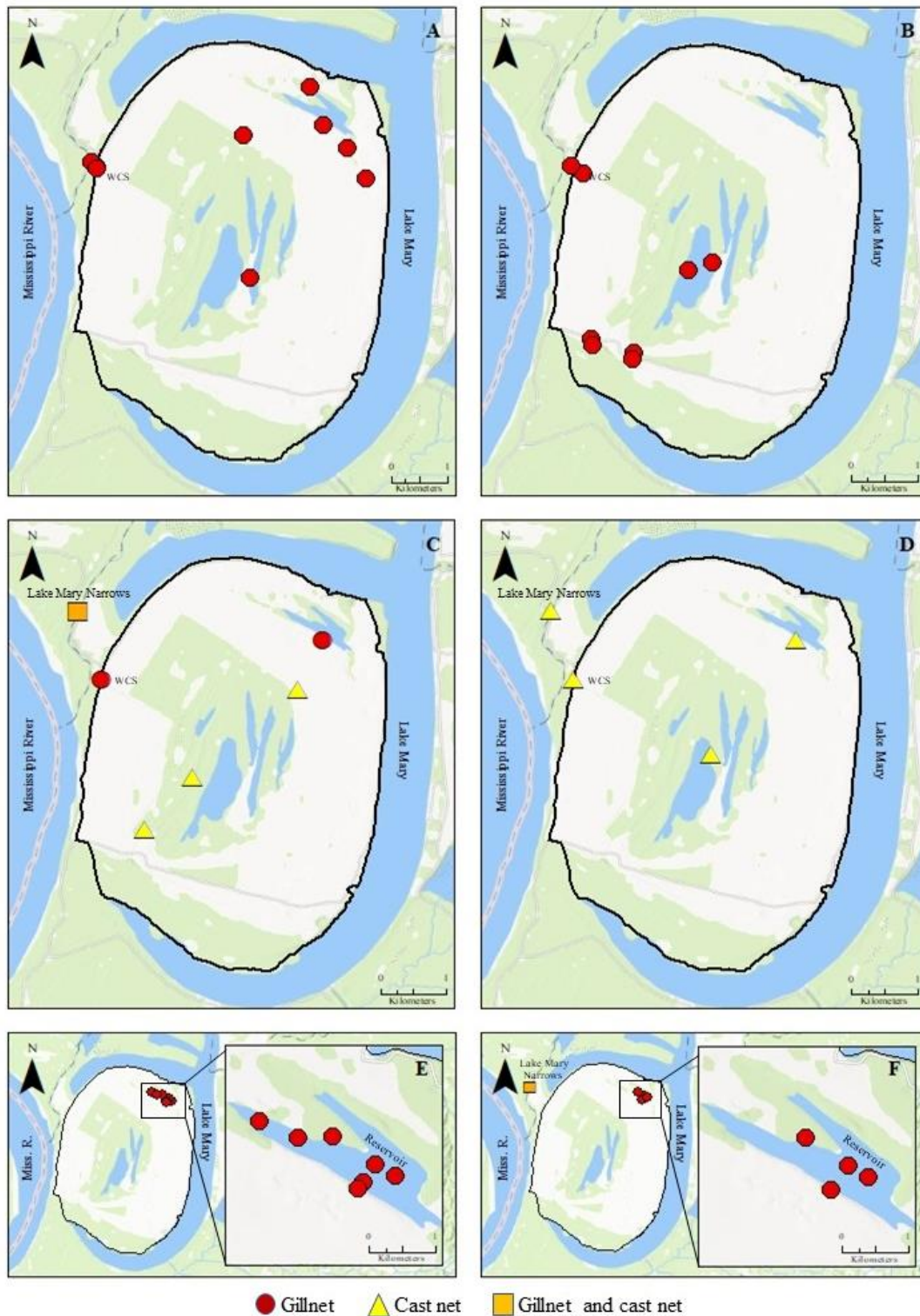
As is characteristic of dynamic floodplain systems, water levels within Loch Leven varied across sampling events. In order for the Mississippi River flood pulse to inundate Loch Leven, water height at the United States Geological Survey (USGS) gauge at Natchez, Mississippi (USGS Gauge 07290880) must exceed 9.45 m. Therefore, the six sampling events were classified as high-water (11 and 18 June; gauge height above 12.5 m), falling-water (9 and 21 July; gauge height between 8.5 m and 12.5 m), and low-water refuge (17 September and 13 November; gauge height below 8.5 m) samplings. During high-water events permanent water features within Loch Leven, such as the reservoir, were hydrologically connected to the floodplain. Falling-water sampling events were characterized by water receding off the floodplain and confined to canals and low-elevation areas. Low-water refuge sampling primarily

focused on the reservoir (approximately 42 ha in surface area during low-water) in the northeast of the site. Floodplain lakes, similar to the reservoir, retain water during the dry season and provide low-water refuge for fishes, including gars (Bonvillain et al. 2008).

### Fish Collection and Processing

Due to rapidly changing water levels, multiple gear types were necessary to safely and effectively sample fishes on the floodplain. Fishes were sampled between 0600 and 1900 hours CST using monofilament gillnets (21.3 m long, 1.2 m depth, 5 cm bar mesh; 21.3 m long, 1.2 m depth, 7 cm bar mesh; 45.7 m long, 1.2 m depth, 9 cm bar mesh; 15.2 m long, 1.2 m depth, 13 cm bar mesh) and cast nets (1.8 m diameter; 1.3 cm bar mesh), depending on water-level and topography. Nets were deployed in various habitats, with shallow, vegetated locations being preferred, although not always accessible (Figure 2). Each gillnet was equipped with two to three foam buoys across the float (top) line and one concrete weight on either corner of the lead (bottom) line. If flow was present, efforts were made to deploy gillnets perpendicular to the direction flow. Once deployed, each gillnet soaked for approximately 45 to 90 minutes. Depending on catch, gillnets were either left in the same location or moved elsewhere, with each net moved 2 to 3 times per sampling event (Figure 2). Cast nets were used during falling-water and low-water refuge sampling to collect fishes from areas with hazardous terrain or limited accessibility and were cast 1 to 3 times at each location depending on catch.

During the six sampling events at Loch Leven in 2020, all fishes collected in gillnets and cast nets were identified to species. Individuals of non-focus species were counted and total length (cm) of the first 30 individuals of each species was measured. Life stage (YOY vs. non-YOY and adult) was determined based on total length. Photographs were also taken to document



**Figure 2.** Loch Leven sampling locations, by trip, represented by red circles (gillnet sampling), yellow triangles (cast net sampling), and orange squares (gillnet and cast net sampling). A) 11 June 2020, B) 18 June 2020, C) 9 July 2020, D) 21 July 2020, E) 17 September 2020, F) 13 November 2020.

each species before release. Fishes that could not be identified in the field were photographed and vouchered on ice for additional analysis in the laboratory.

For each gar collected, total length (cm) was measured. Gars smaller than 45 cm total length were classified as YOY, while gars greater than 45 cm total length were grouped as non-YOY and adults. A small section of tissue, approximately 2.5 cm x 2.5 cm, was also removed from the caudal fin of each gar (Fredrickson 2020). While many trophic studies have used white muscle tissue to determine the composition of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in fishes (Pinnegar and Polunin 1999; Fisher et al. 2001; Roach et al. 2009), fin tissue has been reliably substituted as a non-lethal alternative for muscle in many freshwater fish species (Maitland and Rahl 2021), including Alligator, Longnose, and Spotted Gars (Keppeler 2019; Fredrickson 2020). Therefore, caudal fin tissue was used as a surrogate for muscle throughout this study.

#### Stable Isotope Analysis

Due to low position in the food web, Grass Shrimp *Palaemonetes* spp. and small bivalves were collected as baseline organisms (Cabana and Rasmussen 1996; Post 2002). Grass Shrimp were collected from the ring levee near the WCS and the reservoir using a dipnet on 11 and 18 June 2020 and kept alive for 24 hours. Bivalves were collected by hand from the reservoir on 13 November 2020 and kept on ice until returning to the laboratory. Attempts were made to collect baseline organisms during other sampling events, but excessive flooding followed by rapidly receding water and lack of vegetation made this infeasible. Whole shrimp (Snow et al. 2020) and soft tissue from bivalves were dried in a drying oven at 60°C for a minimum of 48 hours (Fredrickson 2020). Baseline organisms were homogenized by sampling event into a fine powder.

Caudal fin tissue was kept frozen until processing, then rinsed with deionized water to

remove traces of blood or dirt. Fin tissue was then dried in a drying oven at 60°C for a minimum of 24 hours. Once thoroughly dried, each tissue sample was pulverized to a fine powder using a mortar and pestle. Each ground tissue sample, weighing a minimum of 1.0 mg, was then individually placed in a sterile plastic vial. Fin tissue and baseline organisms were sent to the Cornell University Stable Isotope Laboratory (Ithaca, NY) for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  analysis. Samples were analyzed on a Finnigan MAT Delta Plus IRMS coupled to an elemental analyzer (Carlo Erba NC2500). Isotope values are expressed using delta ( $\delta$ ) notation and are defined as per mil (‰) deviation from the International Atomic Agency standards (atmospheric nitrogen for  $\delta^{15}\text{N}$  and Vienna Pee-Dee Belemnite for  $\delta^{13}\text{C}$ ). Deviation from the standard was calculated using the equation:

$$[(R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}] \times 1000 = d_{\text{sample-standard}}$$

### Water Quality

During each sampling event, water temperature (°C), specific conductivity ( $\mu\text{S}/\text{cm}$ ), dissolved oxygen (mg/L), and percent dissolved oxygen (%) were measured at a minimum of three locations (with the exception of the Narrows) using a handheld YSI 556 MPS (Yellow Springs Instruments, Yellow Springs, OH). If conditions allowed, Secchi depth (m) was also measured. Water depth was measured during high-water using a depth finder and visually estimated during low-water refuge sampling.

### Data Analysis

One-way analysis of variance (ANOVA), followed by a Tukey's post hoc analysis when necessary, was used to test for differences in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of fin tissue collected from Longnose, Shortnose, and Spotted Gars on the Loch Leven floodplain and riverine habitats (WCS and the Narrows). One-way analysis of variance (ANOVA) was also used to test for

differences in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of fin tissue from adult gars, YOY gars, and baseline organisms. Differences in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values between first and last sampling events were also tested using one-way analysis of variance (ANOVA). A linear regression was used to model the rate at which  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  change in fishes collected from riverine habitats (WCS and the Narrows) compared to floodplain habitats, followed by an analysis of co-variance (ANCOVA) to test statistical significance. All statistical analyses were carried out using “R” statistical software packages with alpha set at 0.5 for all tests.

For statistical analyses gars collected from the Narrows and the location of the WCS were grouped as “riverine habitat” gars. Stable isotope samples collected from gars at these locations represent individuals feeding in a riverine habitat. Gars collected from sampling locations within the ring levee, including floodplain lakes and the reservoir, were classified as “floodplain” gars and represent individuals feeding in seasonally inundated floodplain habitat.

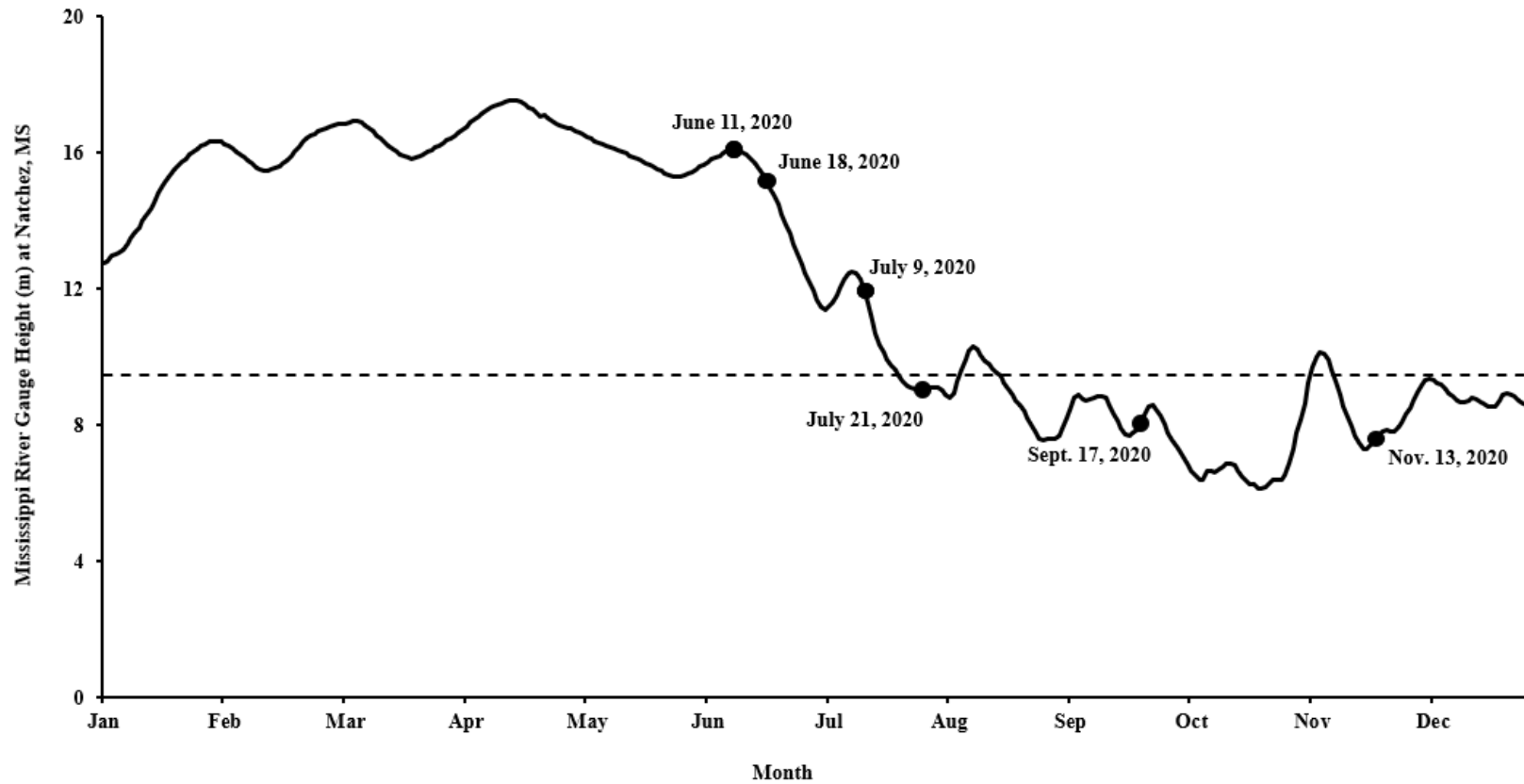
## Results

Six sampling events took place at Loch Leven between 11 June and 13 November 2020. Due to the nature of the floodplain and unusually high water on the Mississippi River, water levels at Loch Leven fluctuated throughout the sampling period. During sampling events, water at the United States Geological Survey (USGS) gauge at Natchez, Mississippi (USGS Gauge 07290880), ranged from 6.13 m to 16.09 m in height. For water to inundate Loch Leven, water height at Natchez, Mississippi, must exceed 9.45 m. When sampling began in June, Loch Leven had been inundated for over 6 months and water height exceeded 9.45 m for 43 consecutive days during the sampling period. Highest water levels at Natchez were recorded in June (max = 16.09 m) and lowest water levels in October (min = 6.13 m; Figure 3).

During high-water sampling, Loch Leven was thoroughly inundated, and water was flowing onto the floodplain at Jackson Point Road and through several breaches in the ring levee. Permanent water features, such as the reservoir and canals, were indistinguishable and fishes would have moved freely throughout the floodplain. Water depths at floodplain sampling locations ranged from 1.21 m to 5.10 m.

The flood pulse was receding from the floodplain during falling-water sampling and was largely confined to canals and the reservoir. As water flowed out of floodplain and drained into the Mississippi River, fishes either moved off the floodplain via the 1 m pipe or were concentrated in canals, the reservoir, and other low-elevation areas. Hazardous topography prevented accurate water depth measurements.

By September, floodplain topography had been deliberately altered by The Nature Conservancy to improve drainage and allow heavy machinery to access the site for restoration purposes. Water was confined to a 42-hectare reservoir creating refuge habitat for many fishes



**Figure 3.** Mississippi River gauge height (m) at Natchez, Mississippi (USGS Gauge 07290880), and corresponding sampling events. Dashed line represents gauge height (9.45 m) at which water begins to inundate the Loch Leven floodplain via the water control structure. In 2020, water height at Natchez exceeded 9.45 m for 206 consecutive days before dropping in July.

The reservoir had an estimated depth of 1.5 m to 2 m.

### Fish Assemblage

A total of 1,044 fishes representing 24 species from nine families (Table 1; Appendix A) were collected from the interior floodplain (n = 428) and riverine habitats (n = 617) using gillnets and cast nets. The most abundant species collected was Gizzard Shad *Dorosoma cepedianum* (n = 591), accounting for 56.6% of the fishes collected, followed by Shortnose Gar (n = 96) and Black Crappie *Pomoxis nigromaculatus* (n = 65; Table 1). Mean total length of fishes varied depending on species (Table 2) and life stage.

High-water sampling resulted in the fewest number of fishes (n = 117; Table 1). Catch was dominated by adult Longnose Gars (n = 32), Shortnose Gars (n = 28), and Skipjack Herring *Alosa chrysochloris* (n = 21). The greatest number of individuals (n = 665) was collected during falling-water sampling on 21 July 2020 and the greatest diversity of species (n = 14) was collected on 9 and 21 July 2020 (Table 1). During falling-water sampling events, YOY Gizzard Shad (n = 536), Black Crappie (n = 64), and Bluegill *Lepomis macrochirus* (n = 56) were the most abundant species and were collected using cast nets (Table 1; Appendix A). YOY Longnose (n = 2), Spotted (n = 9), and Alligator Gars (n = 2) were also collected using cast nets on 21 July (Table 1; Appendix A). In September and November, fish were largely confined to the reservoir or cut off from the floodplain in the Narrows. A total of 131 fishes were collected (n = 15 riverine, 116 floodplain) across both sampling events. Catch within the reservoir was primarily composed of adult Shortnose Gars (n = 32), Spotted Gars (n = 23), and Gizzard Shad (n = 20). Shortnose Gars (n = 7), Gizzard Shad (n = 6), and Longnose Gars (n = 2) were the only species collected from the Narrows.

**Table 1.** A total of 1,044 fishes (adults and YOY), representing 24 species and 9 families were collected from Loch Leven floodplain and riverine habitats between 11 June and 13 November 2020.

Common Name	Family	Species Name	High-Water		Falling-Water		Refuge		Total
			11 June	18 June	9 July	21 July	17 Sept.	13 Nov.	
Silverside	Atherinopsidae	<i>Menidia</i> spp.	-	-	-	2	-	-	2
Black Buffalo	Catostomidae	<i>Ictiobus niger</i>	-	-	-	-	4	3	7
Smallmouth Buffalo	Catostomidae	<i>Ictiobus bubalus</i>	1	1	-	-	2	-	4
Black Crappie	Centrarchidae	<i>Pomoxis nigromaculatus</i>	-	-	39	26	-	-	65
Bluegill	Centrarchidae	<i>Lepomis macrochirus</i>	-	-	13	43	-	-	56
Green Sunfish*	Centrarchidae	<i>Lepomis cyanellus</i>	-	-	-	1	-	-	1
Largemouth Bass	Centrarchidae	<i>Micropterus salmoides</i>	-	1	10	5	-	-	16
Longear Sunfish	Centrarchidae	<i>Lepomis megalotis</i>	-	-	1	-	-	-	1
Redear Sunfish	Centrarchidae	<i>Lepomis microlophus</i>	-	-	3	19	-	-	22
White Crappie	Centrarchidae	<i>Pomoxis annularis</i>	-	1	-	-	1	1	3
Gizzard Shad	Clupeidae	<i>Dorosoma cepedianum</i>	5	10	16	534	14	12	591
Skipjack Herring	Clupeidae	<i>Alosa chrysochloris</i>	5	16	7	-	-	-	28
Common Carp	Cyprinidae	<i>Cyprinus carpio</i>	-	-	1	1	1	4	7
Golden Shiner	Cyprinidae	<i>Notemigonus crysoleucas</i>	-	-	1	-	-	-	1
Blacktail Shiner*	Cyprinidae	<i>Cyprinella venusta</i>	-	-	1	-	-	-	1
Blue Catfish	Ictaluridae	<i>Ictalurus furcatus</i>	-	-	-	-	2	1	3
Channel Catfish	Ictaluridae	<i>Ictalurus punctatus</i>	2	-	2	2	8	1	15
Flathead Catfish	Ictaluridae	<i>Pylodictis olivaris</i>	-	-	-	-	1	-	1
Alligator Gar	Lepisosteidae	<i>Atractosteus spatula</i>	-	-	-	2	0	-	3
Longnose Gar	Lepisosteidae	<i>Lepisosteus osseus</i>	19	13	15	4	4	6	61
Shortnose Gar	Lepisosteidae	<i>Lepisosteus platostomus</i>	17	11	14	15	10	29	96
Spotted Gar	Lepisosteidae	<i>Lepisosteus oculatus</i>	6	7	9	9	9	14	54
Yellow Bass	Moronidae	<i>Morone mississippiensis</i>	1	-	-	-	-	-	1
Freshwater Drum	Sciaenidae	<i>Aplodinotus grunniens</i>	-	1	-	2	1	2	6
<b>Total</b>			<b>56</b>	<b>61</b>	<b>132</b>	<b>665</b>	<b>57</b>	<b>73</b>	<b>1,044</b>

\*species collected only from Lake Mary Narrows

**Table 2.** Mean ( $\pm$  SD) total length (cm) and range of total lengths (cm) of non-focus species collected from Loch Leven floodplain and riverine habitats, 11 June to 13 November 2020.

Common Name	n	Total Length (cm)	Range of Total Length (cm)
Silverside			
Adult and non-YOY	2	5.50 $\pm$ 0.57	5.10 – 5.90
Black Buffalo			
Adult and non-YOY	7	55.51 $\pm$ 14.75	33.40 – 78.00
Smallmouth Buffalo			
Adult and non-YOY	4	34.35 $\pm$ 11.70	25.40 – 51.10
Black Crappie			
Adult and non-YOY	3	22.20 $\pm$ 0.61	21.50 – 22.60
YOY	53	7.12 $\pm$ 1.10	4.80 – 10.00
Bluegill			
Adult and non-YOY	23	12.62 $\pm$ 3.79	10.10 – 28.90
YOY	32	7.80 $\pm$ 1.75	4.70 – 10.00
Green Sunfish			
YOY	1	4.00	-
Largemouth Bass			
Adult and non-YOY	3	16.43 $\pm$ 9.60	10.30 – 27.50
YOY	13	9.11 $\pm$ 0.70	7.90 – 10.00
Longear Sunfish			
YOY	1	9.60	-
Redear Sunfish			
YOY	21	7.69 $\pm$ 1.70	3.10 – 10.40
White Crappie			
Adult and non-YOY	3	25.10 $\pm$ 3.98	20.50 – 27.50
Gizzard Shad			
Adult and non-YOY	49	28.23 $\pm$ 6.96	11.90 – 38.90
YOY	41	6.09 $\pm$ 1.910	3.50 – 9.60
Skipjack Herring			
Adult and non-YOY	20	24.24 $\pm$ 3.43	21.30 – 33.50
Common Carp			
Adult and non-YOY	7	45.06 $\pm$ 22.98	11.60 – 70.00
Golden Shiner			
Adult and non-YOY	1	9.10	-

Table 2 Continued

<b>Common Name</b>	<b>n</b>	<b>Total Length (cm)</b>	<b>Range of Total Length (cm)</b>
Blacktail Shiner			
Adult and non-YOY	1	9.50	-
Blue Catfish			
Adult and non-YOY	3	40.43 $\pm$ 0.85	39.60 – 41.30
Channel Catfish			
Adult and non-YOY	15	36.10 $\pm$ 8.46	23.60 – 50.30
Flathead Catfish			
Adult and non-YOY	1	46.70	-
Yellow Bass			
Adult and non-YOY	1	16.80	-
Freshwater Drum			
Adult and non-YOY	6	24.13 $\pm$ 4.76	17.80 – 29.50

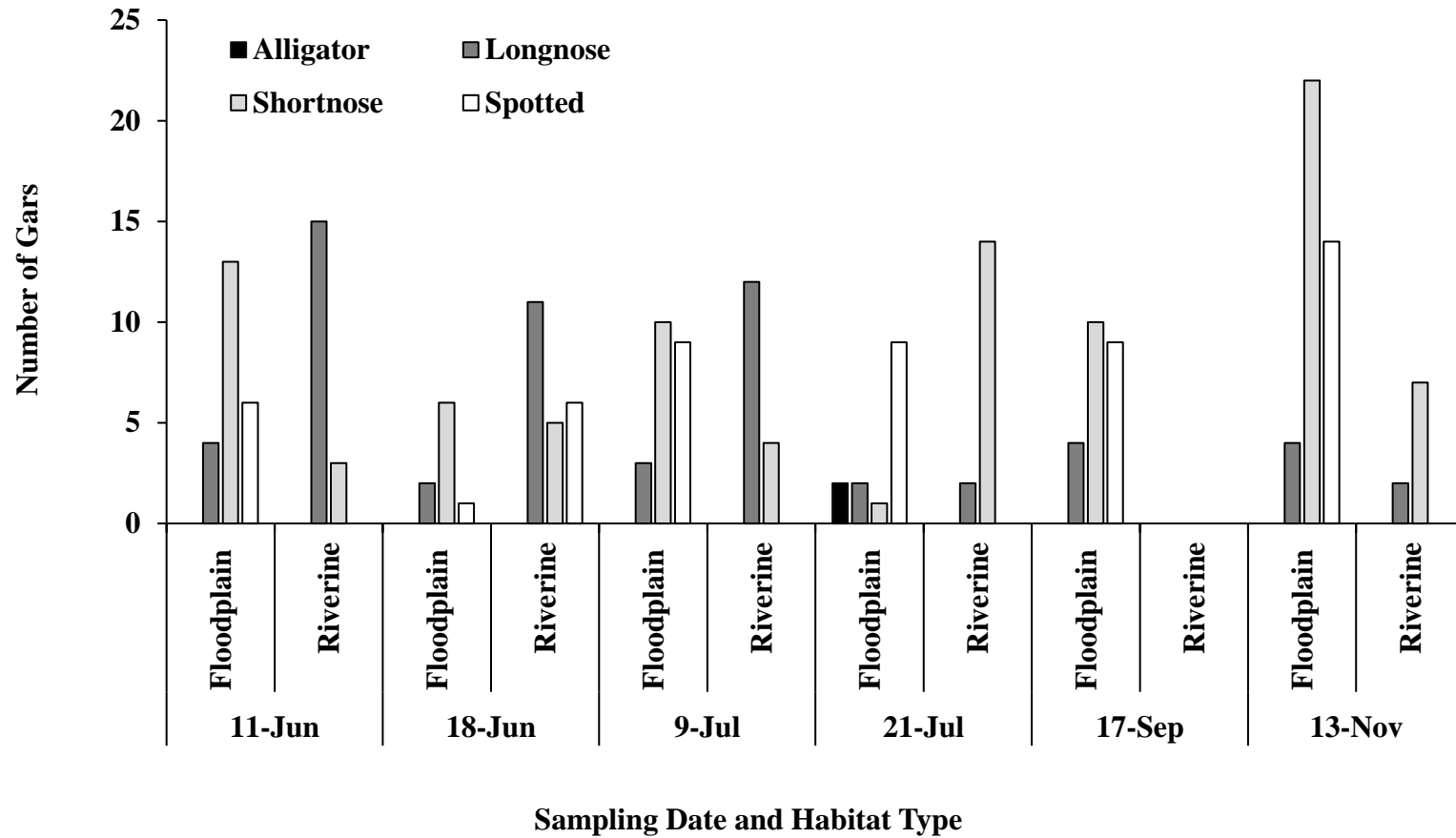
### Focus Species: Gars

A total of 213 gars, representing all four species native to the lower Mississippi River, were collected from riverine habitats and the Loch Leven floodplain during 2020 sampling. Shortnose Gar was the most abundant gar species collected ( $n = 96$ ), followed by Longnose ( $n = 61$ ), Spotted ( $n = 54$ ), and Alligator Gar ( $n = 2$ ; Table 1). The greatest number of gars ( $n = 49$ ) were collected on 13 November 2020 using a combination of gillnets and cast nets during low-water refuge sampling (Table 1; Figure 4; Appendix A). Individuals representing all four species were collected during only one of the six sampling events. Alligator Gar was the only species not collected during every sampling event (Figure 4), however several large Alligator Gars, approximately 1.5 m in length, were observed breaching during high-water sampling. While the majority of gars (32.71%) were collected from the reservoir, 28.04% were collected from the WCS (Figure 5). Longnose Gars were predominately collected from riverine habitats, while Shortnose and Spotted Gars were more common in backwater habitats (Figure 6).

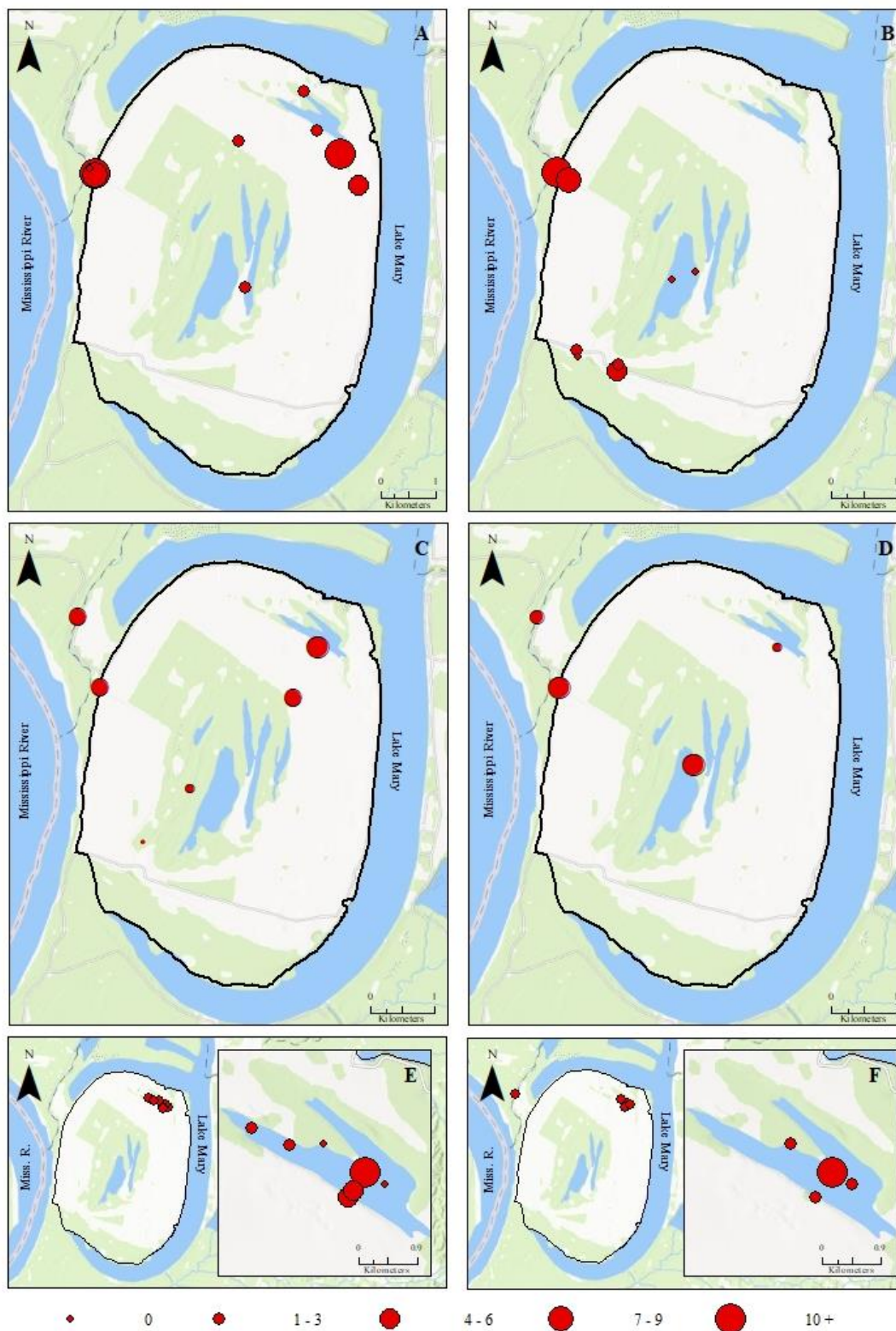
Longnose ( $F$ -ratio = 0.002,  $df = 1$ ,  $P = 0.969$ ) and Shortnose Gars ( $F$ -ratio = 1.76,  $df = 1$ ,  $P = 0.188$ ), had similar mean total lengths between riverine and floodplain habitats, while Spotted Gars ( $F$ -ratio = 6.559,  $df = 1$ ,  $P = 0.014$ ) collected on the floodplain were larger than those from riverine habitats (Table 3).

### Stable Isotope Analysis

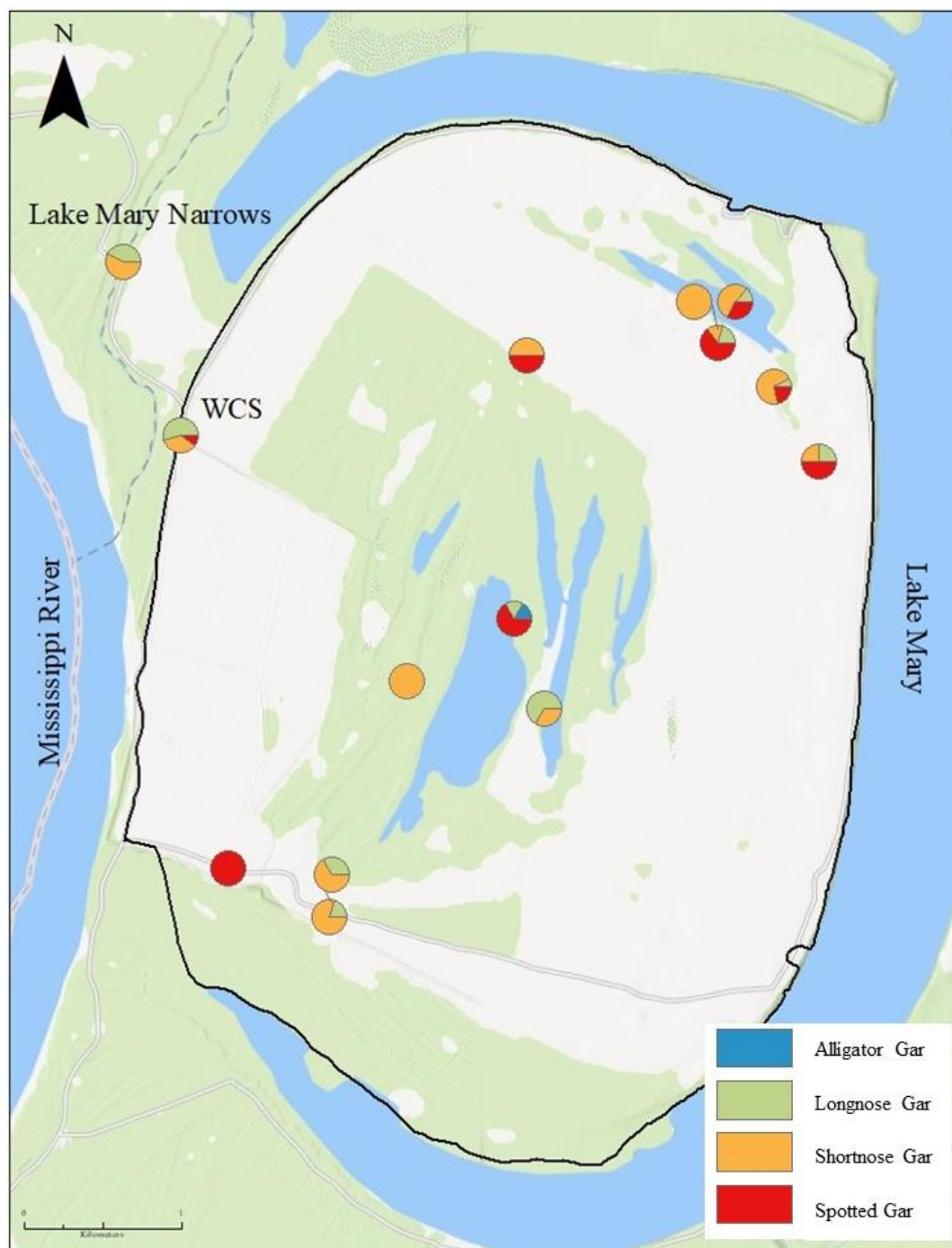
Between 11 June and 13 November 2020, fin tissue was collected from 87 Shortnose, 55 Longnose, 54 Spotted, and 2 Alligator Gars for a total of 198 fin tissue samples (Table 4). Baseline organisms were collected on three sampling events on 11 June (*Palaemonetes* spp.,  $n = 3$ ), 18 June (*Palaemonetes* spp.,  $n = 4$ ), and 13 November (bivalves,  $n = 4$ ). Baseline organisms were homogenized by date for a total of 3 baseline tissue samples (Table 4).



**Figure 4.** Trip totals for Alligator, Longnose, Shortnose, and Spotted Gars collected from Loch Leven floodplain and riverine habitats across six sampling events, 11 June and 13 November 2020. Riverine habitat sampling did not occur on 17 September 2020. Alligator Gar was the only species not collected during every sampling event.



**Figure 5.** Graduated symbols represent number of gars collected from various sampling locations at Loch Leven in 2020. A) 11 June (n = 42), B) 18 June (n = 31), C) 9 July (n = 38), D) 21 July (n = 30), E) 17 September (n = 24), F) 13 November (n = 49).



**Figure 6.** Distribution of gar species collected from Loch Leven riverine and floodplain habitats from 11 June to November 2020. Pie charts represent proportion of Alligator, Longnose, Shortnose, and Spotted Gars collected at generalized locations.

**Table 3.** Mean ( $\pm$  SD), total length (cm), and range of total lengths (cm) of Longnose (Adult/YOY), Shortnose (Adult), Spotted (Adult/YOY), and Alligator Gars (YOY) collected from Loch Leven floodplain and riverine habitats, 11 June to 13 November 2020.

Species	Location	n	Total Length (cm)	Range of Total Length (cm)
Longnose Gar (Adult)				
	Floodplain	16	98.19 $\pm$ 18.65	67.90 – 126.00
	Riverine	40	98.03 $\pm$ 10.80	63.00 – 122.40
	All Locations	56	98.08 $\pm$ 13.33	63.00 – 126.00
Longnose Gar (YOY)				
	Floodplain	2	31.80 $\pm$ 6.51	27.20 - 36.40
Shortnose Gar (Adult)				
	Floodplain	62	66.48 $\pm$ 4.98	55.50 – 84.70
	Riverine	33	65.07 $\pm$ 4.81	55.40 – 76.50
	All Locations	95	65.99 $\pm$ 4.94	55.40 – 84.70
Spotted Gar (Adult)*				
	Floodplain	39	64.83 $\pm$ 7.85	49.80 – 87.50
	Riverine	6	56.50 $\pm$ 2.17	55.00 – 60.50
	All Locations	45	63.72 $\pm$ 7.87	49.80 – 87.50
Spotted Gar (YOY)				
	Floodplain	9	27.07 $\pm$ 3.31	22.10 – 32.40
Alligator Gar (YOY)				
	Floodplain	2	31.45 $\pm$ 0.92	30.80 – 32.10

\*Represents difference between riverine and floodplain habitats

**Table 4.** Mean ( $\pm$  SE)  $\delta^{15}\text{N}$  (‰) and  $\delta^{13}\text{C}$  (‰) values of fin tissue collected from adult and/or young of the year Alligator (n = 2), Longnose (n = 55), Shortnose (n = 87), Spotted Gars (n = 54), and baseline organisms of Grass Shrimp (n = 2) and bivalves (n = 1) in floodplain and riverine habitats.

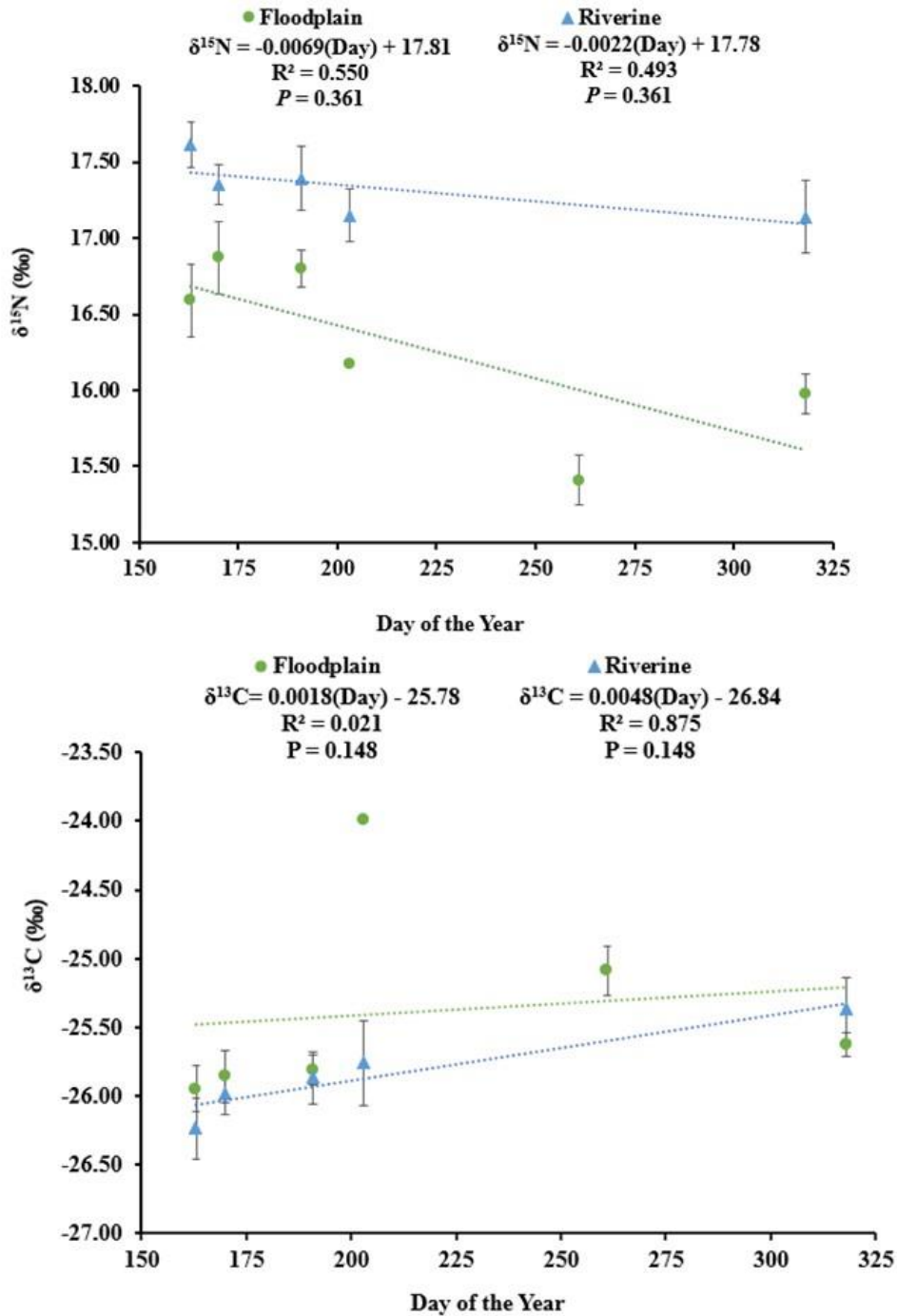
Common Name	n		Overall		Floodplain		Riverine	
	FP	R	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
Longnose Gar								
Adult	15	38	$17.44 \pm 0.11$	$-26.29 \pm 0.09$	$17.10 \pm 0.23$	$-26.39 \pm 0.19$	$17.58 \pm 0.11$	$-26.25 \pm 0.11$
Young of Year	2	-	-	-	$16.34 \pm 0.12$	$-28.12 \pm 0.60$	-	-
Shortnose Gar								
Adult	55	32	$16.60 \pm 0.08$	$-25.60 \pm 0.08$	$16.34 \pm 0.10$	$-25.62 \pm 0.07$	$17.05 \pm 0.09$	$-25.57 \pm 0.18$
Young of Year	-	-	-	-	-	-	-	-
Spotted Gar								
Adult	39	6	$15.97 \pm 0.15$	$-25.35 \pm 0.11$	$15.75 \pm 0.15$	$-25.33 \pm 0.13$	$17.42 \pm 0.21$	$-25.42 \pm 0.10$
Young of Year	9	-	-	-	$16.40 \pm 0.11$	$-28.53 \pm 0.18$	-	-
Alligator Gar								
Adult	-	-	-	-	-	-	-	-
Young of Year	2	-	-	-	$16.49 \pm 0.21$	$-28.55 \pm 0.13$	-	-
Grass Shrimp	1*	1*	$13.45 \pm 0.04$	$-27.55 \pm 0.12$	13.48	-27.66	13.41	-27.43
Bivalves	1*	-	-	-	11.14	-28.50	-	-

FP = Floodplain; R = Riverine

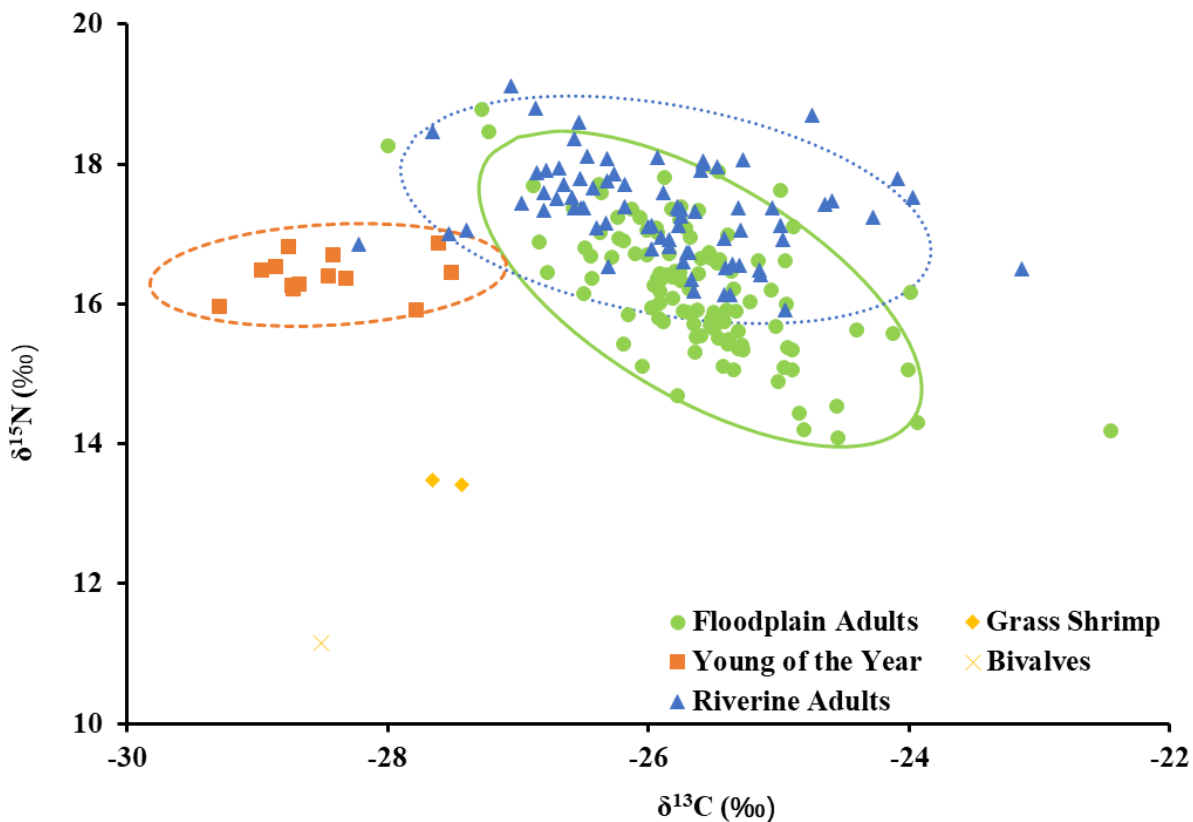
\*Represents samples homogenized by date

When differences in species were not accounted for, the value of  $\delta^{15}\text{N}$  for adult gars ranged from 15.92 to 19.12‰ in riverine habitats and 14.09 to 18.78‰ on the floodplain. Mean  $\pm$  SE  $\delta^{15}\text{N}$  was  $17.37 \pm 0.08\text{‰}$  for gars in riverine habitats and  $16.25 \pm 0.09\text{‰}$  for gars on the floodplain. A one-way ANOVA indicated adult gars collected in riverine habitats were more enriched in  $\delta^{15}\text{N}$  ( $F$ -ratio = 79.42,  $df = 1$ ,  $P < 0.001$ ) relative to floodplain gars. Comparing the first sampling event on 11 June 2020 and the last sampling event on 13 November 2020, there was no change in mean  $\delta^{15}\text{N}$  of gars collected from riverine habitats ( $F$ -ratio = 3.17,  $df = 1$ ,  $P = 0.089$ ; Figure 7). However, mean  $\delta^{15}\text{N}$  of gars collected from the floodplain had significantly decreased by 13 November 2020 ( $F$ -ratio = 5.93,  $df = 1$ ,  $P = 0.018$ ), with the lowest mean  $\delta^{15}\text{N}$  value ( $15.41 \pm 0.16\text{‰}$ ) recorded on 17 September 2020 (Figure 7). A linear regression followed by an ANCOVA indicated the rate of change in  $\delta^{15}\text{N}$  was similar between riverine ( $R^2 = 0.493$ ) and floodplain habitats ( $R^2 = 0.550$ ;  $F$ -ratio = 1.094,  $df = 4$ ,  $P = 0.361$ ; Figure 7).

The value of  $\delta^{13}\text{C}$  for adult gars ranged from -27.66 to -23.97‰ in riverine habitats and -28.22 to -22.46‰ on the floodplain. Mean  $\pm$  SE  $\delta^{13}\text{C}$  was  $-25.90 \pm 0.09\text{‰}$  for gars in riverine habitats and  $-25.63 \pm 0.07\text{‰}$  for gars on the floodplain (Figure 8). One-way ANOVA indicated overall adult gars collected in riverine habitats were depleted in  $\delta^{13}\text{C}$  ( $F$ -ratio = 5.35,  $df = 1$ ,  $P = 0.022$ ) when compared to adult gars collected on the Loch Leven floodplain. While the mean  $\delta^{13}\text{C}$  values of gars from the floodplain did not change between the first sampling and last sampling events ( $F$ -ratio = 3.15,  $df = 1$ ,  $P = 0.08$ ), mean  $\delta^{13}\text{C}$  of riverine gars increased by 13 November 2020 ( $F$ -ratio = 3.166,  $df = 1$ ,  $P = 0.016$ ; Figure 7). As a result, there was no difference in  $\delta^{13}\text{C}$  between riverine and floodplain gars following floodplain inundation ( $F$ -ratio = 1.48,  $df = 1$ ,  $P = 0.232$ ). A linear regression followed by an ANCOVA revealed the rate of change was also similar between riverine ( $R^2 = 0.875$ ) and floodplain habitats ( $R^2 = 0.021$ ).



**Figure 7.** Linear regression of mean  $\delta^{15}\text{N}$  (‰; top) and  $\delta^{13}\text{C}$  (‰; bottom) over time in adult gars (all species) collected from Loch Leven floodplain and riverine habitats, 11 June (Day 163, n = 21 floodplain, 15 riverine), 18 June (Day 170, n = 9 floodplain, 22 riverine), 9 July (Day 191, n = 23 floodplain, 15 riverine), 21 July (Day 203, n = 1 floodplain, 15 riverine), 17 September (Day 261, n = 22 floodplain, 0 riverine) to 13 November (Day 318, n = 33 floodplain, 9 riverine) of 2020.



**Figure 8.** The  $\delta^{15}\text{N}$  (‰) and  $\delta^{13}\text{C}$  (‰) values with 95% confidence ellipses representing fin tissue from floodplain collected adult gars ( $n = 113$ , mean  $\delta^{15}\text{N} = 16.25 \pm 0.09$ ‰, mean  $\delta^{13}\text{C} = -25.63 \pm 0.07$ ‰), adult gars collected from riverine habitats ( $n = 72$ , mean  $\delta^{15}\text{N} = 17.37 \pm 0.08$ ‰, mean  $\delta^{13}\text{C} = -25.90 \pm 0.09$ ‰), young of the year gars ( $n = 13$ , mean  $\delta^{15}\text{N} = 16.41 \pm 0.08$ ‰, mean  $\delta^{13}\text{C} = -28.47 \pm 0.15$ ‰), and composite samples of baseline organisms, Grass Shrimp ( $n = 2$ , mean  $\delta^{15}\text{N} = 13.45 \pm 0.04$ ‰, mean  $\delta^{13}\text{C} = -27.55 \pm 0.12$ ‰) and bivalves ( $n = 1$ ,  $\delta^{15}\text{N} = 11.14$ ‰,  $\delta^{13}\text{C} = -28.50$ ‰).

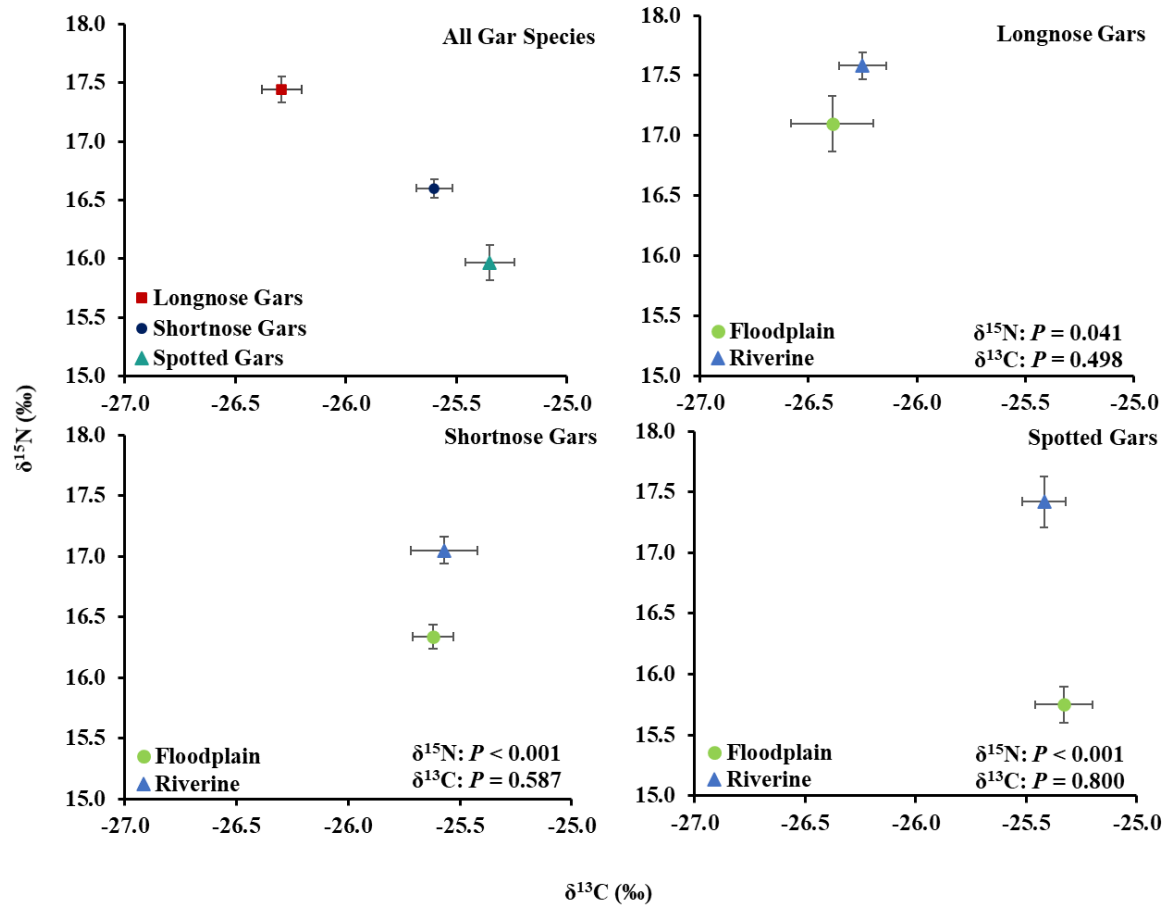
( $F$ -ratio = 1.720,  $df$  = 4,  $P$  = 0.148; Figure 7).

When species was taken into consideration, adult Longnose ( $F$ -ratio = 4.41,  $df$  = 1,  $P$  = 0.041), Shortnose ( $F$ -ratio = 20.37,  $df$  = 1,  $P$  < 0.001), and Spotted Gars ( $F$ -ratio = 19.15,  $df$  = 1,  $P$  < 0.001) collected from riverine habitats were significantly more enriched  $\delta^{15}\text{N}$  in compared to adults of the same species collected on the floodplain (Figure 9; Table 5). However,  $\delta^{13}\text{C}$  values of Longnose ( $F$ -ratio = 0.47,  $df$  = 1,  $P$  = 0.498), Shortnose ( $F$ -ratio = 0.30,  $df$  = 1,  $P$  = 0.587), and Spotted Gars ( $F$ -ratio = 0.07,  $df$  = 1,  $P$  = 0.800) were similar when comparing individuals collected in riverine verses floodplain habitats (Figure 9; Table 5).

Adult Longnose Gars were characterized by a mean  $\delta^{15}\text{N}$  value of 17.44‰ and mean  $\delta^{13}\text{C}$  value of -26.29‰ (Figure 9; Table 4). A one-way ANOVA, followed by Tukey's post hoc analysis, revealed adult Longnose Gars were more enriched in  $\delta^{15}\text{N}$  compared to Shortnose ( $F$ -ratio = 37.97,  $df$  = 2,  $P$  < 0.001) and Spotted Gars ( $F$ -ratio = 37.97,  $df$  = 2,  $P$  < 0.001) (Figure 9). Longnose Gars also exhibited significant  $\delta^{13}\text{C}$  depletion compared to Shortnose ( $F$ -ratio = 24.36,  $df$  = 2,  $P$  < 0.001) and Spotted Gars ( $F$ -ratio = 24.36,  $df$  = 2,  $P$  < 0.001; Figure 9).

Shortnose Gars had a mean  $\delta^{15}\text{N}$  value of 16.60‰ and mean  $\delta^{13}\text{C}$  value of -25.60‰, while Spotted Gars were lower in  $\delta^{15}\text{N}$  and higher in  $\delta^{13}\text{C}$ , exhibiting a mean  $\delta^{15}\text{N}$  value of 15.97‰ and mean  $\delta^{13}\text{C}$  value of -25.35‰ (Figure 9; Table 4). A one-way ANOVA followed by a Tukey's post hoc analysis, indicated Shortnose Gars were more enriched in  $\delta^{15}\text{N}$  ( $F$ -ratio = 37.07,  $df$  = 2,  $P$  < 0.001) than Spotted Gars (Figure 9). However, differences in  $\delta^{13}\text{C}$  values ( $F$ -ratio = 24.36,  $df$  = 2,  $P$  = 0.131) of Shortnose and Spotted Gars were similar (Figure 9).

Of 198 fin clips collected, 13 were collected from young of the year (YOY) gars ( $n$  = 2 Alligator, 2 Longnose, 9 Spotted Gars) on the floodplain. YOY had a mean  $\delta^{15}\text{N}$  value of



**Figure 9.** Biplots of mean ( $\pm$  SE)  $\delta^{15}\text{N}$  (‰) and  $\delta^{13}\text{C}$  (‰) values of fin tissue collected from adult Longnose ( $n = 15$  floodplain, 38 riverine), Shortnose ( $n = 55$  floodplain, 32 riverine), and Spotted Gars ( $n = 39$  floodplain, 6 riverine) in riverine and floodplain habitats.

**Table 5.** Matrix of pair-wise ANOVA comparisons for mean  $\delta^{15}\text{N}$  (‰) and  $\delta^{13}\text{C}$  (‰) of Longnose, Shortnose, and Spotted Gars by location. The  $p$ -values of  $\delta^{15}\text{N}$  (‰) comparisons are represented above the diagonal. The  $p$ -values of  $\delta^{13}\text{C}$  (‰) comparisons are represented below the diagonal.

	Longnose - R	Shortnose - R	Spotted - R	Longnose - FP	Shortnose - FP	Spotted - FP
Longnose - R	-	0.008*	0.831	0.041*	0.000*	0.000*
Shortnose - R	0.000*	-	0.489	0.980	0.000*	0.000*
Spotted - R	0.023*	0.922	-	0.419	0.001*	0.000*
Longnose - FP	0.498	0.001*	0.005*	-	0.008*	0.000*
Shortnose - FP	0.000*	0.753	0.461	0.001*	-	0.001*
Spotted - FP	0.000*	0.302	0.800	0.000*	0.128	-

FP = Floodplain, R = Riverine

\*Indicates difference between pairs

16.41‰ and a mean  $\delta^{13}\text{C}$  value of -28.47‰ (Table 4). A one-way ANOVA revealed there was no difference in the  $\delta^{15}\text{N}$  ( $F$ -ratio = 0.12,  $df = 2$ ,  $P = 0.886$ ) and  $\delta^{13}\text{C}$  ( $F$ -ratio = 0.47,  $df = 2$ ,  $P = 0.640$ ) values of different gar species as young of the year.

Furthermore, the mean  $\delta^{15}\text{N}$  of YOY gars was similar to that of adult gars collected on the floodplain ( $F$ -ratio = 0.35,  $df = 1$ ,  $P = 0.557$ ; Figure 8; Table 4). However, the mean  $\delta^{13}\text{C}$  value of YOY gars was significantly lower than that of adult gars collected on the floodplain ( $F$ -ratio = 157.70,  $df = 1$ ,  $P < 0.001$ ; Figure 8; Table 4). The  $\delta^{13}\text{C}$  value of YOY gars was most similar to that of baseline organisms ( $F$ -ratio = 3.04,  $df = 1$ ,  $P = 0.103$ ), which had a  $\delta^{13}\text{C}$  value ranging from -28.50 to -27.43‰ (Figure 8; Table 4).

The isotopic signature of Grass Shrimp collected from riverine habitats ( $\delta^{15}\text{N} = 13.41$ ‰;  $\delta^{13}\text{C} = -27.43$ ‰) were similar to Grass Shrimp collected on the floodplain ( $\delta^{15}\text{N} = 13.48$ ‰;  $\delta^{13}\text{C} = -27.66$ ‰; Table 4). Bivalves were depleted in  $\delta^{15}\text{N}$  (11.14‰) and  $\delta^{13}\text{C}$  (-28.50‰) relative to Grass Shrimp (Figure 8; Table 4).

Trophic position values are reported in Appendix C.

### Water Quality

Water depth at sampling locations during high-water ranged from 1.2 m to 5.1 m (Figure 3). Reservoir water depth had an estimated range of 1.0 m to 2.5 m. Due to site conditions, water depth could not be measured or estimated during falling-water. Temperature (°C), conductivity (µS/cm), dissolved oxygen (mg/L), percent dissolved oxygen (%), and Secchi depth for the interior Loch Leven floodplain and riverine sites are reported in Tables 6 and 7, respectively.

**Table 6.** Mean ( $\pm$  SD) temperature ( $^{\circ}\text{C}$ ), specific conductance ( $\mu\text{S}/\text{cm}$ ), dissolved oxygen ( $\text{mg}/\text{L}$  and %), and Secchi depth ( $\text{cm}$ ) of Loch Leven interior floodplain, 11 June to 13 November 2020. Secchi depth was not measured on 11 June 2020.

Parameter	High-Water		Falling-Water		Reservoir	
	11 June	18 June	9 July	21 July	17 Sept.	13 Nov.
Temperature ( $^{\circ}\text{C}$ )	$28.87 \pm 0.53$	$29.29 \pm 0.55$	$29.54 \pm 1.42$	$30.44 \pm 1.70$	$28.37 \pm 0.30$	$21.70 \pm 0.12$
Conductance ( $\mu\text{S}/\text{cm}$ )	$343.00 \pm 0.82$	$348.17 \pm 6.77$	$397.25 \pm 33.58$	$451.00 \pm 39.15$	$352.00 \pm 1.73$	$302.00 \pm 0.00$
Dissolved Oxygen ( $\text{mg}/\text{L}$ )	$11.69 \pm 0.79$	$8.44 \pm 1.28$	$5.91 \pm 1.55$	$3.99 \pm 1.12$	$4.78 \pm 0.20$	$8.65 \pm 0.41$
Percent Dissolved Oxygen (%)	$134.87 \pm 14.51$	$113.02 \pm 14.74$	$78.61 \pm 21.26$	$53.70 \pm 16.05$	$61.67 \pm 2.59$	$98.57 \pm 4.54$
Secchi Depth ( $\text{cm}$ )	-	$82.5 \pm 24.42$	$28.60 \pm 18.57$	$29.33 \pm 9.45$	$26.67 \pm 2.52$	$15.00 \pm 0.00$

**Table 7.** Temperature (°C), specific conductance (µS/cm), dissolved oxygen (mg/L and %), and Secchi depth (cm) of Loch Leven riverine habitats (WCS and the Narrows), 9 July to 13 November 2020.

<b>Parameters</b>	<b>9 July</b>	<b>21 July</b>	<b>13 Nov.</b>
Temperature (°C)	29.48	31.19	21.90
Conductance (µS/cm)	381	344	185
Dissolved Oxygen (mg/L)	5.15	6.77	9.47
Percent Dissolved Oxygen (%)	67.70	92.10	103.60
Secchi Depth (cm)	30	27	20

## Discussion

The primary purpose of this study was to compare resource use by gars in floodplain and riverine habitats, as represented by stable isotopes of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ . Based on previous studies, which suggest gars depend on floodplains for spawning, feeding, and nursery habitat (Snedden et al. 1999; Robertson et al. 2008; Buckmeier et al. 2013; Kluender et al. 2016), I hypothesized that Alligator, Longnose, Shortnose, and Spotted Gars would use the Loch Leven floodplain extensively. Due to the high productivity of floodplains (Junk et al. 1989; Zeug and Winemiller 2007), I also hypothesized that the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of gar fin tissue would be different between floodplain and riverine habitats. Because other piscivorous fishes have been documented feeding at lower trophic levels following floodplain inundation (Roach et al. 2009), I specifically predicted that  $\delta^{15}\text{N}$  would be depleted in gars collected from the floodplain when compared to riverine habitats. My study indicated that gars collected on the floodplain were depleted in  $\delta^{15}\text{N}$ . However, Longnose Gars were more enriched in  $\delta^{15}\text{N}$ , when compared to Shortnose or Spotted Gars. This suggests that Longnose Gars have a more specific diet or may be less reliant on floodplain resources relative to other gar species.

Regarding  $\delta^{13}\text{C}$ , I hypothesized that gars collected on the floodplain would be depleted in  $\delta^{13}\text{C}$  relative to riverine habitats, based on research indicating floodplain-dependent fishes exhibit  $\delta^{13}\text{C}$  depletion (Hamilton 1992, Fry 2002). However, because  $\delta^{13}\text{C}$  exhibits little to no change from prey to predator (Vander Zanden and Rasmussen 2001; Post 2002), I also hypothesized that any change in  $\delta^{13}\text{C}$  would be minimal, but detectable. While my results indicated overall that gars collected from the floodplain were enriched in  $\delta^{13}\text{C}$ , failing to support my hypothesis, an ANOVA comparing the  $\delta^{13}\text{C}$  of riverine and floodplain habitats during the last sampling event indicated  $\delta^{13}\text{C}$  abundance of riverine and floodplain gars was similar following

floodplain inundation. Furthermore, when species was taken into consideration,  $\delta^{13}\text{C}$  in Longnose, Shortnose, and Spotted Gars was similar between riverine and floodplain habitats, suggesting the difference in  $\delta^{13}\text{C}$  may not be ecologically relevant. While these results failed to support my hypothesis, the collection of YOY gars with depleted  $\delta^{13}\text{C}$  signatures partially supports my hypothesis that gars collected on the floodplain would be depleted in  $\delta^{13}\text{C}$  relative to gars collected from riverine habitats.

The secondary purpose of this study was to establish a baseline fish assemblage for Loch Leven prior to restoration. Determining the presence and absence of fish species at Loch Leven allows restoration managers to identify shifts in assemblage after restoration. My study identified 24 species, representing 9 families, within the Loch Leven floodplain and riverine habitats.

#### Loch Leven Fish Assemblage

As a dynamic floodplain system, water levels within Loch Leven fluctuate in response to the annual flood pulse (Junk et al. 1989). In a typical year, water from the Mississippi River inundates associated floodplains in early spring and recedes by late summer or early fall (Baker et al. 1991). As water inundates the floodplain, seasonally available habitat becomes accessible to large-river fishes for spawning and foraging (Zeug and Winemiller 2007; Kluender et al. 2016; van der Most and Hudson 2017). When spawning coincides with the flood pulse, YOY spawned on the floodplain move into backwater nursery habitats and exploit abundant resources (Zeug and Winemiller 2007). As a result, several life stages of fish may be present on the floodplain simultaneously. This was observed at Loch Leven, with adults and YOY of several species collected (e.g., Spotted Gar, Bluegill, Gizzard Shad).

Fish assemblage is also strongly influenced by hydrology (Phelps et al. 2015; Pander et al. 2019). At Loch Leven, Skipjack Herring and Longnose Gar, which exhibit preferences for

main channel habitats (Baker et al. 1991; Robertson et al. 2008), were collected in greatest abundance during high-water events. During falling-water sampling, the catch was dominated by YOY Gizzard Shad, Black Crappie, and Bluegill. Adults of these species are common to abundant in seasonally inundated floodplains (Baker et al. 1991).

#### Focus Species: Gars

Gars were found on the floodplain during all sampling events. Shortnose Gars are abundant in the lower Mississippi River and common in wetland habitats (Baker et al. 1991). Of the four gar species, Shortnose Gar was the most common species collected at Loch Leven, followed by Longnose, Spotted, and Alligator Gars. Longnose Gars are most abundant in riverine habitats (Zeug and Winemiller 2007; Robertson et al. 2008) and were primarily collected near the WCS and in the Narrows. Catch of adult Longnose Gars also noticeably decreased as water levels within the floodplain receded. Spotted Gars are often found in shallow backwater habitats during periods of floodplain inundation (Zeug and Winemiller 2007; Robertson et al. 2008; Walker et al. 2013) and have been observed using floodplain lakes as refuge habitat during low-water (Bonvillain et al. 2008). At Loch Leven, Spotted Gars were primarily collected from similar habitats, such as the interior floodplain and reservoir. Only 6 of 54 individuals were collected from riverine habitats. While literature on Shortnose Gar habitat preference is lacking, Longnose and Spotted Gars occupied expected habitat niches as suggested by previous research.

Relative to other gar species in the lower Mississippi River, the Alligator Gar is less abundant (Baker et al. 1991). The collection of only two Alligator Gars (YOY) compared to other gar species reflects this observation. However, several large Alligator Gars were observed breaching on the floodplain during high-water. Alligator Gars also have frequently been observed spawning approximately 40 kilometers upstream on the St. Catherine Creek National

Wildlife Refuge floodplain (Allen et al. 2014; Kimmel et al. 2014). Furthermore, areas within Loch Leven have been identified as suitable spawning habitat for Alligator Gars (Allen et al. 2014) and the presence of YOY indicates that mature Alligator Gars successfully spawned on the Loch Leven floodplain in 2020.

The timing of gar reproductive activity varies by species, but typically occurs in spring and coincides with warming water temperatures and floodplain inundation (Snedden et al. 1999; Zeug and Winemiller 2007). This provides an opportunity for YOY gars to move into slack-water nursery and forage habitats (Zeug and Winemiller 2007). In addition to the YOY Alligator Gars collected at Loch Leven, YOY Longnose and Spotted Gars were also collected on the floodplain. Because all YOY gars were caught on the interior floodplain as water receded, it is unlikely the YOY were spawned outside of Loch Leven. This would imply mature Longnose and Spotted Gars, in addition to Alligator Gars, were spawning at Loch Leven in spring of 2020. Although YOY Shortnose Gars were not collected, given similarities in life history to other gar species (Walker et al. 2013), it is likely that Shortnose Gars also spawned on the floodplain.

#### Stable Isotope Analysis

The value of  $\delta^{15}\text{N}$  is positively correlated with the relative trophic position of aquatic organisms, with lower values being associated with organisms that feed at a lower trophic level (Minagawa and Wada 1984; Zeug and Winemiller 2008). Low  $\delta^{15}\text{N}$  values in fishes can also be indicative of feeding outside the main-channel or in backwater habitats (Fry 2002, Roach et al. 2009; Zeug et al. 2009). During high-water, when floodplain habitat becomes accessible, consumers may also exhibit greater niche breadth (Azevedo et al. 2021) and diet variability (Fisher et al. 2001). This may contribute to lowering of trophic position of fishes. At Loch Leven, Longnose, Shortnose, and Spotted Gars collected on the floodplain exhibited depletion of

$\delta^{15}\text{N}$  relative to gars collected from riverine habitats. This suggests that gars on the floodplain are feeding at lower trophic position and potentially capitalizing on diverse floodplain resources. Roach et al. (2009) observed similar results in Largemouth and Smallmouth Bass, which were depleted in  $\delta^{15}\text{N}$  following floodplain inundation. Gut content analysis supports a diverse diet, finding crayfish, other aquatic and terrestrial invertebrates, and amphibians in the stomachs of Shortnose and Spotted Gars collected from floodplains (Walker et al. 2013). Gars collected from off-channel habitats also have a low occurrence of empty stomachs relative to gars collected from main channels (Robertson et al. 2008), further implying that gars exploit floodplain forage habitat.

The abundance of  $\delta^{15}\text{N}$  in gars collected from the Loch Leven floodplain also followed a trend similar to that of other studies, with fishes collected later in the year being depleted in  $\delta^{15}\text{N}$  (Roach et al. 2009; Fredrickson 2020). Between the first and last sampling events at Loch Leven, mean  $\delta^{15}\text{N}$  significantly decreased in floodplain gars. However, mean  $\delta^{15}\text{N}$  in gars collected from riverine habitats did not significantly differ between the first and last sampling event. Significant depletion of  $\delta^{15}\text{N}$  in floodplain gars over time may be due to opportunistic feeding on abundant floodplain resources, rather than seasonal changes. The lowest  $\delta^{15}\text{N}$  values in floodplain gars, observed on 17 September 2020, likely reflects prey consumed in June or July, when YOY fishes were beginning to increase in abundance on the floodplain (Vander Zanden 2015). By November, the  $\delta^{15}\text{N}$  of floodplain gars had increased. Given a tissue turnover rate of two months, isotopic signatures observed in November reflect prey consumed in July or August (Vander Zanden 2015), when YOY fishes would have created a resource pulse for gars (Piazza and La Peyre 2012).

Mean  $\delta^{15}\text{N}$  values also differed among the three most abundant gar species. Longnose Gars exhibited the greatest  $\delta^{15}\text{N}$  enrichment, followed by Shortnose Gars and Spotted Gars. This implies that Longnose Gars feed at a higher trophic position relative to Shortnose Gars, while Shortnose Gars feed at a higher trophic position relative to Spotted Gars. Other studies which have included, although not focused on, gars have made similar observations (Zeug and Winemiller 2008, Roach et al. 2009). Even as YOY, Longnose Gars are enriched in  $\delta^{15}\text{N}$  relative to Shortnose and Spotted Gars (Snow et al. 2020). This may reflect Longnose Gar preference for main-channel habitats (Robertson et al. 2008). Similarly,  $\delta^{15}\text{N}$  depletion in Spotted Gars, a common characteristic of floodplain fishes, may reflect the tendency of Spotted Gars to occupy backwater habitats (Snedden et al. 1999; Fry 2002; Roach et al. 2009). Although Shortnose Gar research is lacking, available literature suggests a unique diet that may include more terrestrial and aquatic macroinvertebrates compared to other gar species (Vokoun 2000; Walker et al. 2013; Snow et al. 2020). While this study cannot provide dietary specifics, the  $\delta^{15}\text{N}$  value of Shortnose Gars from Loch Leven indicate Shortnose Gar diet varied from that of Longnose and Spotted Gars.

The abundance of  $\delta^{13}\text{C}$  in tissue can indicate the ultimate source of dietary carbon and preferred habitat of an organism (Peterson and Post 1987; Post 2002; Zeug and Winemiller 2008). Low  $\delta^{13}\text{C}$  is characteristic of fishes feeding on floodplains, in unconfined rivers, or backwater habitats (Hamilton et al. 1992; Fry 2002; Zeug and Winemiller 2008), while higher  $\delta^{13}\text{C}$  values are found in fishes feeding in channelized rivers, such as the Mississippi River (Fry 2002). Contrary to these findings, when all species of adult gars from Loch Leven were pooled,  $\delta^{13}\text{C}$  was lower in adult gars collected from riverine habitats relative to the floodplain. Although the difference in  $\delta^{13}\text{C}$  was significant, the difference between means was relatively small

(0.27‰). Catch comparisons of adult gar species suggests higher  $\delta^{13}\text{C}$  in riverine habitats may be influenced by the abundance of Longnose Gars. Longnose Gars had the lowest mean  $\delta^{13}\text{C}$  value of the three species and more Longnose Gars were caught in riverine habitats than on the floodplain. Furthermore, when species was taken into consideration, difference in  $\delta^{13}\text{C}$  between riverine and floodplain gars was no longer significant.

YOY gars collected from the floodplain were similar in  $\delta^{15}\text{N}$  to adult gars, suggesting YOY and adult gars exploit similar food resources. Exploratory gut content analysis revealed several Loch Leven YOY gars had consumed YOY centrarchids and cyprinids, a likely explanation for similarities in  $\delta^{15}\text{N}$  between adult and YOY gars. Despite probable overlap in diet, YOY were significantly depleted in  $\delta^{13}\text{C}$  relative to adult gars. The  $\delta^{13}\text{C}$  signature of YOY reflected that of baseline organisms collected on the floodplain. Snow et al. (2020) observed similar  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  trends in YOY gars relative to baseline invertebrates and recorded  $\delta^{13}\text{C}$  signatures of YOY Longnose, Shortnose, Spotted, and Alligator Gars near to that of Grass Shrimp (Snow et al. 2020). Given that  $\delta^{13}\text{C}$  depletion can be indicative to floodplain resource use (Hamilton et al. 1992; Fry 2002) and YOY gars most likely originated on the floodplain, low  $\delta^{13}\text{C}$  values could indicate significant dependence on floodplain resources in early life stages. YOY gars are secondary consumers, rapidly transitioning to piscivory by 50 to 60 mm, depending on species (Echelle 1968; David et al. 2015; Snow et al. 2020). Based on the total lengths of Loch Leven YOY gars and associated stomach contents, all individuals had transitioned to piscivory at the time of capture. As offspring developed on the floodplain, the  $\delta^{13}\text{C}$  of floodplain prey items would have been readily incorporated in tissues. While the enrichment of  $\delta^{13}\text{C}$  is typically less than 1% from prey to consumer (Post 2002), accelerated tissue turnover and rapid growth in YOY may cause  $\delta^{13}\text{C}$  to be incorporated at a faster rate (Grey

2001; Vašek et al. 2016), resulting in a low  $\delta^{13}\text{C}$  signature similar to that of floodplain organisms (Hamilton et al. 1992; Fry 2002, Zeug and Winemiller 2008).

### Biases and Limitations

Rapidly changing water levels across Loch Leven required use of multiple gear types, depending on topography and water depth. During falling-water, steep banks at multiple sampling locations made it hazardous to successfully deploy gillnets. This resulted in the use of cast nets, which had a smaller bar mesh compared to gillnets. Although collecting adult gars was the primary goal of sampling and adult gars were successfully collected with both gear types, it should be acknowledged that gillnets generally exclude small-bodied fish species and YOY. Thus, the number of small-bodied fish species and YOY within Loch Leven could be higher than observed in this study. For a more comprehensive understanding of the overall fish assemblage, I recommend intentionally targeting small-bodied fishes and YOY through the use of cast nets in nearshore habitats during high-water or gillnets with smaller bar mesh.

Unseasonally high water levels on the Mississippi River and changes in water level also limited the collection of baseline organisms. Some aquatic macroinvertebrates, such as the Grass Shrimp collected at Loch Leven, depend on vegetated habitat. However, Loch Leven was inundated from mid-October 2019 to July of 2020, causing terrestrial vegetation to die, thereby reducing habitat for aquatic macroinvertebrates. Of the baseline organisms that were collected, the stable isotope composition of Grass Shrimp collected from riverine and floodplain habitats were nearly identical. Therefore, any potential, artificial enrichment of nitrogen from anthropogenic sources, such as soil or fertilizer (Cabana and Rasmussen 1996), most likely influenced the riverine and floodplain habitats equally, and would not have impacted the relative trophic position of gars. Although consistency in the collection and species of baseline organisms

would have allowed trophic position to be calculated and artificial enrichment to be addressed with greater certainty, recent stable isotope research within a river-floodplain system has omitted the use of baseline organisms altogether (Azevedo et al. 2021). This suggests that baseline organisms may only be essential when comparing fishes across geographically or hydrologically distinct systems, where  $\delta^{15}\text{N}$  values may vary substantially.

Lastly, this study was constrained by COVID-19 travel and safety restrictions. Travel restrictions implemented in early spring prevented sampling events in April and May of 2020. Sampling in April and May would have allowed the six sampling trips to be spaced out more evenly, possibly capturing an earlier isotope signature as gars entered the floodplain in early spring to spawn (Snedden et al. 1999; Buckmeier et al. 2017; Smith et al. 2019). Early spring sampling may have also resulted in the capture of mature Alligator Gars, for which data was lacking in this study. Alligator Gars have been observed spawning at St. Catherine's Creek Wildlife Refuge, north of Loch Leven, in mid-April (Allen et al. 2014; Kimmel et al. 2014). Therefore, April and May sampling is recommended to close gaps in isotope data and improve Alligator Gar collection.

#### Broader Implications and Future Recommendations

This study applies the work of Keppeler et al. (2019) and Fredrickson (2020) and demonstrated that fin tissue can be successfully supplemented for white muscle in gar trophic ecology studies. Successful use of fin clips in place of muscle tissue provides a dependable, non-lethal method for evaluating the trophic ecology of vulnerable gar populations. As suggested by Fredrickson (2020), the methodology used in this study could be applied to endangered and at-risk populations of gars.

Future work should focus on the collection of gars in spring and winter to fully understand the relationship of trophic position and river-floodplain connectivity. Spring sampling may also increase the likelihood of successfully capturing mature Alligator Gar with gillnets. Due to the collection of YOY gars at Loch Leven, future work should also focus on identifying patterns of YOY floodplain use and better understanding changes in the isotopic composition of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in YOY.

### Conclusions

The  $\delta^{15}\text{N}$  depletion in the fin tissue of gars collected on the floodplain confirms that periodic floodplain inundation provides forage resources unavailable to gars in the main channel. As opportunistic feeders, gars may be exploiting abundant and diverse resources on the Loch Leven floodplain, resulting in  $\delta^{15}\text{N}$  depletion and a potentially lower trophic position (Fry 2002, Roach et al. 2009; Walker et al. 2013). Floodplain foraging by gars and  $\delta^{15}\text{N}$  depletion highlight the importance of river-floodplain connectivity. This study also suggests that the extent to which gars exploit floodplain forage resources may differ amongst species, with isotopic composition and habitat preferences supporting extensive floodplain use by Spotted Gars and possibly Shortnose Gars (Snedden et al. 1999; Robertson et al. 2008; Walker et al. 2013). Few studies have focused on the relationship between river-floodplain connectivity and temporal shifts in fish trophic position (Roach et al. 2009; Zeug et al. 2009). This study contributes to the growing body of literature that aims to better understand the importance of river-floodplain connectivity and may better inform restoration, conservation, and land management decisions.

Lastly, the collection of YOY gars confirms that Alligator, Longnose, and Spotted Gars spawn at Loch Leven. As apex predators (Warren and Burr 2014) historically eradicated as nuisance species, some gar populations may be vulnerable to extirpation and in need of

conservation (Scarnecchia 1992; David et al. 2018). This is especially true for the Alligator Gar, a species which is long-lived and slow to mature (Ferrara 2001), thereby increasing susceptibility to population decline (Buckmeier et al. 2017). As the restoration of Loch Leven continues to evolve, the importance of such habitat to the life history and reproductive success of gars should be taken into consideration.

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## Appendix A: Fish Collection

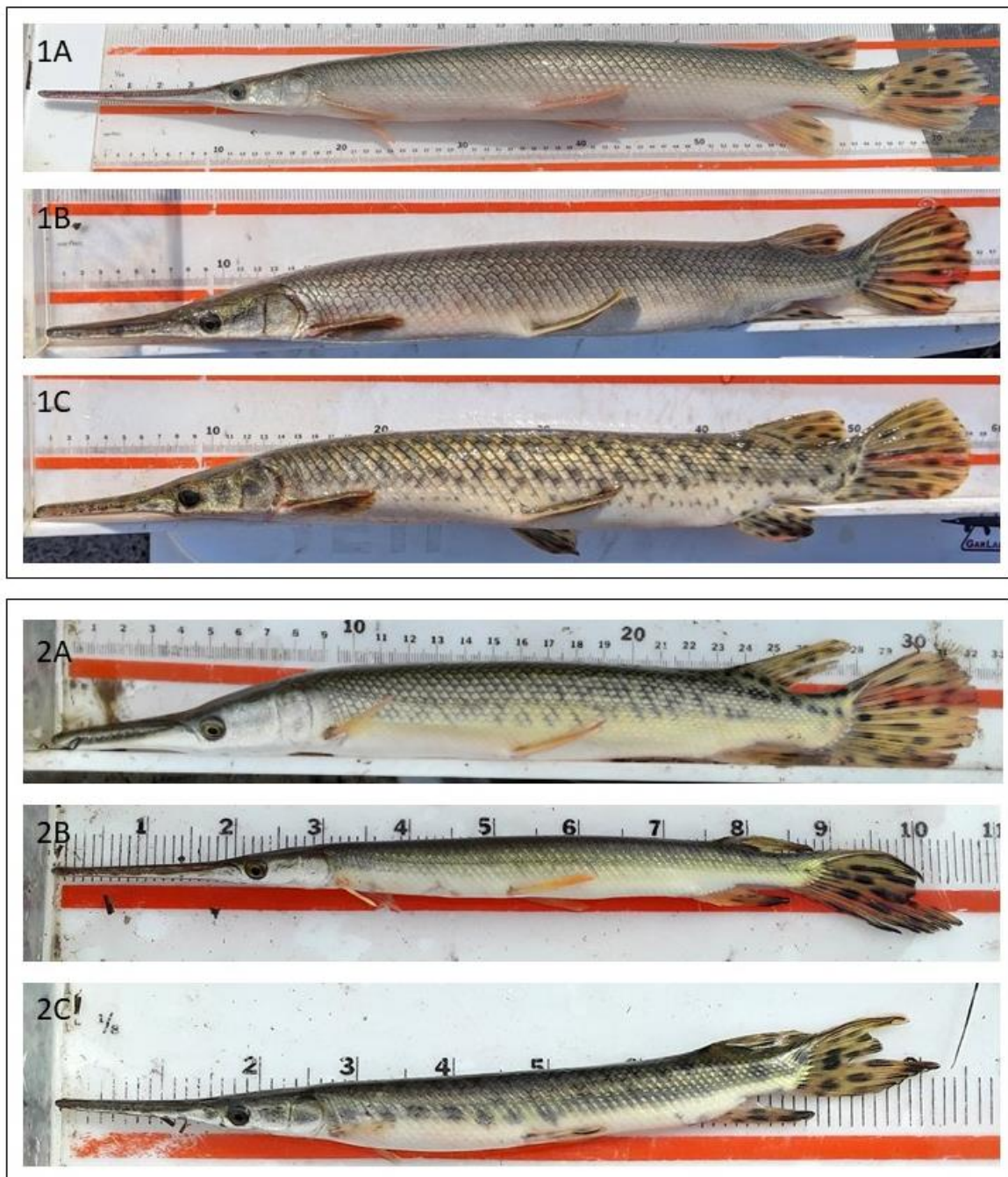
**Table A.1.** Fish species and number of individuals collected from Loch Leven riverine and floodplain habitats using 5 cm, 7 cm, 9 cm, and 13 cm bar mesh gillnets, 11 June to 13 November 2020. The number of gillnets used on a given date with the same size bar mesh is listed in parentheses. Total time each net size was fished is listed in hours. Gear type for some fishes (n = 13 Gizzard Shad, 7 Skipjack Herrings, 6 Longnose Gars, and 2 Shortnose Gars) was unknown.

Species	11 June		18 June		9 July		17 Sept.		13 Nov.		Total
	5 cm	7 cm	5 cm	7 cm	5 cm	7 cm	9 cm	13 cm	9 cm	13 cm	
	(2)	(2)	(2)	(2)	(1)	(1)	(2)	(1)	(2)	(1)	
	11.45	14.10	10.62	11.98	0.33	1.30	4.92	9.15	3.45	5.23	
	hrs.	hrs.	hrs.	hrs.	hrs.	hrs.	hrs.	hrs.	hrs.	hrs.	
Black Buffalo	-	-	-	-	-	-	2	2	2	1	7
Smallmouth Buffalo	-	1	-	1	-	-	2	-	-	-	4
Black Crappie	-	-	-	-	1	-	-	-	-	-	1
Largemouth Bass	-	-	-	1	-	-	-	-	-	-	1
White Crappie	-	-	-	1	-	-	1	-	1	-	3
Gizzard Shad	3	2	5	5	1	-	13	1	12	-	42
Skipjack Herring	3	2	14	2	-	-	-	-	-	-	21
Common Carp	-	-	-	-	-	-	-	1	-	4	5
Blue Catfish	-	-	-	-	-	-	2	-	1	-	3
Channel Catfish	-	2	-	-	1	1	7	1	1	-	13
Flathead Catfish	-	-	-	-	-	-	1	-	-	-	1
Longnose Gar	4	15	-	13	3	6	3	1	4	2	51
Shortnose Gar	3	14	-	11	1	2	10	-	25	-	66
Spotted Gar	3	3	1	6	8	-	7	2	13	1	44
Yellow Bass	1	-	-	-	-	-	-	-	-	-	1
Freshwater Drum	-	-	-	1	-	-	1	-	2	-	4
<b>Total</b>	17	39	20	41	15	9	49	8	61	8	267
	56		61		24		57		69		

**Table A.2.** Fish species and number of individuals collected from Loch Leven riverine and floodplain habitats using cast nets (1.3 cm bar mesh). Gear type for some fishes (n = 13 Gizzard Shad, 7 Skipjack Herrings, 6 Longnose Gars, and 2 Shortnose Gars) was unknown.

Species	11 June	18 June	17 Nov.	Total
	7 casts	28 casts	8 casts	
Silverside	-	2	-	2
Black Crappie	38	26	-	64
Bluegill	13	43	-	56
Green Sunfish	-	1	-	1
Largemouth Bass	10	5	-	15
Longear Sunfish	1	-	-	1
Redear Sunfish	3	19	-	22
Gizzard Shad	2	534	-	536
Skipjack Herring	-	-	-	0
Common Carp	1	1	-	2
Golden Shiner	1	-	-	1
Blacktail Shiner	1	-	-	1
Blue Catfish	-	-	-	0
Channel Catfish	-	2	-	2
Flathead Catfish	-	-	-	0
Alligator Gar	-	2	-	2
Longnose Gar	-	4	-	4
Shortnose Gar	9	15	4	28
Spotted Gar	1	9	-	10
Yellow Bass	-	-	-	0
Freshwater Drum	-	2	-	2
<b>Total</b>	80	665	4	749

## Appendix B: Species Photographs



**Figure B.1.** Adult (top) and YOY (bottom) gars collected at Loch Leven during 2020 sampling events: 1a) adult Longnose Gar, 1b) adult Shortnose Gar, 1c) adult Spotted Gar, 2a) YOY Alligator Gar, 2b) YOY Longnose Gar, and 2c) YOY Spotted Gar.



**Figure B.2.** Fish species collected during Loch Leven 2020 sampling events. A) Blue Catfish, B) Bluegill, C) Largemouth Bass, D) Gizzard Shad, E) Black Crappie, F) Smallmouth Buffalo, G) Common Carp, H) Redear Sunfish, I) Blacktail Shiner, J) Black Buffalo, K) Flathead Catfish, L) Channel Catfish, M) Freshwater Drum, N) Golden Shiner, O) Yellow Bass, P) Skipjack Herring, Q) Silverside sp., and R) White Crappie. Longear Sunfish and Green Sunfish were also collected, but not pictured.

### Appendix C: Trophic Position

**Table C.1.** Mean ( $\pm$  SE) trophic position for YOY and adult gars collected from Loch Leven riverine and floodplain habitats, 11 June to 13 November 2020.

Species		Grass Shrimp			Bivalves		
		Combined	Riverine	Floodplain	Combined	Riverine	Floodplain
Longnose Gar	Adult	3.18 $\pm$ 0.03	3.22 $\pm$ 0.03	3.09 $\pm$ 3.09	3.85 $\pm$ 0.03	3.89 $\pm$ 0.03	3.75 $\pm$ 0.07
	YOY	2.85 $\pm$ 0.05	-	2.85 $\pm$ 0.05	3.53 $\pm$ 0.03	-	3.53 $\pm$ 0.03
Shortnose Gar	Adult	2.93 $\pm$ 0.02	3.07 $\pm$ 0.03	2.85 $\pm$ 0.03	3.61 $\pm$ 0.02	3.74 $\pm$ 0.03	3.53 $\pm$ 0.03
Spotted Gar	Adult	2.74 $\pm$ 0.05	3.17 $\pm$ 0.06	2.68 $\pm$ 0.04	3.42 $\pm$ 0.05	3.85 $\pm$ 0.06	3.36 $\pm$ 0.04
	YOY	2.87 $\pm$ 0.04	-	2.87 $\pm$ 0.04	3.55 $\pm$ 0.03	-	3.55 $\pm$ 0.03
Alligator Gar	YOY	2.90 $\pm$ 0.10	-	2.90 $\pm$ 0.10	3.55 $\pm$ 0.03	-	3.55 $\pm$ 0.03

### **Biographical Sketch**

Kristie Rae Ellis was born in Kenosha, Wisconsin in 1992. She was home educated until 2009, when she began taking science courses at LakeView Technology Academy. After graduating from LakeView Technology Academy, Kristie Rae attended Carthage College. While in college, she was a Supplementary Instructor for an introductory GIS course and interned with the Water Quality Lab at the John G. Shedd Aquarium in Chicago, Illinois. Kristie Rae wrote her undergraduate thesis on the impacts of urbanization and water quality on northern leopard frog *Lithobates pipiens* populations. After graduating from Carthage College in 2015, Kristie Rae received a job working with the Wisconsin Department of Natural Resources conducting electrofishing surveys. She later accepted a position conducting ichthyoplankton surveys throughout the Midwest and Virginia and herpetofauna monitoring in Michigan. In 2018, she began working on several restoration projects, including the North Mill Creek Restoration in Illinois. Kristie Rae was accepted into Nicholls State University Marine and Environmental graduate program in August 2019. While at Nicholls State University, she participated in coastal restoration projects and interned with Applied Ecological Services in Milwaukee, Wisconsin.

## Curriculum Vitae

**KristieRae Ellis**

ekristierae@gmail.com

### Education

**Nicholls State University, Thibodaux, LA**

**August 2019 – August 2021**

M.S. Marine and Environmental Biology

Research: Trophic ecology of gars (*Lepisosteidae*) in a pre-restored Mississippi River floodplain

**Carthage College, Kenosha, WI**

**September 2011 – May 2015**

Environmental Science, focus Conservation and Ecology

Research: The impacts of urbanization and water quality on northern leopard frog (*Lithobates pipiens*) populations

### Work Experience

**Research Assistant,**

**Nicholls State University, Thibodaux, LA**

**August 2019 – August 2021**

Responsibilities: Sampled adult fish using gillnets and electrofishing. Used fish caudal fin tissue for stable isotope analysis and prepared isotope samples. Experience with jon boats, outboard motors, generators, and ATVs

**Teaching Assistant**

**Nicholls State University, Thibodaux, LA**

**August 2019 – May 2021**

Responsibilities: Instructed and assisted with introductory biology labs both virtually and in-person

**Restoration Field Crew Member,**

**Applied Ecological Services, West Allis, WI**

**June 2018 – July 2019**

Responsibilities: Installed soil lifts and planted native vegetation for stream and floodplain restoration projects. Used targeted herbicide application, prescribed burns, and mechanical removal to manage invasive species in wetland, prairie, and forest habitats.

**Biological Technician II and Crew Leader**

**EA Engineering, Science, and Technology, Inc., Deerfield, IL**     **February 2016 – June 2018**

Responsibilities: Led field crew in the collection of ichthyoplankton samples; analyzed ichthyoplankton samples for larval fish and eggs. Conducted adult fish surveys using bottom trawling, seining, and boat electrofishing. Collected benthic samples using a Ponar grab sampler, dip net, and Hester-Dendy sampler; identified macroinvertebrates.

**Water Resources Management Specialist (Seasonal)**

**Wisconsin Department of Natural Resources Waukesha, WI**

**June – September 2015**

Responsibilities: Sampled fish communities using backpack and barge electrofishing. Conducted quantitative habitat evaluations.

## **Water Quality Intern**

**John G. Shedd Aquarium, Chicago, IL**

**February – May 2015**

Responsibilities: Used water quality meters and chemical titration methods to measure water quality parameters in freshwater and marine aquarium habitats. Monitored ion fluctuations in a Lake Michigan harbor using ion chromatography.

## **Presentations**

American Fisheries Society Annual Meeting, September 2020, “Floodplain restoration and gar conservation”.

Nicholls State University Research Expeaux, March 2021, “Fish species diversity and habitat use in a restored Mississippi River floodplain with a focus on gars (Lepisosteidae)”.

## **Awards**

Catina Brandt Outstanding Graduate Student in Marine and Environmental Biology 2021

## **Related Skills**

- Freshwater fish identification
- Electrofishing
- Trawling
- Gillnetting
- Seine netting
- Larval fish collection
- Trailering boats (up to 12ft) and enclosed trailers
- Operating small jon boats with outboard motors
- Driving ATVs and UTVs
- Generator operation
- Water quality monitoring
- YSI calibration
- Statistical analysis
- R Statistical Software
- SAS Statistical Software
- ArcGIS Software
- Stable isotope preparation
- Native and invasive wetland plant identification (Midwest)
- Herpetofauna identification
- Macroinvertebrate sampling
- Macroinvertebrate identification