

Completion Report to:
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Reproduction of Asian Carp in the Upper Mississippi River

Submitted By

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Project Highlights:

Egg densities (all fish taxa) across sampling sites peaked during June 25th and August 8th in 2019. Densities of age-0 fishes (yolk-sac larvae, mesolarvae, and juveniles) were highest during the July 8th and July 18th sampling sessions in 2019. Peak densities of age-0 fish in 2019 occurred immediately following the first peak in egg densities (June 25th). Age-0 fishes from 2019 were identified to family, with *Cyprinidae*, *Catostomidae*, and *Centrarchidae* representing the most abundant families, and peak densities occurred during July 18th, April 26th, and July 18th, respectively. Invasive age-0 Asian carp were not collected at any site or date in 2019; however, unsafe boating conditions due to major flooding from May 1st – June 26th prohibited sampling, and Asian carp reproduction may have occurred during that period. Average chlorophyll *a* concentrations ($\mu\text{g/L}$) were highest within the Rock, Wapsipinicon, and Iowa Rivers and lowest within the Des Moines River at Keosauqua, within pool 15 of the Mississippi River, and both upstream and downstream of the Wapsipinicon River.

Introduction

Ecological communities worldwide are becoming more uniform through the introduction and subsequent establishment of non-native species into novel areas through anthropogenic activities (Rahel 2002). Intentional introductions commonly occur to provide societal benefits such as food, recreation, and biological control (Pimentel et al. 2000). Additionally, advancements in transportation and worldwide commerce have increased unintentional introductions (Rahel 2002). A single non-native species can alter ecosystem structure and function and have costly economic consequences (Macisaac 1996, Pimentel et al. 2005, Weber and Brown 2009), but in the United States, approximately 50,000 non-native species introductions have occurred with varying success and impacts (Pimentel et al. 2005, Sagoff 2005). Economic losses due to non-native introductions are estimated at US\$120 billion a year; however, actual costs are likely much higher because monetary costs associated from species extinctions, loss of ecosystem services, and aesthetic values are not easily assessed

(Pimentel et al. 2005). Likewise, ecological costs may be much greater than economic costs but are difficult to quantify and assess because of lag times between invasion and empirically confirmed impacts to the environment (Gido and Brown 1999, Stohlgren and Schnase 2006).

Non-native fishes are one of the most introduced groups of aquatic animals that have resulted in negative ecological effects in riverine ecosystems (Gozlan 2008). Rivers naturally provide an invasion highway for fishes to expand from the point of introduction. Furthermore, modifications to rivers for navigation have connected previously separate waterways, facilitating inter-basin movement and the spread of invaders into additional novel habitats (Leuven et al. 2009). For example, the Mississippi River Basin covers approximately 40% of the lower 48 U.S. states with thousands of river miles (USACE 2011) and connects to the previously separated Great Lakes basin via the Chicago Area Waterway System, the Ohio-Erie Canal, and other man-made structures (USACE 2014).

At least 83 non-native fishes have become established in the Upper Mississippi River Basin (UMRB) as a result of dispersal from other basins or by direct introduction from anthropogenic activities (Rasmussen 2002). Two of the more recent and widely recognized invaders to the UMRB are Silver Carp (*Hypophthalmichthys molitrix*) and Bighead Carp (*H. nobilis*; collectively referred to as Bigheaded Carp). These species have become abundant and threaten the integrity of the UMRB and any connected aquatic ecosystems (Irons et al. 2009). Asian carp were imported during the 1970s into the United States for food consumption and biological control in aquaculture facilities (Freeze and Henderson 1982). In the 1970s, individuals thought to have escaped during flooding events were observed in several rivers within Arkansas. Due to their high reproductive capabilities and long distance migrations (DeGrandchamp et al. 2008), these fish quickly became established and now inhabit more than 20 states throughout the Mississippi, Missouri, Ohio, and Illinois river basins (Kolar et al. 2007, Baerwaldt et al. 2013, Deters et al. 2013). By the mid-1980s, Asian carp were caught in the pooled sections of the UMRB (Kolar et al. 2007) with the first observations of Asian carp in Iowa occurring in 1986 when Silver Carp were captured in the UMRB below lock and dam 19 (LD19) near Keokuk (Irons et al. 2009). A year later, Bighead Carp were captured near the mouth of Yellow Springs Creek north of Burlington, IA (Irons et al. 2009). Since the initial observations in

Iowa, Asian carp adults have been sighted in several additional UMRB tributaries in Iowa such as the Des Moines, Skunk, Iowa, and Cedar rivers (Bruce 1990, United Press International 2011, Irons 2012, Camacho 2016, Sullivan 2016).

Currently, southeastern Iowa appears to be on the leading edge of Asian carp expansion in the UMRB. Substantially higher adult catch rates of both Silver and Bighead Carp occur below LD19 than above, suggesting this structure and other lock and dams on the Mississippi River may serve as a partial migration barriers (Wilcox et al. 2004). For example, the UMRB water level is regulated at each dam in order to maintain a navigation channel by reducing or eliminating the amount of water discharged, leaving passage through the locks as the only means of fish movement during low river discharge periods. However, dam gates are lifted during higher discharge events that facilitate fish passage (Garvey et al. 2010). It is also during these high discharge events that Asian carp exhibit some of their highest movement rates, especially during annual spring runoff and associated peak discharge events when temperatures are below or within the spawning optimum, suggesting movement may be associated with spawning migration behavior (Jennings 1988, Peters et al. 2006, DeGrandchamp et al. 2008). Furthermore, Asian carp can quickly make long distance migrations (DeGrandchamp et al. 2008), indicating that these fish are capable of dispersal into new locations.

Although Asian carp may be able to navigate lock and dams on the UMRB, pooled sections between these structures may provide unsuitable spawning habitats for these species. Asian carp are highly fecund (up to 3.5 million eggs per female; Garvey et al. 2006) and have short gestation periods (Chapman and George 2011). Thus, only a few adult individuals may be needed to quickly establish an abundant population (Crawley et al. 1986). Despite adult Asian carp being detected above LD19 up to St. Paul, MN, USA, their populations have remained low, suggesting reproduction may be limited in these reaches. Pooled sections associated with lock and dams exhibit reservoir-like characteristics that are more lentic in nature resulting in lower Asian carp reproduction than in unregulated sections where lock and dams are absent (Lohmeyer and Garvey 2009). In contrast, established Asian carp populations in tributary systems, such as the Illinois River, can have high recruitment and adult populations have

increased exponentially in abundance within a decade (Sass et al. 2010). Yet, Asian carp abundance in the tributaries of the UMRB are much lower compared to the Illinois River and much less is known regarding Asian carp reproduction ecology within these systems, which may be limiting adult population abundance.

Successful Asian carp spawning depends on adults finding suitable habitat of sustained, high flow or increasing discharge when water temperatures are between 17 and 30°C (Kolar et al. 2007). Continuous river flow of at least 25 km may be necessary to suspend the semi-buoyant eggs for a 24 h period or until larvae successfully hatch (Krykhtin and Gorbach 1981, George and Chapman 2013, Murphy and Jackson 2013). In most areas of the UMRB, reaches between dams with sufficient sustained velocities of 0.3 to 3.0 m/s and turbulence to keep eggs in suspension do not exist or are poorly suited for egg survival (Lohmeyer and Garvey 2009). However, age-0 Asian carp have been documented in tributaries such as the Cache River (a tributary to the Ohio River; Burr et al. 1996) and the Illinois River (a tributary to the Mississippi River; DeGrandchamp et al. 2007). Additionally, tributaries are associated with Asian carp spawning activity in their native range in the Yangtze River (Yi et al. 1988) and in varying capacities in the Missouri River (Schrank et al. 2001) and Illinois River (DeGrandchamp et al. 2007) where they are introduced. Successful establishment and reproduction in tributaries could provide sources of recruitment for pooled sections of the UMRB and other areas of poor reproduction.

Successful expansion and establishment of Asian carp populations within the UMRB depends on the ability of adults to find adequate conditions of temperature and long fetches of sustained, high flow. Despite perceived poor conditions for successful Asian carp reproduction in the Upper Mississippi River, tributaries could provide adequate conditions for reproduction, resulting in population expansion along the leading edge of the invasion. Reproductive success on an invasion front can increase exponentially through time (Chick and Pegg 2001, Sass et al. 2001) until establishment occurs (Hayer et al. 2014). Some systems where Asian carp are starting to establish can have sporadic reproduction (Irons et al. 2011), leading to either a surge or decline in the population. Evaluating factors affecting reproduction and recruitment in tributaries of the Mississippi River in association with annual variation in environmental

conditions is needed to better understand Asian carp population dynamics in these systems and potentially develop management strategies for these invasive fishes. By understanding more about factors affecting reproduction and recruitment within the tributaries of the UMRB, potential increases of Asian carp presence, or newly established residence in UMR could be detected early. Spatial and temporal distribution of Asian carp eggs and larvae will help to locate spawning habitat, determine reproductive cues, and provide insight between environmental variables and reproduction.

Project Objectives:

- 1) Evaluate Asian carp reproduction in pools 14, 15, 16, 17, 18, 19, 20 and the contribution of the Wapsipinicon, Rock, Iowa, Skunk, and Des Moines rivers to Asian carp reproduction (egg and age-0 fish densities).

Methods:

Fish eggs, age-0 fishes, and chlorophyll *a* were sampled in 2019 at 18 locations (Figure 1) approximately every 10 days depending upon river conditions from late April until mid-August (12 sessions, 18 sites, 3 habitats equating to 54 tows per session, or 648 ichthyoplankton tows per year). Sampling sites are abbreviated to distinguish tributary sites from sites within the Mississippi River upstream and downstream from the tributary site (Table 1). Sampling was not conducted when water levels were too high or low for boat access, or inclement weather prevented safe boating (May – late June, 2019). Ichthyoplankton tows (0.5 m diameter net, 500 μm mesh) were conducted at the surface at a constant boat speed relative to the shoreline up to four minutes depending on debris load. A General Oceanics flowmeter (Model 2030R) was attached in the mouth of the net to estimate volume (m^3) of water filtered during each tow. Three tows were conducted at each site parallel to river flow: the first tow was in the main thalweg for drifting eggs and larvae (<24 hours post fertilization), the second tow occurred near channel borders where water velocity is moving downstream slower than the thalweg, and the third was in an adjacent backwater area for mobile larvae and juveniles (>24 hours post

fertilization). After each tow, ichthyoplankton net contents were rinsed toward the cod end, placed in sample jars, and preserved in 95% ethanol. Chlorophyll *a* was sampled in conjunction with each tow for eggs/age-0 fish by filtering 50 – 100 mL of water through a GF/F Whatman® glass fiber filter (47-µm porosity) that was placed on ice in the field and frozen in the laboratory. Chlorophyll *a* was extracted with 90% acetone and quantified using an Aquafluor Handheld Fluorometer (Turner Designs) to obtain chlorophyll *a* concentrations (µg/L).

In the laboratory, eggs and age-0 fishes (yolk-sac larvae, mesolarvae, and juveniles) were separated from debris. Samples containing $\geq 1,000$ age-0 fish ($n=1$) were subsampled so that at least 25% (minimum 100 fish) were identified. All age-0 fishes have been identified to the lowest possible taxa using morphometric and meristic characteristics described in literature (Auer 1982). Asian carp larvae are difficult to distinguish among species and are being identified to genus using meristic and morphometric characteristics (Chapman 2006, Chapman and George 2011). Age-0 fishes were first categorized as yolk-sac larvae, larvae, or juveniles based on fin development and complete absorption of the yolk-sac. Fish recognized as having a full complement of fins are categorized as juvenile fish. Data and figures presented here includes all age-0 fishes without distinguishing ontogenetic categories.

Results and Discussion:

Chlorophyll *a*

Chlorophyll *a* concentrations within each of the major tributaries to the Mississippi River were consistently higher than within the Mississippi River throughout 2019, with the highest being the backwater of the Rock River mouth ($99.24 \mu\text{g/L} \pm 29.76 \text{ SE}$), the channel border of the Wapsipinicon River mouth ($94.05 \mu\text{g/L} \pm 17.41 \text{ SE}$), and the channel border of the Iowa River mouth ($92.81 \mu\text{g/L} \pm 28.13 \text{ SE}$; Figure 2). Within tributary mouths, chlorophyll *a* concentrations tend to decrease with latitude from the Wapsipinicon River to the Des Moines River. Interestingly, the opposite trend presents itself within the Mississippi River proper, albeit in a less obvious manner (Figure 2). While chlorophyll *a* concentrations within tributaries may be more

dependent on the local watershed, chlorophyll *a* within the main-stem UMR will likely be the cumulative result of tributary contributions as well as its own productivity.

Eggs and Age-0 Fishes

A total of 366 ichthyoplankton tows were completed in 2019. Eggs were collected during every sampling session for a total of 709 eggs in 2019. The largest number of eggs were collected during the sixth sampling event (August 8th – August 9th; 10.48 per 100m³ ± 3.27 SE), with a slightly smaller pulse of eggs occurring earlier in the year from June 25th – June 27th. Eggs were captured at all sites and habitats with the exception of sites within the mouths of the Skunk or Wapsipinicon rivers (Figure 3.) A total of 248 tows were conducted in the Mississippi River that collected a total of 565 eggs. An additional 120 tows were taken within the tributary mouths that captured 144 eggs (Keosauqua = 34 eggs, Des Moines = 49 eggs, Skunk = 0 eggs, Iowa = 40 eggs, Rock = 21 eggs, Wapsipinicon = 0 eggs). Egg density appeared highest in the Mississippi River at Pool 17 and lowest within the mouths of the Wapsipinicon and Skunk rivers (Figure 4). Across all tributary sites and habitats, the highest egg density (18.45 per 100m³ ± 10.73 SE) was within the thalweg of the Iowa River (Figure 5); however, the highest overall egg density was within the channel border habitat of Pool 17 of the Mississippi River (41.19 per 100m³ ± 21.29 SE).

A total of 17,233 age-0 fishes were captured in 2019. The highest densities of age-0 fishes were collected July 8th (229.28 per 100m³ ± 116.02 SE) and July 18th (228.55 per 100m³ ± 70.17 SE; Figure 3). Peaks in age-0 fish density occurred during the two sessions immediately following one of the two main peaks in egg density in 2019 (Figure 3). The sampling session with the lowest density of age-0 fishes captured in 2019 (19.24 per 100m³ ± 9.72 SE) occurred during the first sampling session on April 26th.

Age-0 fishes were sampled from every river in 2019. The majority of age-0 fishes were captured either upstream (8,988 fish) or downstream (5,817 fish) of tributaries than within tributary mouths (506 fish) in 2019 (Figure 4). Densities of age-0 fishes relative to their

association with a tributary (upstream, downstream, or within) varied in 2019. Densities of age-0 fishes were higher upstream than downstream of the Wapsipinicon, Rock, and Iowa rivers, but the opposite trend presented itself with the Skunk and Des Moines rivers (Figure 4). Across sites, densities of age-0 fishes tended to be highest within the backwater or channel border habitats throughout 2019 (Figure 5).

Ichthyoplankton from 2019 have been identified to family except for non-native carp which were identified to genus. In 2019, no age-0 Asian carp were collected across all sites, habitats, and sessions. However, this does not necessarily mean that Asian carp did not reproduce in the UMR in 2019; nearly two months (April 26th – June 25th) of major flooding occurred in the UMR that prevented sampling. Given that age-0 Asian carp have been detected in the UMR every year from 2014-2018, it is likely that an undetected successful Asian carp spawning event occurred during this flooding period.

Cyprinidae were the most abundant age-0 fishes collected followed by *Catostomidae*, *Centrarchidae*, *Clupeidae*, and *Sciaenidae* (Figure 6). Other taxa present but at lower densities include *Cyprinus carpio*, *Percidae*, *Lepisosteidae*, *Atherinopsidae*, *Moronidae*, and *Esocidae* (Figure 6). Age-0 *Cyprinidae* density peaked during July 8th (72.75 per 100m³ ± 39.37 SE) and July 18th (72.72 per 100m³ ± 22.46 SE) sampling sessions (Figure 6). Age-0 *Centrarchidae* density also peaked during the same period as *Cyprinidae* in 2019 (July 8th 1.97 per 100m³ ± 0.61 SE; July 18th 2.11 per 100m³ ± 0.85 SE; Figure 6).

Publications resulting from this project to date:

Sullivan, CJ, MJ Weber, and CL Pierce. In revision. Spatial variation in Silver Carp populations across their existing distribution in North America. *Ecology of Freshwater Fish*.

Sullivan, CJ, CA Camacho, MJ Weber, and CL Pierce. In revision. Spatial variation in Grass Carp populations across their existing distribution. *Journal of Fish and Wildlife Management*.

Camacho, CA, CJ Sullivan, MJ Weber, and CL Pierce. In press. Morphological identification of Bighead Carp, Silver Carp, and Grass Carp eggs using random forests machine learning classification. *North American Journal of Fisheries Management*.

Erickson, RA, JL Kallis, AA Coulter, DP Coulter, R MacNamara, J Lamer, WW Bouska, K Irons, L Solomon, MJ Weber, MK Brey, †C Sullivan, GG Sass, JE Garvey, and DC Glover. In review. Demographic variability of two invasive species along an invasion gradient: Bighead and silver carps in the Illinois, Ohio, and Mississippi rivers, USA. *Fisheries Research*.

Camacho, CA, CJ Sullivan, MJ Weber, and CL Pierce. In revision. Suitability of an Upper Mississippi River Tributary for Asian carp reproduction. *North American Journal of Fisheries Management*.

Fritts, AK, BC Knights, J Amberg, JH Larson, JJ Amberg, C Merkes, T Tajjoui, SE Butler, MJ Diana, DH Wahl, MJ Weber, and JD Waters. 2019. Development of a quantitative PCR method for screening ichthyoplankton samples for bigheaded carps. *Biological Invasions* 21: 1143-1153.

Whitledge GW, B Knights, J Vallazza, JH Larson, MJ Weber, JT Lamer, QE Phelps, and JD Norman. 2019. Identification of Bighead Carp and Silver Carp early-life environments and inferring Lock and Dam 19 passage in the Upper Mississippi River: insights from otolith chemistry. *Biological Invasions* 21: 1007-1020.

Sullivan, CJ, CA Camacho, MJ Weber, and CL Pierce. 2019. Influence of river discharge on Grass Carp occupancy dynamics in south-eastern Iowa rivers. *River Research and Applications* 35: 60-67.

Sullivan, CJ, MJ Weber, CA Camacho, DH Wahl, R Columbo, and CL Pierce. 2018. Factors regulating year-class strength of Silver Carp throughout the Mississippi River basin. *Transactions of the American Fisheries Society* 147: 541-553.

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Sullivan, CJ. 2016. Asian Carp population characteristics and dynamics in the Mississippi River watershed. MS thesis, Iowa State University.

Camacho, C. 2016. Asian Carp reproductive ecology along the Upper Mississippi River invasion front. MS thesis, Iowa State University.

In prep

Camacho, CA, CJ Sullivan, MJ Weber, and CL Pierce. In prep. Asian Carp reproduction in the tributaries of the Upper Mississippi River. To be submitted to River Research and Application.

Cozzola, A, NA Tillotson, CJ Sullivan, MJ Weber, and CL Pierce. In prep. Spatiotemporal variation in Silver Carp populations across the leading edge of the invasion of the Upper Mississippi River. To be submitted to Biological Invasions.

Matthews, A, MJ Weber, and CL Pierce. In prep. Bigheaded carp reproduction output is associated with environmental influences but not adult abundance. To be submitted to Biological Invasions.

Matthews, A, MJ Weber, and CL Pierce. In prep. Assessment of random forest model ability to identify fish eggs based on morphological characteristics. To be submitted to Canadian Journal of Fisheries and Aquatic Sciences.

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Table 1. Sampling site codes for 2019 sampling seasons. See Figure 1 for sampling locations.

Site Codes	Site Names
UMR-UPW	Upper Mississippi River, Upstream of the Wapsipinicon River
UMR-DNW	Upper Mississippi River, Downstream of the Wapsipinicon River
WAP-MTH	Mouth of the Wapsipinicon River
UMR-P15	Upper Mississippi River, Pool 15
UMR-UPR	Upper Mississippi River, Upstream of the Rock River
UMR-DNR	Upper Mississippi River, Downstream of the Rock River
ROC-MTH	Mouth of the Rock River
UMR-P17	Upper Mississippi River, Pool 17
UMR-UPI	Upper Mississippi River, Upstream of the Iowa River
UMR-DNI	Upper Mississippi River, Downstream of the Iowa River
IAR-MTH	Mouth of the Iowa River
UMR-UPS	Upper Mississippi River, Upstream of the Skunk River
UMR-DNS	Upper Mississippi River, Downstream of the Skunk River
SKK-MTH	Mouth of the Skunk River
UMR-UPD	Upper Mississippi River, Upstream of the Des Moines River
UMR-DND	Upper Mississippi River, Downstream of the Des Moines River
DSM-MTH	Mouth of the Des Moines River
DSM-KQA	Des Moines River at Keosauqua

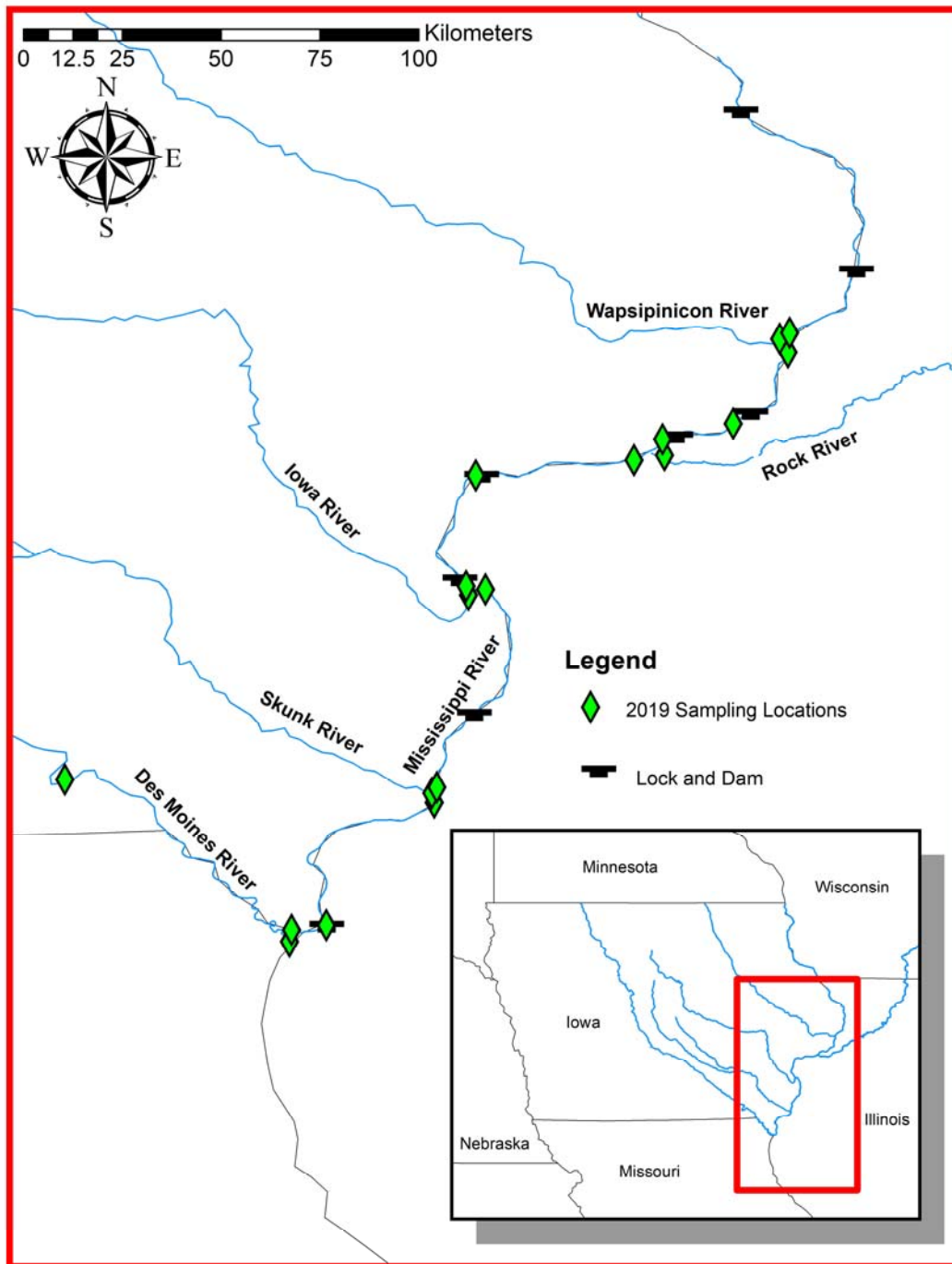


Figure 1. Map of study area on the southeast border of Iowa with the 18 sampling sites of chlorophyll *a*, fish eggs, and age-0 fishes indicated by green diamonds. Mississippi River lock and dams within the sampling reach in black.

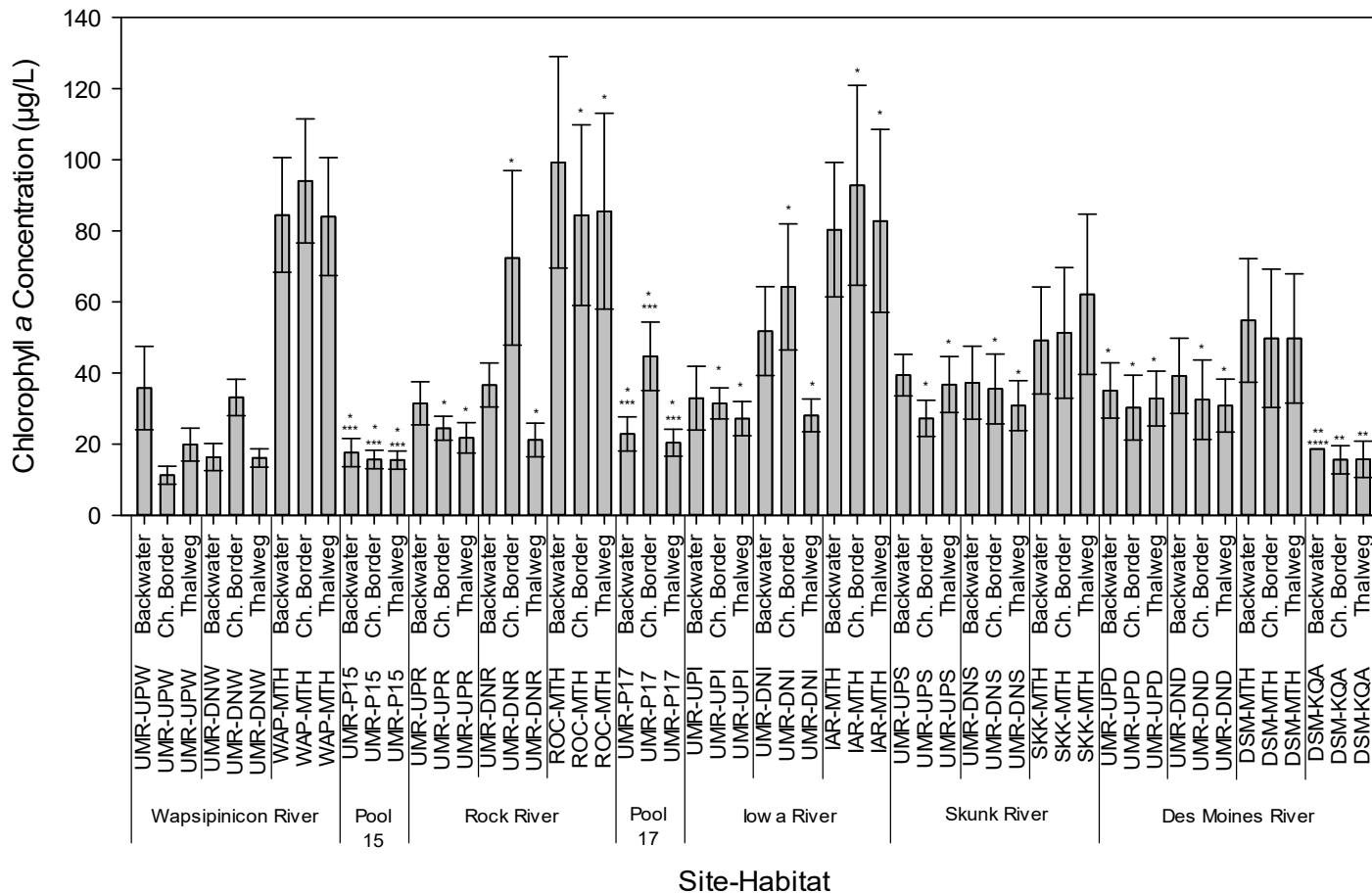


Figure 2. Chlorophyll *a* (mean ± SE) of each site collected between April 26th and August 19th, 2019.

* Not during the August 8th sampling session due to equipment malfunctions.

** The Des Moines River at Keosauqua was not sampled due to low flows and unsafe boating conditions.

*** The Mississippi River at Pool 15 and Pool 17 were not sampled on April 26th due to major flooding.

**** The backwater habitat of the Des Moines River at Keosauqua was only sampled once (April 26th) due to habitat availability after flooding.

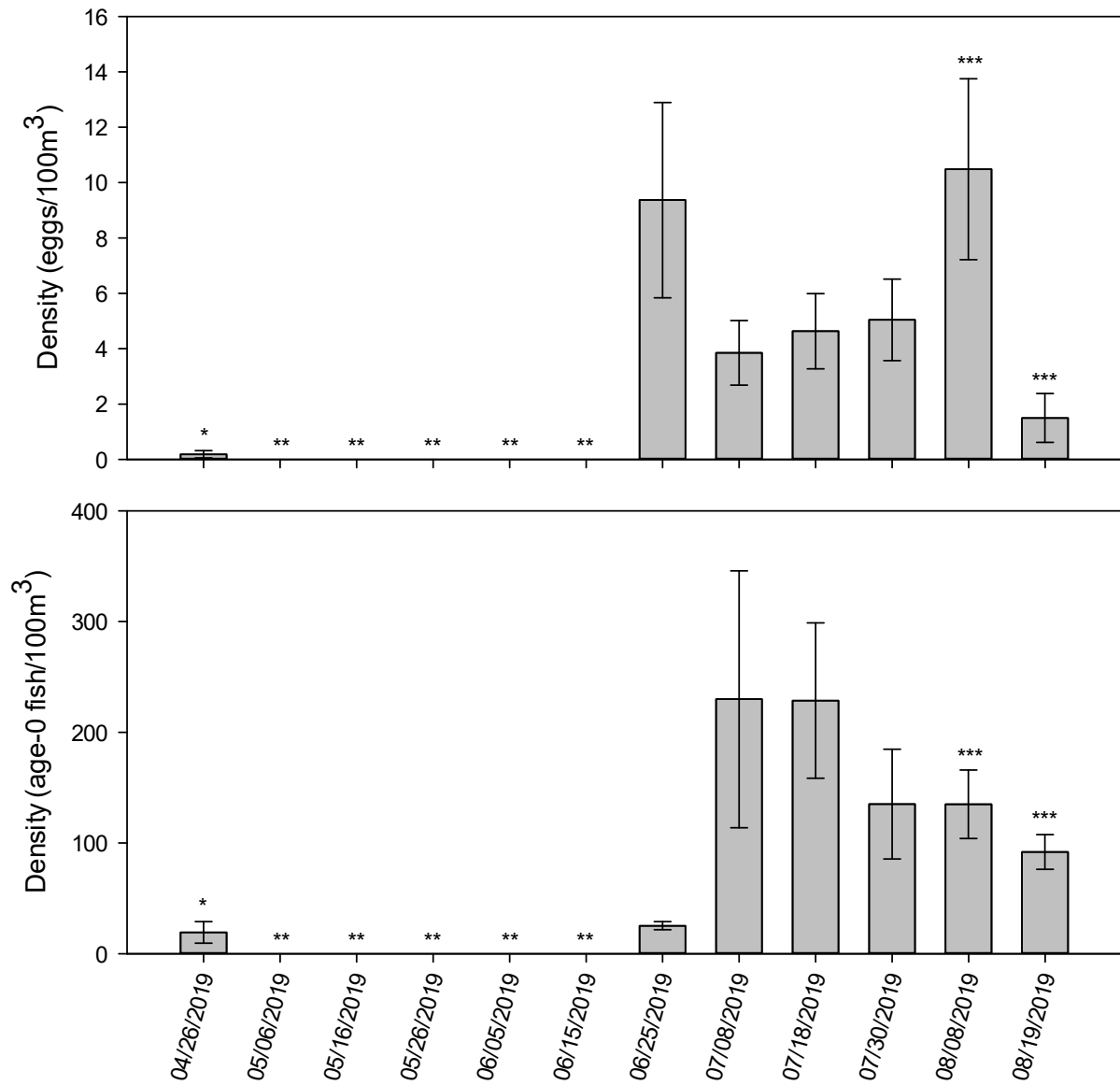


Figure 3. Density (mean \pm 1 SE) of fish eggs (top) and age-0 fishes (bottom) collected between April 26th and August 19th, 2019.

* The Mississippi River at Pool 15 and Pool 17 were not sampled on April 26th due to major flooding.

**None of the sites were sampled from May 6th – June 15th 2019 due to major flooding.

*** The Des Moines River at Keosauqua was not sampled due to low flows and unsafe boating conditions.

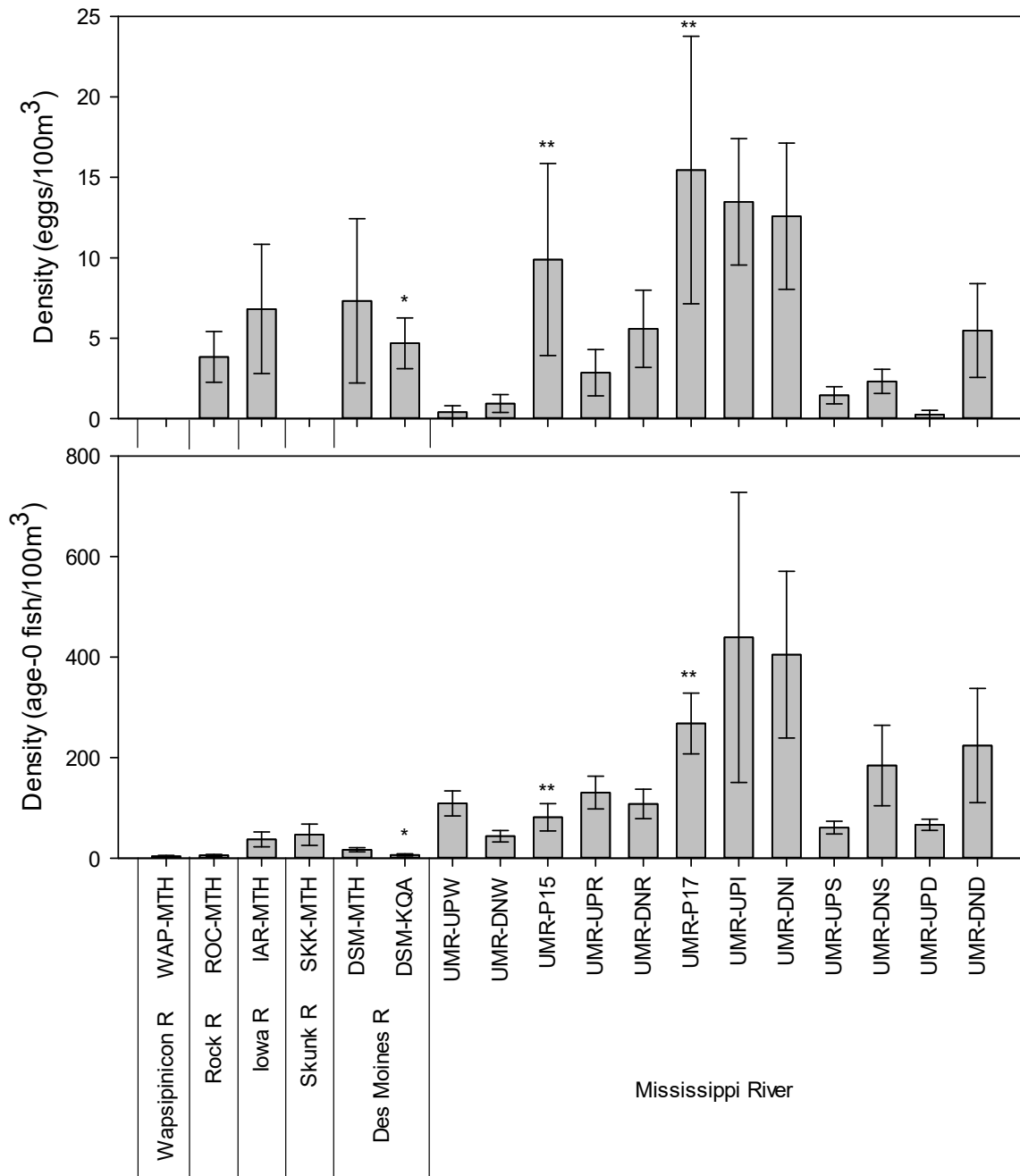


Figure 4. Density (mean \pm 1 SE) of fish eggs (top) and age-0 fishes (bottom) collected from each site during 2019. Site codes are defined in Table 1.

* The Des Moines River at Keosauqua was not sampled during the last two sampling events due to low flows and unsafe boating conditions.

** The Mississippi River at Pool 15 and Pool 17 were not sampled on April 26th due to major flooding.

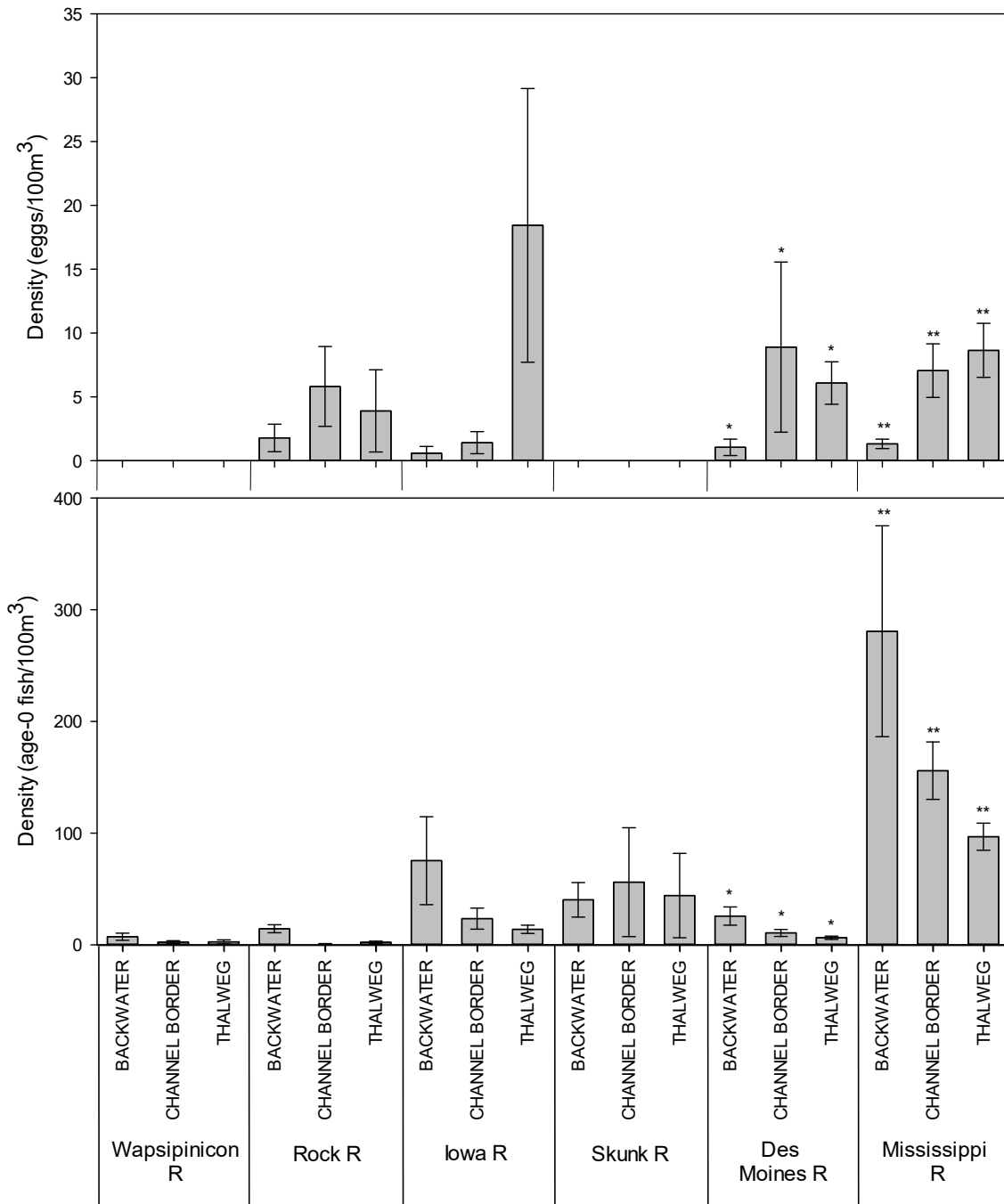


Figure 5. Density (mean \pm 1 SE) of fish eggs (top) and age-0 fish (bottom) by habitat from the Wapsipinicon (WAP-MTH), Rock (ROC-MTH), Iowa (IAR-MTH), Skunk, Des Moines river (DSM-MTH) and Keosauqua (DSM-KQA), and all sites sampled within the Mississippi river from UMR-UPR down to UMR-DND during 2019. A list of sites can be found on Table 1.

* The Des Moines River at Keosauqua was not sampled during the last two sampling events due to low flows and unsafe boating conditions.

** The Mississippi River at Pool 15 and Pool 17 were not sampled on April 26th due to major flooding.

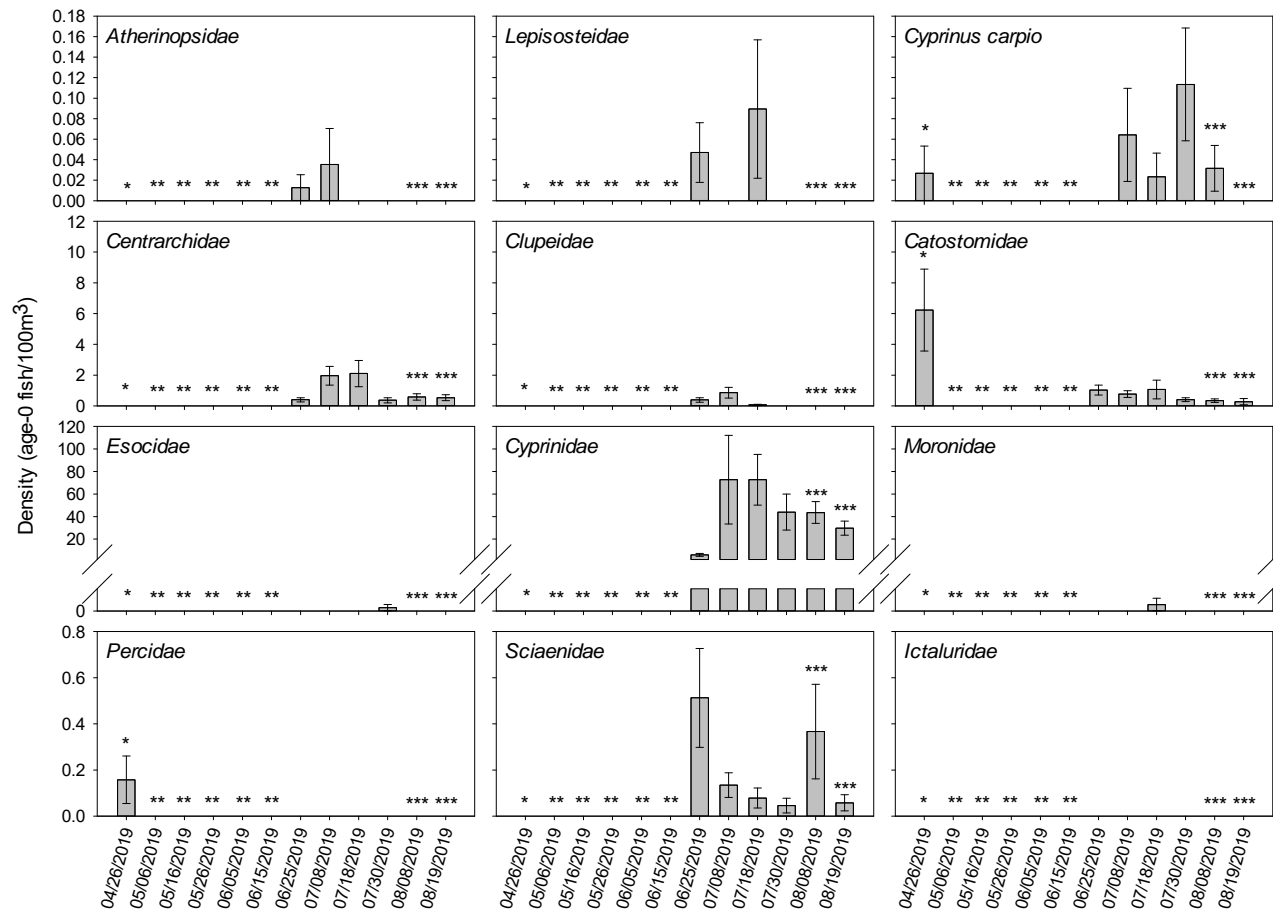


Figure 6. Density (mean \pm 1 SE) of age-0 fishes by family from each sampling session in 2019.

* The Mississippi River at Pool 15 and Pool 17 were not sampled on April 26th due to major flooding.

**None of the sites were sampled from May 6th – June 15th 2019 due to major flooding.

*** The Des Moines River at Keosauqua was not sampled due to low flows and unsafe boating conditions.