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A microchemical analysis of native fish passage through Brandon Road Lock and Dam, Des Plaines River, Illinois

Claire Snyder
Southern Illinois University Carbondale, snyderce2@gmail.com

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A MICROCHEMICAL ANALYSIS OF NATIVE FISH PASSAGE THROUGH

BRANDON ROAD LOCK AND DAM, DES PLAINES RIVER, ILLINOIS

by

Claire Snyder

B.A., Whitman College, 2012

A Thesis

Submitted in Partial Fulfillment of the Requirements for the
Master of Science Degree

Department of Zoology

in the Graduate School

Southern Illinois University Carbondale

August 2019

THESIS APPROVAL

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Approved by:

Dr. Gregory W. Whitledge, Chair

Dr. James Garvey

Brent Knights

Graduate School

Southern Illinois University Carbondale

June 28, 2019

AN ABSTRACT OF THE THESIS OF

Claire Snyder, for the Master of Science degree in Zoology, presented on June 28, 2019 at Southern Illinois University Carbondale.

TITLE: A MICROCHEMICAL ANALYSIS OF NATIVE FISH PASSAGE THROUGH

BRANDON ROAD LOCK AND DAM, DES PLAINES RIVER, ILLINOIS

MAJOR PROFESSOR: Dr. Gregory W. Whitledge

Modifications to Brandon Road Lock and Dam (BRLD), located on the Des Plaines River in northeastern Illinois, have been proposed to prevent the upstream transfer of aquatic invasive species, particularly Asian carps, into the Great Lakes Basin. These modifications, including the installation of an electric barrier, acoustic fish deterrent, and air bubble curtain, are designed to completely eliminate all upstream fish passage and may negatively impact native fish populations in the Des Plaines River by reducing upstream movement and potentially fragmenting populations. BRLD is situated just 21 km upstream of the Des Plaines River mouth, and fish are only able to pass upstream via the lock chamber. Fish species richness within the Des Plaines River watershed has increased over the last 35 years. It has been suggested that the majority of new species to the upper Des Plaines River have migrated upstream past Brandon Road Lock and Dam (BRLD), from the Illinois, Kankakee, and lower Des Plaines rivers. However, documentation of emigration needed to support that contention is lacking and there is limited knowledge of the current rate of BRLD passage by native species. To assess native fish passage through the lock, a microchemical study was conducted using fin rays from fish collected from the Des Plaines, Illinois, and Kankakee Rivers. The edge of each fin ray, which contained the most recently deposited material, was assumed to contain a microchemical signature reflective of residency in the river where the fish was sampled. Fin ray edge strontium:calcium ratio (Sr:Ca) was used to define taxonomic and river-specific signature ranges

for four taxonomic groups: centrarchids, catostomids, ictalurids, and lepisosteids. Fin ray edge Sr:Ca data were input into a random forest classification model, and the classification accuracy of fish to their river of capture based on their fin ray edge Sr:Ca was > 97% in each taxonomic group. The classification model was then applied to the entire fin ray of each fish sampled upstream of Brandon Road to infer retrospective environmental history. Upstream BRLD lock passage was suggested by the presence of Sr:Ca signatures indicative of prior downstream residency in the Illinois or Kankakee rivers in a fish sampled upstream of BRLD. Results indicated some evidence of downstream residency that suggested upstream BRLD lock passage for centrarchids, catostomids, ictalurids, and lepisosteids, ranging from 15 – 37% of individuals sampled depending on taxa. An additional 19 – 80% of individuals within each taxonomic group were classified as fish with uncertain downstream residency, whereby the possibility of BLRD lock passage could not be rejected, but there was higher uncertainty in establishing downstream residency in the Illinois or Kankakee rivers. The impact of BRLD modifications and passage restriction on Des Plaines River fish populations is unknown and merits further investigation.

ACKNOWLEDGEMENTS

USGS DISCLAIMER

DEDICATION

To Steve Pescitelli, who thought this whole thing was a good idea (and rightly so).

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A MICROCHEMICAL ANALYSIS OF NATIVE FISH PASSAGE THROUGH

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INTRODUCTION

Riverine fish often move between different environments to satisfy life history

requirements, including movements associated with spawning, dispersal, foraging,

overwintering, or avoiding sub-optimal environmental conditions (Abell et al. 2018; Johnson and

Noltie 1996; Laughlin et al. 2016; Page and Johnston 1990). Impeding fish movement with

artificial barriers at any life stage may have consequences that range in severity. On an individual

level, fish that are prevented or delayed from moving to optimal habitats can experience declines

in reproductive output due to delayed spawning, spawning in sub-optimal habitats, or reduced

energy allocated to reproduction (McLaughlin et al. 2013; Pratt et al. 2009). Survival may also

decline if fish are unable to reach temperature, oxygen, or flow refuges during times of

environmental stress or disturbance (McKay et al. 2013). On a population scale, loss of

ecosystem connectivity caused by artificial barriers can isolate populations, leading to reductions

in gene flow and access to habitats used for reproduction and making populations more

vulnerable to disturbances or local extirpations (Graf 1999; Nislow et al. 2011; Pringle 2003).

Although artificial barriers can prove detrimental to native fish movement, they may

serve an intended or unintended purpose of preventing the spread of aquatic invasive species

(AIS) (Lubejko et al. 2017). Invasive species have been identified as one of the primary dangers

to worldwide biodiversity (Jansson et al. 2007; McLaughlin et al. 2013) and are responsible for a

wide range of detrimental ecological, social, and economic impacts, which in concert threaten

native ecosystems and the interests of stakeholders that rely on them. Because river networks

provide only single or limited pathways for aquatic species movement versus more numerous

mechanisms for dispersal over land or air by terrestrial organisms, the installation of artificial

barriers at critical locations can be effective at blocking AIS movement (McKay et al. 2013).

However, it is vital to consider the trade-offs of barriers specifically targeting AIS; resource

managers may need to determine whether the benefits of reducing further AIS range expansion

outweigh the impacts. Major potential negative impacts include, but are not limited to: reducing

native species connectivity, alteration of flow regime, water quality degradation, and habitat

degradation (Bunn and Arthington 2002; Jansson et al. 2007; Nislow et al. 2011). Improving

river connectivity to increase the success of a certain species may also come at the cost of

leaving the same species vulnerable to impacts of invasion (Fausch et al. 2009; McKay et al.

2013; Rahel 2013).

The trade-offs between native fish movement and restricting invasive species passage are

of particular concern in the case of invasive carps from eastern Asia. Introduced to the United

States in the 1970s for use in commercial aquaculture ponds, the fish soon escaped containment

and began invading the Mississippi River and its tributaries (Kelly et al. 2011). In particular,

Silver Carp *Hypophthalmichthys molitrix* and Bighead Carp *H. nobilis*, together called bigheaded

carps, spread quickly as a result of their high fecundity, early maturity, rapid dispersal, and

tolerance of a broad range of environmental conditions. To combat the threat of bigheaded carps

expanding their range into the Great Lakes, the United States Army Corps of Engineers

(USACE) installed a series of electric dispersal barriers on the Chicago Sanitary and Ship Canal,

a man-made channel southwest of Chicago, Illinois that acts as one of the key connection points

between the Mississippi River and Great Lakes watersheds (USACE 2014). The barriers are

located approximately 40 km from Lake Michigan and create an electric field in the water which

acts as a deterrent to fish passage (Bryant et al. 2016). To reduce the probability of bigheaded carps challenging the electric dispersal barriers, a contracted harvest program was implemented by the Illinois Department of Natural Resources in the Illinois River and lower Des Plaines River with commercial fishermen, resulting in the removal of 90,469 Bighead Carp and 681,743 Silver Carp between 2010 and 2017 (ACRCC 2017a). Additionally, a barrier fence was constructed along the section of the Des Plaines River that flows parallel to the Chicago Sanitary and Ship Canal to prevent invasive fish from bypassing the electric barrier in the event of flooding (USACE 2014).

To further reduce the possibility of upstream bigheaded carp passage along the Chicago Area Waterway System (CAWS) toward Lake Michigan, the USACE recommended the enhancement of Brandon Road Lock and Dam (BRLD) as an additional control point (USACE 2018). Brandon Road Lock and Dam is located on the Des Plaines River approximately 16 km downstream of the Chicago Sanitary and Ship Canal electric dispersal barriers and at the leading edge of the bigheaded carp invasion front (ACRCC 2018). Completed in 1933, the dam is 728.8 m long and 10.4 m high. Because of the height of the dam, upstream fish passage at BRLD occurs exclusively through the lock chamber, which is 182.9 m long and 33.5 m wide (USACE 2017). The recommended plan, which was approved by the commanding general of USACE in May 2019 (USACE 2019), includes a suite of technologies designed to eliminate upstream AIS passage at BRLD, including the installation of an engineered channel on the downstream approach to the lock, an electric barrier within the engineered channel, an acoustic deterrent, an air bubble curtain, and a flushing lock as well as non-structural controls such as continued overfishing and public outreach (USACE 2018). In concert, these controls are intended to

prevent upstream transit of swimming and floating Mississippi River AIS while still maintaining navigation.

The impact of barrier enhancement at BRLD on the movement of native fishes into the upper Des Plaines River is unknown but will depend on the extent to which native fishes utilize the lock chamber for upstream passage. Brandon Road Lock and Dam is located just 21 km upstream of the mouth of the Des Plaines River (USACE 2014), so any fish moving upstream to access the 198 km upper Des Plaines River (i.e. the portion of the river upstream of BRLD) must first pass through the lock chamber at BRLD (Pescitelli 2015). Upstream fish passage through the lock chamber at BRLD has been suggested in several reports. Acoustic telemetry studies by USACE detected five tagged Common Carp *Cyprinus carpio* moving upstream through BRLD from the Dresden Island Pool into the Brandon Road Pool (ACRCC 2017a). Additionally, a 2013 Des Plaines River basin survey by the Illinois Department of Natural Resources (IDNR) documented 50 native fish species in the Des Plaines River upstream of BRLD, a substantial increase in fish species richness compared to the 28 species present in the first basin survey in 1983. Species richness increased in each of the four basin surveys conducted between 1997 and 2013 (Altenritter et al. 2019; Pescitelli 2015). The 2013 IDNR basin report proposed that, although some immigrant species could have traveled from the CAWS or Lake Michigan, it is more likely that new riverine species migrated from the lower Des Plaines, Illinois and Kankakee rivers and therefore passed upstream through BRLD (Pescitelli 2015). Due to the high efficacy of the electric dispersal barriers at preventing fish passage (Parker et al. 2015), immigration into the Des Plaines River from the CAWS would likely be reduced after the first barrier became operational in 2002, though some passage may still occur in small fish during barge transit (Davis et al. 2016; Parker et al. 2015). Although upstream BRLD passage has been proposed as a

mechanism for the increasing species richness in the Des Plaines River, documentation of emigration needed to support that contention is lacking (Pescitelli 2015).

Microchemical analysis of calcified structures in fishes represents a potential means of identifying individual fish captured in the upper Des Plaines River that have passed upstream through the lock chamber at BRLD. This technique utilizes trace element concentrations in water bodies which are incorporated into calcified structures in fish like otoliths and fin rays. Hard part trace element concentrations are strongly correlated with ambient water trace element concentrations, and trace element analysis of these structures can allow for the examination of movement patterns and environmental history throughout a fish's lifetime, provided the locations have chemically-distinct water signatures and the fish resides in the locations long enough to incorporate the location-specific signatures into its hard structures (Campana and Neilson 1985; Elsdon and Gillanders 2004; Pracheil et al. 2014). The use of fin rays for analysis has proven a viable non-lethal alternative to otoliths, and several studies have shown its efficacy in retrospectively documenting fish environmental life histories, including in the Illinois and Kankakee rivers (Pracheil et al. 2014; Rude et al. 2014; Smith and Whitledge 2011).

Microchemistry has been used to infer lock and dam passage in other studies (Schaffler et al. 2015; Whitledge et al. 2019). Limited data indicate that requisite differences in water chemistry (specifically strontium (Sr):calcium (Ca) ratio) exist among the Des Plaines, Illinois, and Kankakee rivers (G.W. Whitledge, unpublished). In addition, Sr:Ca differences in otoliths from fish collected in the Des Plaines and Illinois rivers and in fin rays of fish sampled in the Illinois and Kankakee rivers have been reported (Smith and Whitledge 2010; Whitledge 2009). This suggests an ability to use microchemistry techniques to identify fish captured in the Des Plaines River upstream of BRLD that had previously resided in the Illinois or Kankakee rivers

and therefore had passed upstream through BRLD. Therefore, the objectives of the study were to 1) identify differences in water Sr:Ca between the Des Plaines, Illinois, and Kankakee rivers and confirm inter-annual persistence, 2) determine whether water Sr:Ca differences between rivers were also reflected in fish fin rays of select native species, 3) quantify fin ray Sr:Ca signatures that reflected fish residency in each of the three rivers, 4) examine Des Plaines River fish captured upstream of BRLD for evidence of prior Illinois or Kankakee River residency and therefore BRLD passage, and 5) determine the relative abundance of fish captured upstream of BRLD that showed evidence of BRLD passage.

METHODS

Study area

This study was conducted in northeastern Illinois near the confluence of the Des Plaines and Kankakee rivers, which forms the headwaters of the Illinois River. The study area encompassed 31 km of the Des Plaines River immediately upstream of BRLD, 35 km of the Kankakee River upstream of the Wilmington Dam, and 18 km of the Illinois River downstream of Dresden Island Lock and Dam (Figure 1). The Des Plaines River study reach extended from BRLD to the site of the former Hofmann Dam, which was removed in 2012. The Illinois River study reach encompassed the Marseilles pool, and its uppermost extent at Dresden Island Lock and Dam is located approximately 2.4 km downstream of the confluence of the Des Plaines and Kankakee rivers. The upstream extent of the Kankakee River study reach was marked by the Kankakee Dam and the downstream extent was at the Wilmington Dam, located approximately 16.7 km upstream of the river mouth and confluence with the Des Plaines River.

Fish were not sampled between BRLD, Wilmington Dam, and Dresden Island Lock and Dam. This was done for multiple reasons. First, an anticipated inability to distinguish between

Des Plaines Sr:Ca water signatures upstream vs downstream of BRLD meant that only fish captured upstream of BRLD could be used to confirm lock passage events, as a fish sampled from downstream of BRLD that contained evidence of residency in the Illinois or Kankakee rivers may or may not have ever been upstream of BRLD. Secondly, the goal of collecting fish from the Illinois and Kankakee rivers was to establish resident Sr:Ca signatures for each river, which would be compared to Des Plaines signatures of residency established by sampling upstream of BRLD. Keeping two dams between each study reach increased the likelihood of encountering Illinois and Kankakee resident fish and reduced the probability of finding recent immigrants with different Sr:Ca signatures which could confound the ability to define true Sr:Ca signatures of residency for each reach.

The Des Plaines River flows 214 km from southeast Wisconsin to northeast Illinois, where it changes from a small forested stream to a suburban river and ultimately to an industrial waterway. Its mean annual discharge is approximately 108 m^3/s at 25 km from the mouth (U.S. Geological Survey 2019a). Although some reaches of the Des Plaines River are relatively unaltered and protected within preserves, many have experienced heavy development. It is a low gradient stream whose fish assemblage is characterized primarily by cyprinids, centrarchids, and cyprinodontids, as well as substantial numbers of catostomids and ictalurids (Pescitelli 2015). The Des Plaines River shares dozens of species in common with the neighboring Illinois and Kankakee rivers, including centrarchids, catostomids, ictalurids, and lepisosteids (Benke and Cushing 2011; Pescitelli 2015; Pescitelli and Widloe 2017).

The Kankakee River flows 214 km from northwestern Indiana to northeastern Illinois, with a discharge of approximately 138 m^3/s at 17 km from the river mouth (U.S. Geological Survey 2019b). Though the agricultural Indiana section has been highly channelized, the 95 km

Illinois section, though dammed in places, has been relatively unaltered in comparison, with little change in the fish assemblage or water quality since 1975 (Pescitelli and Widloe 2017). Conversely, the entire 439 km Illinois River has been highly modified. With a mean annual discharge of 649 m^3/s at 100 km from the river mouth (Benke and Cushing 2011), the Illinois River is a larger order river than the Des Plaines and Kankakee and is maintained for commercial shipping though its extent. The Illinois River has seen significant increases in native fish species richness from 1957 to 2009 as well as significant increases in mean relative fish abundance from 1976 to 2009 (McClelland et al. 2012). These improvements have been attributed to wastewater treatment, habitat enhancement projects, and increased water quality regulation following the passage of the Clean Water Act in 1972 (Gibson-Reinemer et al. 2017; Parker et al. 2016). This followed decades of low fish diversity and abundance after the opening of the Sanitary and Shipping Canal and the rerouting of pollution and wastewater effluent from the CAWS beginning in 1900 (McClelland et al. 2012).

Water and fish sampling

Water samples for strontium and calcium were collected at least every other month at sites on the Des Plaines, Kankakee, and Illinois rivers in October 2017 and between April and October 2018 (Figure 1). Water sampling locations on the Des Plaines River included sites upstream and downstream of BRLD. Each water sample was collected in a 20 ml vial using acid-cleaned polypropylene syringes with a Whatman Puradisc 0.45 µm polypropylene filter using methods described in Shiller (2003) and refrigerated until analysis. Additional water data were used from previous samples collected using the same methods between 2013 and 2017 and were included in the analysis (G.W. Whitledge, unpublished).

Fish were collected using 60 pulse per second (pps) DC boat electrofishing and gill nets (76.2 mm, 88.9mm, 101.6mm, 108 mm, and 127mm mesh) in October 2017 and April-November 2018 (Figure 1). The following species were selected for study: Smallmouth Buffalo *Ictiobus bubalus,* Bigmouth Buffalo *Ictiobus cyprinellus*, Black Buffalo *Ictiobus niger*, River Carpsucker *Carpoides carpio*, Quillback *Carpoides cyprinus*, Largemouth Bass *Micropterus salmoides*, Smallmouth Bass *Micropterus dolomieu*, Channel Catfish *Ictalurus punctatus*, and Longnose Gar *Lepisosteus osseus*. Each of these species has been documented in each of the three study rivers; however, only Largemouth Bass and Smallmouth Bass were documented upstream of BRLD by IDNR in 1983 and even then, Smallmouth Bass abundance was low (Pescitelli 2015), suggesting that most of the target species may have entered the upper Des Plaines after 1983 by passing through the BRLD lock from the Illinois or Kankakee rivers. None of the catostomid or lepisosteid target species have been documented upstream of the site of the former Hofmann Dam (Pescitelli 2015). Target species were determined following discussions with IDNR personnel after examining trends in fish species richness and abundance data over multiple Des Plaines River basin surveys (S. Pescitelli, personal communication).

Total length and capture location were recorded for each fish sampled. Using pliers, the left leading pectoral fin ray of each fish was removed by making a transverse cut across the fin ray as proximal to the fish's body as possible (Abell et al. 2018; Rude et al. 2014). For Channel Catfish, the articulating process was removed with the spine. Fin rays were stored in scale envelopes until laboratory analysis. After fin ray removal, each fish was released at its capture location. Fish sampling and fin ray collection procedures were conducted in accordance with Southern Illinois University Institutional Animal Care and Use Committee protocols 15-009 and 18-010.

Laboratory analysis

Water samples were sent to the Center for Trace Analysis at the University of Southern Mississippi. Samples were acidified to pH 1.8 using ultrapure (Seastar Basline) HCl and allowed to sit for at least one week. Samples were then diluted 11x in ultrapure (Seastar Baseline) 0.16 M HNO_3, which contained 2 ppb scandium, indium and thorium as internal standards. External certified reference standards were also prepared using the same HNO_3 used for sample dilutions. Samples were analyzed for strontium and calcium concentrations using a Thermo-Finnigan Element 2 (Thermo Fisher Scientific, Waltham, MA, USA) inductively coupled plasma mass spectrometer. Elemental concentrations were converted to Sr:Ca ratios (mmol/mol).

Fin rays were embedded in Epo-fix epoxy (Electron Microscopy Sciences, Hatfield, PA) and sectioned in the transverse plane along the base of the fin ray using a low-speed ISOMET saw (Buehler Inc., Lake Bluff, IL) to a thickness of 0.7 mm. Channel Catfish spines were sectioned across the articulating process rather than the base of the spine, as spines more frequently contain a central lumen, resulting from reabsorption of bone grown during early life; thus, fin rays containing a central lumen will not contain a complete record of the fish's environmental history (Tzadik et al. 2017). Fin ray sections were sanded using 800 and 1000 grit sandpaper wetted with deionized water and polished with lapping film to expose the fin ray core and annuli. Sanded and polished sections were mounted on acid-washed glass microscope slides with double-sided tape and stored in acid-washed polypropylene Petri dishes until analysis for elemental concentrations (Norman 2013; Smith and Whitledge 2011).

Concentrations of Sr and Ca in fin ray sections were analyzed using a Thermo X-Series 2 inductively coupled plasma mass spectrometer paired with an LSX-266 laser (CETAC Technologies, Omaha, NE). A transect was ablated along each fin ray or spine section from the

core (the area reflecting early life history) to the edge (which contains the most recently accrued material) using a beam diameter of 25 μm, a scan rate of 5 μm/s, a pulse rate of 10 Hz, an energy level of 35%, and a 40 second gas blank before and after each sample. Reference materials with a $CaCO_3$ matrix (MACS-3, United States Geological Survey) and a bone meal standard (NIST 1486, National Institute of Standards and Technology) were run every 10-20 samples to correct for potential instrument drift. Elemental concentrations of Sr and Ca were calculated from isotopic counts of Ca^{43} and Sr^{86} and were converted to Sr:Ca ratios (mmol/mol) in relation to distance (µm) along the laser transect using Microsoft Excel macros. Sr:Ca data along the laser transect were smoothed using a 25 µm increment moving average (Allen et al. 2009).

Statistical analysis

To detect fish movement among rivers using hard part chemistry, persistent differences in water chemistry must exist among study rivers. Therefore, differences in mean water Sr:Ca among rivers using water Sr:Ca data from 2013-2018 were assessed using a generalized linear model (gamma distribution, log link) followed by Tukey's adjusted pairwise comparisons. Water chemistry data from sites in the upper Des Plaines and the lower Des Plaines were compared to determine whether water Sr:Ca differed upstream and downstream of BRLD.

To assess lock passage by fish captured in the Des Plaines River upstream of BRLD using fin ray Sr:Ca, it was necessary to define river-specific fin ray Sr:Ca ranges to allow for interpretation of the record of a fish's environmental history contained within its fin ray. To characterize river-specific Sr:Ca fin ray signatures, mean Sr:Ca from the outermost 25 μm of the laser ablation transect across the sectioned fin ray from each fish was assumed to represent a Sr:Ca signature indicative of residency of the river where the fish was captured (Smith and Whitledge 2011; Zeigler and Whitledge 2010). This number was chosen as it represents the

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beam diameter of the laser and therefore the minimum possible resolution for distance along the transect. Species within the same family were combined for analysis (Smith and Whitledge 2011, Rude et al. 2014, Laughlin et al. 2016), resulting in four different taxonomic groups: a catostomid group composed of carpsuckers and buffaloes; a centrarchid group composed of Smallmouth Bass and Largemouth Bass, an ictalurid group composed of Channel Catfish, and a lepisosteid group composed of Longnose Gar.

A generalized linear mixed model (glmm) (gamma distribution, log link) with fixed effects of taxonomic group, river of capture, and their interactions and a random effect of individual fish was used to estimate mean edge Sr:Ca for each fish (Kéry 2010). Input to the model consisted of the five smoothed Sr:Ca values that comprised the final 25 μm of the transect of each individual fish and the fish's taxonomic group and river of capture, and the model output was a single modelled Sr:Ca mean edge value for each fish. A dispersion formula (taxonomic group*river + river) was applied to meet assumptions of normality. The glmm was utilized rather than simply taking an average of Sr:Ca values across the last 25 μm of each fish transect in order to provide a better estimate of the central tendency of the values within the last 25 µm of each transect and because the smoothed fin ray edge Sr:Ca values were not normally distributed in several river and taxonomic groupings both within and across individuals.

To characterize a Sr:Ca signature range for resident fish from each river, outliers were identified and removed from each taxonomic group after the predictive edge model was run using an upper range of the seventy-fifth percentile of the modelled fin ray edge Sr:Ca means plus 1.5 * the interquartile range, and a lower range of the twenty-fifth percentile of the modelled fin ray edge Sr:Ca means minus 1.5 * the interquartile range (Navidi 2008). This was done to account for the influence of fish in each river that may have been recent immigrants, which may

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not have had time to accrue a Sr:Ca fin ray edge signature reflective of residency and therefore may contribute Sr:Ca signatures inconsistent with their river of capture. After outliers were removed, the generalized linear mixed model used above was run a second time to predict mean edge Sr:Ca for each fish, again using the input of multiple Sr:Ca values from the edge of the fin ray.

The generalized linear mixed model performed after outlier removal was followed by Tukey's adjusted pairwise comparisons to examine differences in mean fin ray edge Sr:Ca among taxonomic groups and rivers. Pairwise comparisons indicated that there were differences among taxonomic groups, but those differences were not consistent by river of capture. While fin ray edge Sr:Ca differed significantly among the three rivers within each taxonomic group, it did not always differ in the same river among taxonomic groups, or between different taxonomic groups in different rivers. Therefore, the classification model groupings were subsequently developed by individual taxonomic group rather than by combining all taxonomic groups.

Random forest classification (Breiman 2001) was performed in order to assess classification accuracy of assigning individual fish to the river in which they were collected using mean fin ray edge Sr:Ca. The training data set utilized edge data from all fish collected from each of the three study rivers. The predicted mean fin ray edge values for each fish were used as the independent variable, and river of capture designations for each fish were used as the dependent *a priori* grouping (i.e., response variable). Prior to use in the classification model, the river of capture designation for Illinois and Kankakee fish was combined into a single category. This simplified classification into Des Plaines River vs Illinois-Kankakee categories, as it was not necessary to determine specific river residency downstream of BRLD. Within each taxonomic group, some overlap in Sr:Ca signature between Des Plaines River and Illinois-

Kankakee was present even after the removal of outliers (i.e. potential recent immigrants). Therefore, any fish with an edge Sr:Ca signature in the overlap zone was reclassified with a river of capture designation of "uncertain". This ultimately resulted in three possible river of capture designations in the model: Des Plaines River, Illinois-Kankakee, and uncertain.

Four hundred trees were generated for each taxonomic group using aggregated bootstrap sampling of the training data. "River of capture" was used as a single node. Within each tree, the model used the bootstrapped sample to predict (i.e., classify to river of capture) the data not included in the bootstrap and generated an out-of-bag (OOB) error, which indicated the ability of the tree to appropriately classify a fish back to its correct river of capture designation based on its predicted edge Sr:Ca signature. The OOB errors from each of the 400 trees were aggregated into a single OOB estimate of error rate, which was used to assess the overall classification accuracy of the model (Liaw and Weiner 2002).

To identify individual fish captured upstream of BRLD that showed evidence of upstream passage, fin ray Sr:Ca data from entire individual fish transects (i.e., test data set) were input into each taxonomic group's specific random forest classification model, which then provided river classifications for the entire life history of each fish captured in the Des Plaines River upstream of BRLD. In reviewing Des Plaines fish classifications for Illinois-Kankakee Sr:Ca signatures that indicated upstream BRLD passage, a continuous Illinois-Kankakee Sr:Ca signature of at least 35 µm was required in order to suggest that a fish had passed upstream. This minimum threshold was selected to avoid spurious inferences of movement based on single or very few data points; a consistent signature of that length was long enough to suggest that the signature was not machine error and was also slightly higher than the 25 µm minimum resolution in which a precise estimate of Sr:Ca could be obtained based on the scan rate (5 µm/s), beam diameter (25

μm) of the laser, and the time interval between data points (~0.5 s). A fish with an Illinois-Kankakee Sr:Ca signature of fewer than 35 μm or containing an uncertain Sr:Ca signature of 35 μm or greater was classified as a fish with uncertain downstream residency. Fish with exclusively Des Plaines River Sr:Ca signatures or uncertain Sr:Ca signatures of 35 μm or fewer were classified as fish without evidence of passage. Relative frequencies of fish classified into each passage category (i.e., evidence suggesting passage, uncertain downstream residency, and no evidence of passage) were calculated for each taxonomic group.

Fish collected in the Illinois and Kankakee rivers were not assessed for upstream BRLD passage. It was not possible to identify BRLD passage (upstream or downstream) for fish caught in the Illinois or Kankakee rivers due to an anticipated (and later confirmed) lack of difference in water Sr:Ca in the Des Plaines upstream and downstream of BRLD. If a fish sampled in the Illinois or Kankakee rivers contained Sr:Ca values indicative of prior residency in the Des Plaines River, it would not be possible to determine whether that individual had been upstream or downstream of BRLD during the time(s) that it was in the Des Plaines River. However, detection of Illinois-Kankakee Sr:Ca signatures in fish collected in the Des Plaines River upstream of BRLD would provide confirmation of upstream passage. Therefore, the determination of upstream BRLD passage depended on both the collection location of the fish (i.e., upstream of BRLD) as well as the presence of Illinois-Kankakee signatures earlier in the fish's life history.

All statistical analyses were performed using R Studio (R version 3.5.1, R Core Team 2018). Package glmmTMB (Brooks et al. 2017) was used to model the mean edge value for fish, and package randomForest (Liaw and Wiener 2002) was used to build and run the random forest

classification model. All assumptions of parametric statistics were assessed and met or addressed, and all statistical analyses were evaluated at $\alpha = 0.05$.

RESULTS

Water chemistry

The Des Plaines River had the highest mean water Sr:Ca (1.988 ± 0.072 mmol mol^{-1} [mean ± SE]), the Kankakee River had the lowest mean Sr:Ca (0.949 ± 0.020 mmol mol^{-1}), and the Illinois River had an intermediate mean water Sr:Ca (1.564 ± 0.087 mmol mol^{-1}) (Figure 2). The upper and lower Des Plaines River had similar Sr:Ca (upper Des Plaines: 1.981 ± 0.097 mmol mol^{-1} [mean ± SE], lower Des Plaines: 2.01 ± 0.047 mmol mol^{-1}), and Tukey's adjusted pairwise comparisons indicated that mean water Sr:Ca did not differ between the two Des Plaines River reaches (z ratio = 0.191, $P = 0.998$). All other pairwise comparisons among rivers were statistically significant (z ratio = 3.019 – 12.171, $P \leq 0.014$ for all pairwise comparisons). Although the Des Plaines, Illinois, and Kankakee rivers had significantly different mean water Sr:Ca, ranges of water Sr:Ca for the Des Plaines and Illinois rivers partially overlapped (Figure 2).

Fin ray edge chemistry relationships

A total of 458 fish were collected, including 208 centrarchids, 127 catostomids, 75 ictalurids, and 48 lepisosteids (Table 1). Sixteen fish (eight centrarchids, four catostomids, and four lepisosteids) were identified as outliers based on their fin ray edge Sr:Ca and were removed from models used to define river-resident signatures (i.e., glmm for mean fin ray Sr:Ca edge value estimation and subsequent random forest training datasets). The centrarchids included two Des Plaines fish, three Illinois fish, and three Kankakee fish; the catostomids included one Illinois fish and three Kankakee fish; and the lepisosteids included one Des Plaines fish and three

Kankakee fish. Differences in mean estimated fin ray edge Sr:Ca among rivers followed a similar pattern as water Sr:Ca, with the highest fin ray edge Sr:Ca in Des Plaines River-captured fish, the lowest fin ray edge Sr:Ca in Kankakee River fish, and intermediate fin ray Sr:Ca in Illinois River fish (Table 2). As with water Sr:Ca, there was some overlap in the range of fin ray edge Sr:Ca data between fish from the Des Plaines and Illinois rivers in each taxonomic group. In the centrarchids, there was also partial overlap in the ranges of fin ray edge Sr:Ca for Des Plaines River and Kankakee River fish.

Tukey's adjusted pairwise comparisons indicated some separation of mean fin ray edge Sr:Ca among taxonomic groups and rivers (Figure 3). Within each taxonomic group, mean fin ray edge Sr:Ca consistently differed among the three rivers (t ratio = 4.155 – 20.326, $P \leq 0.002$ for all pairwise comparisons). However, mean fin ray edge Sr:Ca within each river differed significantly for some pairs of taxonomic groups, but not others. Additionally, mean fin ray edge Sr:Ca for a taxonomic group in a particular river did not always differ significantly from mean fin ray edge for another taxonomic group from a different river. Because of this, fin ray edge Sr:Ca data from each of the taxonomic groups were analyzed separately in subsequent classification models and assessment of upstream lock passage.

Random forest classification

Prior to testing the random forest models for accuracy of assigning fish to their river of capture, 129 (of 200) centrarchids, 21 (of 123) catostomids, 8 (of 75) ictalurids, and 6 (of 44) lepisosteids were *a priori* reclassified with a river of capture designation of "uncertain" due to their mean estimated fin ray Sr:Ca edge signature falling within the fin ray Sr:Ca overlap zone of the Des Plaines and Illinois-Kankakee classification categories. The random forest classification model for each taxonomic group assigned fish to their river of capture based on estimated fin ray

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edge values with a high degree of accuracy (Table 3). The overall OOB estimates of error rate for centrarchids, catostomids, ictalurids, and lepisosteids were 1.5%, 1.6%, 2.7%, and 2.3%, respectively. Errors in classification were produced exclusively from incorrect assignments regarding the uncertain category; no Des Plaines River fish were misclassified as Illinois-Kankakee or vice versa.

Assessment of upstream BRLD passage in Des Plaines River fish

Fin ray Sr:Ca data from 200 fish captured in the Des Plaines River upstream of BRLD were examined for evidence of prior occupancy of the Illinois or Kankakee rivers by these individuals, including 114 centrarchids, 25 catostomids, 41 ictalurids, and 20 lepisosteids. Any Des Plaines River fish previously removed as outliers based on mean fin ray edge Sr:Ca (i.e., in the glmm for mean fin ray Sr:Ca edge value estimation and random forest training data) were included for analysis (three centrarchids and one lepisosteids). These fish were removed due to unusual fin ray edge Sr:Ca signatures and were therefore not appropriate to use to characterize a Des Plaines River Sr:Ca fin ray signature of residency but, as potential recent immigrants, were important to include in the assessment of potential upstream lock passage at BRLD. Some individuals in each taxonomic group contained consistent Illinois-Kankakee Sr:Ca signatures in their life history that suggested upstream BRLD lock passage, ranging from 15% (lepisosteids) to 37% of individuals (ictalurids) (Figure 4). Of the 114 centrarchids that had Sr:Ca signatures suggesting lock passage, 33% were Smallmouth Bass and 67% were Largemouth Bass. Of the seven catostomids that had Sr:Ca signatures suggesting lock passage, 57% were River Carpsuckers, 29% were Smallmouth Buffaloes, and 14% were Bigmouth Buffaloes. Most fish classified as passers had one or more sections of the laser ablation transect with a consistent Illinois-Kankakee Sr:Ca signature that was much longer than the 35 µm minimum. Indeed, some

fish had consistent Illinois-Kankakee designations for the majority of the laser ablation transect, up to 865 µm. In fact, 90% of centrarchids, 86% of catostomids, 87% of ictalurids and 100% of lepisosteids that had evidence suggesting upstream passage had consecutive Illinois-Kankakee signatures that exceeded, rather than simply met, 35 µm in length.

Additional fish from each taxonomic group were classified as fish with uncertain downstream residency (Figure 4). The range of fish classified as uncertain downstream residency was much broader than that of fish with evidence suggesting passage, ranging from 19% (ictalurids) to 80% of individuals (catostomids). The percentages of fish classified as uncertain downstream residency in each taxonomic group did not necessarily reflect the size of the uncertain zone: centrarchids had double the degree of overlap between Des Plaines and Illinois-Kankakee compared to catostomids but a much lower percentage of uncertain downstream residency classifications (32% and 80% for centrarchids and catostomids, respectively).

DISCUSSION

BRLD passage

Even when considering the lowest estimates of passage, all taxonomic groups contained individuals with Illinois-Kankakee Sr:Ca signatures that suggested upstream BRLD passage. This is the first evidence suggesting upstream passage through BRLD for all of the study's taxonomic groups, though it has been suggested by previous IDNR basin surveys, which recorded the presence of new species in the upper Des Plaines River which likely passed upriver through the BRLD lock (Pescitelli 2015). Although lock passage has not been documented in native species at BRLD prior to this study, several of the target species in this study have been reported passing through lock and dam structures elsewhere on the Illinois Waterway and Upper Mississippi River System (UMRS), of which BRLD is a part (ACRCC 2017b; Tripp et al. 2014;

Wilcox et al. 2004). Wilcox et al. (2004) identified Bigmouth Buffalo, Smallmouth Buffalo, Channel Catfish, Largemouth Bass, and Smallmouth Bass as species known to move through UMRS dams, and Longnose Gar and Quillback Carpsuckers were identified as probable migrants within the system. Later studies examined passage specifically through UMRS lock chambers and found evidence of upstream lock passage in Bigmouth Buffaloes, among other native species not included in this study (ACRCC 2017b; Tripp et al. 2014). Lock passage has been observed to occur less frequently than dam passage in some lock and dam structures along the Illinois Waterway and UMRS, including Lock and Dam 26 on the Mississippi River and Starved Rock Lock and Dam on the Illinois River (ACRCC 2017b; Lubejko et al. 2017; Tripp et al. 2014); this may stem from additional difficulties associated with lock passage, including turbulent flow, lack of directional flow to orient fish to upstream, and irregularity in frequency and timing of lock operations (Wilcox et al. 2004). However, fish have also been shown to use the lock chamber for upstream movements at structures where it is the only available option: multiple species have been observed passing upstream through the lock chamber of Lock and Dam 19 on the Mississippi River, which, like at BRLD, is the exclusive means of upstream transit (ACRCC 2017b).

Species within each of the taxonomic groups in this study have been documented making intentional upstream migrations of varying distances, often associated with movement into smaller tributaries and backwaters for spawning or movement between seasons (Butler and Wahl 2011; Cooke et al. 2005; Curry and Spacie 1984; Johnson and Noltie 1996; Langhurst and Schoenike 1990; Lucas and Baras 2001; Pellett et al. 1998). These migrations suggest a possible motivation to challenge and pass through locks and dams, including BRLD. Despite biological motivations and studies that have reported upriver movements, only a few studies have examined

lock passage in some of the study target species. This is a topic in need of further study, which

could help to assess factors influencing passage rates at BRLD and elsewhere.

Microchemistry as a tool

The underlying differences in water Sr:Ca between the three study rivers were reflected

in the fin ray Sr:Ca signatures of fish in the study and followed the same trends reported in prior

studies (Smith and Whitledge 2010; Whitledge 2009). The intermediate water and fin ray Sr:Ca

signatures in the Illinois River reflect the river's source at the confluence of the high-Sr:Ca Des

Plaines River and the low-Sr:Ca Kankakee River. Despite some overlap in water and fin ray

Sr:Ca signatures, fish fin ray Sr:Ca within each taxonomic group consistently separated

significantly among all three rivers based on the Tukey groupings. These differences enabled the

use of microchemistry as an effective tool to retrospectively infer fish residencies and

movements among the study rivers. In particular, the use of microchemistry facilitated the

examination of lock passage using a larger sample size of fish from within a larger geographic

distribution than would have been possible in a short time frame with other approaches like

telemetry, which requires costly receiver arrays and transponders and may take many years to

acquire the amount of data that a single fin ray can provide when analyzed with microchemistry.

Microchemistry, though it necessitates complex laboratory analysis, requires minimal tools in the

field, can track lock and dam passage without the need to sample directly at the lock site, and can

provide many years of data from a single sampling event.

The similarity of Des Plaines water Sr:Ca signatures upstream and downstream of BRLD

precluded a number of other analyses associated with passage. Passage events could not be

linked to age: the age that a fish acquired a Des Plaines signature would only indicate residency

in the river, and not necessarily BRLD passage, as a fish may reside in the lower Des Plaines

River for an unknown amount of time prior to passing upstream of BRLD. Similarly, the number of passage events could not be assessed, as a fish moving past BRLD multiple times may acquire signatures of the Illinois or Kankakee if it moved downstream for a sufficient period of time, or it may only have a static Des Plaines signature if it stayed within the river. Furthermore, analysis by natal origin was untenable, as a fish identified with a natal origin of Des Plaines could have originated upstream or downstream of BRLD.

In this study, the use of microchemistry to distinguish movement patterns was applicable across taxa. However, differing relationships among taxonomic groups within each river necessitated separate analysis by taxa rather than a combined analysis using all fish. The ability to jointly analyze taxa varies by study, with some studies reporting no difference between taxa (Rude et al. 2017; Zeigler and Whitledge 2010), and others requiring separate analysis (Hamer and Jenkins 2007; Swearer et al. 2003). This may be due in part to the fact that some species incorporate Sr:Ca signatures into their fin rays differently than others (Campana 1999). Therefore, any study that examines multiple taxa should test for differences amongst taxa prior to analysis.

Though water and fin ray chemistry signatures differed among the three study rivers, they were not completely distinct. While the differences among rivers were almost certainly driven by differing geology of the Des Plaines and Kankakee watersheds (Pracheil et al. 2014), variability of Sr:Ca (as evidenced by water Sr:Ca standard error) within the Des Plaines River, and the Illinois River downstream by extension, resulted in some overlap in the range of water Sr:Ca among rivers. It is possible that the variability in the Des Plaines was driven by changes in river discharge; additional water samples are needed throughout the Des Plaines River watershed in future years across multiple seasons and flows to determine water chemistry patterns with higher

resolution. An additional source of overlap between fin ray Sr:Ca river signatures could stem from the presence of recent immigrants in the sample, which may bias the characterization of river-specific Sr:Ca fin ray signatures. All efforts were made to remove outlier fish from the glmm for mean fin ray Sr:Ca edge value estimation and the training datasets for the random forest classification, but without knowing if a collected fish has been in an area long enough to obtain a resident Sr:Ca signature, it is possible that some non-resident fish were included in the data. The length of time needed to acquire a signature of residency in a fin ray is variable and depends on the fish's growth rate, which can be influenced by a number of physiological and environmental factors including age, temperature, and condition (Hamer and Jenkins 2007; Morais and Daverat 2016; Sturrock et al. 2015).

Despite overlap in signatures, the random forest model was able to classify fish in the training dataset to their correct river of capture with at least 97% classification accuracy across all taxa. The addition of the uncertain category reflecting the fin ray overlap zone greatly increased classification accuracies and is suggested for use in microchemistry-based classification models in systems where microchemical signatures in water bodies are distinct, but not completely so. The combination of the Illinois and Kankakee rivers into a single category also increased the classification accuracy of the random forest model, as it no longer had to contend with any overlap between the Illinois and Kankakee Rivers. The combining of downstream rivers did preclude additional examination of specific river downstream residency; however, given the overlap between rivers (i.e. the presence of the uncertainty category), it is unlikely this could have been examined with sufficient resolution to draw conclusions. The error in classifying fish to river of capture suggests that the error in classifying retrospective environmental history for fish caught in the Des Plaines River was likely of similar magnitude.

However, because the OOB error of classification was less than 3% across taxonomic groups, the evidence suggesting passage or lack thereof was probably not substantially affected by this low level of classification error. Additional techniques that are often used in microchemistry studies to provide increased resolution and distinction of river signatures where overlap exists (i.e. stable isotope analysis or the use of additional trace elements) were not applied in this study due to limited differences between study rivers or the inability for other markers to provide additional differentiation (Whitledge 2009).

Other rivers in the area could act as potential sources of fish to the Des Plaines River. The CAWS is the nearest system, entering the Des Plaines River upstream of BRLD. The CAWS and Lake Michigan, which flows into the CAWS in multiple locations, have intermediate Sr:Ca signatures between the Des Plaines and Illinois rivers (Whitledge 2009, and unpublished) Therefore, intermediate fin ray signatures might be expected in fish traveling from the CAWS, which could confound estimates of passage, as fish would not have to pass through BRLD to get into the Des Plaines River from the CAWS. However, it is unlikely that fish from the CAWS substantially contributed to estimates of fish with evidence suggesting passage. First, the Chicago Sanitary and Ship Canal electric dispersal barriers are located approximately 9.6 km upstream of the confluence of the CAWS with the Des Plaines River. These barriers are in place to prevent fish passage, though some small fish may be able to pass under certain conditions (Davis et al. 2017; USACE 2014). The Lockport Lock and Dam is also located on the CAWS 1.6 km upstream of the confluence with the Des Plaines and may act as an additional barrier, though telemetry studies have indicated some downstream fish passage through the structure as well as the adjacent Lockport Controlling Works (ACRCC 2017a). Fish surveys by the Illinois Department of Natural Resources have documented all target species in this study in the CAWS

upstream of the electric barriers, and all target species except Bigmouth Buffalo, River Carpsucker, and Quillback Carpsucker in the Lockport Pool (ACRCC 2017a). However, given the intermediate water Sr:Ca signatures in the CAWS, it is likely that any fish from the CAWS would have fin ray Sr:Ca signatures that fell within the overlap zone between the Des Plaines and Illinois-Kankakee and would therefore be classified as uncertain. Thus, it is possible that a limited number of CAWS fish contribute to the estimates of fish with uncertain downstream residency but less likely they would be included as fish with clear, consistent Illinois-Kankakee signatures that suggested downstream residency and upstream passage.

Other nearby rivers include the DuPage River, a tributary to the Des Plaines River downstream of BRLD, and the Fox River, a tributary to the Illinois River. The DuPage River has water chemistry Sr:Ca intermediate of the Kankakee and Illinois rivers (Whitledge 2009, and unpublished); therefore, any fish passing from the DuPage River into the Des Plaines River would likely contain signatures consistent with the Illinois-Kankakee category and appropriately classified as fish with evidence suggesting passage. The Fox River has the highest water Sr:Ca of any regional river (Whitledge 2009) which would be expected to result in fish residing in the Fox River having similarly high fin ray Sr:Ca as Des Plaines River fish. However, since the confluence of the Fox River with the Illinois River is 53 km from the source of the Illinois River, it is likely that a fish migrating from the Fox River to the Des Plaines River would reside in the Illinois River long enough to incorporate a fin ray Sr:Ca signature indicative of Illinois River residency and would therefore again be appropriately categorized as fish with evidence suggesting passage.

Lastly, tributaries within the upper Des Plaines River could also be a source of additional fish. Of the target species, however, only Smallmouth Bass, Largemouth Bass and Channel

Catfish have been observed in upper Des Plaines River tributaries during IDNR basin surveys, suggesting the tributaries are either not appropriate habitat for catostomids and lepisosteids, or that those taxonomic groups have not yet expanded their range upstream to access those areas. Channel Catfish have been only been found in small numbers in upper Des Plaines River tributaries, suggesting those are not prime habitat for that species either. Any fish sampled in this study that had been present in upper Des Plaines River tributaries would likely have similar fin ray Sr:Ca to fish in the mainstem, as the water Sr:Ca is derived from underlying geology. Water samples taken from Salt Creek, the largest tributary in the upper Des Plaines, matched that of the mainstem.

While it was applied to large fish in this study, microchemistry can also be used to study small-bodied fishes (Rude et al. 2017) and would be appropriate for the Des Plaines River, as new species to the river have included small fish such as the Rosyface Shiner *Notropis rubellus* (Pescitelli 2015) . This is another strength of the tool, as it is difficult to study small fish with techniques like telemetry. However, telemetry studies on larger-bodied native fish passage would be an excellent complement to microchemistry studies in order to confirm passage events. As telemetry arrays are already in place above, below, and within the lock chamber at BRLD (ACRCC 2018), the only work that would need to be done is tag implantation for target species.

Factors affecting estimates of passage

Although all taxonomic groups were shown to pass BRLD, confounding factors exist that could underestimate BRLD passage. Of particular note are temporal factors. Detecting a successful BRLD passage relies on fish acquiring a signature of downstream residency in their fin rays. This is not always guaranteed, particularly if a fish resides in a river for a short period of time. The rate of signature acquisition depends on a number of factors, including fish age,

growth rate, and ambient water temperature (Pracheil et al. 2014). Short-term residency in (i.e. rapid movements between) study rivers may not be detectable if a fish was not present in the system long enough to acquire a Sr:Ca signature. Even if Sr:Ca residency signatures are acquired, they must also be retained. Fin rays and spines may be vascularized and resorbed from the core during growth or times of stress, which can erase early life signatures (Tzadik et al. 2017). Any resorption or non-acquisition of Illinois-Kankakee Sr:Ca signatures could lead to an underestimation of passage. Several fin rays with a central lumen were observed during this study, particularly in lepisosteids. Overall, when examining Des Plaines River-captured fish (i.e. the only fish where the fin ray core was sampled), 2% of centrarchids. 4% of catostomids, 20% of ictalurids, and 85% of lepisosteids had a central lumen present of varying size, which could indicate a potential loss of early life history signatures. In each case, the central lumen encompassed a portion of the first growth season (age-0), not typically the age at which fish make deliberate upstream migrations. Though several papers have commented on the difficulty of aging lepisosteids based on pectoral fin rays (Buckmeier et al. 2018; King et al. 2018; Stein et al. 2018), none have reported the high degree of vascularization observed in this study. Should this pattern be consistent across other lepisosteid populations, caution is recommended if using pectoral fin rays in microchemistry studies focusing on natal origin analysis, as this information may often be lost or obscured.

Spatial considerations play an additional role in potential underestimation of passage. As anticipated, the similar water Sr:Ca in the Des Plaines River upstream and downstream of BRLD compelled the use of fish collected exclusively from upstream of BRLD to confirm upstream lock passage, as any fish captured downstream of BRLD with retrospective Des Plaines River Sr:Ca signatures could indicate residency exclusively downstream of BRLD rather than passage.

However, it is possible that fish from the Illinois or Kankakee rivers had passed. Similarly, it is possible that any fish sampled upstream of BRLD with exclusively Des Plaines classifications had also passed after residing only in the lower Des Plaines River but not the Illinois or Kankakee rivers. Furthermore, the use of the uncertainty category increased classification accuracy, but it also may have reduced estimates of movement. Only Des Plaines River fish with clear, consistent Illinois-Kankakee signatures were classified as fish with evidence suggesting passage. Any Des Plaines fish with consistent uncertain signatures were classified as fish of uncertain downstream residency. It is likely that some uncertain classifications were, in reality, fish that had resided downstream in the Illinois or Kankakee rivers. Without the use of supplementary techniques, however, passage was unable to be assessed in the fish of uncertain downstream residency. Therefore, the estimates of passage reported in this paper can be viewed as a conservative estimate.

While there was evidence of passage across all taxonomic groups in this study, passage patterns should only be assumed to hold true for this particular sample; care should be taken when extrapolating the results to Des Plaines River fish populations, due to unknown differences in passage patterns not only across time scales outside of the fin ray record represented in this study, but also in other individuals not sampled within the study reaches and in other reaches of the upper Des Plaines River not studied here. Future research is needed to determine whether the patterns of passage observed here are consistent with the entire upper Des Plaines populations, and whether those patterns are consistent across years and taxonomic groups. *Ecological and management considerations*

The Des Plaines River has seen increasing connectivity over the years. Twelve dams have been removed upstream of BRLD, and the remaining two low-head dams, which can act as fish

passage barriers at low flows, are also scheduled to be removed (Altenritter et al. 2019; USACE

2013), leaving BRLD the sole barrier to upstream passage into the upper Des Plaines and its

tributaries. Across the United States, dam removal is occurring at unprecedented rates, with

1,355 dams removed in the last 30 years and 168 of those in 2017 and 2018 alone (Thomas-Blate

2019). BRLD, on the other hand, is proposed for heavy modifications to prevent the spread of

AIS and is also important for maintaining a navigation channel. This highlights the importance

of the trade-offs between connectivity and invasive species control: there is no solution that will

be appropriate in every case, and different stakeholders may have different priorities, which may

vary by system. Globally, in fact, at least 3,700 major dams were proposed or under construction

as of 2015, which would reduce the number of large, free-flowing rivers around the world by

more than 20% (Zarfl et al. 2015). Any barrier construction, enhancement, or removal comes

with its own set of ecological, economic, and social considerations, which should be fully

examined and addressed as much as possible throughout the planning and implementation

processes.

Some evidence of upstream passage through BRLD by native fish was found in this

study, and the goal of the installation of barrier technologies at BRLD is to eliminate upstream

passage. Despite this reality, it is unclear what the impacts of restricting passage at BRLD will be

on Des Plaines River fish populations. The mechanism and degree to which BRLD passers

contribute to the Des Plaines River, and whether that contribution is consistent across years and

taxa, is undetermined and warrants further study. Should passage be eliminated, it also unknown

whether all fish populations in the Des Plaines River are capable of self-sustaining without

access to recruitment sources downstream (Altenritter and Casper 2018). There is a need for

further study on population-level implications of restricted passage. The nearby DuPage River

offers a case study to potential impacts of a barrier that eliminates upstream passage. With an impassable dam 1.6 km from the river mouth, the DuPage River has seen increasing species richness below the dam since 1983 but plateauing richness above the dam (Altenritter et al. 2019), suggesting that new fish are moving into the river mouth but are unable to pass upstream due to the barrier. Future research will be needed to determine whether this pattern may also apply to the upper Des Plaines River post-installation of barrier technologies at BRLD. Microchemistry could be one of many tools used to assess the impacts of barrier installation: fewer immigrants from the Illinois or Kankakee rivers would be expected upstream of BRLD if passage is no longer possible. Further research would be needed to capture passage rates of the populations at large, both pre- and post-installation of barrier technologies. Microchemistry may also prove a useful technique in assessing the efficacy of any mitigation efforts such as stocking or translocating fish; if fish are stocked or translocated from sites with unique water chemistry, that signature can act as a natural marker that can later be identified (Rude et al. 2014; Wolff et al. 2013).

Although the priority of the BRLD enhancement project is to limit upstream AIS passage, it is important to consider the unintended consequences of restricting passage for native species into a river system that has seen increases in species richness, habitat connectivity, and restoration efforts. This study suggests that native fish passage should be further studied, possibly including additional microchemistry, to inform ecological assessments and mitigation related to the planned barrier enhancement at BRLD.

Table 1. Counts of fish sampled by taxonomic group from each river.

| Taxonomic group | River of capture | | | Total |
	Des Plaines	Illinois	Kankakee	
Centrarchids	114	49	45	208
Catostomids	25	60	42	127
Ictalurids	41	14	20	75
Lepisosteids	20	13	15	48

Table 2. Mean and range of modelled fin ray edge Sr:Ca (mmol/mol) for fish from each taxonomic group sampled in the Des Plaines, Illinois, and Kankakee rivers. Outliers (recent immigrants) were removed prior to calculating means and ranges.

| Taxonomic group | Fin ray edge Sr:Ca (mmol/mol) by river | | | | | |
| | Des Plaines | | Illinois | | Kankakee | |
	Mean	Range	Mean	Range	Mean	Range
Centrarchids	0.460	0.304-0.609	0.350	0.239-0.491	0.280	0.186-0.352
Catostomids	0.561	0.454-0.717	0.410	0.315-0.511	0.254	0.126-0.402
Ictalurids	0.469	0.353-0.607	0.325	0.265-0.379	0.229	0.160-0.305
Lepisosteids	0.615	0.489-0.735	0.476	0.390-0.548	0.320	0.261-0.399

Table 3. Classification accuracy of the random forest training dataset using modelled fin ray edge Sr:Ca values. Values reported represent number of fish assigned to location categories (Des Plaines River, Illinois and Kankakee rivers, or uncertain) based on fin ray edge Sr:Ca.

| Source Location	Assigned location			Classification accuracy (%)
	Des Plaines	Uncertain	Illinois-Kankakee	
Centrarchids				
Des Plaines	33	1	0	97
Uncertain	1	128	0	99
Illinois-Kankakee	0	1	36	97
Catostomids				
Des Plaines	17	1	0	94
Uncertain	0	20	1	95
Illinois-Kankakee	0	0	84	100
Ictalurids				
Des Plaines	35	1	0	97
Uncertain	0	7	1	88
Illinois-Kankakee	0	0	31	100
Lepisosteids				
Des Plaines	15	1	0	94
Uncertain	0	6	0	100
Illinois-Kankakee	0	0	22	100
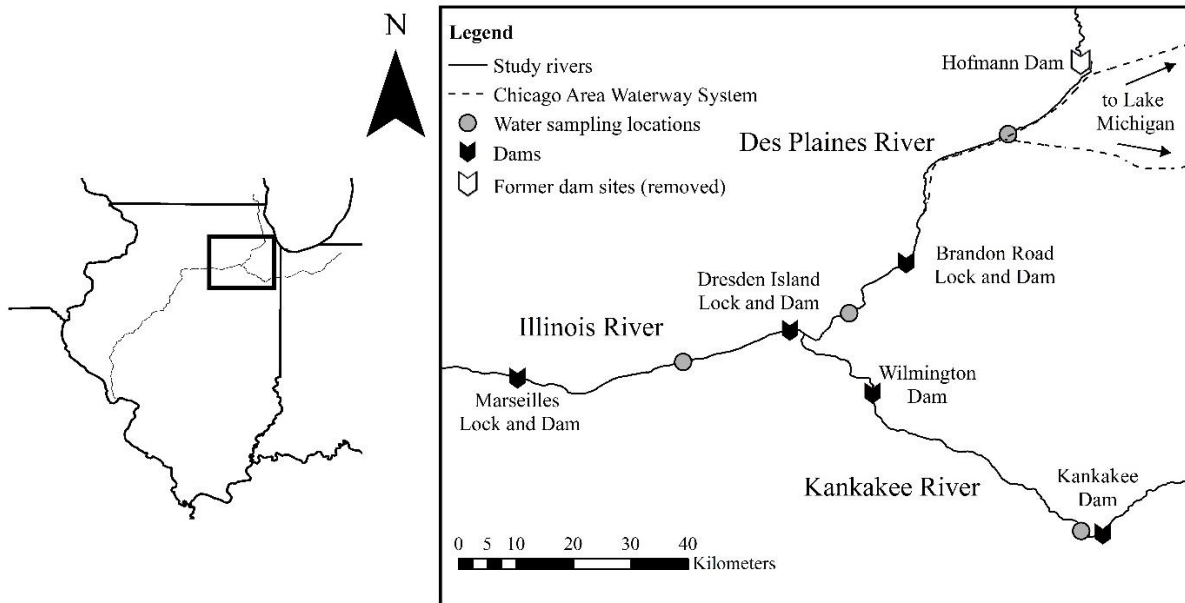
Figure 1. Map of the study area in northeastern Illinois. The river reaches between Brandon Road Lock and Dam, Dresden Island Lock and Dam and the Wilmington Dam were not sampled for fish, nor was the Chicago Area Waterway System.
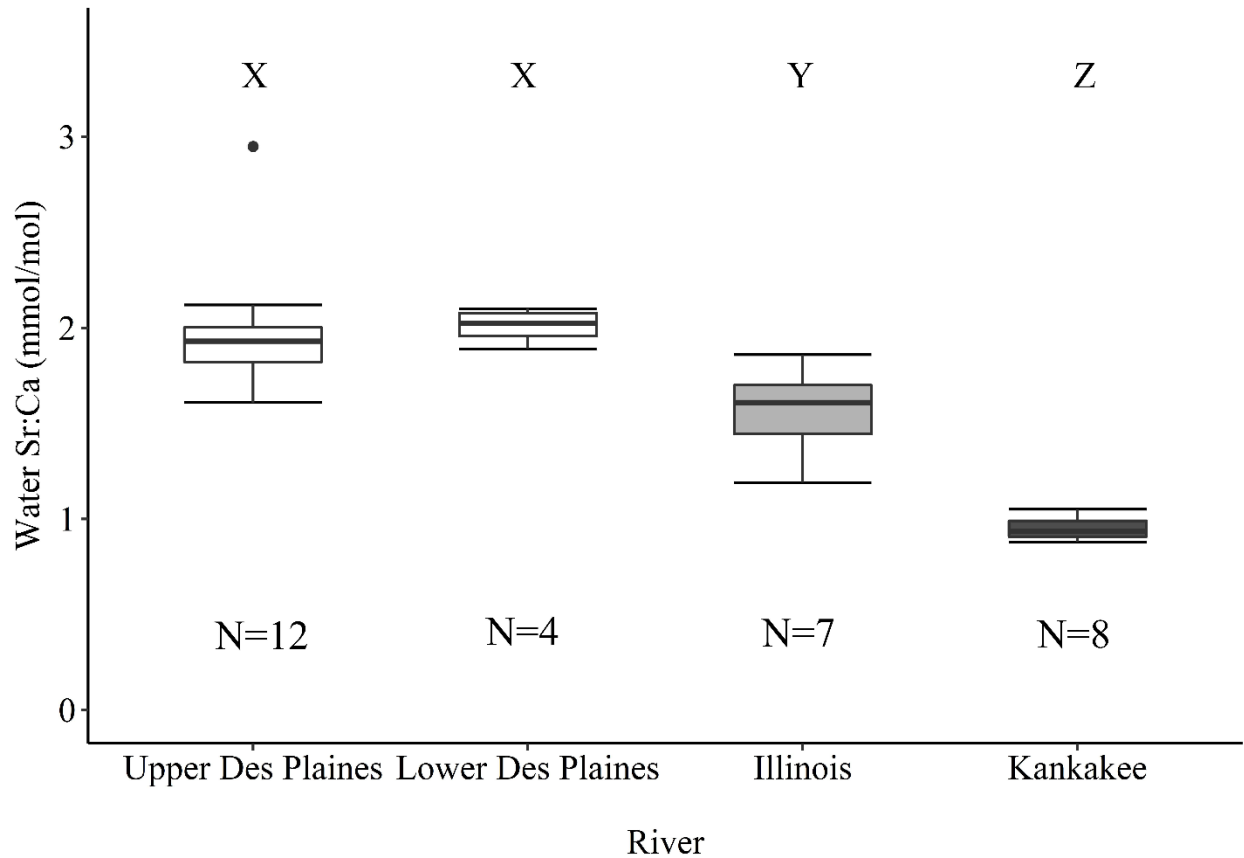
Figure 2. Boxplot of ranges, medians, and inter-quartile ranges for water Sr:Ca from the upper Des Plaines River (upstream of Brandon Road Lock and Dam (BRLD)), lower Des Plaines River (downstream of BRLD), Illinois River, and Kankakee River. Mean water Sr:Ca differed among locations that do not bear the same letter above boxplots ($P < 0.05$). Samples were collected in October 2017 and every other month from April to October 2018 and supplemented with additional sporadic samples from 2013-2017.
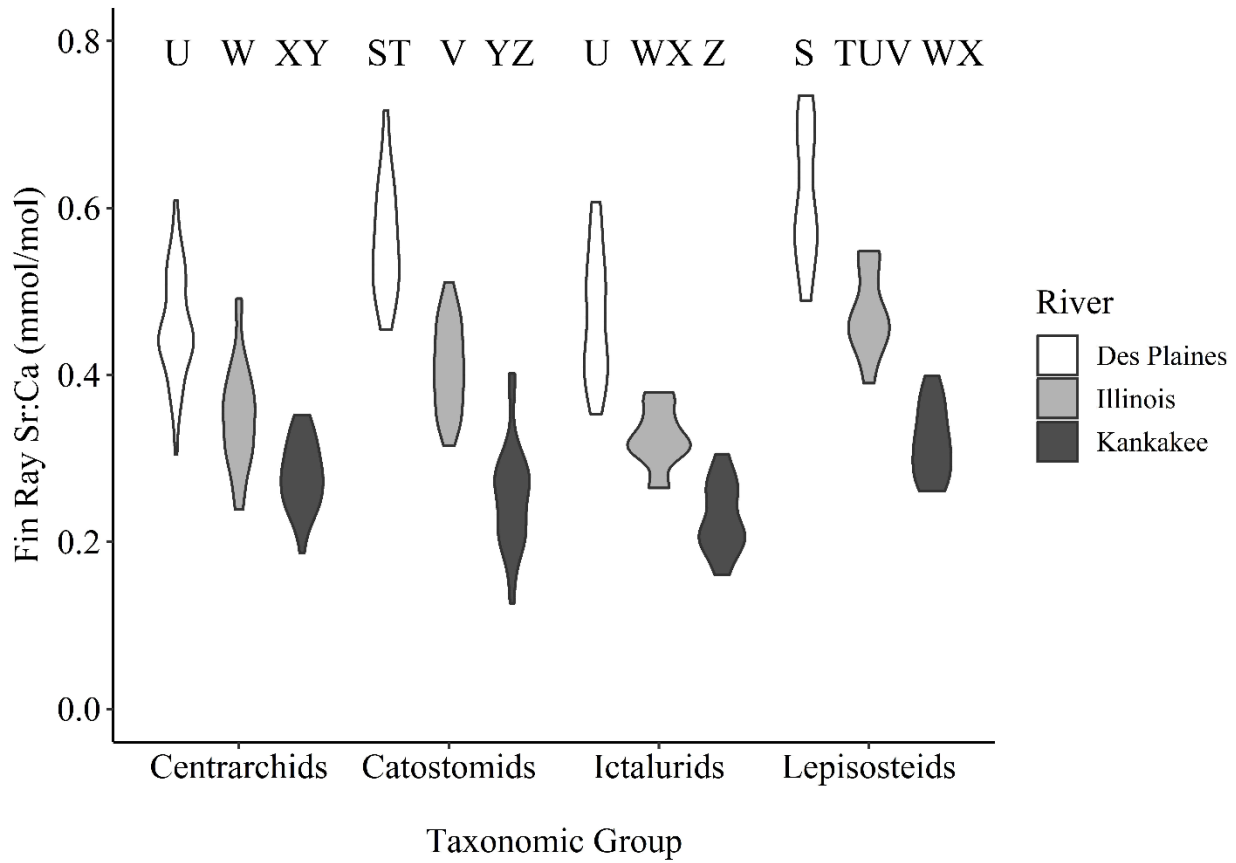
Figure 3. Violin plot showing distributions of fin ray edge Sr:Ca values from fish in each of four taxa sampled from the Des Plaines, Illinois, and Kankakee rivers. Mean fin ray edge Sr:Ca differed among groups that do not bear the same letter above plots ($P < 0.05$).
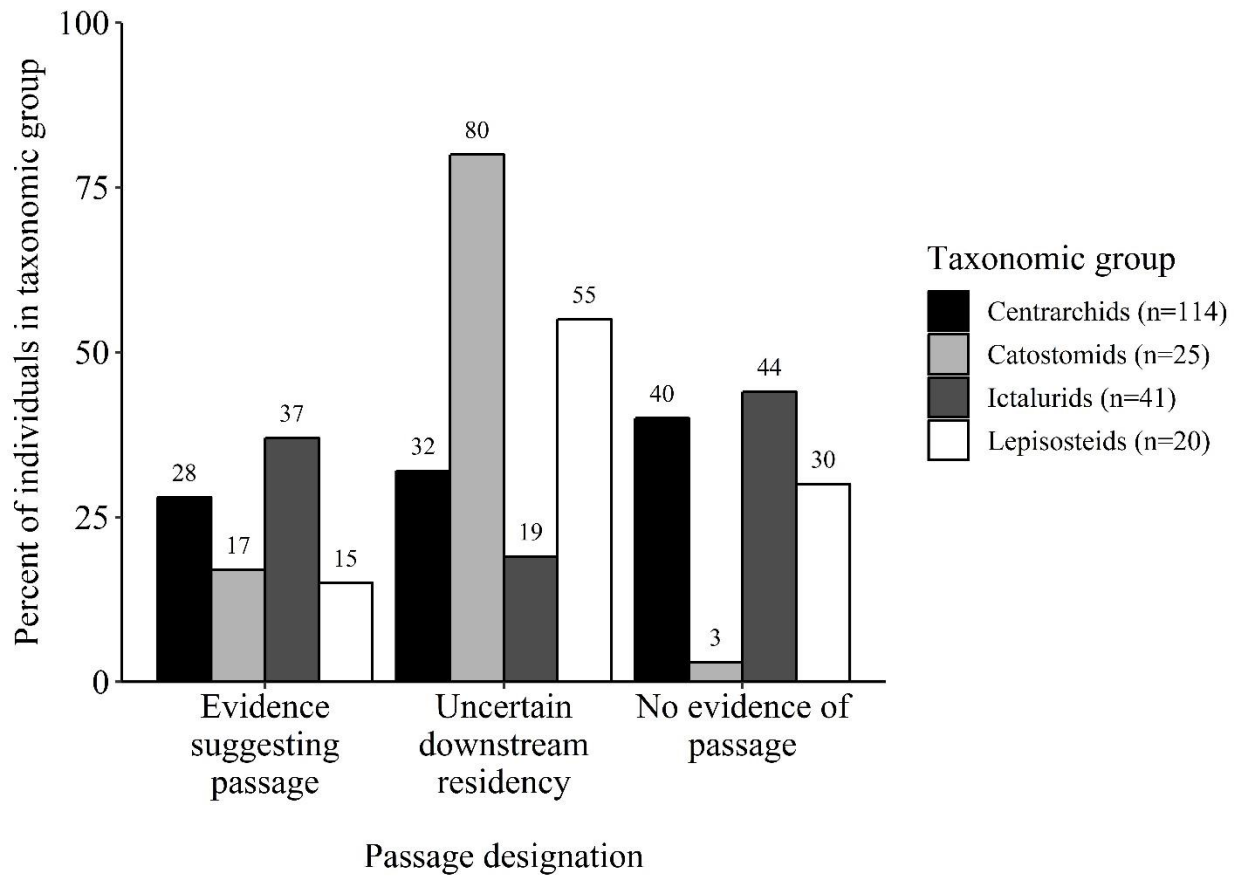
Figure 4. Histogram reflecting upstream BRLD passage frequencies for each taxonomic group, calculated from proportions of classifications derived from the random forest model.

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VITA

Graduate School
Southern Illinois University

Claire E. Snyder

snyderce2@gmail.com

Whitman College
Bachelor of Arts, Environmental Studies-Biology, May 2012

Thesis Title:
A microchemical analysis of native fish passage through Brandon Road Lock and Dam, Des Plaines River, Illinois

Major Professor: Gregory W. Whitledge