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ARTICLE

INVESTIGATION OF FRESHWATER MUSSEL GLOCHIDIA PRESENCE ON ASIAN CARP AND NATIVE FISHES OF THE ILLINOIS RIVER

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ABSTRACT

Densities of introduced Asian carp (Silver Carp and Bighead Carp) in the Illinois River Basin are among the highest in the world. Asian carp have been reported to serve as hosts for *Sinanodonta woodiana* in their native territories, but no research has been conducted on the potential for Silver or Bighead Carp to host North American freshwater mussels. Our objectives were 1) to examine the presence of glochidia on native and non-native fishes from the Illinois River Basin, 2) to determine an optimal concentration and duration of potassium hydroxide (KOH) exposure for increasing transparency of preserved fish gills to more effectively detect the presence of glochidia and parasites, and 3) identify parasite burdens. Fifteen fish species (12 native and 3 non-native) were collected from the Illinois River Basin during summer of 2014. Preserved fins and gills of native and non-native fishes were examined for glochidia and parasite infections. We determined that a 20 min 5% KOH bath was optimal for increasing gill transparency. We recovered 242 glochidia from 5 native fish species: Bluegill, Largemouth Bass, Smallmouth Bass, Freshwater Drum, and Sauger. Based upon morphometric data, we were able to identify the glochidial larval stage of 5 groups of freshwater mussels: Group A-Lilliput, Group B- Threeridge, Group C- Deertoe or Fawnsfoot, Group D- Threehorn Wartyback, and Group E- Fragile Papershell. We did not locate glochidia on any of the non-native fish species. Future research should include the use of laboratory host trials to elucidate if Asian carp could serve as successful host fishes for native mussels or if they are recruitment sinks, a possibility that could have a major impact on the future stocks of currently imperiled freshwater mussels.

KEY WORDS - Unionoida, parasites, *Hypophthalmichthys molitrix*, *Hypophthalmichthys nobilis*, *Cyprinus carpio*

INTRODUCTION

Freshwater mussels have experienced substantial declines in their populations worldwide over the past century (Starrett 1971; Lydeard et al. 2004). However, there have been positive developments in some locations where mussel populations have been able to recolonize areas from which they had previously been extirpated (Sietman et al. 2001). Unfortunately, the success of some of these recolonizations may be undermined by emerging threats. The introduction of Silver Carp (*Hypophthalmichthys molitrix*) and Bighead Carp (*Hypophthalmichthys nobilis*), hereafter referred to collectively as Asian carp, into North America has had a substantial negative impact on native fish species, including declines in condition indices and population size (Irons et al. 2007; Crimmins et al. 2015). However, little research has been conducted to evaluate how Asian carp are affecting other aquatic organisms.

The life cycle of freshwater mussels is complex and unique among bivalves. Larval mussels (glochidia) are released by the adult female and must attach to gills or fins of a suitable host fish (Kat 1984; Barnhart et al. 2008). If it is an appropriate host, glochidia remain attached to the fish for several days to several weeks and metamorphose into juvenile mussels. Juveniles are released from the host and fall to the benthic substrates to continue their life cycle as free-living organisms. Glochidia can also attach to non-suitable hosts but the fishes' immune systems eventually reject the glochidia, which fall from the fish and perish (Kat 1984).

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Asian carp have been reported to serve as fish hosts to freshwater mussels in their native range (Djajasasmita 1982; Girardi and Ledoux 1989; Domagala et al. 2007). However, no research has been conducted on the potential for Silver Carp and Bighead Carp to host North American freshwater mussels or to determine if they serve as recruitment "sinks." Asian carp densities in Illinois River Basin are among the highest in the world (~2,500 per river km; Sass et al. 2010), and glochidia may inadvertently attach to carp. If glochidia do not metamorphose, Asian carp may be reproductive sinks that prevent glochidia from attaching to suitable native host fishes.

We conducted a study to evaluate the potential effects of invasive Asian carp on native freshwater mussels. Our primary objective was to investigate the presence of glochidia on native and non-native fishes throughout the Illinois River Basin. Special emphasis was placed on examining three species of carp (Silver, Bighead, and Common Carp (*Cyprinus carpio*)), as these species may have the potential to intercept glochidia and interfere with glochidial attachment to suitable native fish hosts. Secondary objectives were to identify other parasite burdens carried by native and non-native fishes, as well as determining an optimal concentration and exposure time for potassium hydroxide (KOH) solution to increase clarity of preserved fish gills to more effectively locate and identify encysted glochidia.

METHODS

Fishes were collected during May, June, and July of 2014, which coincides with peak glochidial release for most freshwater mussel species in the Illinois River Basin (Watters et al. 2009). We targeted native fishes that coincidentally occur in microhabitat with Asian carp, as well as natives that are proven hosts for a variety of common mussels in the basin. We also collected Common Carp, a non-native species that has existed in the river since the 1800's and has been reported to be a marginal host for three North American freshwater mussel species (Lefevre and Curtis 1910; Hove et al. 2011a; Hove et al. 2014). Fishes were collected from the Upper Illinois River (Dresden and Marseilles reaches), the Middle Illinois River (La Grange Reach), as well as from several major tributaries to the Illinois River (Kankakee, Spoon, Mackinaw, and Sangamon rivers, and Salt Creek of the Sangamon).

Fishes were collected using a combination of pulsed-DC boat electrofishing, hoop netting, and fyke netting and were then returned to the laboratory for analysis. Smaller fish were preserved whole in 95% ethanol, while gills and fins were removed from larger fish (e.g., invasive carp) and were either preserved in 95% EtOH or were frozen. All Animal Care and Use protocols for fish collection, anesthetization, and euthanasia were followed (University of Illinois IACUC #14023). Freezing gills is not believed to influence detection of glochidia (Cunjak and McGladdery 1991). Fish were identified to species and then gill and fin tissues were examined for glochidia and other parasites under a Leica S8 APO stereomicroscope (Leica Microsystems, Wetzlar, Germany). When glochidia were found, they were counted, photographed with a Leica DMC 2900 digital camera (Leica Microsystems, Wetzlar, Germany), and measured for their length (parallel to hinge), height (perpendicular to hinge), and hinge length using Leica scale bar measurements in ImageJ (version 1.46r). Glochidia were left in the gill tissue unless they released from the tissue during processing. Efforts were made to orient all of the glochidia onto a level plane prior to being photographed, and only glochidia with suitable orientations were used for measurements. To ensure accuracy of our measurements, a subset of glochidia were measured by using three different techniques (Leica scale bar measurements in ImageJ, micrometer photograph measurements in ImageJ, and Leica measurements using the Leica measurement software). Efforts were made to identify the glochidia using morphological features to the lowest taxonomic level possible. Based upon morphometric data and recent mussel community information from the Illinois River, we used a bivariate plot and simple qualitative overlap to determine the most-likely identity of each glochidium, as our sample size was small (Kennedy and Haag 2005). Morphometric data were derived from Waller (1987), Hoggarth (1999), Williams et al. (2008) and references therein, Watters et al. (2009), Hove et al. (2012), Hove et al. (2015) and M.C. Hove (Macalester College, personal communication). When other types of parasites were encountered, the parasite type and infection intensity was also recorded for all of the native and non-native fishes. We also documented the occurrence of telangiectasia, a condition in which the blood vessels within the lamellae of the gill filaments burst and blood pools in the lamellae tips, causing the gills to have cyst-like structures along the gill margins that superficially resemble parasitic infections. We recorded the occurrence of these structures and compared the probability of occurrence in native versus non-native fishes using logistic regression (R Core Package 2015).

Potassium hydroxide study for gill clarification optimization

We completed an experiment to determine the most effective processing treatment for detection of gill parasites. Gill size was determined by measuring from dorsal to ventral margin of an entire gill arch removed from the fish; small, medium, and large size classes were approximately 10mm (i.e., Spotfin Shiner, *Cyprinella spiloptera*), 10-50mm (i.e., Smallmouth Bass, *Micropterus dolomieu*), and greater than 50mm (i.e., Silver Carp), respectively. Three concentrations of KOH were used: 2%, 5%, and 10% KOH. Transparency and intactness of the gills were evaluated at six different time points: 1, 5, 10, 20, 30, and 60 minutes. A black and white grid (cell size of 10×10 mm) was used to record transparency by determining when the transition line from black to white was visible through the gill filament. Transparency was noted for tips or edges of gill filament, mid-vein of filament, and whole filament. Intactness was determined by picking up the gill and recording if parts of it began to disintegrate.

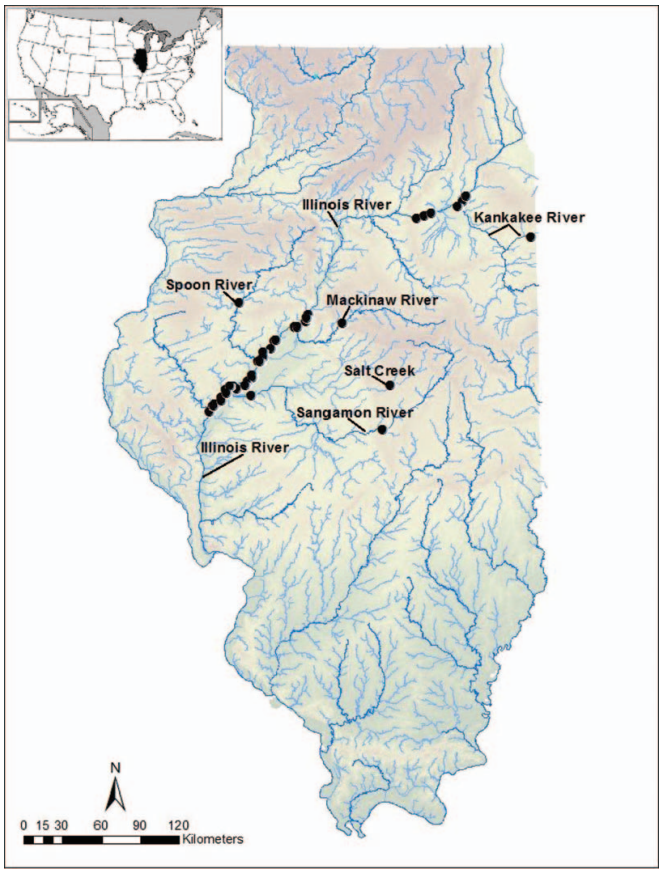

Figure 1: Fish collection sites on the Illinois River and its tributaries, Illinois, USA.

RESULTS

Fishes were collected from 66 sites between 20 May 2014 and 22 July 2014 (Figure 1). We analyzed a total of 435 fishes for this study, including 145 non-natives (92 Silver Carp, 3 Bighead Carp, and 50 Common Carp) and 290 native fishes (12 species from 5 families) that are known to be host fish for the larval stage of at least one freshwater mussel species (Table 1). Of the total number of native fish collected, 10.7% were infected with glochidia, and five native fish species were infected. Infection rates among these species ranged from 12.5-100%. We recovered 242 glochidia from the 5 native fish species: Bluegill (*Lepomis macrochirus*), Largemouth Bass (*Micropterus salmoides*), Smallmouth Bass, Freshwater Drum (*Aplodinotus grunniens*), and Sauger (*Sander canadensis*) (Table 2). We identified the glochidial larval stage of 5 groups of freshwater mussels: Group A-Lilliput (*Toxolasma parvum*), Group B- Threeridge (*Amblema plicata*), Group C- Deertoe (*Truncilla truncata*) or Fawnsfoot (*Truncilla donaciformis*), Group D-Threehorn Wartyback (*Obliquaria reflexa*), and Group E- Fragile Papershell (*Leptodea fragilis*) (Table 3, Figure 2).

We recovered 9 types of non-glochidial parasites on 10 fish species. These parasites included: anchor worms (*Lernaea* sp.), black grub (*Neascus*), white grub (*Posthodiplostomum minimum*), yellow grub (*Clinostomum* sp.), monogenean trematodes (*Dactylogyrus* sp.), digenean trematode (*Bolbophorus* sp.), copepods, leeches, and nematodes. Telangiectasia were documented in eight fish species, and this phenomenon occurred more frequently in non-native fishes (Silver Carp and Common Carp) (Figure 3, Table 4). Non-native fishes were 4.3 times more likely to have telangiectasia than native fishes (95% confidence interval = 2.9 to 7.9 times more likely).

Potassium hydroxide study for gill clarification optimization

The optimal concentration of KOH was determined to be a 5% KOH bath for 20 minutes. This concentration provided maximum transparency without causing excessive gill tissue deterioration. Smaller fishes and frozen Silver Carp gill specimens reached a sufficient level of transparency in less

Table 1. Native and non-native fishes collected in the Illinois River and its tributaries during May, June, and July of 2014.

Common name	Scientific Name	Tributaries	Upper Illinois	Middle Illinois
Gizzard Shad	*Dorosoma cepedianum*	0	1	0
Red Shiner	*Cyprinella lutrensis*	0	0	93
Spotfin Shiner	*Cyprinella spiloptera*	5	31	0
Common Carp	*Cyprinus carpio*	0	27	22
Silver Carp	*Hypophthalmichthys molitrix*	53	0	39
Bighead Carp	*Hypophthalmichthys nobilis*	1	0	2
Spottail Shiner	*Notropis hudsonius*	0	0	9
Bluntnose Minnow	*Pimephales notatus*	0	5	0
Bullhead Minnow	*Pimephales vigilax*	0	9	31
Green Sunfish	*Lepomis cyanellus*	0	1	0
Bluegill	*Lepomis macrochirus*	0	29	28
Smallmouth Bass	*Micropterus dolomieu*	0	4	0
Largemouth Bass	*Micropterus salmoides*	0	14	5
Sauger	*Sander canadensis*	0	1	0
Freshwater Drum	*Aplodinotus grunniens*	0	0	24

Table 2. Native fish species from the Upper and Middle Illinois River (ILR) with glochidia presence. N = number of native fish collected.

Fish species	Upper ILR	Middle ILR	Total N	% infested	No. glochidia	Mean glochidia/fish
Bluegill	16	3	57	33%	178	9.4
Smallmouth Bass	1	0	4	25%	1	1.0
Largemouth Bass	7	0	19	37%	8	1.1
Sauger	1	0	1	100%	38	38.0
Freshwater Drum	0	3	24	13%	17	5.7

time, but the intactness of their gills was not negatively affected after 20 minutes of KOH exposure.

DISCUSSION

We did not recover glochidia from any non-native fishes in this study. This result was not entirely unexpected for a number of reasons. First, if Asian carp were intercepting glochidia but were not suitable hosts, it is likely that the glochidia would slough from the carp within 1-4 days (Arey 1932a; Zale and Neves 1982a; Waller and Mitchell 1989). To document attachment, we would need to have collected fishes within a short period of time (i.e., <4 days) after they encountered the glochidia. Second, the Illinois River was in flood stage for most of the summer during 2014. Any glochidia that would have been present in the system, particularly in the water column, would have been more dilute than in a regular flow year. The flooding may have contributed to us not finding glochidia on any of our pelagic species (e.g., native cyprinids or Silver Carp). We also found no evidence of natural infestation on the benthic dwelling Common Carp. The gills of this species were rather difficult to process; the densely packed gill filaments of the Common Carp began to deteriorate in the KOH bath more quickly than any other species. This phenomenon could have decreased our ability to detect glochidia on Common Carp. Further, they are only considered marginal hosts for three native mussel species in Illinois that are considered host generalists: Rock Pocketbook (*Arcidens confragosus*), Flutedshell (*Lasmigona costata*), and Giant Floater (*Pyganodon grandis*) (Lefevre and Curtis 1910; Hove et al. 2011a; Hove et al. 2014). Thus glochidia would be subject to sloughing in a short time period in this case as well.

The majority of the native species that were infested with glochidia were collected in the Upper Illinois River, where recent surveys have seen a considerable rebound in mussel populations over the last decade (INHS Mollusk Collection; http://wwx.inhs.illinois.edu/collections/mollusk; accessed 1 July 2015). This recovery may be a response to improved water quality conditions brought forth by the Clean Water Act of 1972, as an extensive mussel survey in 1966 revealed extremely low mussel populations (Starrett 1971). Fifty-five percent of the Bluegill and 50% of Largemouth Bass collected from the Upper Illinois River were infected with glochidia. The best fit mussel species for identified glochidia are from the tribes Lampsilini and Amblemini. Lampsiline species typically have high fecundity, shorter life spans, and are host specialists that utilize only a single host fish species or a few species within a genus or family. Fragile Papershell, Deertoe, and Fawnsfoot are Freshwater Drum specialists and cannot metamorphose on any other fish species. Lilliput likely use *Lepomis* spp. and potentially darters as hosts (Mermilliod *in* Fuller, 1978; Hove, 1995; Watters et al. 2005). Threehorn Wartyback are known to metamorphose on cyprinids, but marginal metamorphosis success on fishes from several families has also been observed (Watters et al. 1998, B.R.

Table 3. Mean (±1 SE) and ranges (in parentheses) for measurements of glochidia recovered from naturally infested fishes of the Upper and Middle Illinois River (ILR). N = number of glochidia identified from each reach. Best fit species were assigned based upon established glochidial measurements from the literature and knowledge of the current mussel assemblage present in the ILR. Fish species = species from which the glochidia were obtained.

Glochidia group	Height (μm)	Length (μm)	Hinge (μm)	N, Upper ILR	N, Middle ILR	Total N	Best fit species	Fish species
A	176 ± 1 (163-188)	158 ± 1 (146-171)	87 ± 1 (78-97)	52	0	52	Lilliput	Bluegill, Sauger
B	208 ± 1 (206-210)	187 ± 1 (182-190)	134 ± 1 (131-136)	3	2	5	Threeridge	Bluegill, Largemouth Bass, Freshwater Drum
C	54 ± 1 (52-58)	57 ± 3 (49-64)	36 (36-36)	0	7	7	Deertoe, Fawnsfoot	Freshwater Drum
D	263 ± 7 (251-281)	251 ± 1 (243-259)	147 ± 3 (139-154)	0	15	15	Threehorn Wartyback	Bluegill
E	77	71	45	0	1	1	Fragile Papershell	Freshwater Drum

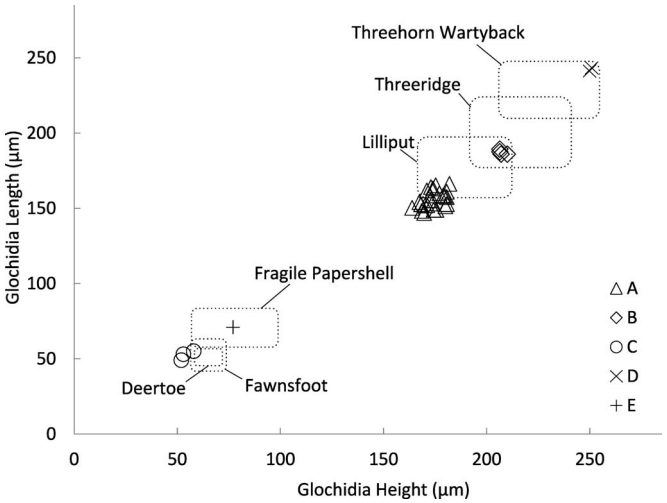

Figure 2. Bivariate plot of glochidia height and length, with assumed groups labeled as A, B, C, D, or E. Published size ranges for known glochidia are represented by labeled dotted lines.

Bosman, Texas Tech, personal communication). Amblemines are typically longer-lived, slower to reach sexual maturity, and can be host specialists or generalists (Watters et al. 2009). The Threeridge is a host generalist that can utilize many fish species, which is a factor that likely contributes to its dominance among mussel fauna within the Illinois River (Haag 2012).

We used a combination of morphological measurements and current species distribution to determine the most likely species assignment of attached glochidia. There were additional species that did overlap morphometrically with our glochidia but were eliminated based on known status of freshwater mussels in Illinois. Specifically, Spike (*Elliptio dilatata*) and Spectaclecase (*Margaritifera monodonta*) both have similar measurements as our designated Groups B and C, respectively, but have not been recovered alive from the Illinois since the early 20th century (Starrett 1971, Sietman et al. 2001). However, there have been recent discoveries of species considered extirpated, such as Scaleshell (*Leptodea leptodon*) (Illinois Natural History Survey Mollusk Collection; http://wwx.inhs.illinois.edu/collections/mollusk; accessed 1 July 2015), thus we cannot completely rule out the possibility that Spike or Spectaclecase may be attached to native fishes in the Illinois River.

Glochidia size can vary widely within a species throughout its range (Hove et al. 2011b, 2012, and M.C. Hove Macalester College, personal communication). An optimal situation would be to collect gravid females from the same waterbody and compare known glochidia morphology with the unknown glochidia from natural infestations. This was not an option in this study due to the flooding/sampling conditions at the time of fish collection; additionally, the availability of preserved specimens with mature glochidia from the Illinois River is also very limited, given the recent recolonization of this water body. Future research involving naturally infested glochidia should strive to include the collection of brooding females and glochidia from the areas of interest to allow for more accurate morphological comparisons.

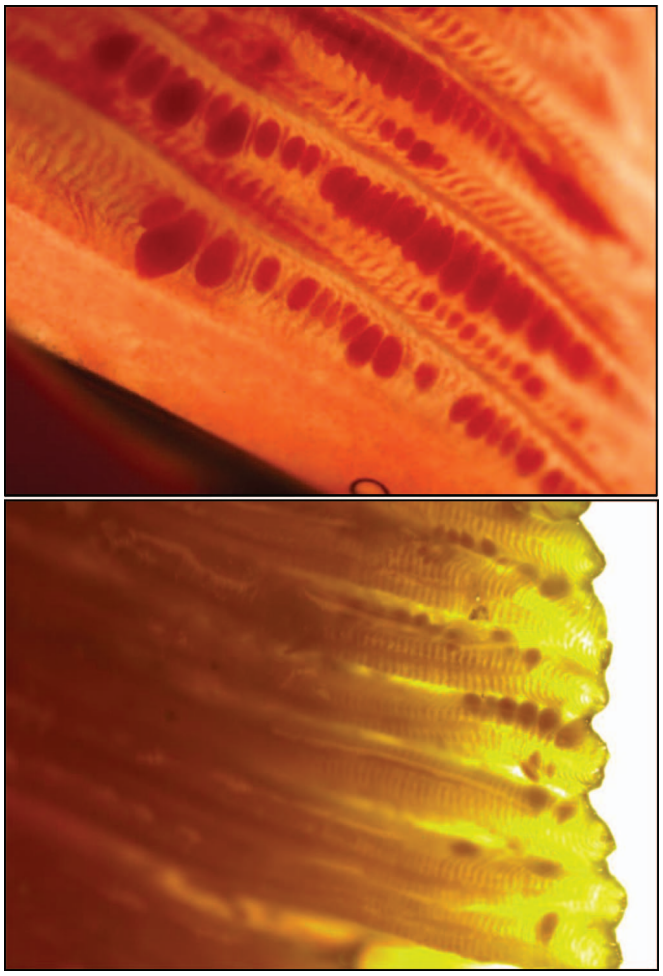

Figure 3. The occurrence of telangiectasia in gill filaments on Silver Carp. Top photo is of a freshly collected gill and the bottom photo is a gill that had been preserved in 95% EtOH.

In much of the literature with wild-caught fish, thousands of fish were examined to elucidate glochidial infestation levels (Zale and Neves 1982b; Neves and Widlak 1988; Weiss and

Table 4. Occurrence of telangiectasia on native and non-native fishes in the Illinois River (ILR) and its tributaries. Total N = total number of fish collected per species.

Fish species	Tributaries	Upper ILR	Middle ILR	Total N
Red Shiner	–	–	16	93
Common Carp	–	–	16	49
Silver Carp	7	–	39	92
Bullhead Minnow	–	–	1	40
Bluegill	–	–	3	57
Smallmouth Bass	–	2	–	4
Largemouth Bass	–	–	1	19
Freshwater Drum	–	–	6	24

Layzer 1995; Boyer et al. 2011). Thus, the fact that we found glochidia on a large percentage of the relatively low numbers of fishes examined during this study suggests that the Illinois River mussel community is recovering. However, it also means we would need to examine a substantial number of Asian carp to truly rule out the possibility that carp are not intercepting glochidia, since we only examined 145 carp during this study.

Silver, Bighead, and Common Carp did not carry many parasites, but Silver and Common Carp did have a substantially higher occurrence of telangiectasia hemorrhages compared to native fishes. This is a novel finding that is not well reported in the literature. It is unknown as to why non-native fishes may experience a higher degree of hemorrhages. There was also a higher occurrence of this condition in main stem fishes compared to tributaries, despite the fact that the fishes were collected with the same protocols and electrofishing settings. Variation in conductivity between the tributaries and the main stem may have contributed to the pattern; conductivity in the tributaries was generally lower than that in the main stem and pulsed DC-electrofishing can be affected by different conductivities (M.W. Fritts and R.M. Pendleton, Illinois Natural History Survey, C. Morgeson, Eastern Illinois University, personal communication). This may contribute to the increased presence of hemorrhages, but ultimately the cause of this phenomenon remains unresolved and will need additional research.

Asian carp are voracious filter-feeding consumers that can filter particles (e.g., plankton and algae) as small as 10-20 μm (Jennings 1988; Smith 1989; Vörös 1997). This feeding behavior introduces the potential for Asian carp to be consuming glochidia, an area of research that is currently unstudied. We focused our laboratory efforts on the filament structure of the gills, because this is the most likely location for glochidial gill-attachment in native fishes (Arey 1932b). However, the Asian carp could have been collecting glochidia with the fused, sponge-like gill rakers and subsequently ingesting the glochidia. Further studies should consider examining the gill rakers in addition to the filaments. It is unlikely that we would be able to detect glochidia in the stomach contents, but use of advance dietary studies, such as stable isotope analysis, may shed light on the potential for Asian carp to be ingesting glochidia.

Future work should include conducting laboratory host trials to evaluate the physiological ability of Asian carp to successfully transform any of our native mussel species, focusing on both host specialists and host generalists. This would be an efficient method to determine whether the Asian carp could successfully metamorphose glochidia to the juvenile life stage. If they are not suitable hosts, we could determine the period before untransformed glochidia are sloughed.

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