

REGULAR ARTICLE

INVASIVE BLACK CARP AS A RESERVOIR HOST FOR THE FRESHWATER MOLLUSK PARASITE *ASPIDOGASTER CONCHICOLA*: FURTHER EVIDENCE OF MOLLUSK CONSUMPTION AND IMPLICATIONS FOR PARASITE DISPERSAL

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ABSTRACT

Black Carp (*Mylopharyngodon piceus*) has invaded the Mississippi River and is a potential threat to native mollusks. During prior diet research, we discovered that the fluke *Aspidogaster conchicola*, a mollusk parasite, occurs regularly in the gastrointestinal tract of Black Carp. The fluke remains in fish intestines for extended periods after the fish has consumed its host. Flukes were found in 33% of the wild Black Carp examined, and numbers ranged from 1 to 802, with no pattern evident across seasons of fish capture. Treating the flukes as indicators of prior mollusk consumption, we adjusted the percent occurrence of mollusks from 26.6% to 54.1%, indicating that the previously reported incidences for bivalves (22.8%) and gastropods (16.5%) in the diet of wild Black Carp are likely to be underestimated. Based on percent occurrences in Black Carp, larger fish (>791 mm) had significantly higher fluke occurrence (42.6%) and fish captured from lentic habitats had significantly greater fluke-adjusted mollusk occurrence (87.5%). These diet-occurrence estimates, coupled with the presence of gravid *A. conchicola* and evidence of their continued viability in Black Carp intestines, indicate that these fish retain evidence of mollusk consumption for extended periods after evacuation of the gastrointestinal tract. Consequently, Black Carp has the potential to disperse this parasite to other mollusks.

KEY WORDS: invasive carp, reservoir host, fluke parasites, mollusks, diet

INTRODUCTION

Aspidogastreaan trematodes, such as the common and widespread *Aspidogaster conchicola* Von Baer (Trematoda: Aspidogastridae), are regularly encountered parasites in freshwater unionid mussels and gastropods in the United States (Hendrix et al. 1985; Alves et al. 2015). This group of flukes can reside as a secondary occurrence in vertebrate species that feed on their hosts, including fish and turtles (Fulhage 1954; Rohde 1972, 2002). Currently, however, direct

infection of vertebrate hosts by eggs or larval stages is unknown in this group of flukes. Compared with digenetic flukes with life cycles that include intermediate hosts, this fluke has a more ancestral life cycle (Olson et al. 2003) with direct development that requires only one host for maturation. Infection occurs when a mollusk ingests the egg stage during feeding on benthic substrates (i.e., grazing by snails) or—in unionid mussels—through their filtering apparatus (Huehner 1984). Autoinfection also has been suggested in this species, where the entire ontogenetic development may occur within one host individual (Williams 1942; Rohde 1973, 1994). Nonciliated fluke larvae (aspidocidia; Huehner and Etges

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1977) hatch immediately after uptake and begin maturation, most often residing in the pericardial cavity, renal cavity, or viscera of the host mollusk (Huehner and Etges 1981); however, under certain conditions, the eggs or young larvae may become encapsulated or encysted in certain body tissues (Pauley and Becker 1968). This fluke possesses a ventral adhesive disk (opisthaptor) used as a holdfast, a sensory organ, and a source for secretion of digestive enzymes (Bakker and Diegenbach 1974; Fredricksen 1980). Adult flukes feed on the hemolymph and epithelial cells of the host by ingestion through the mouth (Bakker and Davids 1973; Huehner et al. 1989). Fish and other vertebrates consume infected mollusks and act as facultative hosts for *A. conchicola* (i.e., reservoir hosts), where adult flukes remain in the intestinal tract for extended periods after tissues of the mollusk host are digested (Rohde 1972; Evtushenko et al. 1994). The fluke *A. conchicola* has been reported in the intestinal tract of several freshwater fish species worldwide (Alves et al. 2015), including Grass Carp (*Ctenopharyngodon idella*) and Black Carp (*Mylopharyngodon piceus*) in Eurasia, and most recently, in wild-caught Black Carp of the Mississippi River Basin (Poulton et al. 2019). In Eurasia, among the parasites found in Zebra Mussels (*Dreissena* spp.), *A. conchicola* is the only fluke species that is also native to North America (Molloy et al. 1997).

The Black Carp is one of four invasive species that are commonly referred to as Asian carps; the others are Bighead Carp (*Hypophthalmichthys nobilis*), Silver Carp (*Hypophthalmichthys molitrix*), and Grass Carp (Kocovsky et al. 2018). These fish are receiving increasing attention in the United States because of their potential adverse effects on native species and aquatic ecosystems (Chapman and Hoff 2011; Nico and Neilson 2019; USFWS 2019). Black Carp was imported to the United States in the 1970s and 1980s to control digenetic trematodes in aquaculture by consuming their snail hosts; the intent was to improve the quality of aquaculture-produced food fishes (Venable et al. 2000; Ledford and Kelly 2006). Black Carp is characterized by molariform pharyngeal teeth adapted to crush mollusk shells during feeding (Liu et al. 1990; Shelton et al. 1995; He et al. 2013). The known molluscivorous habits of Black Carp—in combination with the expanding geographic range of wild fish within the Mississippi River Basin (Kroboth et al. 2019)—have led to concern that they may threaten populations of native and imperiled unionid mussel species in the United States (Nico et al. 2005; DeVaney et al. 2009; Nico and Jelks 2011; Hodgins et al. 2014). Most of the research available on Black Carp has been based on aquaculture studies. For example, Nico et al. (2005) summarized accounts for trematode parasites that reside in Black Carp muscle or liver tissue and use mollusks as intermediate hosts, but further noted the lack of information on parasites infecting wild populations. Diet, diseases, parasites, and ecological consequences of establishment have been identified as key components in the ongoing assessments of Black Carp invasion risk into the Great Lakes region (D.A.R. Drake, Fisheries and Oceans Canada, Burlington, Ontario,

Canada, personal communication) and Mississippi River Basin (ACRCC 2019; USFWS 2019).

Understanding the frequency and extent of mollusk consumption in Black Carp is important for evaluating both diet composition and risk of invasion to native mollusks as well as for assessing effects on invasive mollusks. Bivalves and gastropods in the diet are most easily identified through their shells or shell fragments, but sometimes they cannot be identified or counted because these fish partially regurgitate mollusk shell material or expel fragments without swallowing them. During recent examination of wild Black Carp diets (Poulton et al. 2019), the fluke *A. conchicola* was commonly encountered in the gastrointestinal tract, even in fish that contained no other evidence of mollusk consumption. The ecological significance of this discovery was unknown at the time of that publication, but we recognized the need to further investigate the presence of these flukes in Black Carp and their importance to freshwater mollusks. As a reservoir host, Black Carp may share the same life stage of *A. conchicola* with the primary host mollusks, but also could serve as a source of infective organisms (Haydon et al. 2002) in addition to providing evidence that primary hosts were consumed in the diet some time before capture. Here, we report the specific interpretation of fluke incidence in the gastrointestinal tract from the original 109 wild Black Carp examined (Poulton et al. 2019), including the treatment of flukes as indicators of previous mollusk consumption and the potential role of Black Carp as a vector for dispersal of *A. conchicola*. Specifically, our study goals were to (1) report the abundance (infection rate) of *A. conchicola* flukes in wild Black Carp and any seasonal patterns in their numbers among fish examined, (2) provide an alternate method in estimating percent occurrence of mollusks in Black Carp diet based on the presence of flukes, and (3) test the significance of fluke and mollusk occurrence among fish of different sizes and the habitats where fish were captured. We also review the current ecological knowledge on *A. conchicola* flukes as related to freshwater mollusks, including longevity, viability, host pathways, potential damage to host tissues, and parasitic transmission. We also discuss the potential role of Black Carp as a vector of this mollusk parasite and summarize our study findings considering the risk to mollusks within the current range of Black Carp in the Mississippi River Basin. Our intent is to improve the understanding of linkages between wild Black Carp and mollusk hosts and to provide critical information for mollusk conservation efforts in riverine systems.

FIELD-SITE DESCRIPTION

Wild Black Carp, captured year-round from the Mississippi River Basin during 2009–2017, were acquired through collaborative research efforts involving commercial fisherman, federal and state agencies, and universities. They were used for multiple research projects, including distribution monitoring, life history, genetics, determination of origin, and age and growth. Black Carp were captured with hoop nets (51%; the

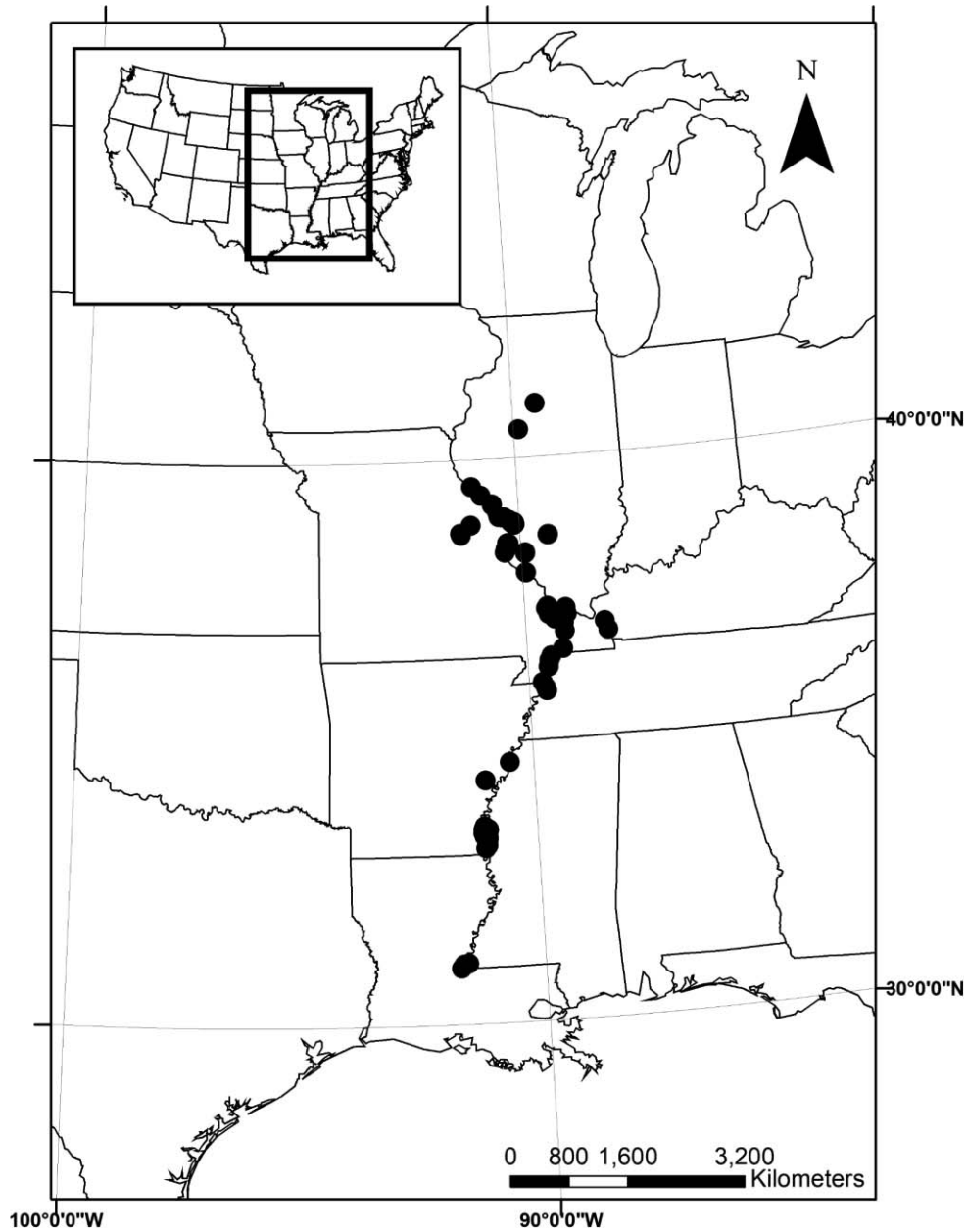


Figure 1. Capture locations of Black Carp (*Mylopharyngodon piceus*) examined in this study from the Mississippi River Basin in 2009–2017 ($n = 109$).

others were captured with various or unreported methods) by commercial fishing activities in riverine and backwater habitats. The geographic range of samples included the Mississippi River mainstem and the Atchafalaya, Cumberland, Illinois, Kaskaskia, Ohio, and White River basins (Fig. 1). Specific fish capture locations, methods, and dates are available at <https://doi.org/10.5066/P9K88CWF>.

METHODS

Gastrointestinal tract samples were dissected from 109 wild-caught Black Carp and preserved in formalin for diet analysis. Laboratory preparation methods, taxonomic identifi-

cation of contents, and taxa-specific data analysis for these samples are detailed in the recently published diet study (Poulton et al. 2019). Flukes removed from these samples were stained and mounted on glass slides for species identification via electron microscopy, based on descriptions by Bailey and Tompkin (1971), Hathaway (1971), Halton and Lyness (1971), and Huehner and Etges (1977). To investigate any seasonal patterns in flukes, we compared fluke abundance (number of flukes found in individual fish) across seasons (astronomical winter, spring, summer, and fall). To provide additional comparisons of mollusks and flukes present in the gastrointestinal tract, we used frequency of occurrence based on diet data (Poulton et al. 2019). This occurrence is defined as

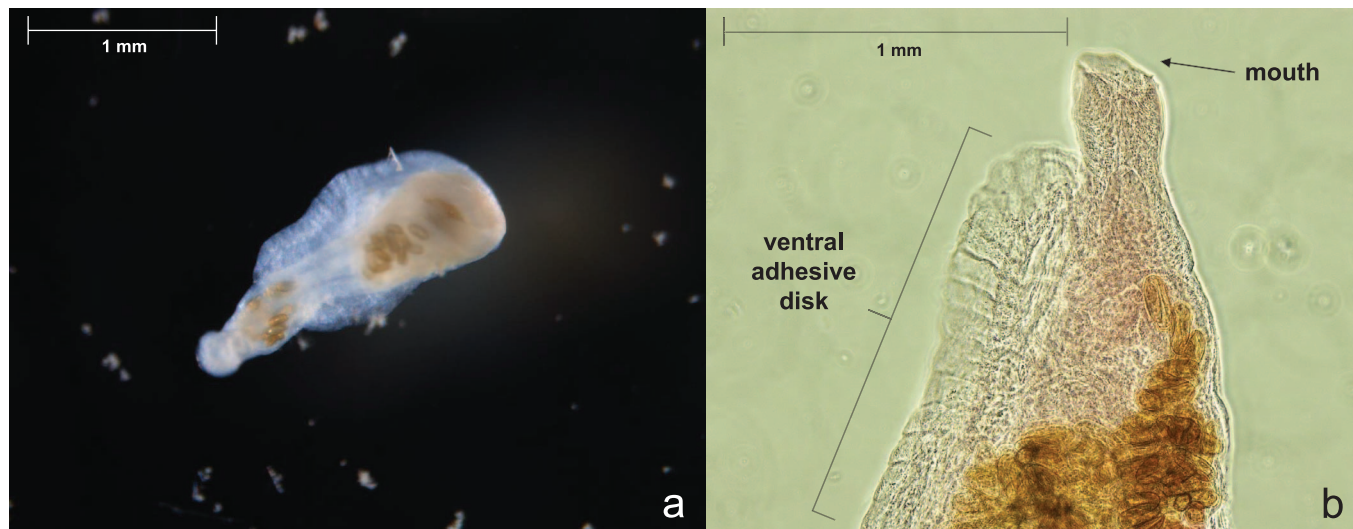


Figure 2. The fluke *Aspidogaster conchicola* found in the gastrointestinal tract of Black Carp (*Mylopharyngodon piceus*) captured from the Mississippi River Basin in 2009–2017. The images represent (a) dorsal view, showing gravid fluke with eggs, and (b) lateral view, showing buccal funnel (mouth) and opisthaptor (adhesive disk). Photograph© (a) Anne Herndon, (b) Jennifer Bailey.

the relative number of fish (percentage of total examined) that contained each specific diet group or taxa (percent incidence of Buckland et al. 2017). This diet measurement, as used for inventory of preserved gastrointestinal contents in this study, is based on presence–absence data only and does not take into account the abundance of diet items (i.e., in samples or the environment) or the timing of feeding, ingestion, or digestion (i.e., when items were ingested or gut evacuation rates).

For comparisons and statistical analyses, we recalculated percent occurrence for mollusks among diet samples, accounting for the presence of flukes as an indicator of prior mollusk consumption (=fluke-adjusted mollusks). We accomplished this by using the original mollusk occurrence estimate of 26.5% (Poulton et al. 2019) and adding percent occurrence estimates for the following: (1) fish containing *A. conchicola* flukes only and (2) fish containing flukes co-occurring with nonmollusk diet items. To investigate whether fluke occurrence differed among fish size classes or capture locations, we compared fluke classes (percent occurrence of flukes and fluke-adjusted mollusks) across two fish sizes (above and below the median total length, in millimeters) and two fish capture location habitats (lotic, mainstem flowing water habitats, including side channels and chutes; and lentic, off-channel areas, including backwater sloughs, oxbows, or reservoirs). Because of nonnormality of the data and bias associated with fish capture, we used nonparametric Kruskal–Wallis/Mann–Whitney U-tests ($\alpha = 0.05$) for these comparisons (Excel formatted for analysis; Microsoft, Redmond, WA, USA). Based on this information, we reinterpreted the contribution of mollusks to Black Carp diets and provide discussion of implications related to the risk of Black Carp invasion on native freshwater mollusks and the potential of *A. conchicola* dispersal.

RESULTS

The fluke *A. conchicola* (Fig. 2) was present in 36 (33%) of the 109 wild Black Carp examined. The abundance of flukes within individual fish (mean = 50.1, SE = 23.1) varied widely from 1 to 802, and seasonal means ranged from 3.4 to 122.4, with the highest mean in fish captured during spring (Fig. 3). All five fish with more than 100 flukes in their gastrointestinal tract were captured during May–September, but we found no significant differences in fluke abundances in Black Carp across seasons (Fig. 3). This fluke was the only diet item found in the gastrointestinal tracts of 18 fish and co-occurred with nonmollusk diet items in 12 additional fish (Fig. 4). Only 6 of the 36 fish containing flukes had shell remains of mollusks; the other 30 fish were added to the calculation of percent mollusk occurrence to account for prior ingestion of fluke-infected mollusk prey items, resulting in an estimated mollusk occurrence of 54.1%. Fluke percent occurrence did not differ significantly between capture habitats (Table 1), but was significantly higher in larger fish above the median of 791 mm total length (42.6%, $P = 0.04$). Fluke-adjusted mollusk occurrence did not differ significantly between the two Black Carp size classes (Table 1), but was significantly higher in fish captured from the lentic vs lotic habitats (87.5%, $P = 0.005$).

DISCUSSION

In general, the ecology and effects of intestinal flukes in freshwater fish are more poorly understood than those that infect other tissues or organs, carry human diseases, or require intermediate hosts for development. Because of the widespread distribution, more ancestral life cycle, and extensive list of host species, *A. conchicola* is relatively well known among fish trematodes, although it has no known medical importance to humans. Black Carp was reported as a reservoir host for *A.*

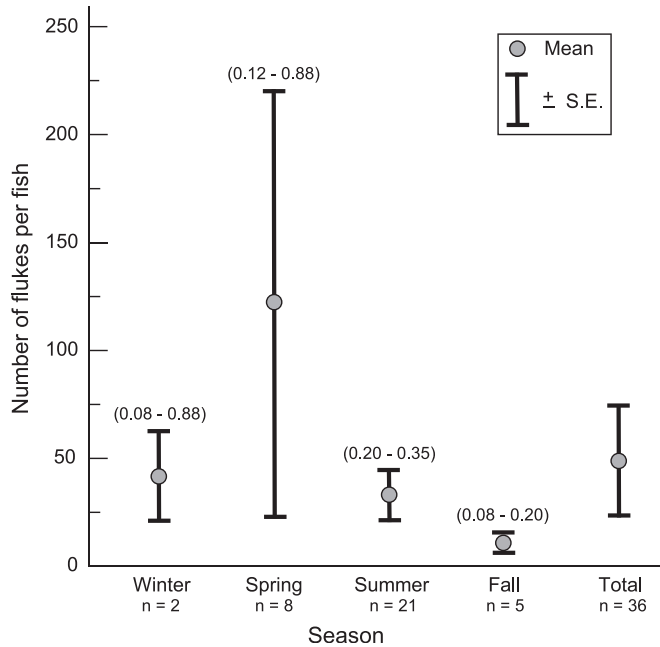


Figure 3. Fluke abundance by season of fish capture (mean number of *Aspidogaster conchicola* per fish examined, ± 1 SE) for Black Carp (*Mylopharyngodon piceus*) captured from the Mississippi River Basin in 2009–2017. Only the 36 fish containing flukes are included, and range in *P* values for seasonal comparisons are given in parentheses (Kruskal–Wallis/Mann–Whitney U-tests, $\alpha = 0.05$ significance level).

conchicola, resulting from consumption of infected unionid mussels (*Cristaria plicata*) in the Amur River, China (Evtushenko et al. 1994; Nico et al. 2005). According to the aspidogastrea summary provided by Alves et al. (2015), *A. conchicola* has the highest number of known host associations among this group of flukes, with freshwater bivalves and gastropods making up 78% of the total reported (122), although this number may be conservative because endosymbionts infecting imperiled species of mollusks are often overlooked (Brian and Aldridge 2019). Aspidogastrea flukes, including the closely related genus *Cotylogaster*, are commonly found in the gastrointestinal tract of benthic-feeding freshwater fishes native to the Mississippi River Basin (Alves et al. 2015), including Blue Catfish (*Ictalurus furcatus*) and

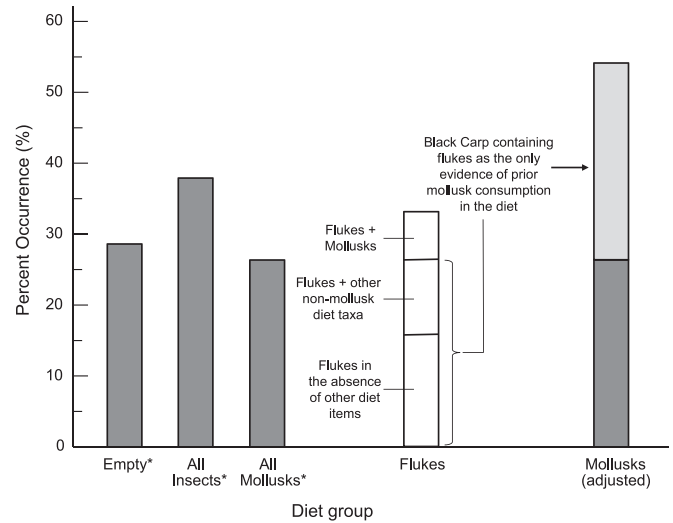


Figure 4. Percent occurrence ($n = 109$) of diet groups found in gastrointestinal tracts of Black Carp (*Mylopharyngodon piceus*) captured from the Mississippi River Basin in 2009–2017 (empty = no identifiable contents). Data with an asterisk (*) shown for comparison are from Poulton et al. (2019), and flukes (*Aspidogaster conchicola*) are further subdivided from data in that publication.

Freshwater Drum (*Aplodinotus grunniens*), that also occasionally consume mollusks. Specific U.S. records of *A. conchicola* in fish only included Shorthead Redhorse (*Moxostoma macrolepidotum*) and Common Carp (*Cyprinus carpio*; Alves et al. 2015); thus, our discovery of this fluke in wild Black Carp represents the first U.S. report for this fish species. The occurrence frequencies of *A. conchicola* across populations of other fish species are unknown and thus not available for comparisons. Because *A. conchicola* infects a wide variety of mollusk taxa, we were unable to determine which infected mollusk group(s) was consumed by individual fish.

The fluke *A. conchicola* is known to obtain a higher infection intensity (number of flukes per host individual) than other aspidogastrea species, with as many as 1,545 reported in one individual mollusk (Nelson et al. 1975). Some researchers have reported that flukes are more prevalent in mollusk hosts during winter (Bailey and Tompkin 1971; Halton and Lyness 1971). However, the variable infection intensity for host mollusk taxa and individuals (Huehner and

Table 1. Statistical comparisons of occurrence (percent incidence in the diet) for flukes (*Aspidogaster conchicola*) and mollusks (*, adjusted for fluke presence) based on fish size (total length [TL], median = 791 mm) and capture habitat (lotic, mainstem flowing water habitats, including side channels and chutes; and lentic, off-channel areas, including backwater sloughs, oxbows, or reservoirs) for 109 Black Carp (*Mylopharyngodon piceus*) from the Mississippi River Basin in 2009–2017 (Kruskal–Wallis/Mann–Whitney U-tests, $\alpha = 0.05$ significance level).

| Parameter | Comparison | Fluke Occurrence | <i>P</i> | Mollusk Occurrence* | <i>P</i> |
|----------------------|---------------------------------|------------------|----------|---------------------|----------|
| Fish size (TL; mm) | At or below median ($n = 55$) | 23.6 | 0.04 | 47.3 | 0.20 |
| | vs Above median ($n = 54$) | 42.6 | | 59.3 | |
| Fish capture habitat | Lentic ($n = 16$) | 18.8 | 0.32 | 87.5 | 0.005 |
| | vs Lotic ($n = 93$) | 35.5 | | 47.3 | |

Etges 1977; Huehner 1984; Carney 2015) precludes us from relating fluke numbers in Black Carp samples to seasonality in mollusk ingestion rates, diet abundances among prey items, or specific mollusk taxa consumed in the diet. Therefore, we are reporting fluke abundances in Black Carp merely as a reference for comparison across seasons of fish capture and for any future studies that may examine abundance of *A. conchicola* in other vertebrate host species. There are no documented reports in the literature that this fluke species causes harm to fish, although trematodes in general are known to feed on the mucosal layers lining the intestinal tract (Roberts and Janovy 2000; Rohde 2005). A closely related fluke species, *Aspidogaster limacoides* Diesing, that is a common mollusk parasite in Europe is known to significantly damage gut tissues in host fish (Rahanandeh et al. 2016), and this observation may warrant further studies. Similarly, little is known about whether infections of *A. conchicola* can significantly reduce the fitness or viability of host mollusks or their populations. Some have reported changes in tissue histopathology (Michelson 1970) and variability of effects across host species and infection sites (Bakker and Davids 1973; Danford and Joy 1984). Pauley and Becker (1968) documented metaplasia in host tissues with high rates of fluke infection, and Benz and Curran (1997) concluded that *A. conchicola* infections would likely cause adverse effects in host unionid mussels. Pavluchenko and Yermoshyna (2017) also reported an increase in the cardiac index and reduced function in ciliated membranes of the filtering apparatus in unionids as infection rates increased. The ecological significance of *A. conchicola* infection in host mollusks and the extent to which infection might affect their populations, imperilment status, or vulnerability as a prey item are currently unknown. The potential of *A. conchicola* to adversely affect the integrity of host tissues and mollusk fitness in combination with our results suggests that further research on the sublethal effects of *A. conchicola* infections is needed, especially considering the widespread nature of this parasite among freshwater mollusks.

Among the 109 Black Carp examined for diet items (Poulton et al. 2019), the 30 fish containing flukes without other evidence of mollusk consumption (i.e., shell fragments or mollusk structures) likely fed upon mollusks sometime before capture, because mollusk ingestion is the only known pathway for *A. conchicola* infection in vertebrates. The time between mollusk ingestion and fish capture is unknown and cannot be easily approximated with our data, especially because gut evacuation rates are poorly known for Asian carps in general (Nico et al. 2005; Chapman and Hoff 2011). Based on the literature, we conclude that the presence of these flukes is a viable indicator of previous mollusk consumption in Black Carp. Although insects were reported at a higher incidence in diet samples (37.6%, Poulton et al. 2019), our fluke-adjusted mollusk occurrence of 54.1% is consistent with diet accounts for both cultured and wild fish given by Nico et al. (2005) and the common description of Black Carp as a molluscivore. It is likely that the previously reported percent occurrences for

gastropods (16.5%), unionid mussels (13.7%), and all bivalve mollusks (22.8%) in the diet of wild Black Carp are lower than actual contributions to the diet because the presence of *A. conchicola* flukes was not accounted for in those estimates. Therefore, omitting these fish from the calculation of mollusk occurrence undervalues both the importance of these organisms in the diet and the risk of Black Carp invasion to freshwater mollusks.

Studies on the longevity, viability, and development of *A. conchicola* within and outside mollusk hosts were partially summarized by Huehner and Etges (1977). This fluke species can survive in water or saline solution for 2–5 wk in the laboratory (Van Cleave and Williams 1943; Rohde 1972), indicating that these flukes can remain viable outside the host for extended periods. However, these flukes are not motile in their adult (sexually mature) stage, with transmission and subsequent infection occurring through the egg, larval, or both forms (Rohde 1972; Bakker and Davids 1973). The *A. conchicola* flukes we found in Black Carp varied in size and growth phase, as described by Huehner and Etges (1977), but their development and maturation while residing within the intestinal tract of host fish have not been evaluated. Fredricksen (1980) suggested that some aspidogastrea fluke species probably continue their growth within the vertebrate hosts after the infected mollusks are consumed. Evtushenko et al. (1994) noted *A. conchicola* remained in the intestine of Black Carp after digestion of the host tissues, but did not specify fluke viability or a specific period. Our data showing 16.5% of fish examined contained this fluke in the absence of other diet items (Fig. 4) imply that the flukes remain in the gastrointestinal tract for extended periods after mollusk consumption, digestion of other diet items, and evacuation of mollusk shell fragments. This also implies that these fish may not have eaten for several hours or days before capture and that *A. conchicola* can withstand the enzyme activity associated with digestion. We recognize that commonly used methods for reporting gastrointestinal contents in fish, such as those outlined in Buckland et al. (2017) for qualitative presence–absence data such as ours, often assume a prey item has been recently consumed within the gut evacuation time period. Although our modified approach to calculating mollusk percent occurrence is nonstandard and beyond this definition, the extended presence of these flukes in a reservoir host such as Black Carp represents evidence of prior mollusk consumption regardless of time period or gut evacuation rate.

The habitats that Black Carp currently occupy within the invaded range of the Mississippi River Basin can be partially inferred by capture information and determination of prey habitats in combination with percent occurrence data for mollusks and *A. conchicola* flukes. Diet information associated with feeding zones and modes of prey capture (Poulton et al. 2019) indicate Black Carp feed on mollusks that are sediment dwelling or attached to hard substrates, both of which are well represented among the diet items consumed by these fish (Poulton et al. 2019) and known mollusk species host fish infected by *A. conchicola* (Alves et al. 2015). When

flake presence in the gastrointestinal tract is accounted for in mollusk estimates, larger Black Carp (above the median total length for this study of 791 mm) and those captured in lentic environments have significantly higher occurrences (Table 1). Furthermore, of the 18 fish containing *A. conchicola* flukes as the only diet item present, all were collected in lotic environments, indicating that these fish had spent more time evacuating gut contents (i.e., while in hoop nets before retrieval or during transit between feeding locations). Conversely, fish containing mollusks, flukes, or both and captured in lentic habitats may have been actively feeding or had ingested mollusks more recently, as supported by our significantly higher adjusted mollusk occurrence estimates of 87.5% (Table 1) and the significantly greater diet taxa richness in fish collected from these environments (Poulton et al. 2019). However, many of the Black Carp that we examined were acquired from commercial fishers; therefore, the bias associated with season and gear preferences of these activities (including variation in time spent in capture gear such as hoop nets) warrants consideration when interpreting these results. Further investigations related to Black Carp movements and habitat use in riverine systems are underway and are needed to thoroughly assess the effects of Black Carp invasion on assemblages or specific taxa of mollusks.

The ecological importance of Black Carp as a potential carrier or transmitter of *A. conchicola* has not been investigated. Literature suggests that transmission pathways of aspidogastrea flukes among and between mollusk populations are associated with the environmental requirements of host unionids (such as current velocity and substrate factors) and that the dispersal of egg and nonciliated larval stages of *A. conchicola* may be passive (Huehner 1984; Carney 2015). These papers did not specify the mode of fluke transmission or the role of fish carriers, but Ferguson et al. (1999) recognized the passage of fluke eggs through the feces of mollusk-eating turtles as a component of aspidogastrea life cycles. Many of the flukes that we found in Black Carp were gravid (Fig. 2), and laboratory studies have shown that eggs of *A. conchicola* are immediately infective upon uptake by a mollusk host (Huehner and Etges 1972, 1977). If viable *A. conchicola* eggs or larvae pass through the digestive tract of Black Carp during waste evacuation, these fish may be dispersing the fluke to other feeding sites within riverine habitats suitable for colonization of host mollusks. Considering the presence of gravid flukes and their apparent extended longevity in the gastrointestinal tract, it seems likely that Black Carp may play a role in the dispersal of *A. conchicola*, both directly to mollusks and indirectly to other benthic dwelling fishes that feed on mollusks. To date, there are no literature reports that eggs or larvae of *A. conchicola* can infect fish directly. Viability of *A. conchicola* eggs passing through the digestive tract of fish have not been studied; thus far, dispersal by Black Carp can only be inferred by our observance of gravid flukes and the proximity of these fish to the mollusk habitat where they forage.

The invasion and expansion of Black Carp in the United

States could deplete native unionid mollusks, a group with documented *A. conchicola* infections. Literature, including recent and ongoing efforts to evaluate Black Carp invasion risks (ACRCC 2019; USFWS 2019), has highlighted adverse effects on native freshwater mollusks as being the primary concern. The list of *A. conchicola* mollusk hosts in the United States (Alves et al. 2015) contains approximately 61 freshwater mussels and 3 gastropods within the currently known range of wild Black Carp in the Mississippi River Basin (Kroboth et al. 2019), including unionids classified as threatened (1), endangered (3), or of special concern (14) based on Williams et al. (1993). To date, Black Carp diet includes six taxa of unionids and two gastropod families from this list (Poulton et al. 2019), as well as *Corbicula* and *Dreissena*, both of which are documented *A. conchicola* hosts in Europe and Canada, respectively. Additional Black Carp are being examined for diet analysis, and we expect the number of mollusk taxa documented in the diet of wild fish (and listed as *A. conchicola* hosts by Alves et al. [2015]) to increase as they further expand their range in the United States. Among the eight freshwater fish hosts of aspidogastrea flukes listed by Alves et al. (2015) that are present in the United States, four are native species and three of them (Shorthead Redhorse, Blue Catfish, and Freshwater Drum) occasionally feed on mollusks. The other four are nonnative species and are known hosts of *A. conchicola*: Common Carp and the three invasive species Black Carp, Grass Carp, and Round Goby (*Neogobius melanostomus* (Pallus)) that occur in the same habitats of riverine ecosystems where mollusks are common. Kelly et al. (2009) theorized that parasitic spillback can occur when newly invasive hosts lead to increases in native parasites, which ultimately may cause higher infection burdens for native hosts. Given that the transmission of aspidogastrea flukes potentially may involve multiple pathways that include both invasive mollusk hosts (*Dreissena* and *Corbicula*) and invasive fish hosts that are currently expanding their ranges in the United States (Black Carp, Grass Carp, and Round Goby), a future increase in *A. conchicola* fluke infections in native unionid mussels and gastropods is theoretically possible, especially considering the high densities that these invasive species can attain (Graney et al. 1980; Schloesser et al. 1996; Johnson et al. 2005; Higgins and Vander Zanden 2010; Sass et al. 2014; Sullivan 2016). These relationships also add another dimension to the knowledge of mussel–fish host associations as described in papers related to unionid mussel life history (Schwalb et al. 2013) and shared parasitism among multiple hosts (Brian and Aldridge 2019). Although *A. conchicola* is widespread, Black Carp acting as a reservoir host could enhance their density or, ultimately, increase infection rates or occurrences within mollusk individuals or populations.

Reservoir–host pathways are important for controlling emerging diseases of domestic animal and wildlife populations (Daszak et al. 2000), but many of these pathways have not been well characterized for species that do not infect humans. Our assessment of *A. conchicola* flukes present in Black Carp

indicates that this fish species is a reservoir host that shares a reciprocal pathway with the freshwater mollusks that it consumes in its diet. Although the capture bias associated with our fish and the qualitative nature of presence–absence diet data preclude us from determining ingestion timing, feeding rates, or specific taxa consumed, the omission of these flukes from mollusk occurrence estimates would seriously undervalue their importance in the diet of wild Black Carp. Our data show that *A. conchicola* flukes remaining in the gastrointestinal tract provide an indication of mollusk consumption even after the digestion of host tissues and the fragments of their shells have been expelled or evacuated as waste. Recently published diet data for wild Black Carp in the United States (Poulton et al. 2019) suggest that this species has a more opportunistic and insectivorous diet than prior species descriptions (Nico et al. 2005) or that their application in aquaculture as a gastropod biological control would suggest (Venable et al. 2000; Ledford and Kelly 2006). Poulton et al. (2019) also noted the ability of Black Carp to expel mollusk shell fragments orally after ingestion and the resulting difficulty in identification of mollusk taxa in diet samples. This limits our ability to determine the potential effects of Black Carp on specific imperiled freshwater mollusk populations without further advances in collection methods and determination of habitats where Black Carp and potentially threatened mollusk taxa co-occur. Not only do mollusks provide a greater relative contribution to their diet than was previously determined (Poulton et al. 2019) but also wild Black Carp currently occupying the Mississippi River Basin are carrying gravid *A. conchicola* flukes with them as they expand their geographic distribution and could be a source of infections to mollusks while they move between habitats and forage in areas with suitable conditions for parasite transmission.

Collectively, literature and our additional interpretation of *A. conchicola* flukes in Black Carp indicate that the effects of this fish and that of fluke infections on native freshwater mollusks warrant further study in several areas of research. Although McElwain (2019) recently discussed the low likelihood that eukaryotic organisms such as flukes would be responsible for undiagnosed die-offs or other declines of unionid mussels, the fact that parasite infection rates are poorly known, especially for rare and imperiled mollusks, may lead to additional research and improvements in parasite detection for these taxa. Relatively few imperiled species are included among the mollusk hosts infected by *A. conchicola* (Alves et al. 2015), but as noted by Brian and Aldridge (2019), endosymbionts have not been adequately surveyed in rare taxa and dead specimens are not available for examination. We suspect that other rare mollusks not yet reported may also be hosts of this fluke, but development of nonlethal detection methods may be needed to accurately assess infection rate, occurrence, and population viability within mollusk assemblages. Furthermore, laboratory studies are needed to determine declines in mollusk fitness with high rates of infection and to quantify any negative cellular, tissue-, or organ-level effects this fluke might have on hosts. Additional knowledge on reservoir–host pathways associated with *A. conchicola*

flukes, particularly the longevity and development of *A. conchicola* within reservoir hosts, would also be helpful in characterizing environmental and habitat conditions favorable for parasitic transmission between Black Carp and mollusks. Although Black Carp are not intentionally ingesting *A. conchicola* during their foraging behavior, they may facilitate dispersal of a parasite that readily infects the mollusks they frequently consume in the wild.

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