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Population Genetics and Distribution of the Oriental Weatherfish, Misgurnus anguillicaudatus, in Chicago Area Waterways

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LOYOLA UNIVERSITY CHICAGO

POPULATION GENETICS AND DISTRIBUTION OF THE ORIENTAL WEATHERFISH, *MISGURNUS ANGUILLICAUDATUS*, IN CHICAGO AREA WATERWAYS

A THESIS SUBMITTED TO
THE FACULTY OF THE GRADUATE SCHOOL
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Remember kids, the only difference between screwing around and science, is writing it down.
– Adam Savage

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ABSTRACT

Oriental Weatherfish (*Misgurnus anguillicaudatus*), native to Southeast Asia, are freshwater fish belonging to the family Cobitidae (loaches). They are benthic fish with the ability to breathe atmospheric air, and exhibit an unusual behavior of swimming vertically in the water column in response to changes in barometric pressure. Oriental Weatherfish appear to be successful invaders to North American waterways. Within the last century they have been sighted in freshwater systems throughout the United States, yet the distribution and source of this invasion are largely unknown. This study investigates the distribution patterns, surveying methods, and population genetics of the Oriental Weatherfish within Illinois and Indiana waterways. These data were collected in 2013 and 2014 and supplemented with publically available data to provide the most up-to-date map of the current distribution in Illinois (IL) and Indiana (IN). Catch rates were calculated and compared across gear types and sampling sites to assess different survey methods. Sequence data from cytochrome c oxidase I (COI) and the control region (D-Loop) were compared among specimens collected from multiple sites throughout IL and IN with those available on GenBank. Results from this study suggest a single introduction to IL and IN before 1987, with a subsequent range expansion. This study is the first to genetically examine this weatherfish population. Data suggest that the population is genetically identical to the weatherfish introduced into Australia before 1984, and that these populations were derived from the same native population in Asia.

CHAPTER I

INTRODUCTION

Invasive Species

The introduction of species by humans to novel environments is not a new phenomenon, and as methods of human movement have become more sophisticated, the species we take with us spread farther and faster than ever before. This movement of species can be the result of accidental or purposeful releases. In the United States, an estimated 50,000 non-native species have been introduced (Pimentel et al. 2005), but this problem is not restricted to a given region. Species introductions are continually occurring and affect all continents. For example, the black rat (*Rattus rattus*) is a native of India and Southeast Asia and was spread as far as Great Britain by the early first century (Engels 1999). The black rat has since spread to several other countries, including the United States where it was estimated that their total population is around 1 billion (USDA 2001). The estimated cost of damages caused by the rat is around $19 billion per year just in the United States (Pimentel et al. 2005). This is largely an accidental release, as the rats are typically found aboard ships, which subsequently carry them between regions (Harper & Bunbury 2015). The Cane Toad (*Rhinella marina*), a native to the Americas, has been a deliberate release. The species was brought to places such as Australia and other Pacific Islands in the early 20th century in the hope that it could help rid areas of agricultural pests. While these introductions have been successful in some aspects, they can prove to be detrimental to native fauna that were not the intended target (Freeland 1984). Typically, non-native species have considerable impact on extirpation rates of native fauna, alter food webs, and alter the life history traits of native species (Ricciardi 2001).

As more species are introduced and expand their range, the cost of controlling these invaders will continue to rise. Currently, it is estimated that $125 billion per year is spent in the US on control efforts (Allendorf & Lundquist 2003). These control methods are not always effective and might not even be possible if the species in question provides some economic gain or societal benefit. This is especially prevalent in instances such as establishing a sport fishery, transporting valued food crops, or trying to control for a harmful invasive by using another invasive (Cambray 2003). The control of invasive species is often difficult and futile once they've become established. Many contend that the best way to truly combat an invasive species is prevention of the introduction through education (Allendorf & Lundquist 2003; Cambray 2003).

Great Lakes Invasives

Locally, as of 2006 the Great Lakes are home to more than 180 invasive species (Ricciardi 2006). Some of these organisms, Round Gobies (*Neogobius melanostomus*) and Zebra Mussels (*Dreissena polymorpha*), were transported accidentally via the ballast water of ocean going vessels that entered the Great Lakes through the Saint Lawrence Seaway (http://www.noaa.gov/). Goldfish and *Tilapia,* which are found in the pet and food trades, are found in the Chicago River (Widloe et al. 2014). Many other fish species have been released into waterways via careless individuals or those discarding unwanted pets. For example, during a routine sampling event, the Illinois Department of Natural Resources (ILDNR) found a rather large Silver Arowana (*Osteoglossum bicirrhosum*)**,** a native of South America and popular aquarium fish, in the North Shore Channel in Chicago (Zeigler et al. 2014). The most probable way this species could get into those waters would be from an aquarist release, but the exact route is unknown. Still other introductions are the result of prayer releases, which are practices of freeing captive animals into the wild as a virtuous act (Severinghaus & Chi 1999).

Depending on the location of the introduction, invasive species can have different threat levels to the lakes and their surrounding tributaries. An area of great concern is Lake Michigan, specifically because of the connections with the rivers and channels of the Chicago Area Waterways (CAWS). Construction of the Chicago Sanitary and Shipping Canal (CSSC) created a direct connection between the Great Lakes basin and the Illinois River and, by extension, the Mississippi River Basin. This waterway allows species to move between the two systems with relative ease, and it is facilitating the movement of invasive species into a much wider area than they were initially released. The Round Goby and quagga mussels (*Dreissena bugensis*) are just two recent invaders that have moved from the lakes to the rivers (Grigorovich et al. 2008; Irons et al. 2006, Malone 2016). Coming from the river side, Silver and Bighead Carp (*Hypophthalmichthys molitrix* and *H. nobilis*), collectively called Asian Carp, are poised to move from the river into the lake. These species and several others are known to disrupt ecosystem processes and degrade habitats in areas where they are found. It has become a primary goal of state and federal agencies to prevent their further spread (Ricciardi & Maclsaac 2000; Higgins & Zander 2010; Conover et al. 2007).

There has been some success with control efforts aimed to stop or reduce the spread of invasive species in the Great Lakes over the years. These efforts vary in scope and species selectiveness. The stocking of an invader to prey on a different invader has been tried with moderate success and interesting consequences. For example, salmon were stocked to control the invasive alewife population in the Great Lakes. They reduced alewife numbers, and also created a valuable commercial fishery (Fenichel et al. 2010). Chemical controls have been introduced that specifically target a species during pivotal points in its life cycle, in most cases before the species reaches reproductive maturity (Aldridge et al. 2006; Christie & Goddard 2003). One example is the use of lampricides to control the invasive Sea Lamprey (*Petromyzon marinus*) population that inhabit the Great Lakes. These lampricides target the larval stages of the lamprey before they have been recruited to the adult, parasite stage (Jones et al. 2003). Electric barriers have even been erected to stop or deter movements into or between water systems (Verrill & Berry 1995; Swink 1999; Sparks et al. 2010). These deterrents range in both species selectiveness and effectiveness, and even though they are in place, the creation of newer, updated technologies is necessary for stopping the spread of the next Great Lakes non-native as they continue to be introduced.

Weatherfish

One of the invaders introduced to the Great Lakes Basin, including the Chicago River, is *Misgurnus anguillicaudatus*, or the Oriental Weatherfish (Figure 1). The Oriental Weatherfish (termed weatherfish from here on) is a peculiar benthic freshwater fish that belongs to the ostariophysan family Cobitidae (loaches). The weatherfish gets its common name from its unusual ability to detect and respond to changes in barometric pressure by swimming frantically and standing on end (Sterba 1973). Unlike most fishes, it can burrow into and hide in soft substrates, breathe atmospheric air when necessary, and survive for extended periods of time out of water (Koetsler & Urquhart, 2012).

Figure 1. Male (top) and female (bottom) Oriental Weatherfish, *Misgurnus anguillicaudatus* (www.michigan.gov/invasives)

Cobitids as a group are native to Asia, Europe, and North Africa, but not to North America. The weatherfish is native to eastern Asia from Siberia to Northern Vietnam, including Japan. However, they are increasingly found in regions that they are not native to, and as a result, could be competing with or disrupting native fishes. In North America, they are found in disjunct (isolated) populations throughout the United States (Berg 1965), and their effects in these isolated areas are unknown. They have also been found in Australia since 1984 (Allen 1984) and starting in 2001, the first recorded wild population was found in the northern part of Italy (Razzetti et al. 2001). The effects of these newer introductions could be less obvious, but could prove harmful. This new European population could be particularly destructive to the currently declining European Weatherfish (*Misgurnus fossillis*), as it would likely compete with it for food and habitat resources.

Multiple hypotheses have been offered to explain the introduction of the weatherfish into freshwater systems of the United States. Devick (1991) proposed that, because the weatherfish is an important food resource in their native Asia, they were brought here by Asian immigrants in the 19th and early 20th centuries. Early introductions might have been the result of immigrants bringing food sources they were culturally familiar with and releasing them into local waterways in hopes of creating a stock for their culinary dishes. Another possible cause is the escape or intentional release of fish from the pet trade or home aquaria. The exact reason for and number of weatherfish introductions are unknown in many areas. However, various methods (genetic analysis and habitat surveys) can provide estimates of range size or probable windows of when, where, and how many introductions have taken place. Many of these methods were used in this study.

According to Brock (1960), the weatherfish was first introduced to the Hawaiian Islands at the end of the 19th century, and as such, was the first sighting of them in the Western hemisphere. Populations of weatherfish have since been found in sixteen more states (Figure 2), with Illinois and Indiana being among the more recent invasions (Nico et al. 2016b; Laird & Page 1996). The disjunct distribution of the weatherfish across the U.S. is suggestive of multiple introductions in different locations. In most regions, it has been assumed that the populations would remain relatively localized and not expand far from their introduction points (Laird & Page 1996), but this claim has not been supported empirically. However, in some areas with weatherfish populations there is some contradictory evidence suggesting that these fish are expanding their ranges (Schmidt & Schmidt 2014; Franch et al. 2008; Simon et al. 2006). Illinois is one such place that this species is potentially expanding its range, but because no detailed study on weatherfish distributions in this area had been done, this could not be confirmed.

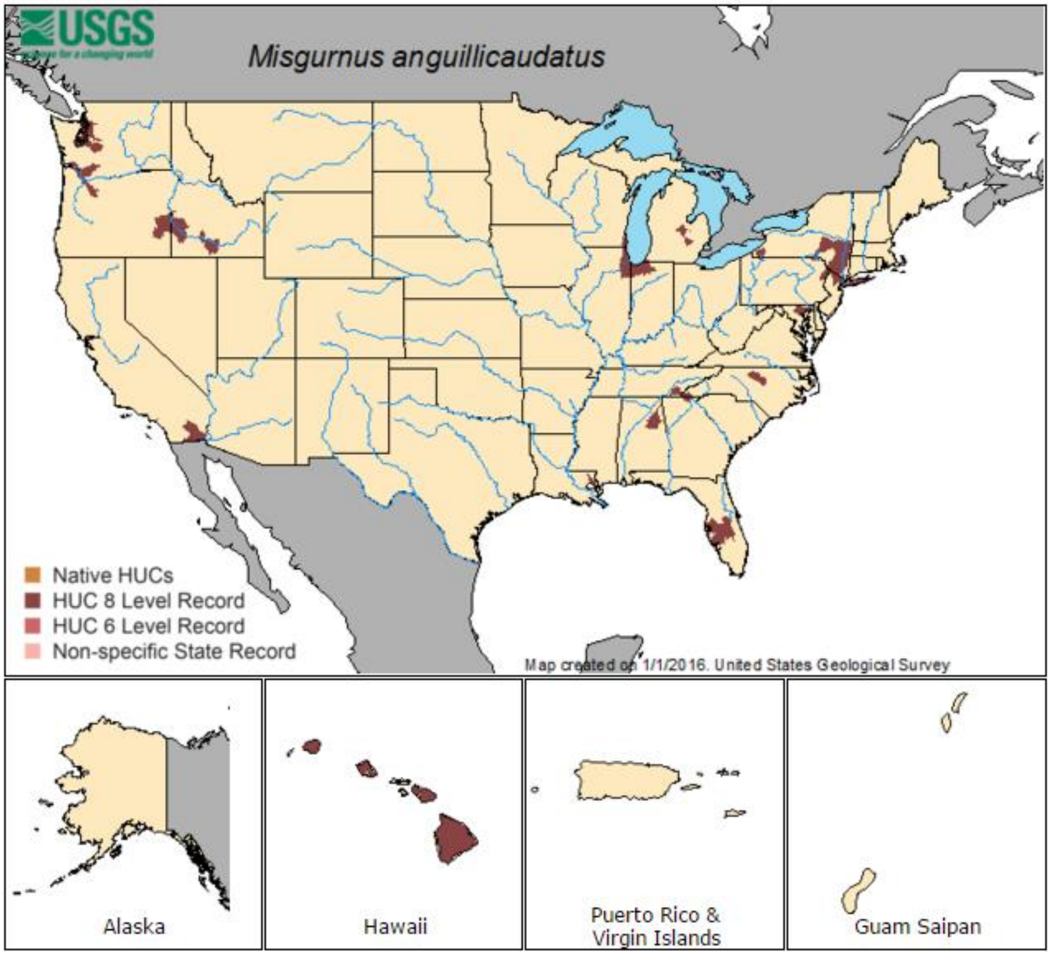

Figure 2. Current United States distribution of the Oriental Weatherfish as reported by the US Geological Survey. Maroon areas indicate weatherfish collection records. http://www.usgs.gov/

As the weatherfish is relatively new to freshwater systems of the United States, it is not surprising that few studies (Tabor et al. 2001; Koestler and Urquhart 2012; Norris 2014) have addressed its invasion biology, life-history, or effects on native fauna. Such studies will be vital for formulating a baseline that can be used for conservation strategies to limit expansion of its range and to minimize its detrimental effects on native species. Other invasive fish species of the Great Lakes are known to have had negative effects on native species, altered native fishes' life history, and have forced several species to the brink of local extinction (Janssen and Jude 2001; Steinhart et al. 2004)**.** In the case of the weatherfish, it is important to understand how many separate invasions into U.S. waterways have occurred, what water bodies have already been successfully colonized, where the fish have come from, the vector(s) of introduction, how it disperses into new areas, and what is the likelihood of competition with native fishes.

This Thesis

This research examines the current distribution of weatherfish in the Chicago Area Waterways (CAWS) and IL Waterways (IWW) while evaluating effective sampling techniques. The genetic similarity of weatherfish throughout this region is also determined. In Chapter 2, I gathered all publically available weatherfish records from 1987 to 2012 and used these data to establish a known range for the weatherfish in the CAWS and IWW up until the time of my study. I sampled the waterways within and outside this range in 2013 and 2014 with active and passive collecting techniques, and gathered other agencies' sampling effort information during that time to determine the current distribution of the weatherfish. Totally, 21 locations covering over 360 miles of rivers and streams within the CAWS and IWW system were sampled personally or by various state and federal agencies and universities. I used the number of hours of effort to establish Catch Per Unit Effort (CPUE) and to determine the most effective method of sampling weatherfish in different environmental conditions. I hypothesized that the weatherfish expanded its range to areas outside of the known range that was determined using historical occurrence data. I also hypothesized that active gear type will have an increased catch rate compared to passive. Results from Chapter 2 provide an updated range map for this species. Results from this study will also enable management officials to more accurately target this species if specific removal programs were to be implemented.

In Chapter 3, I used molecular techniques to isolate DNA and determine the sequences of the mitochondrial control region (D-Loop) and cytochrome c oxidase subunit I (COI) loci.

Different areas along the CAWS and IWW were examined in an effort to determine if these loci exhibit unique haplotypes in those areas, and if they could be attributed to separate introductions. Fifty-four samples were collected from 12 different locations throughout the range of the weatherfish. The fish from each of these 12 locations was examined for genetic similarities to each other. A genetic network was created for each locus, showing each location and the number of haplotypes found at that locus. This information was used to help determine genetic similarities across the entire watershed in an effort to determine if the weatherfish's population is the result of natural expansion or multiple introductions into the CAWS and IWW. These sequences were also compared with those available on GenBank in an effort to determine a possible ancestral locality for the Midwest Chicagoland population. I hypothesized that the sample sites will share many or all of the haplotypes present in the system, indicating that the weatherfish invasion is the result of only one or a few, closely timed releases that spread from approximately the same location on the CAWS. This would result in a roughly genetically homogeneous population throughout its entire Illinois/Indiana range. I predicted this population can trace its origins to a highly sampled area in its home range, likely China or Japan. These results can help to better understand how this species has and is moving through the system as a whole, and may provide a solution for stopping their spread through the use of optimal control efforts.

CHAPTER II

CURRENT DISTRIBUTION AND EFFECTIVE SAMPLING TECHNIQUES FOR ORIENTAL WEATHERFISH (*MISGURNUS ANGUILLICAUDATUS)* IN ILLINOIS AND INDIANA WATERWAYS

Introduction

The movement and successful transplantation of species into habitats outside their native range has become an increasing environmental problem. Invasive species are one of the leading threats to biodiversity via competition, predation and habitat loss (Richter et al. 1997; Pimentel et al. 2005). Some estimates list non-native species as the second largest cause of extinctions of North American fishes after habitat loss (Clavero & Garcia-Berthou 2005; Miller et al. 1989). Invasives can also result in direct economic loss due to environmental impacts to important crops through competition with economically important natives and the expenditure of capital for control and eradication efforts (IUCN 2009).

More than 180 non-native species are present in the Great Lakes basin (Ricciardi 2006). Many of these are the result of human releases through accidental or purposeful means. These releases can stem from direct input into the lakes such as the stocking of Chinook Salmon (*Oncorhynchus tshawytscha*) (Fuller et al. 2016a) or from release of species into a small feeder tributary with subsequent migration into the lakes. Such was the case with the Round Goby (*Neogobius melanostomus*), which was transported via container ships and released when ballast water was discharged into the St. Clair Riveraround 1990 (Fuller et al 2016b; Jude et al. 1992).

Round Gobies have since spread to all of the Great Lakes and have made their way south to the confluence of the IL and Mississippi Rivers (Irons et al. 2006). Another recent example of an invasive fish species found in the waters around Lake Michigan is the Oriental Weatherfish (*Misgurnus anguillicaudatus*).

Oriental Weatherfish are freshwater fish in the family Cobitidae and are native to eastern Asia, ranging from Siberia to Burma, including Japan (Franch et al. 2008). They have become a popular aquarium fish, resulting in their establishment in at least 10 countries outside of their native range. The first recorded collection in the contiguous United States was in Michigan in 1939 (Mills et al. 1993). Since then, weatherfish have been found in 15 other states, and are assumed to be established in many of them, including Illinois (Nico et al. 2016).

According to Laird & Page (1996) weatherfish were first spotted in Skokie, IL in the North Shore Channel (NSC) in 1987. This body of water connects to not only Lake Michigan, but also the Chicago River, where the flow joins with other tributaries around northeastern IL eventually reaching the Mississippi River. Since their first detection, weatherfish have been found in many of the major rivers with both direct and indirect connections to the NSC. There was, however, no complete listing of all historical weatherfish collections for the Chicago region. It is also unclear just how far their range extends in the waters surrounding Lake Michigan or if this species has made the transition from the river system into the lake.

For this study, records were used to construct the historic range of weatherfish in IL/IN (Figure 3). These data were collected from the United States Geological Survey (USGS), U.S. Army Corps of Engineers (USACE), IL Natural History Survey (INHS), Field Museum of Natural History, IL Department of Natural Resources (ILDNR), U.S. Fish and Wildlife Service (USFWS), and the Shedd Aquarium. Occurrences of weatherfish in more northerly locations, closer to the original collection point (i.e., Skokie, IL), appear chronologically earlier, whereas the southern, more distant locations have occurred more recently. This pattern suggests that the range of weatherfish in IL and IN has been expanding since its initial discovery. It is, however, unclear if this distribution pattern is due to the natural spread of the fish, or if the pattern represents isolated populations resulting from multiple independent invasions.

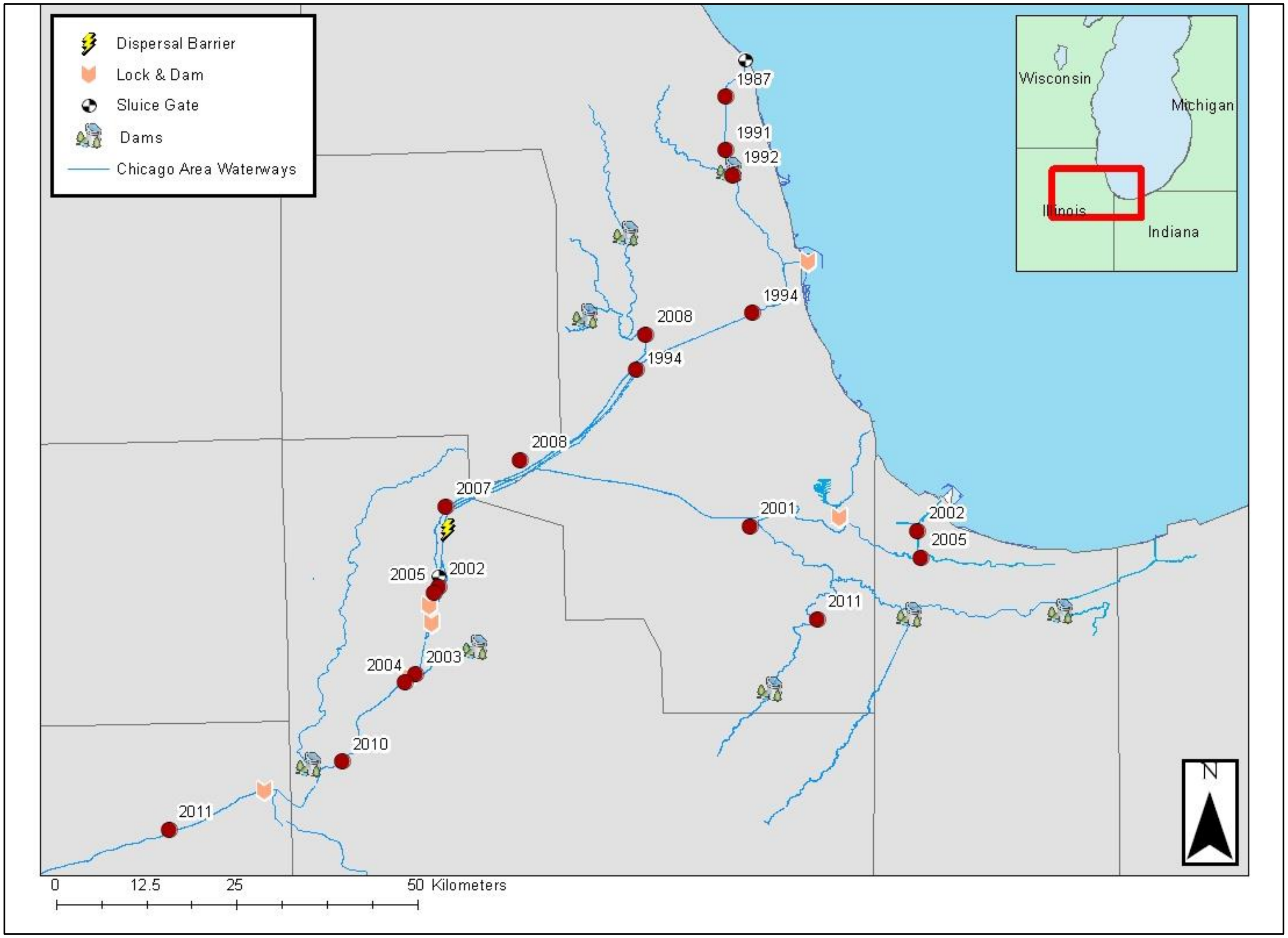

Figure 3. Historic occurrences of Oriental Weatherfish (*Misgurnus anguillicaudatus*) found in Northeastern Illinois and Northwestern Indiana waterways from 1987 - 2012. Years indicate when weatherfish were first collected in an area.

The range expansion of weatherfish into the waterways connected to the Chicago River system, such as the Des Plaines, Kankakee, and Illinois Rivers, will largely be controlled by a number of factors, both natural and anthropocentric. For instance, as of 1994 these fish have been in close proximity to at least two locations in and around Chicago, IL that would give them unfettered access to Lake Michigan. Wilmette Pumping Station and Chicago Lock. Despite this and their close proximity to two additional lake-river transition points, as of 2012 they had not been collected in the lake. This is most likely due to the unsuitable habitat found in the lake. Weatherfish prefer slow moving water with soft, muddy bottoms where they can burrow into the substrate (Koetsler & Urquhart, 2012). Lake Michigan can experience periods of high wave action and typically does not provide suitable cover in terms of vegetation or even bottom debris to help these fish avoid being preyed upon by larger fishes (Meyer & Hinrichs 2000). It has been reported that the European Weatherfish, *Misgurnus fossilis*, cannot burrow into sand (Meyer & Hinrichs 2000), which might also be the case for the Oriental Weatherfish and may explain why Lake Michigan and its sandy bottom is avoided.

Although the lake has so far proven to be unoccupied, the river system that connects to the lake has areas of dense vegetation, soft substrate, and calm backwaters, all features that provide prime habitat for weatherfish. Habitat suitability is the most likely explanation for their continued success in areas throughout and beyond their current range, and may also explain why they could find their way farther into the Illinois and Kankakee rivers or beyond. Within these systems however, the many man-made barriers could prevent range expansion to at least some areas. Potential disruptions to weatherfish dispersal include structures such as dams or locks, all common within the Chicago Area Waterways (CAWS) and IL Waterway (IWW). The presence of these structures might not limit their range expansion in all cases and an accurate assessment of their potential for movement past those structures and habitat sampling would be necessary to determine the presence of weatherfish in any given area of the system. For instance, weatherfish have been found on both sides of several locks throughout the CAWS (Lockport Lock and Dam, Brandon Road Lock and Dam), but have also been found to be on only the downstream sides of others (Chicago Harbor Lock and T.J. O'Brien Lock and Dam). In the upper Illinois River weatherfish were found to be expanding in the Dresden Island Pool in 2010 when three fish were collected during that year approximately 7.5 miles (12.25km) below the Brandon Road Lock and Dam where they were last found in 2004. In 2011 one weatherfish was found in the Marseilles Pool, downstream of the Dresden Island Lock and Dam, 13.5 miles (22km) downstream of the 2010 location. No fish were observed in 2012 in either pool (Figure 3).

This project aimed to assess the current distribution of weatherfish within and outside of their documented range in the Chicagoland area using historical data (1987-2012) as a baseline of where to expect weatherfish (Figure 3). This was done through sampling at known hot spots of weatherfish activity and at several locations that fit their ideal habitat types. In addition to determining an up-to-date distribution of weatherfish, sampling methods were assessed to determine the most effective method(s) to capture weatherfish. These methods can be used to increase capture rates for future monitoring programs. Accurate sampling is imperative to accurately evaluate weatherfish range expansion and, if needed, provide a means of controlling the population through removal from the system.

Materials and Methods

Specimen Acquisition

From early spring to late fall (March – November) 2013 and 2014, Lake Michigan and its tributaries in Illinois and Indiana within and outside the known range of weatherfish were sampled (Table 1, Appendix B). Waterbodies included Lake Michigan, Chicago River (North and South Branches), Chicago Sanitary and Ship Canal (CSSC), the North Shore Channel (NSC), Des Plaines River, Illinois and Michigan Canal (I&M Canal), DuPage River, Cal Sag Channel, the Calumet River, the Grand Calumet River, Little Calumet River, the Illinois River, and several smaller tributaries. These waterways were divided into smaller sampling locations that encompassed specific lengths of waterway (Table 1). These locations correspond in part to areas defined by the Asian Carp Regional Coordinating Committee's Monitoring and Response Workgroup (www.asiancarp.us). The location lengths varied because they are based on boundaries established by the workgroup and/or by natural barriers in the system. These divisions allowed for consistent sampling areas and enabled supplementary catch data and sampling efforts conducted by the workgroup to be incorporated into catch per unit effort analysis (CPUE). Within each location several sampling methods were used to assess the presence of weatherfish. The four main methods of capture were steel-mesh minnow traps, a Smith-Root backpack electroshocker, a Smith-Root boat electroshocker, and mini-fyke nets. These sampling methods were deployed at several points in each location or at different times, termed sampling sites from here on. Each location had several sampling sites, with the number of sites being dependent on habitat features such as access, depth, and flow speeds (Appendix C).

Table 1. Water bodies sampled during 2013 and 2014, denoted by location number and the length of location between designated boundaries. CSSC – Chicago Sanitary and Ship Canal, I&M – Illinois & Michigan Canal

Location Number	***Water Body***	***Start Point***	***End Point***	***Miles (Km)***
1	Lake Michigan Near Shore	North Point Marina	Burns Harbor	78 (125.5)
2	Lake Calumet/Calumet River	Lake Calumet	Lake Michigan	7 (11.3)
3	North Shore Channel/North Branch Chicago River	Wilmette Pumping Station	Chicago Lock	16 (25.7)
4	South Branch Chicago River/CSSC	Harrison Ave	Electric Dispersal Barrier	28 (45.1)
5	Little Calumet River/Cal Sag Channel	T.J. O'Brien Lock	CSSC Confluence	23 (35.7)
6	North Branch Chicago River	North Shore Channel Confluence	Foster Ave	1.25 (2)
7	CSSC (Lockport Pool)	Dispersal Barrier	Lockport Lock	5.2 (8.4)
8	CSSC/Des Plaines River (Brandon Rd. Pool)	Lockport Lock	Brandon Rd Lock	4.8 (7.8)
9	Lower Des Plaines River (Dresden Island Pool)	Brandon Rd Lock	Dresden Island Lock	15 (24.1)
10	Illinois River (Marseilles Pool)	Dresden Island Lock	Marseilles Lock	26 (41.8)
11	Illinois River (Starved Rock Pool)	Marseilles Lock	Starved Rock Lock	14 (22.5)
12	Upper Des Plaines River	Cermak Rd	CSSC Confluence	30(48.3)
13	Little Calumet River South Arm	Cal Sag Confluence	Burns Harbor	37 (59.5)
14	Grand Calumet River	Calumet River Confluence	Cline Ave	9 (14.5)
15	DuPage River	Channahon Parkway State Park	DuPage Park	1 (1.6)
15	DuPage River	Warrenville Rd	Butterfield Rd	2 (3.2)
16	Kankakee River	Des Plaines River Confluence	Wilmington Dam	10 (16.1)
17	I&M	Hodgkins, IL	Cal Sag Channel	7 (11.3)
18	I&M	I-355	Lock Number 1	6 (9.7)
19	I&M	Brandon Rd Lock	Larkin Ave	1 (1.6)
20	I&M	DuPage River	McKinley Woods	3 (4.8)
21	Iroquois River	Kankakee River Confluence	IL/IN Border	45 (72.4)

Sampling locations were chosen based in part on proximity to previously documented occurrences of weatherfish (Figure 3). The historical locations of weatherfish were provided by the Shedd Aquarium, IL Department of Natural Resources (ILDNR), Indiana Department of Natural Resources (INDNR), United States Geological Survey (USGS), U.S. Fish and Wildlife Service (USFWS), and U.S. Army Corps of Engineers (USACE). Sampling sites outside of the known range (Figure 3)were chosen primarily based on the presence of slow moving or stagnant waters, and bottom substrates consisting of clay, mud, or leaf litter (i.e. how conducive the habitat was for weatherfish) as well as ease of access to the site. Many of the sites were revisited throughout the two-year sampling period to account for the possibility that any initial absence of weatherfish was due to seasonal variations in catchability or weatherfish movements or faulty sampling and was rather due to an actual absence of weatherfish in the area. All sampling locations are detailed in Figure 4. Multiple sampling sites were present within each location.

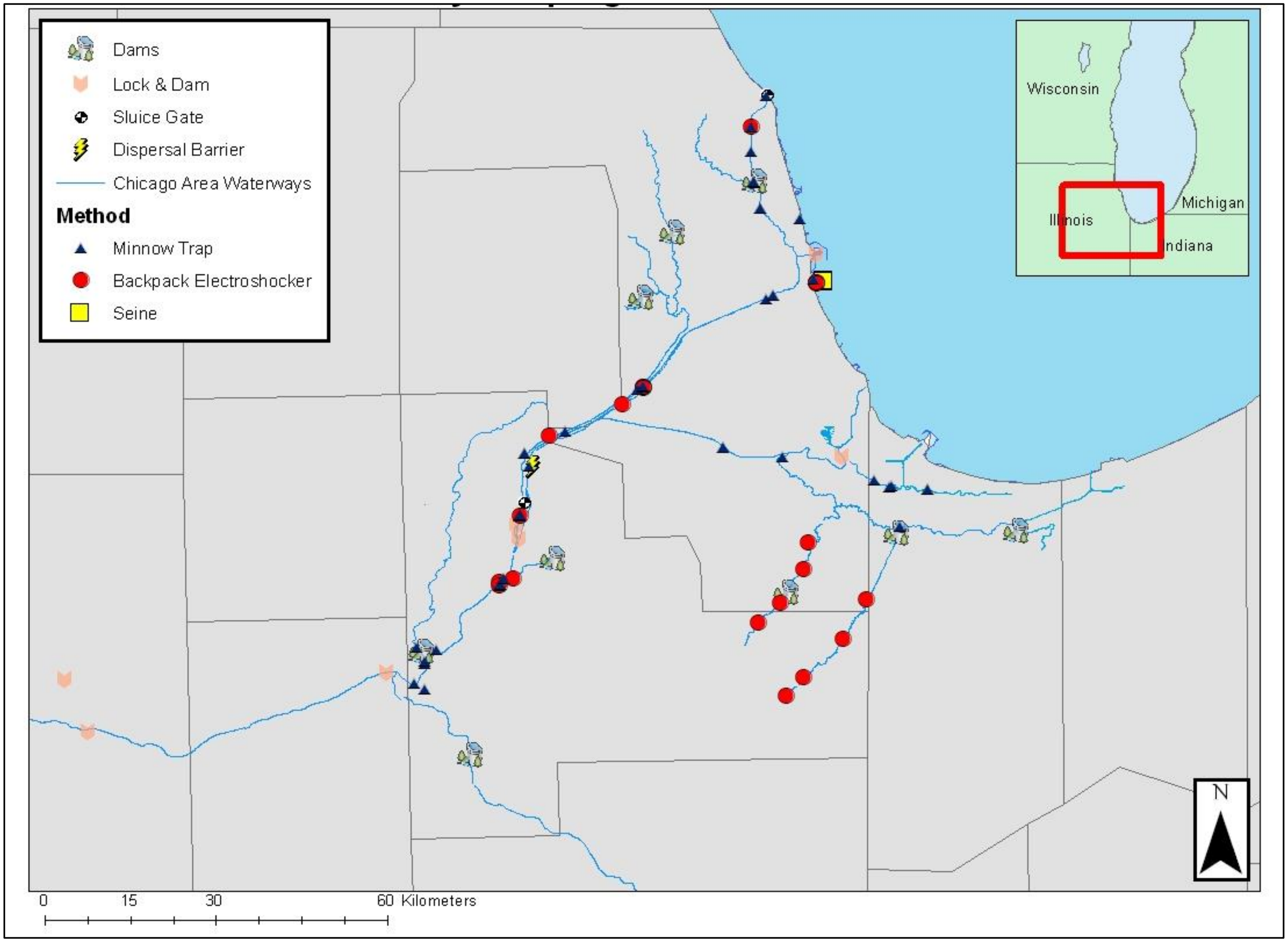

Figure 4. Study sampling locations within the Illinois Waterways and the Chicago Area Waterways between March and October 2013 and 2014.

At sites where minnow traps were used, multiple traps were placed approximately 30 – 50 meters apart with 3 – 5 traps at each location. Traps were vinyl coated, steel-mesh, measuring 42x22 cm with a 2.5 cm opening at each end. The number of traps at each site and the distance between them was dependent on factors such as river access, shore length, boat traffic, and public access. There were multiple instances of tampering due to the public having access to some sites. Trap loss occurred on three of 77 sampling events. If traps were lost, new traps were reset in an area upstream or downstream of the previous site, typically within 0.5 km of the original site for the standard time period described below.

Different types of bait were used with the traps over the course of the collection season, each with varying degrees of success. The baits used included spawn sacs obtained from a local bait store (2-3 per trap), store-bought canned sardines (approx. 15-30g), and commercially available dried dog food (approx. 30g) (Urquhart & Koetsier 2011). There appeared to be no difference in effectiveness of the different baits. Traps were typically set for at least 24 hrs. and up to seven days. Traps were initially checked after one day and if weatherfish were present, that trap was relocated to a new site as described above. If there were no fish within the trap it would be re-baited and reset for an additional time period, typically seven days after the initial deployment for additional assessment of the area. This deployment strategy was an attempt to account for possible variation in weatherfish daily or seasonal movements. If weatherfish were not caught after a sampling event it would likely be because they were not at a site, and not because they were simply missed.

In addition to using baited minnow traps along the known and suspected habitat range of weatherfish, a Smith-Root LR-24 Backpack Electrofisher was used to collect weatherfish from streams. The use of this additional sampling method was dependent on water accessibility, depth, and current flow. It was not used at every site where traps were placed and was sometimes used in lieu of traps depending on time and site constraints. At sites suitable for using the backpack shocker, electroshocking (50-120 volts DC) was conducted for approximately 15 minute runs in the upstream direction of the river access point (approximately 200-300 meters) and in water that typically was less than 1-meter deep.

Supplemental data on weatherfish occurrences spanning the same time period as this study were provided by state and federal agencies, as well as local institutions. The groups involved were Chicago's Shedd Aquarium, INHS, USFWS, ILDNR, INDNR, and the USACE. These groups primarily conducted boat mounted electroshocking and used mini-fyke nets along large spans of the same locations that had been sampled for this study. They were also able to access deeper portions of the rivers and certain sections that were inaccessible during my sampling efforts (Figure 5). They covered a much broader, statewide area as well. Boat-mounted electroshocking was conducted in 15 minute runs and many of these sites were sampled on a monthly or bi-weekly basis between March and November of 2013 and 2014. Mini-fyke nets were used in multiple locations over one or two nights from April to November of the same years. Some groups also conducted backpack electroshocking in 15 minute runs in areas that connect to the main river habitat.

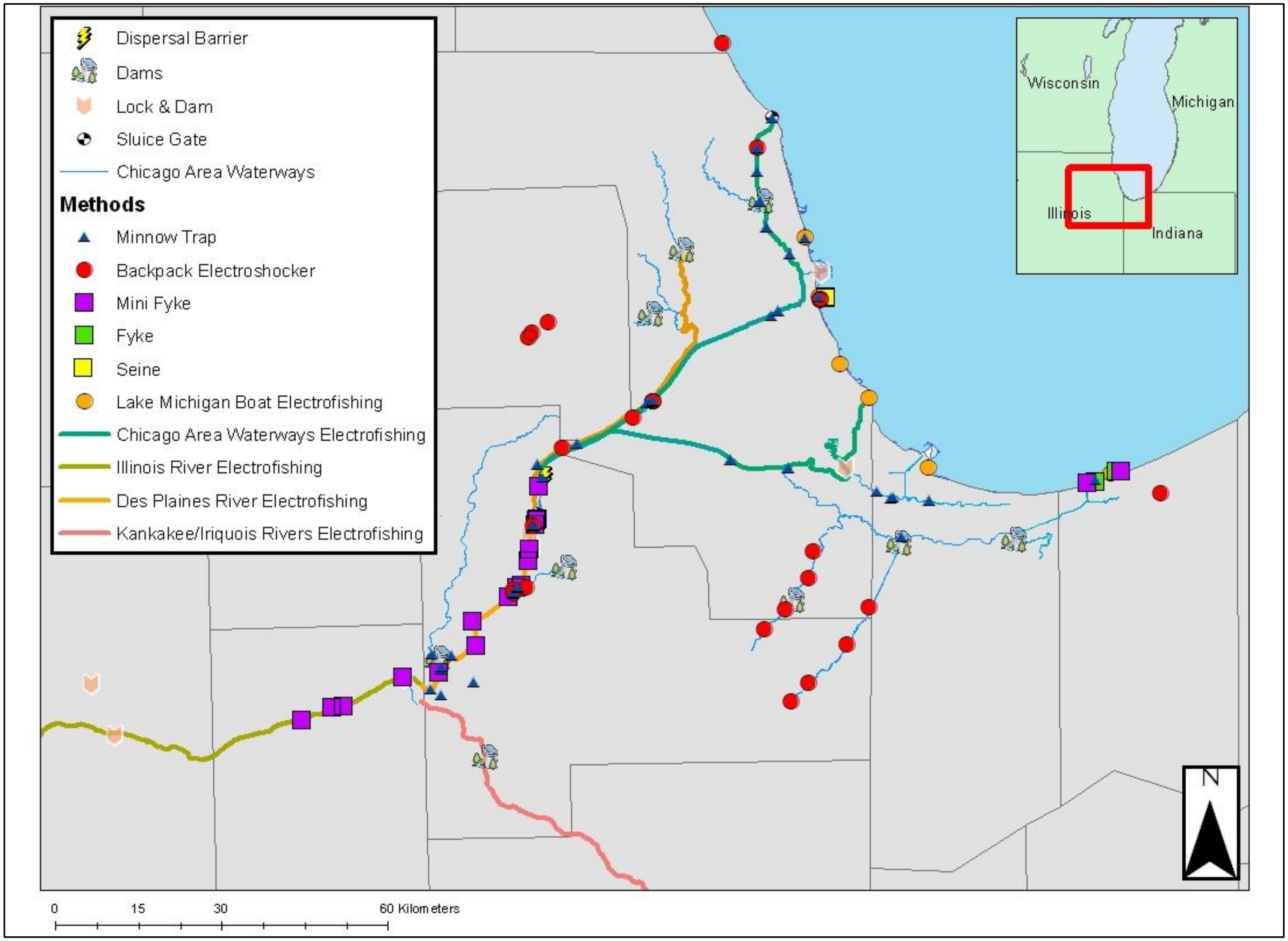

Figure 5. Total sampling effort conducted within Illinois Waterways and Chicago Area Waterways between March and November 2013 and 2014

Study Area

Four rivers and streams separated by dams from the main stem of the CAWS and IWW were sampled: The North Branch (NB) of the Chicago River above its confluence with the NSC, the DuPage River above the confluence with the Des Plaines, Plum Creek above the confluence with the Little Calumet River, and the Kankakee River above the Wilmington Dam in Wilmington, IL. The NB and the DuPage were both sampled downstream and directly upstream of their respective dams using at least 3 baited minnow traps as described above. Upstream areas were sampled during July, 2014 for 22 and 20 continuous days, respectively, with traps being checked and bait being replaced weekly. Also in 2014, Plum Creek and the DuPage River were sampled in four and three locations, respectively, using a backpack electroshocker. These three rivers were sampled to determine if weatherfish had moved past their respective dams into three waterways where they had not been previously known to occur. Kankakee River sampling was conducted as part of the Asian Carp Monitoring Program by USFWS. Although the goal of this program was not to specifically assess the presence of weatherfish, the gear used is appropriate to collect them. All species collected in that program are recorded in the annual Monitoring and Rapid Response Plan Interim Summary Report (www.asiancarp.us). The report also includes other sampling locations in the CAWS and IWW that were used as a secondary source for weatherfish monitoring for this study.

Weatherfish collected in my study were photographed and their lengths and weights recorded. Non-weatherfish captured were identified and released. All weatherfish were euthanized with tricaine methanesulfonate (MS-222) in the field after their wet weights and lengths were recorded. Weatherfish were dissected in the laboratory and the entire digestive tract was preserved for later gut content analysis. The sex of each weatherfish was determined through presence of testes or ovaries/eggs in conjunction with secondary sexual characteristics of the pectoral fin described by Urquhart & Koetsier (2011).

Gear Effectiveness

The entirety of the sampled watershed was divided into 21 locations (Table 1). Twelve of these locations overlap with those used by agencies tasked with monitoring Asian Carp in the CAWS and IWW and the boundary demarcations were kept the same (IDNR & USFWS 2013; IDNR, USACE, & USFWS 2013). The other 9 locations are various tributaries that were sampled as a part of this study and that are connected to the main stem of the CAWS or IWW. They may be partially sampled by other groups at various times, but not nearly as frequently as those sampled as part of the Asian Carp monitoring program.

A variety of gear (described above) was used to obtain weatherfish for this study. The gear used was divided into the two technique categories: passive or active. Passive gear is represented by minnow traps and mini-fyke nets. Boat electrofishing and the backpack electroshocker were the two active gear used for this study. Gear effectiveness was evaluated by calculating a catch per unit effort (CPUE) for each category of sampling gear using the following formula:$(Weatherfish\ Caught)/(Effort\ Hrs) = CPUE$, where weatherfish caught is the number of weatherfish caught in a given month with a particular gear and effort hrs. is the number of hours that gear was used in that same month.

CPUE was compared examining the effects of location, method, and the interaction of the two on catch rates using a two-way ANOVA. The number of fish and hours of effort were pooled by year for each method within each location. CPUE was also compared across methods and location separately using a one-way ANOVA due to a significant interaction between location and gear type. The total number of fish caught in each year and with each method were analyzed using a two-way ANOVA, with differences in method being analyzed by one-way ANOVA. The datasets were analyzed using the Box-Cox function in R and CPUE was natural log transformed for all analyses to meet the assumptions of ANOVA. A Tukey's multiple comparisons test was used to determine statistically significant catch rates among sites. All CPUE analyses were conducted using R v3.3.1 (R Core Team 2013).

Results

Range Expansion

During the study period a total of 272 weatherfish were captured or observed within and outside of the previous known range (Figure 6). These were the result of 31,755.31 hours of effort (16,507 in 2013 and 15,247 in 2014) in the CAWS, the IWW, IN waterways, and their various feeder tributaries (Table 2). Of the 21 locations sampled, 10 had occurrences of weatherfish. The highest was found in the CSSC (Location 7) where over 90 weatherfish were collected over the two years (Table 3). Five locations had capture numbers between 20 and 40 fish. The remaining locations had fewer than 10 weatherfish captured over the two years (Figure 7).

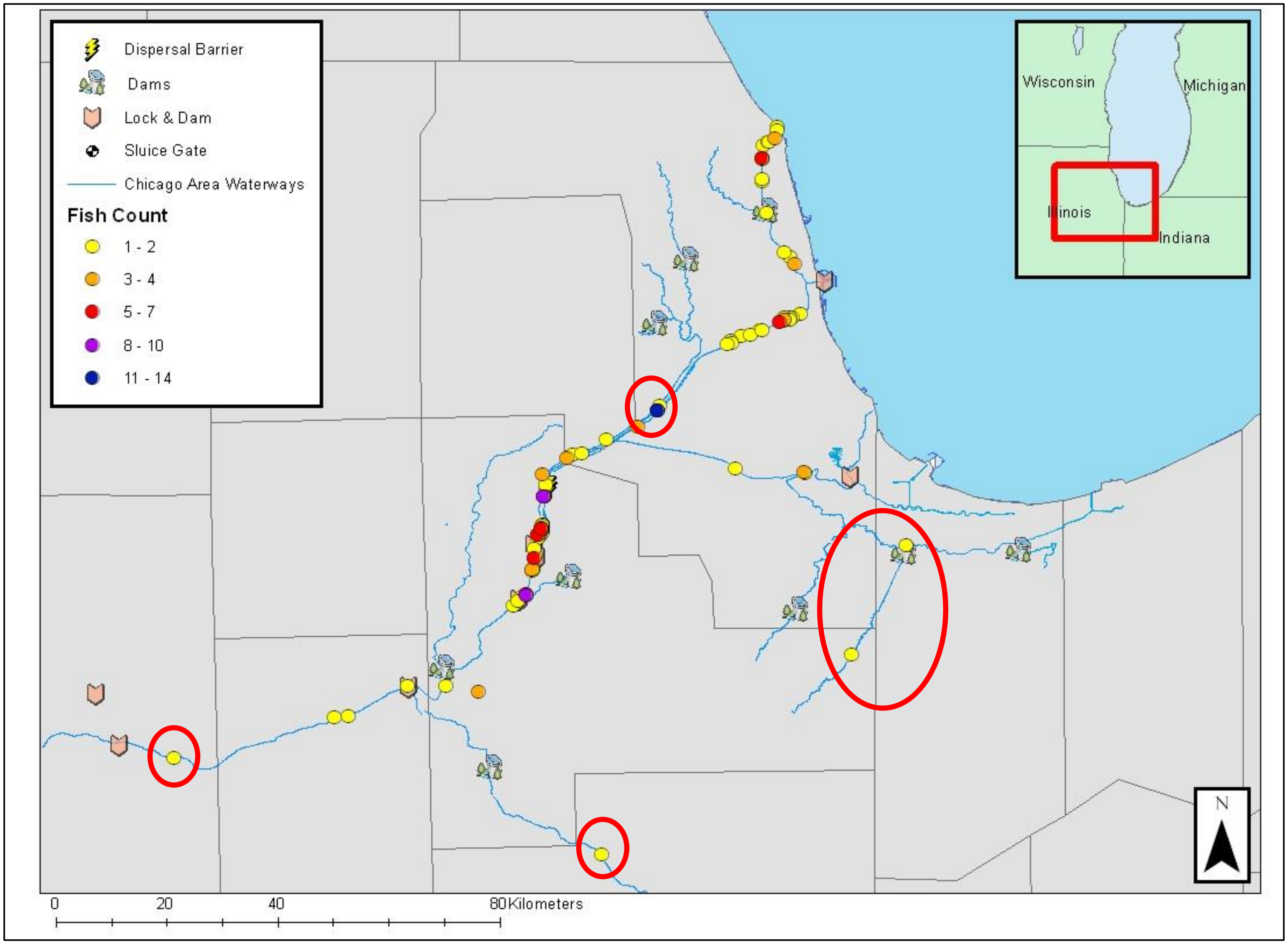

Figure 6. Weatherfish occurrences in the Chicago Area Waterways and Illinois Waterways during 2013 & 2014 indicated by colored dots. Red circles indicate an occurrence in a new watershed and/or outside the 2012 range.

Table 2. Total hours of effort from both study and supplemental data over the 2013 and 2014 sampling seasons. Blank spaces indicate no effort took place. Active – active sampling methods, Passive – passive sampling methods, BS – boat mounted electroshocker, BP – backpack electroshocker, MF – mini-fyke nets, MT – minnow trap.

	2013 Yearly Totals				***2014 Yearly Totals***				***Cumulative Method Totals***				
	Active		Passive		Active		Passive		Active		Passive		
Locations	BS	BP	MF	MT	BS	BP	MF	MT	BS	BP	MF	MT	***Totals***
Location 1	1.67		768	1200	43.23	1.08		2856	44.9	1.08	768	4056	4870
Location 2	38.55			120	40.75				79.3			120	199.3
Location 3	32.75	0.14		1704	20				52.75	0.14		1704	1756.9
Location 4	55.95			384	16.75				72.7			384	456.7
Location 5	42.8			120	7.5			144	50.3			264	314.3
Location 6								696				696	696
Location 7	59		1248		50.75		672		109.75		1920		2029.8
Location 8	54		1248	192	51		672	648	105		1920	840	2865
Location 9	53	1.10	1248	1944	53		1248	2112	106	1.1	2496	4056	6659.1
Location 10	53		1248		62.5		2016		115.5		3264		3379.5
Location 11	4		480		12.25		933		16.25		1413		1429.3
Location 12	7.4			1104	8.15	0.83		984	15.55	0.83		2088	2104.4
Location 13		0.25		216		2		144		2.25		360	362.25
Location 14				648				288				936	936
Location 15						1		1440		1		1440	1441
Location 16	15		1920		15				30		1920		1950
Location 17		0.62		96		0.83				1.46		96	97.46
Location 18				96								96	96
Location 19		0.45								0.45			0.45
Location 20				96.00								96	96
Location 21	8.00				8				16				16
Device Total	425.12	2.56	8160	7920	388.88	5.75	5541	9312	814	8.3135	13701	17232	
Method Totals	427.68		16080		394.63		14853		822.31		30933		
Total Hours	16507.68				15247.63				31755.31				

Table 3. Number of fish captured from study effort and supplemental effort during the 2013 and 2014 collection seasons. Blank spaces indicate no fish were caught. Active – active sampling methods, Passive – passive sampling methods, BS – boat mounted electroshocker, BP – backpack electroshocker, MF – mini-fyke nets, MT – minnow trap.

	2013 Yearly Totals				***2014 Yearly Totals***				***Method Totals***				
	Active		Passive		Active		Passive		Active		Passive		
Location	BS	BP	MF	MT	BS	BP	MF	MT	BS	BP	MF	MT	***Totals***
Location 1													0
Location 2													0
Location 3	4	1		10	9				13	1		10	24
Location 4	25			2	7				32			2	34
Location 5	4							1	4			1	5
Location 6													0
Location 7	9		23		26		35		35		58		93
Location 8	3		12		5		16	2	8		28	2	38
Location 9			2		1		2	3	1		4	3	8
Location 10			2				1				3		3
Location 11													0
Location 12	1			9	1	3		9	2	3		18	23
Location 13						1		1		1		1	2
Location 14													0
Location 15													0
Location 16													0
Location 17		12		10		17				29		10	39
Location 18													0
Location 19													0
Location 20													0
Location 21													0
Device Total	46	13	39	31	49	21	54	16	95	34	93	47	
Method Totals	59		70		70		70		129		140		
Total Fish	129				140				269				

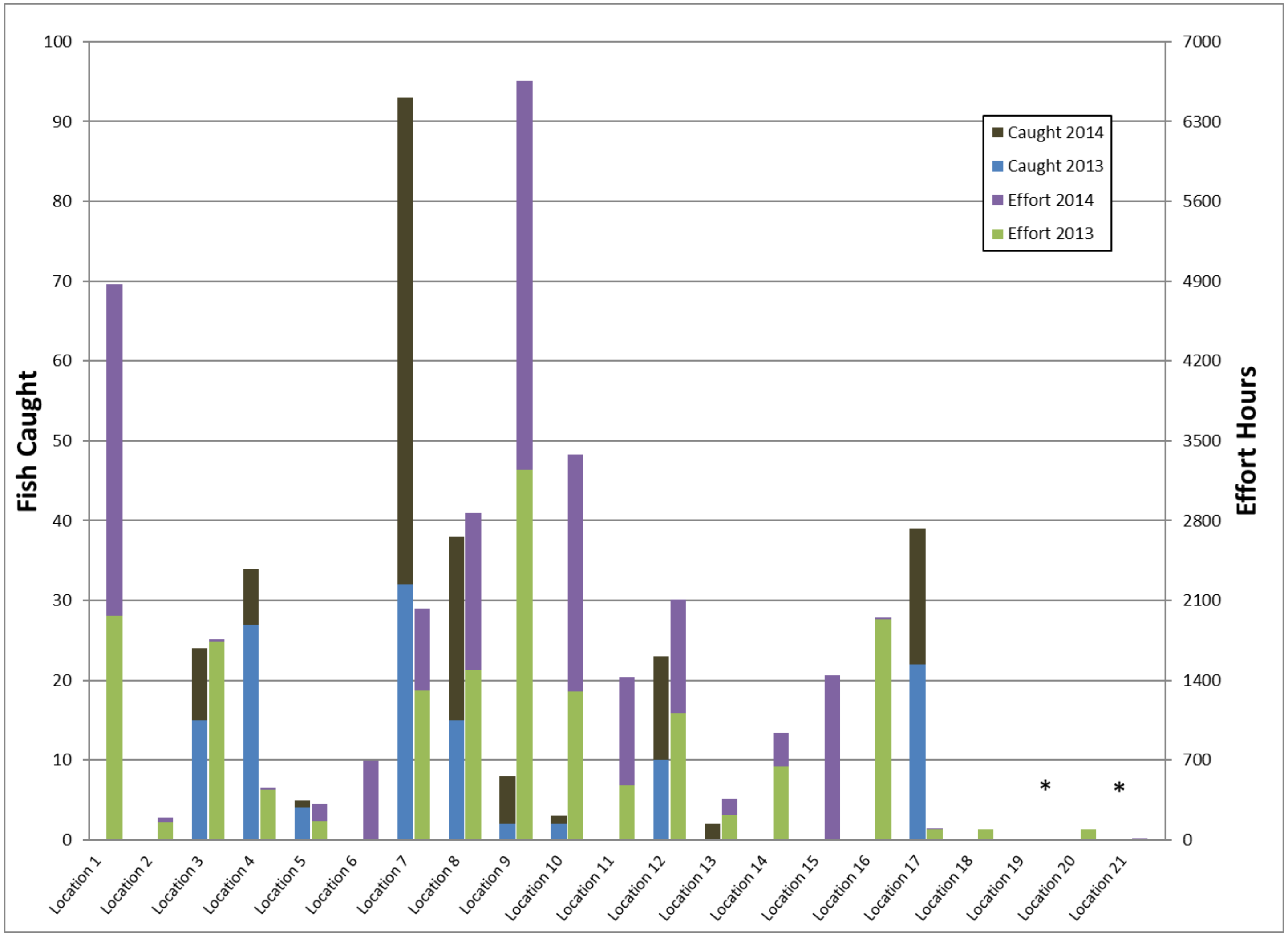

Figure 7. Number of weatherfish captured in relation to effort hours over the 2013 and 2014 sampling seasons broken down by location. * Represents sites where fewer than 20 hours of effort were conducted.

Weatherfish were prevalent in many sites within the historic IL/IN range and showed limited expansion outside the southern and eastern portions of the known range. Weatherfish were collected for the first time in three sites during the study period. Other sites, such as other portions of the I&M Canal and the northern Des Plaines River, experienced no weatherfish incursions or range expansion.

The Marseilles Pool represents the current known population front for the Midwest weatherfish population in the Illinois River. A total of 8 and 4 weatherfish were found in the Dresden Island and Marseilles pools, respectively, in 2013 and 2014. Over 200 electrofishing and 5700 mini-fyke net hours of sampling effort were conducted in these pools**.** Three of the four weatherfish found in the Marseilles Pool were within the previous known range determined in 2012. The fourth fish was found approximately 15 miles (24km) farther downstream than the 2011 location in the Illinois River, where it was captured at a power plant just downstream of Seneca, IL. In the next pool downstream (Starved Rock Pool) there was an effort of ~16 electrofishing hours and over 1400 mini-fyke net hours conducted during the 2013 and 2014 sampling season. No weatherfish were collected during this time.

The eastern-most occurrence of weatherfish in the Little Calumet River watershed was collected in 2011 from North Creek, a tributary of Thorn Creek, that connects to the Little Calumet River. Weatherfish were collected approximately 8 miles (12.9 km) farther east than that previous occurrence in the Little Calumet River in Highland, Indiana during this study.

In 2014, a weatherfish was also found above a water diversion structure in Plum Creek, a tributary of the Little Calumet River watershed. This weatherfish was captured by the USACE 13.5 river miles upstream of the diversion structure that separates Plum Creek from the Little Calumet River. Three additional sites were sampled along Plum Creek using a backpack electroshocker, totaling an hour of active sampling effort. No additional weatherfish were found in this tributary. This diversion structure is just upstream of the location where the weatherfish was found in the Little Calumet River as described above.

Two weatherfish were reported to have been collected in the Kankakee River during 2014. This was reported on the USGS website (http://www.usgs.gov/) as a personal communication. They were captured approximately 13 miles (21km) upstream of the Wilmington Dam in Wilmington, IL. Efforts from this study or from those reported in the Monitoring and Response Workgroup Interim reports for 2013 or 2014 did not collect weatherfish from this area during the same time period.

Four sections of the I&M canal were sampled during this time period, designated locations 17-20 (Table 1). Only one (location 17) had weatherfish collected, the section in Willow Springs, IL. This was the first known recording of these fish in any part of the I&M. This section accounted for 39 of the weatherfish captured during the study period. While it is the first occurrence in this area, given the high volume of fish, it is possible that they had been established in this water body for several seasons and were not recent migrants.

During the study period, both the Chicago River’s North Branch above the confluence with the NSC and the DuPage River were sampled with baited minnow traps during the month of July 2014. No weatherfish were observed or captured in that time at either site. For the North Branch this would indicate that weatherfish have not crossed over the concrete dam that separates the two systems. This failure to colonize has continued since the early 1990’s when weatherfish were first observed in the area below the dam.

Similarly, the DuPage River is also separated from the Des Plaines River, an area known to have weatherfish, by a large dam. There were no weatherfish collected on the upstream side of this dam during the study period.

The upper Des Plaines River has had historic collections of weatherfish as far upstream as Ogden Avenue (approximately 25 miles upstream of the Des Plaines River/CSSC confluence). The river is regularly sampled past the last known leading population front (USFWS 2014) and to date the front does not appear to have advanced. In the two years of this study more than 2100 effort hours (Table 2) were expended in the Des Plaines River. The farthest upstream point of capture for weatherfish was in the Sawmill Creek tributary of the Des Plaines River, about 11 miles (17.7km) downstream of the last known population front on the river.

There are a total of five ingress points that lead from this river system (NSC, Chicago River, and Grand and Little Calumet rivers) into Lake Michigan, three in IL and two in IN. They are the Wilmette Pumping Station, Chicago Lock, and T.J. O'Brien Lock and Dam in IL and Indiana Harbor and Burn's Ditch in IN. By 2012 weatherfish had access to four of these five points. More than 4800 hours of sampling effort occurred in Lake Michigan during 2013 and 2014(Table 2) in areas near access points, but also in other, potentially suitable habitat areas (Figure 5). No weatherfish were found during the sampling period in Lake Michigan.

Despite weatherfish being previously captured in the Grand Calumet River (Location 14) in IN (Simon et al. 2006) and over 900 hours of passive sampling throughout this location, no weatherfish were captured during this study in this location.

Gear Efficacy

A total of 272 weatherfish were collected from IL and IN inland waterways during the 2013 and 2014 field seasons (Table 3). Of these, 47 fish were collected after 718 minnow trap net nights (24 hr. period), 93 after 571 mini-fyke net nights, 95 from 814 hours of boat mounted electrofishing, and 34 from 8.3 hours of backpack electroshocking. A single fish was collected from the permanently stationed impingement net at the LaSalle Power Plant in LaSalle, IL and two were reported by USGS as being caught in the Kankakee River near the Kankakee River State Park through unknown means. The LaSalle and Kankakee captures were excluded from analysis based on the method of capture.

Of the 21 locations sampled, weatherfish were found at 10 locations. The total number of fish captured for 2013 was 59 for active and 70 for passive gears. For 2014, 70 fish were captured for each gear. Total number of fish caught per location can be seen in Figure 7. Number of fish captured did not differ between years or between gear types (p = 0.642, p = 0.943) (Figure 8 & Figure 9). A significant interaction was not present between method and year on the number of fish caught (p = 0.742).

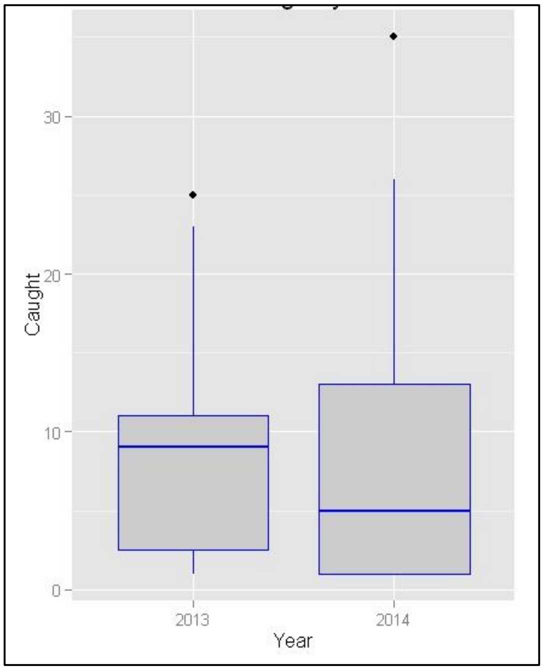

Figure 8. Box and whisker plots of the weatherfish captured in 2013 and 2014. The horizontal line with box indicated the median, boundaries of the box indicated 25th and 75th percentiles, and whiskers indicate lowest and second highest results. Highest results indicated by dots.

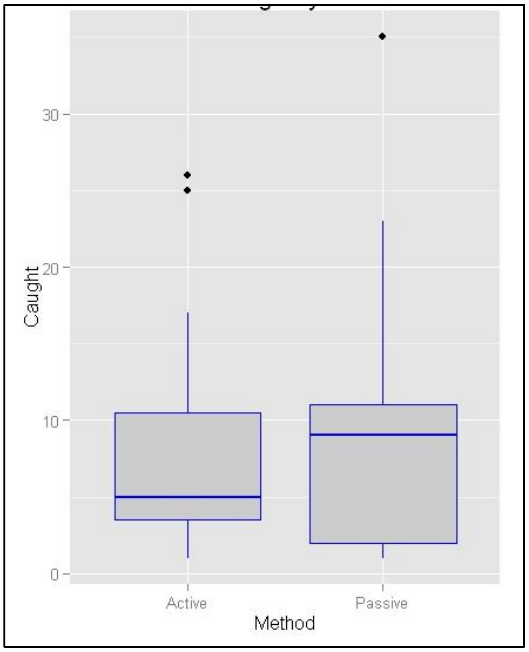

Figure 9. Box and whisker plots of the weatherfish captured using different gear types. The horizontal line with box indicated the median, boundaries of the box indicated 25th and 75th percentiles, and whiskers indicate lowest and second highest results. Highest results indicated by dots.

Pooling the number of fish caught and effort hours within each location for each year, produces a maximum catch rate for active methods of 20.4 fish/hr. in location 17, with a minimum of 0.02 fish/hr. in location 9 (Table 4). For passive sampling the maximum rate was 0.1 fish/hr. also for location 17, whereas the minimum was found in site 10 at 0.0005 fish/hr. The average catch rate for active gear was 2.88 fish/hr., whereas it was 0.02 fish/hr. for the passive gear. A significant interaction occurred between gear type (active and passive) and the site on catch rates (two-way ANOVA, df = 8,11; F =3.11; p=0.0424).

Table 4. Pooled catch rate of weatherfish for both gear types in locations where weatherfish were caught. Blank rows indicated no weatherfish were captured during 2013 and 2014 sampling seasons.

Location	*Year*	*Method*	*Effort Hrs.*	*Fish Caught*	*Caught/Hr.*
Location 03	2013	Active	32.89	5	0.152
Location 03	2013	Passive	1704	10	0.006
Location 03	2014	Active	20	9	0.45
Location 03	2014	Passive			
Location 04	2013	Active	55.95	25	0.447
Location 04	2013	Passive	384	2	0.005
Location 04	2014	Active	16.75	7	0.418
Location 04	2014	Passive			
Location 05	2013	Active	42.8	4	0.093
Location 05	2013	Passive			
Location 05	2014	Active			
Location 05	2014	Passive	144	1	0.007
Location 07	2013	Active	59	9	0.153
Location 07	2013	Passive	1248	23	0.018
Location 07	2014	Active	50.75	26	0.512
Location 07	2014	Passive	672	35	0.052
Location 08	2013	Active	54	3	0.0556
Location 08	2013	Passive	1248	12	0.010
Location 08	2014	Active	51	5	0.098
Location 08	2014	Passive	1320	18	0.014
Location 09	2013	Active			
Location 09	2013	Passive	1248	2	0.002
Location 09	2014	Active	53	1	0.0189
Location 09	2014	Passive	3360	5	0.001
Location 10	2013	Active			
Location 10	2013	Passive	1248	2	0.002
Location 10	2014	Active			
Location 10	2014	Passive	2016	1	0.0005
Location 12	2013	Active	7.4	1	0.135
Location 12	2013	Passive	1104	9	0.008
Location 12	2014	Active	8.98	4	0.445
Location 12	2014	Passive	984	9	0.009
Location 13	2013	Active			
Location 13	2013	Passive			
Location 13	2014	Active	2	1	0.5
Location 13	2014	Passive	144	1	0.070
Location 17	2013	Active	0.622222	12	19.286
Location 17	2013	Passive	96	10	0.104
Location 17	2014	Active	0.833333	17	20.4
Location 17	2014	Passive			

There was a significant difference between method types for catch rates (one-way ANOVA, df = 1, 28; F= 41.96; p<0.001) (Figure 10), active sampling had a higher rate than passive. There was also a significant effect of location on the number of fish caught per hour (one-way ANOVA, df = 9, 20; F=3.10; p= 0.017). A Tukey's multiple comparisons test revealed that fish were captured at a higher rate at location 17 than in all other locations in the system (p<0.001). Location 10 was not significantly different than location 9, and location 9 was not statistically different than location 5. All combinations of locations 3, 4, 5, 7, 8, 12, and 13 were not statically different (Figure 11).

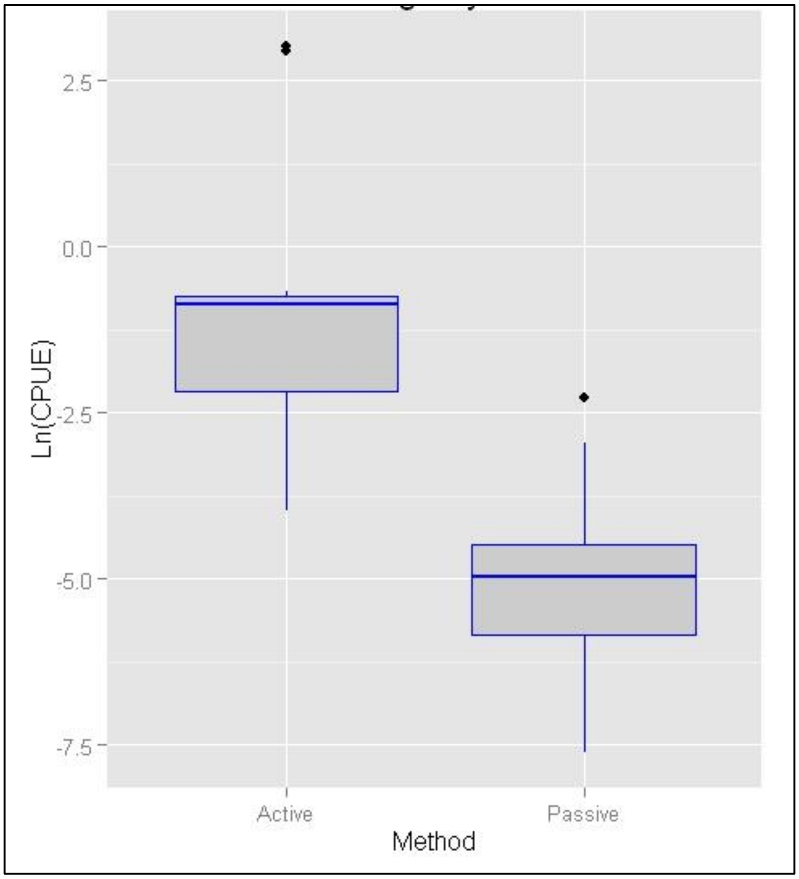

Figure 10. Box and whisker plots of log transformed Catch Per Unit Effort (CPUE) for gear type during 2013 and 2014. The horizontal line with box indicated the median, boundaries of the box indicated 25th and 75th percentiles, and whiskers indicate lowest and second highest results. Highest results indicated by dots. Shows significantly higher CPUE for active effort.

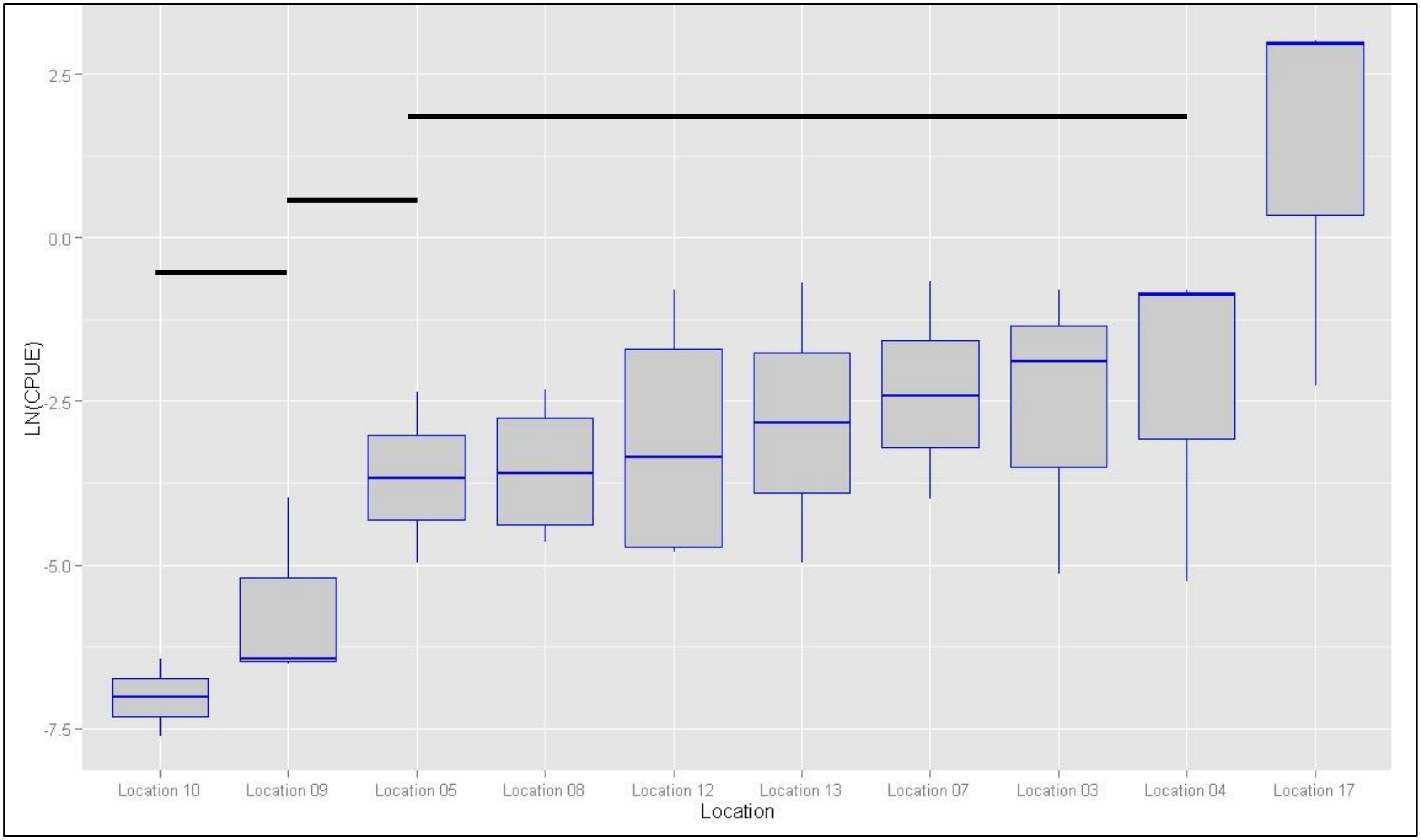

Figure 11. Box and whisker plot of log transformed Catch Per Unit Effort (CPUE) by location. The horizontal line with box indicated the median, boundaries of the box indicated 25th and 75th percentiles, and whiskers indicate lowest and highest results. Black bars over boxes indicate nonsignificant CPUE between locations.

Discussion

Range Expansion

The Great Lakes Basin and the Mississippi River Basin were historically separate watersheds which, in the mid- 1800s, were connected by the I&M canal. It was replaced by the larger Chicago Sanitary and Shipping Canal (CSSC) in the early 1900s (IDNR 2016) and as a result has been fragmented into smaller, mostly unused sections. These sections are often separated from the larger river by old locking structures, previously used for shipping, but which are no longer functional (IDNR 2016). The CSSC was originally intended to prevent sewage carried by the Chicago River from flowing into Lake Michigan, but also provided a valuable shipping link between the lake and the Mississippi River. In providing a link for people, the canal has also provided a path for organisms, both native and nonnative, to move between the two watersheds. The Round Goby (*Neogobius melanostomus*), zebra mussel (*Dreissena polymorpha*), and White Perch (*Morone Americana*) are just a few species moving from the lake into the Mississippi Basin, whereas species like the Bighead and Silver Carp (*Hypophthalmichthys nobilis* and *H. molitrix*), commonly called Asian Carp, are moving from the Mississippi toward the lake (Sparks et al. 2010). The weatherfish is another species using this link to move from the Lake Michigan Basin side toward the rivers and tributaries of the Mississippi River Basin. So far it has done so with seeming success and with unknown ecological impacts.

Weatherfish were first recorded in Chicagoland in 1987 in the North Shore Channel (NSC) and have slowly expanded their range and are now found throughout the NSC, Chicago River, CSSC, Des Plaines River, and the Cal Sag Channel (Figure 3). This study demonstrated that weatherfish continue to occupy those areas and are well-established within the Chicago Area

Waterways (CAWS) and many IL Waterways (IWWs). Based on catch sizes, there are smaller populations within the Illinois, Little Calumet, and possibly the Kankakee rivers. Weatherfish are likely to be found in many of the smaller feeder streams that have an uninterrupted connection with the CAWS or IWW, but the number was not determined in this study. They have been found throughout approximately 152 miles (243km) of the main river system. This number is undoubtedly higher, but it is unknown how many of the smaller streams they occupy and in what densities.

Given their current location within the Illinois River and previous expansion history, these fish are expected to continue expanding through that system past the Marseilles Lock and Dam, extending their range into the Starved Rock Pool of the Illinois River. It is difficult, however, to determine when that will occur. Previous studies conducted in Australia have concluded that weatherfish can expand their range relatively quickly at upwards of 4.5 miles (7.2 km) per year (Lintermans et al. 1990). However, a study by Shultz (1960) determined that in the Shiawassee River in Michigan, their expansion was much slower, around a half mile (0.8 km) per year. During the study period weatherfish were collected around 2 miles (3.2 km) upstream of the Marseilles Dam and 4.5 miles (7.2 km) from the lock. Using historical records, when weatherfish were found on the upstream side of a lock in the CSSC or Des Plaines River they were found on the downstream side within the next two years. With their current population front in the Marseilles pool and their maximum possible expansion rate, it would be highly likely for them to be found consistently within the Starved Rock Pool in a few years.

In contrast to their new collections in the Little Calumet River, weatherfish have been established in the Grand Calumet River since 2001 (Simon et al. 2006). However, during this study they were not collected in this waterway. This lack of capture could be due to the limited access points available for sampling or a low abundance of fish in the areas that were sampled. Previous sampling attempts used active sampling methods and had greater access to the entire system. This location in the present study was only sampled using passive methods because active tools were not available at the time. In many instances during passive sampling there were disturbances short distances upstream or downstream of the sampling point. These disturbances were typically in the form of some type of construction project within or immediately adjacent to the river. This in turn could be driving weatherfish from the area or limiting their time in the water column and as a result limited the chances they would come into contact with the passive sampling devices.

Movement Upstream of Dams

During the study period several locations were sampled upstream of permanent dam structures and were assessed for the presence of weatherfish. Some locations were of particular interest because of dams that separate the main stems of the CAWS and IWW from other large watersheds. The watersheds in question are the DuPage River, the upper North Branch of the Chicago River, Plum Creek, Upper Des Plaines River, and the Kankakee River. The structures that separate the watersheds range from a small flow diversion structure of only a foot (~30.5 cm) or so found on Plum Creek, to a large, concrete dam in excess of 10ft (3m) that separates the IL and DuPage Rivers.

Plum Creek joins with the Little Calumet River via Hart Ditch and within that small section there is a small flow diverter approximately 1 ft. (30.5 cm) in height that was constructed to control flooding in the area (Lazerus 2015). This study found one confirmed weatherfish at a location several kilometers upstream of this structure. The creek was sampled at three other locations using the same method, but no other weatherfish were found. Weatherfish have been found in other tributaries of the Little Calumet River previously in 2001 and 2011 and during the study period a weatherfish was found a few kilometers east of the Plum Creek/Little Calumet River confluence on the downstream side of this dam in the Little Calumet. This suggests that these fish have been present in proximity to the dam for a few years. Since monitoring of this area began in 2007 by USGS, flooding events that submerge this diversion structure have occurred frequently (Figure 12). These flooding events could be the method that the weatherfish are using to traverse this structure.

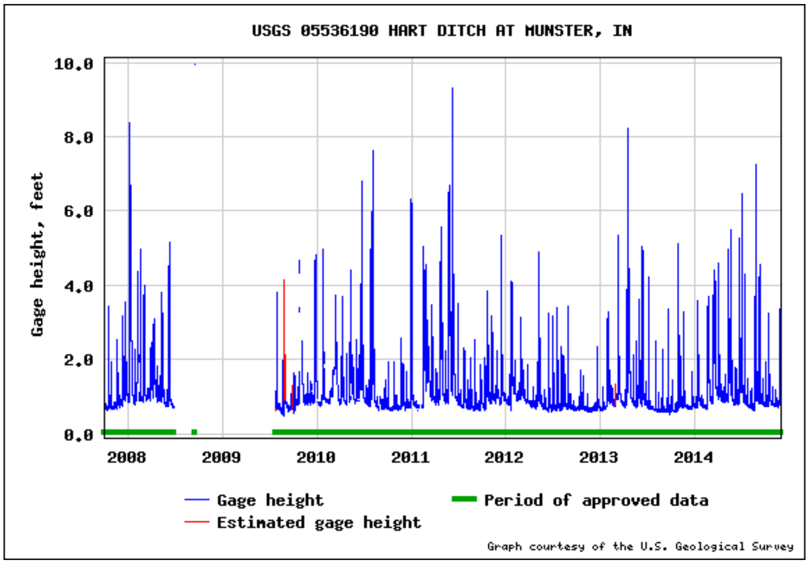

Figure 12. US Geological Survey stream gage 05536190 detailing stream height in Hart Ditch in Munster, Indiana between October, 2007 and December 2014. (waterdata.usgs.gov/nwis/rt)

It has been reported by Fujimoto et al. (2008) that weatherfish are less likely to traverse vertical distances of 19-39 inches (50-100 cm). This flow diverter however is approximately 12 inches (30 cm) in height. This low height and the stream's frequent rises to at least two feet make it plausible for weatherfish, which can move over small vertical structures (Mizutani 2000), to move over the top of the structure into this new watershed. With only one fish being caught however, it is not possible to determine fish abundances in Plum Creek or if they are even related to the main population as a whole. Further study is needed within this watershed to answer these questions.

Although weatherfish were found on the upstream side of the diversion structure leading to Plum Creek, they were not found on the upstream sides of dams that separate the upper North Branch of the Chicago River from the NSC, or the one separating the DuPage and Des Plaines rivers. These two structures create a significant separation between the up and downstream sides, 4ft (1.2m) for the North Branch and 10ft (3m) on the DuPage. Weatherfish have been observed on the downstream side of the North Branch dam beginning in the early 90s and have failed to make the transition to the upstream side. This could be due to the larger vertical migration that is needed and to the lower probability that this dam will be completely submerged during times of increased flow. This structure is large enough that the river would need to have risen at least an additional 8ft (2.4m) or more for it to be submerged. This is possible in the most extreme circumstances, the discharge at those times would be in excess 3000 ft^3/sec (914 m^3/sec) (Figure 13 and Figure 14). No studies have been conducted on swim speed of the weatherfish, but this high discharge rate would be a difficult obstacle for these small fish to pass and could explain why they have not been found above this dam to date.

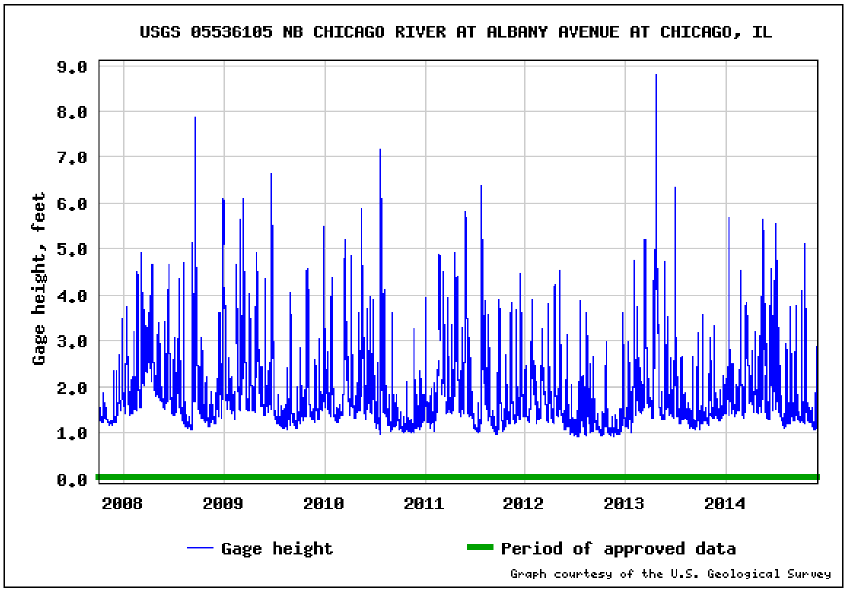

Figure 13. US Geological Survey stream gage 05536105 detailing stream height at Albany Ave in Chicago, IL at the confluence of the North Shore Channel and the Chicago River North Branch from October, 2007 to December, 2014. (waterdata.usgs.gov/nwis/rt)

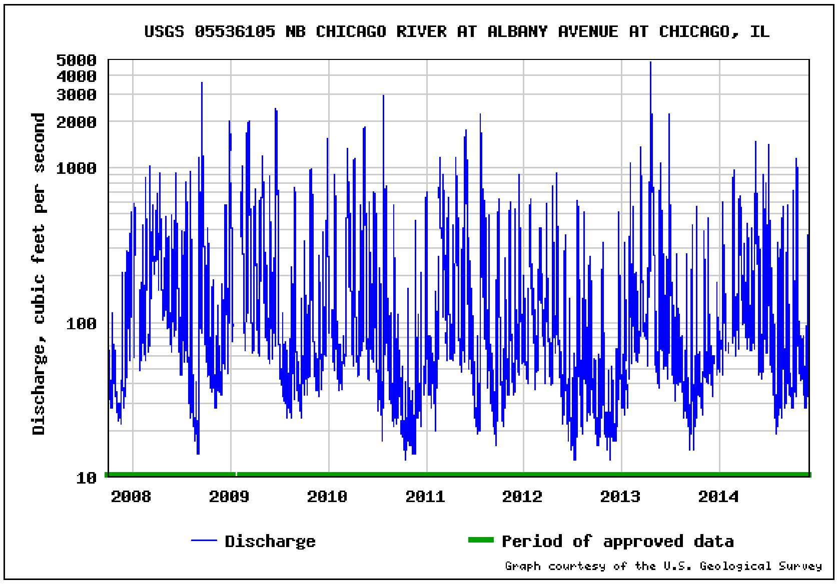

Figure 14. US Geological Survey stream gage 05536105 detailing stream discharge at Albany Ave in Chicago, Illinois at the confluence of the North Shore Channel and the Chicago River North Branch from October, 2007 to December, 2014. (waterdata.usgs.gov/nwis/rt)

Unlike the North Branch, the DuPage River is separated from the Des Plaines River by a 10ft (3m) dam. Under normal circumstances the dam on the DuPage River would most likely be impossible for weatherfish to pass through unaided. Portions of the river upstream were sampled with both active and passive methods and no weatherfish were collected. Although weatherfish were not found upstream of the dam during the sampling period, it is important to note that in 1996 the original dam at this site was breached during a high flow event that caused the two rivers to be connected without any sort of barrier for two years (Hammer & Linke 2003). Weatherfish were not collected in this section of the Des Plaines until 2010 and the lack of weatherfish upstream on the DuPage River indicates that they were likely not present that far downstream on the Des Plaines River to move into the DuPage River during this unique situation. This is corroborated by at least two previous sampling efforts of the DuPage in 2003 and 2012 (Conservation Foundation 2003; Midwest Biodiversity Institute 2014) when the fish assemblages at 13 sites along the DuPage River were surveyed and no weatherfish were found. They are now unlikely to cross this new barrier without human assistance.

The upper Des Plaines River is currently inhabited with weatherfish, but their progress through that system appears to have stopped, as they were not collected any farther north than their previous known extent. This could be due to the presence of several low head dams that existed close to the leading edge of the population in that system. This stoppage may be only temporary. The Army Corps has been tasked with the removal of several of these dams as a way to mitigate flooding farther up river (O'Connell 2015). Several of the downstream dams have been removed and migrations of various fish species have been reported into previously unoccupied areas (USACE communications). It appears the weatherfish have not taken advantage of this new opening, but it could be expected that weatherfish will be among those future species that will move north into the now open habitat.

Lastly, the USGS website had a reported capture of two weatherfish during 2014 upstream of the dam in Wilmington, IL (Nico, et al 2016). This dam is on the Kankakee River approximately 10 miles (16km) upstream of the confluence of the Kankakee and Des Plaines rivers. The reported capture is approximately another 12 miles (19.3km) upstream of the dam (22 miles, 35km, above confluence). Using historical records as benchmarks for the approximate location of the population front in a given year, the closest historical weatherfish capture was approximately 5 miles (8km) upstream of the confluence on the Des Plaines River. In order to reach this location, the weatherfish would have to travel around 30 miles (48km) in four years to reach the reported location in the Kankakee River. Ignoring the movement over this dam which is several feet/meters tall, the weatherfish population would have had to travel over double their reported range expansion rate to reach this location. Additionally, despite being surveyed for a combined 1950 hours, no weatherfish were found along any sampling location in the Kankakee during the study period. In May 2015, there was a 24 hr. Bio-Blitz conducted in the area of the Kankakee River where the captures were reported that did not find weatherfish to be present (ILDNR communications; USACE communications; Shedd Aquarium communications). This could be evidence that weatherfish are actually not present within the Kankakee River watershed and the report was mistaken. It could also mean that if they have made the transition to this new section of river they are localized and in such low abundances that they will rarely come into contact with sampling gear. Future habitat assessments would be needed to monitor this section of river and to make a more accurate assessment of the population front.

Lake Michigan Assessment

Until this study, the weatherfish population in the Chicagoland region was believed restricted to the river system that connected with Lake Michigan. The sampling effort conducted in this study and by other monitoring groups indicate that weatherfish are not present in the lake, confirming previous beliefs. Lake Michigan is repeatedly sampled by various agencies using both active and passive methods in nearshore and deep water areas. Despite over 4800 sampling hours with all gear types during this study, no weatherfish have been collected from the lake.

Since weatherfish were first collected they have been within only a few miles/kilometers of the Wilmette Pumping Station, an inlet point that connects the river to Lake Michigan, yet have failed to transition into the lake. The pumping station has sluice gates that are opened periodically to discharge water into the lake after large rain events. This release of water should not only force water into the lake, but because these gates are unscreened (USACE 2014), any organisms that get caught in the current will be pulled into the lake as well. Yet, there have been no weatherfish observed on the lake side of the gates to date. One hypothesis could be that weatherfish were brought through when these gates were open, but were unable to survive for an extended period of time due to unfavorable habitat conditions.

Aside from the original access point of the pumping station, weatherfish have come into contact with three additional entry points (Chicago Lock, T.J. O’Brien Lock, and Indiana Harbor) and will likely soon meet the fifth (Burn’s Ditch) if their eastward movement through the Little Calumet River continues. The two access points in IN lack any type of barricade between Lake Michigan and the river, whereas the other two have large locks that can prevent transitions between the two water bodies. All access points, even the two entrances with locks (Chicago and T.J. O'Brien Locks) seem to offer little hindrance into the lake given that weatherfish have already moved past three locks on the river. Despite this, it is unclear why they have not used the area of unrestricted flow at the mouth of Indiana Harbor. The design of the locks leading to the lake could provide a more effective barrier because of how the locks are actually constructed.

In general, the design of the lock consists of two sets of large metal gates that control the upstream and downstream movement of vessels. There is variation in design seen in the vertical concrete structure that the gates rest on when closed called a sill. This sill will vary in height depending on how large an elevation change there is between the upstream and downstream sides of the lock chamber. Elevation change could range from 1-2 meters for small changes to ten or more meters for a large change. Brandon Road Lock, one of nine such structures that are found along the river between Lake Michigan and Mississippi River, has a 25ft (7.6 m) elevation change separating the upstream and downstream sides (Figure 15 and Figure 16). This is also one of the three such structures that weatherfish have already moved over to the downstream side, the other two being the Lockport and Dresden Island locks.

Figure 15. Inside the Brandon Rd lock chamber facing upstream direction. Red box indicates sill below lock gates (blue box). Only top portion (~10ft, 3m) of sill shown. Total chamber height is ~41 ft. (12.5m) with ~26ft (8m) vertical sill under the lock gates.

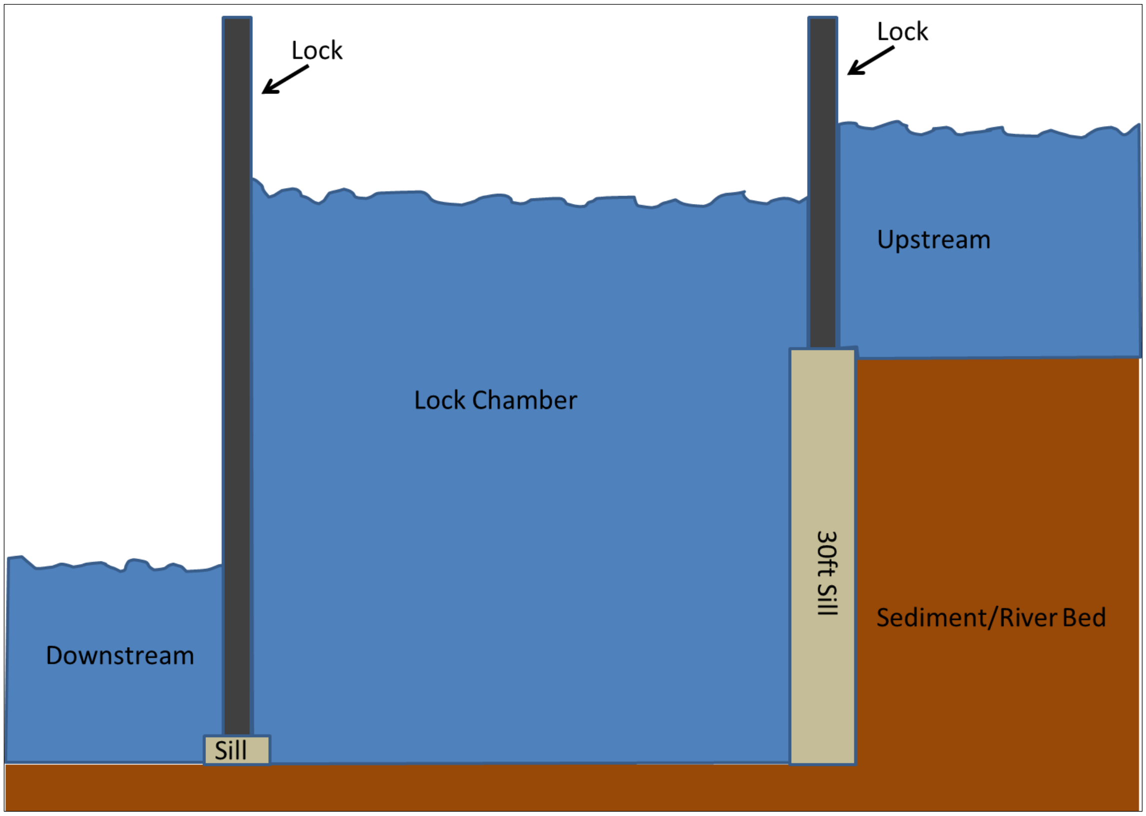

Figure 16**.** Representation of lock structures separating several pools on the river system between Mississippi River and Lake Michigan. Side closest to Lake Michigan is upstream and side closer to the Mississippi River is downstream. (Diagram not to scale).

The two locks that control movement between the river and lake control a much smaller elevation change and as a result have a sill of only 2-3 feet (0.6m- 1m) (Figure 17). However, weatherfish appear to have only been able to pass through the locks on the river and not the ones leading to the lake. This is likely due to the design of the lock and the directionality of weatherfish travel. Moving to the lake through these structures would require weatherfish to make a vertical migration that is close to and possibly above the upper limit observed by Fujimoto et al. (2008). There is also the possibility that because these two lake locks are gravity fed, when the gates open, it could create a strong enough current to push weatherfish far enough away from the opening where they cannot make it into the lock in time before it closes again. No study has established the maximum swimming speed of weatherfish or the amount of flow created when the locks open.

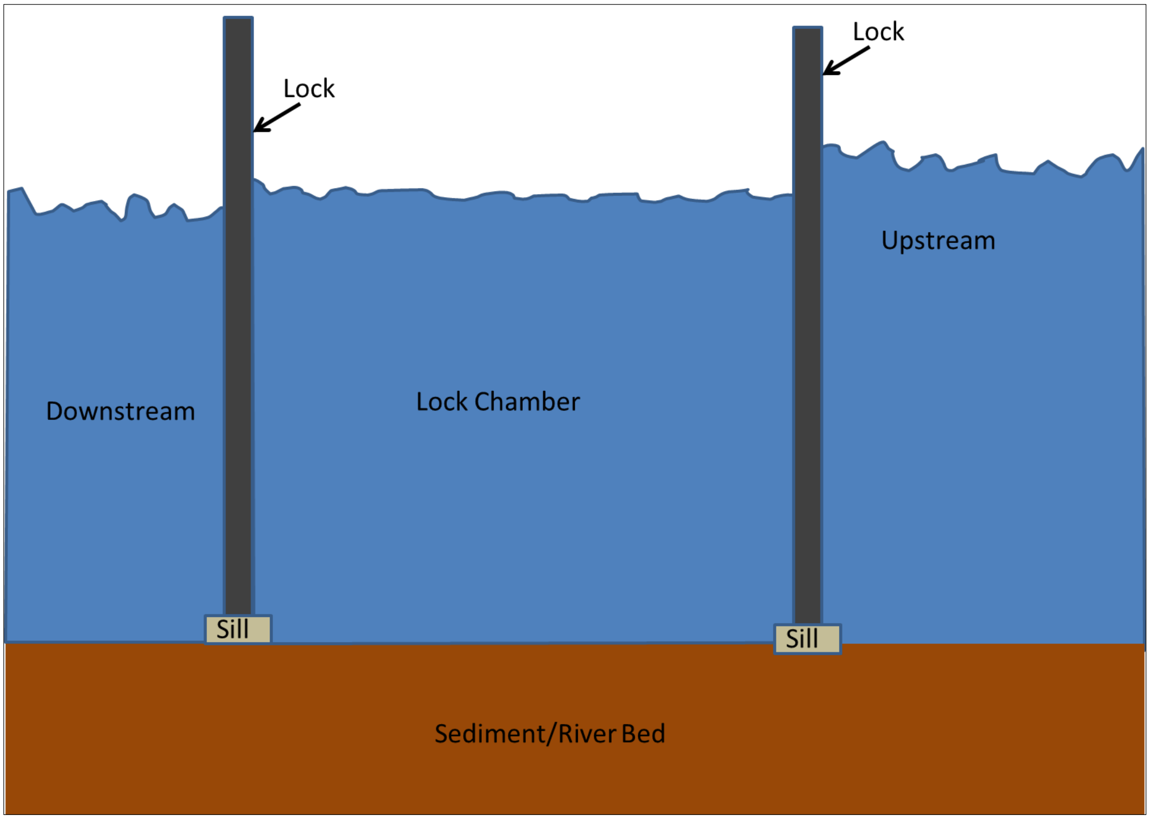

Figure 17. Representation of lock structures that separate Lake Michigan (upstream) from the Chicago River (downstream) or Lake Calumet (upstream) from Little Calumet River (downstream) (Diagram not to scale).

The reason weatherfish have moved through the river locks even though the sills are much larger is because they are moving downstream and have to traverse over a much smaller vertical distance than they would if they were going in the opposite direction. This shorter distance is within the range established by Fujimoto et al. (2008). If they were traveling upstream and came across these structures, it is likely that weatherfish would not make the vertical migration and would only be found on the downstream side. The river system as a whole is also subject to large flow events during rainstorms. During this time, many of the sluice gates are open on the dams, leading to the next, lower pool and can create flows of 8000 ft^3/s ($227m^3/s$) or greater on the CSSC (Straub et al. 2012). This could, in theory, be enough to pull any aquatic life caught in the current into the next pool, aiding in the expansion of the weatherfish’s range through semi-natural means.

Even though locks and sluice gates appear to be effective deterrents for movement into the lake, the last two connections between the river and lake have no structures preventing movement and are free flowing, open channels. To date however, there has been no observation of weatherfish within Indiana Harbor in Lake Michigan even though they were observed in 2005 (Simon et al 2006) to be in the Indiana Harbor Canal, approximately 3 miles (5km) from the lake. Weatherfish have also not been found in close proximity to the last connection point in Portage, IN. Given their current movement patterns through the Little Calumet River, they will likely reach that point within the next few years. Their past trends however would indicate that they will continue to stay within the river and not make the transition to Lake Michigan.

Speculation on Failed Lake Michigan Colonization

If weatherfish are currently able to make it past the lake lock structures, the reason behind their failure to colonize the lake overall is a bit of a mystery. A number of factors such as the availability of food resources, habitat preference/availability, or predator avoidance or some combination therein could be playing a role. If weatherfish were to make the transition to the lake environment there is a possibility that they could persist if the highly variable conditions of the lake are suitable for a sufficiently longtime period for them to become established. Suitable conditions would be, but are not limited to sufficient food sources, burrowing materials/substrate cover, water turbidity, and predator avoidance opportunities.

The majority of the weatherfish diet in Chicago waterways consists of Sphaeriidae (fingernail clams) and Chironomidae (non-biting midges) (Norris 2015). However, their diets within the CAWS were more cosmopolitan and consisted of 19 taxa, including caddisflies

(Trichoptera), leeches (Clitellata), mayflies (Ephemeroptera), and fish eggs (Norris 2015) among others, indicating that they are primarily opportunistic feeders and should not be strongly limited by the type of food resources present in a system. Surveys of insect communities in the river show that representatives of the midge family typically make up less than 10% of the benthic communities, but have been known to be upwards of half the collected taxa in some locations where weatherfish are also found (www.mwrd.org). Some areas of the lake are known to have similar amounts of midges present in the near shore area, sometimes upwards of 20%+ given the right conditions (Smith 2005). In some localities of the lake there are other examples of the weatherfish's diet being present such as fingernail clams and side swimmers (Amphipoda) that could easily supplement the lack of midges (Garza and Whitman 2004; Smith 2005; Kuhns & Berg 1999). These taxa provide an indication that the primary concentration of organisms, as well as some of the infrequent taxa, found in this fish's diet are on either side of the transition point and in similar abundances, which should sustain them if they were to transition into the lake. This is a sign that a lack of a food source is likely not the cause for their failure to establish.

Habitat conditions in most of the near shore area of Lake Michigan at the inlets would be the other major factor that might influence the weatherfish's establishment in the lake. Weatherfish tend to have a preference for silty or muddy substrates, usually accompanied by some form of detritus or vegetation covering in slow moving waters (Schmidt & Schmidt 2014; Schultz 1960; Tabor et al. 2001; Logan et al 1996). This is largely the habitat type they were collected in during the present study. The habitat type is lacking in Lake Michigan except in places such as the harbors. These harbors are also fairly well protected from currents and wave action of the open lake and could mimic backwater areas. Other than Wilmette Harbor however, these locations are not usually close to a river-lake connection, thus making the movement to the harbors a challenge for these fish. The majority of the area between the harbors consists largely of a sandy or rocky substrate (Janssen et al. 2005; Creque et al. 2010), a habitat type that weatherfish are rarely if ever collected from (Schmidt & Schmidt 2014; Meyer & Hinrichs 1999). These unfavorable expanses of habitat could effectively be forming an un-crossable zone between the favorable harbors of the lake and the favorable river inlets forcing the fish to stay in the river where they are already established.

There is the potential for weatherfish to be overlooked in the lake due to sampling bias. Many of the inlet locations have active boating traffic, necessitating them to be deeper than the shallow locations weatherfish were captured from on the river. This could limit the effectiveness of some sampling gear to the point where weatherfish could be unnoticed at that location. This is unlikely given the number of survey hours conducted in the lake, but continued sampling would be needed to assess this claim further.

Currently, the cause(s) for the lack of range expansion of weatherfish into the lake is/are uncertain. Given their hardy nature it should be possible for them to make the transition into the Lake Michigan system via the CAWS. Their current expansion however, seems to be limited to the rivers as they are currently well established within many of them. They have the potential to move through many more kilometers of rivers and streams and given their current path they could expand as far as the Mississippi River and into many of the adjacent waterways. It is recommended that this species' expansion be continually monitored and the risk factors be evaluated.

Gear Efficacy

Catch per unit effort (CPUE) is commonly used to assess fish assemblages in terms of fish relative abundance, community composition, and size-age structure (Hangsleben et al.

2012). It can be used to examine environmental responses of fish, and monitoring fish abundances can be a critical tool for fisheries management (Hangsleben et al. 2012). A variety of gear types can be used to sample a water body, and depending on such factors as season, species, and water chemistry, catch rates will vary for any given species. This study used four gear types that were classified into the categories active (backpack electrofishing and boat mounted electroshocker) and passive (minnow traps and mini-fyke nets). Other methods such as seining, gill nets, trawling, and hoop nets are effective techniques, but were not used in this study.

For this study, the overall number of fish caught was slightly higher using passive methods (active 129, passive 140). This could be an artifact of the passive gear type being deployed for 38 times longer than the active gear (30933 vs. 822.3 hrs.). However, the use of active gear types was more effective than passive in terms of the rate of capture in a given stretch of river. Overall, active gears were able to collect 0.16 fish per hour and passive collected 0.005 fish per hour. This study does not support Wells' (2014) findings that baited minnow traps were the best method for capturing more weatherfish as there was no difference in the number of fish caught between gear types. Gear effectiveness was highly dependent on habitat conditions and time constraints. For example, weatherfish were caught at a higher rate in this study using backpack electrofishing in shallower streams, but baited minnow traps worked better in deeper waters.

The area sampled varied the catch rate, which could be a product of the habitat features influencing the effectiveness of the method. Previous evidence suggests that specific habitat conditions will change the efficacy of any method used. For instance, conductivity, depth, and velocity all are said to play roles in how effective a given gear will be and catch rates will fluctuate depending on said conditions (Hill & Willis 1994; King & Crook 2002). Each location had varying degrees of cover, flow, depth, and substrate type which was reflected in the capture rate at each site and is evident by the significant interaction seen between gear type and location sampled.

The higher rate of active capture could be due to the nature of how each method is deployed in the system. For instance, passive techniques are typically stationary and rely on the likelihood of fish coming into contact with the traps (Portt el al. 2006). Habitat features such as debris, currents, and substrate type could restrict weatherfish access to traps and reduce catch rates. There are also noted examples of species being deterred from a trap if they detect the presence of other certain species (He & Lodge 1990). Conversely, active methods can typically cover more area in less time than passive techniques (Larimore 1954). They are also less limited by the same habitat features that limit the passive methods (Dauble & Gray 1980). Passive techniques have an advantage because they require a lower setup time and manpower commitment, but active methods are a more reliable way to assess the presence of weatherfish given a limited timeframe. In most cases the use of either method would be dependent on the study restrictions and resources.

Weatherfish Overall Assessments

Since weatherfish were first recorded in the North Shore Channel in 1987 they have been found at various points along an additional 150 plus miles (over 241km) of river. They are typically found in areas of the CAWS and IWW where water is shallow and slow moving. The exact density of weatherfish within this system is unknown, but they can be found in high abundances in the NSC, CSSC, I&M Canal, and parts of the Des Plaines River. They are found in lower abundances in the Cal Sag Channel, Grand and Little Calumet rivers, and the Illinois River. They are considered to be established throughout much of the region and are likely to keep expanding to many of the connected tributaries in the future. Based on the results of this study and previous patterns of expansion, it is unlikely that this species will be found in Lake Michigan. They are also unlikely able to move upstream of large structures such as dams without outside interference. Smaller, water diversion structures might not provide an adequate barrier to upstream movement and weatherfish could potentially pass over them given favorable conditions. There is no universal assumption that could be made about these barriers' effectiveness toward upstream movement.

The overall effectiveness of the sampling methods varies by location, but the active gear type can produce more weatherfish per hour of effort in given locations that are conducive to active methods. It is likely necessary that both gear types would be needed for accurate assessment of the weatherfish’s expanding range, as the various habitats found in the area are not typically conducive to any one method.

The weatherfish's unique characteristics, including breathing atmospheric air (Laird & Page 1996), high and low thermal tolerances (Urquhart & Koetsier 2014b), and omnivorous diet (Norris 2015; Urquhart & Koetsier 2014a) mean weatherfish have the potential to become a serious problem throughout the waterway if left unchecked. There is little evidence to suggest the current or future environmental impact this species will have, but there is also no current method for preventing the movement of this species. It is necessary to have continued monitoring of this species to assess the potential limits (if any) to their range expansion and their environmental impact.

CHAPTER III

INTRODUCTION, DISPERSAL, AND RELATIONSHIPS OF THE ORIENTAL WEATHERFISH (*MISGURNUS ANGUILLICAUDATUS*) IN CHICAGO AREA WATERWAYS

Introduction

Invasive species are considered to be one of the biggest threats to global biodiversity and can have major impacts on global economics. The estimated 50,000 non-native species in the United States alone cause an estimated $125 billion in damages each year (Pimentel et al. 2000). Federal and local conservation and management groups are thus tasked with finding ways to inhibit introduction of new species, stop the spreading of invasive species, and prevent reintroduction of invasives once removed. This can be done through actions such as education of the public about the dangers invasive species pose, regulating importation of foreign species, and active removal programs. However, these approaches are often not enough, and new tools are continually being developed to aid in the prevention of non-native colonization.

Increasingly, genetic tools are being used to assess invasive populations. Among other things, these tools are useful in answering questions about a species' place of origin, how many times a species has been introduced, and how populations are interacting through examination of gene flow between populations. For example, genetic analysis of the lionfish (*Pterois miles* and *P. volitans*) invasion off the coast of Florida indicates that the fish originated from a population in Indonesia (Hamner et al. 2007; Betancur et al. 2011). This Florida population has since dispersed to Bahamian waters and can readily be found throughout the Gulf of Mexico (Freshwater et al. 2009). Black Carp (*Mylopharyngodon piceus*) found in the Mississippi River Basin are believed to be the result of multiple introductions. The populations show genetic similarities to several aquaculture stocks in the US (Hunter & Nico 2014). These non-native populations are the result of multiple releases of individuals at various locations along the watershed. This is evident when looking at their distribution pattern, as they have been found in several isolated pockets along the southern and central Mississippi River Basin (Nico & Neilson 2016).

Another benefit of determining the origins of an invasive species is the potential to identify control methods. One control method is the use of biological control programs, through purposeful introduction of natural enemies (predators, parasites, etc.) (Roux & Wieczorek 2009). These natural enemies should be specific enough that they only target the original non-native. It is estimated that 10% of land used for cultivation experiences some type of biological control, and that during the last 120 years the long-term pest control of 165 species has been achieved (Bale et al. 2008; Cock et al 2010). In the Great Lakes region, the introduction of salmon to control an exploding Alewife (*Alosa pseudoharengus*) population has not only dramatically decreased the Alewife population, but has also created a multi-billion-dollar fishery on the lakes (Jacobs et al. 2013).

The Oriental Weatherfish (*Misgurnus anguillicaudatus*) is native to eastern Asia with a range from Siberia to Northern Vietnam, including the island of Japan. During the past several decades these fish have been introduced to many other countries including Australia and parts of Europe (Berg 1965; Allen 1984; Razzetti et al. 2001). In addition, weatherfish have been collected from U.S. waters for almost 90 years (Mills et al. 1993). They have been found in isolated populations in 15 states, and are assumed to be established in many areas (Nico et al. 2016). It is unclear what native areas these invasive populations came from and if they are expanding their ranges in any of the current locations. Although, there have been some studies examining diets and life history features of the weatherfish (Norris 2015; Urquhart & Koetsier 2014; Koetsler & Urquhart 2012), the effects of weatherfish on the environment or native species has not been examined to date. There are also no genetic studies on the U.S. weatherfish, which might help to monitor their movements, determine their place of origin, or determine if a viable control/removal option exists.

It is hypothesized that U.S. weatherfish populations are the result of food supplier or aquaria releases (Laird & Page 1996). It has also been suggested that in many regions throughout the country where these fish are present, that they have and will remain relatively localized (Laird & Page 1996). An example would be the several, apparently geographically distinct populations that have been reported in the Hudson River Valley in New York (Schmidt & Schmidt 2014). If the hypothesis that they remain localized is correct, multiple and independent weatherfish introductions could have occurred in areas such as this where they occupy large sections of a waterway. However, gene flow or relatedness among and within populations has not been tested, so their mode of dispersal and origin source is often unclear, and their overall relatedness is a mystery (Schmidt & Schmidt 2014). If genetic tests were to be conducted, it would give a better understanding of this fish’s capacity for dispersal in places where they are found over a large area.

A similar situation exists in the Chicago Area Waterways (CAWS) and Illinois

Waterways (IWW). Weatherfish were originally found in Chicago's North Shore Channel (NSC) in 1987. Sequential downstream occurrences were reported through 2012, suggesting a steady expansion of over 150 miles (241km) into several rivers and tributaries (Nico et al 2016). However, it has not been determined if these populations are the product of natural expansion, multiple introductions, or some combination of the two. These systems have several lock and dam structures throughout their lengths that could provide barriers to these bottom-dwelling fish, thus inhibiting natural range expansion. As a result, it is possible that the fish found on the upstream and downstream sides of these structures share few genetic similarities. It is also unclear where the ancestral stock(s) for these fish originated. It is possible that they are the product of transfer between the presumed isolated U.S. populations or that they originated from drainages in the native range and do not share a source population with other locations.

The goals of this study are to 1) assess the genetic structure of weatherfish that are present in the Chicago Area Waterways (CAWS) and Illinois Waterways (IWW) and 2) compare the genetic sequence data from this population with those available on GenBank to infer the origins of the IL and IN population(s).

Two mitochondrial genes that have been successfully applied to studies of genetic structure are D-Loop (displacement loop) and COI (Cytochrome Oxidase Subunit I). COI is a locus that, depending on the species, has 5 to 13 subunits, and is involved in the electron transport chain (Denis 1986). Specifically, it is involved in the binding of ATP during the process of cellular respiration (Fukuda et al. 2007). The first three subunits are coded for by the mitochondria, with subunit I being the largest subunit of the complex (Denis 1986). Overall, all three subunits are functionally conserved in many species, i.e. they perform the same or similar functions across several species. Around 75% of the genetic sequence for this loci is the same across mammal genera, meaning that it is one of the more conserved loci of the mitochondrial genome (Saccone et al. 1999). It is conserved because the protein that is eventually made has to maintain a level of functionality for use in the electron transport chain. Within genera of fishes, the percentage is higher. For example, in the flatheads (*Platycephalus*, *Neoplatycephalus*, *Cymbacephalus*) 85 % is conserved, and in the tunas (*Thunnus*) 99% is conserved (Ward et al. 2005). When COI mutations do occur it is at a relatively rapid rate, with enough differences accruing over time so that closely related species can be differentiated. Within a species, these mutations mostly occur in the third position of the codon allowing protein function to remain. As protein function is maintained, COI actually has one of the lowest amino acid rates of change in the mitochondrial genome (Hebert et al 2003). Therefore, all members of a species should have highly similar, if not identical, COI amino acid sequences. This variability makes COI useful as a DNA barcode and for a universal bio identification system. (Hebert et al. 2003). The mutation rate of COI varies among taxonomic groups, ranging from 0.25% per million years (Chevaldonne et al. 2002) in some marine worms to 3.1% - 7.2% in some marine fishes (Horne & Herwerden 2013). Many of these within species base pair changes result in a synonymous substitution that could be unique to a specific region or population of a species thus discriminating an individual population from the entirety of the species (Cox & Herbert 2001; Wares & Cunningham 2001). This will be important for this study because the differences in the COI locus could allow for the determination of source population(s) that have contributed to the Chicagoland population.

By comparison, D-Loop is a non-coding region, and as a result, generally evolves at a faster rate in comparison to evolutionary rates of some coding regions (Tang et al. 2005). The mutation rate of D-Loop has been shown to be up to five times that of the protein encoding regions (Meyer 1993). The evolutionary rate of D-Loop has been estimated to be between 11% and 20% per million years for vertebrate species (Brown et al. 1993) and could be as high as 15% - 38% per million years as was reported in butterfly fishes (McMillan & Palumbi 1997). It has however been shown to evolve slower than some coding regions in some closely related species of Cobitidae (Tang et al. 2005). Lower rates are also reported for other groups such as the salmonid fishes (Bernatchez and Danzmann 1993; Shedlock et al. 1992). These slower rates of evolution are thought to be the product of structural folding constraints or an, as of yet, unknown reason (Tang et al. 2004). In addition to variable mutation rates, D-loop also has a high frequency of insertion/deletion mutations. The generally higher mutation rate of the D-Loop region has made it useful for studying population structure of fishes (Meyer 1993). D-loop coupled with COI should allow for identification of the source population for IL and IN weatherfish and also identify genetic structure within this group, should it exist.

This study compares sequences of the COI and D-Loop loci from weatherfish throughout the Chicagoland region (see Chapter II). The fish from various areas of the waterway were then be assessed for similarities by examining their haplotypes. Fish from areas that share more haplotypes and are geographically close in proximity are likely the product of natural species expansion rather than two separate introductions. Additionally, the sequences were compared to those available on public databases. This information was then used to infer origin(s) of the IL and IN invasive population(s).

Methods

Specimen Acquisition

Weatherfish were collected from early spring to late fall (March - November) of 2013 and 2014, from waterways of the Chicagoland area. They were collected from within and outside of the previously known range of weatherfish from the Chicago River (North and South Branches), Chicago Sanitary and Ship Canal (CSSC), the NSC, Des Plaines River, Illinois & Michigan Canal (I&M Canal), Little Calumet River, the Illinois River, and several smaller, feeder tributaries. Sampling included both active and passive methods as detailed in Chapter 2. The 2014 samples were collected and donated for this study by the U.S. Army Corps of Engineers (USACE), IL Department of Natural Resources (ILDNR), and from the study conducted by Norris (2014).

DNA Extraction and Amplification

Fin clips and muscle tissue (approx. 0.05g) were taken from each weatherfish collected. The DNA from these tissue samples was extracted using a QIAGEN DNeasy Blood and Tissue Kit (Cat. No. 69504). The mitochondrial COI locus and D-Loop locus were amplified using primers and parameters described by Lie et al. (2012) and Tang et al. (2005), respectively (Table 5 and Table 6). Amplified PCR products were sent to University of Washington’s High Throughput Genomics Center for sequencing. Sequences were edited and aligned using Geneious software (v 8.1.8).

Table 5. Parameters described by Liu et al (2012) for the amplification of the mitochondrial COI region of the Oriental Weatherfish.

COI: Primers: (Liu et al. 2012) LCOIa - CCTACCTGTGGCAATCACRCGC HCOI - GTGAATAGGGGGAATCAGTC		
Initial denaturing	95°C	4 min
Denaturing	94°C	1 min
Annealing	56°C	1 min
Extension	72°C	1 min
Cycles	34	
Final extension	72°C	7 min

Table 6. Parameters described by Tang et al. (2005) for the PCR amplification of the mitochondrial D-Loop Control Region of the Oriental Weatherfish.

D-Loop: Primers: (Tang et al. 2005) DL1 – ACCCCTGGCTCCCAAAGC DH2 – ATCTTAGCATCTTCAGTG		
Initial Denaturation	94°C	3 min
Denaturation	94°C	30s
Annealing	52-58°C	45s
Extension	72°C	1 min
Cycles	35	
Final Extension	72°C	8 min

Population Analysis

A total of 21 locations were sampled for this study. Weatherfish used in this study were present in 9 of those locations. The 59 fish used in this study were collected from 14 sampling sites in those 9 locations. Locations are described and shown as maps in Appendix B. Weatherfish collected from the same location, within 2 miles (3.2 km) of each other, or in small tributaries of that location were categorized as being within the same group. Those found greater than 2 miles (3.2 km) apart or in different locations were categorized as being a part of different group. This was done because sampling sites are typically separated by large diversion structures (locks and dams) or long expanses (greater than 2 miles) of less favorable habitat that limit movement between areas. **Table 7** describes the collection site and number of weatherfish caught within each location and their resulting groupings (Figure 18 and Figure 19).

Table 7. Number of weatherfish captured from each location. Broken into groups relative to the location they were collected.

Location	Group Number	Group Name	Number of Weatherfish	Latitude	Longitude
3	1	NSC	6	42.03739	-87.710069
3	2	Goose Island	3	41.910326	-87.656486
3	1	NSC	1	42.008557	-87.710479
4	3	CSSC	2	41.841357	-87.675464
5	4	Cal Sag	1	41.662576	-87.752913
8	5	Brandon Rd.	3	41.501691	-88.104828
10	6	Illinois River	1	41.36288	-88.38018
12	7	Lockport Prairie	8	41.582368	-88.073778
12	8	Romeoville	4	41.656257	-88.064938
13	9	Little Cal	1	41.569185	-87.475909
13	9	Little Cal	1	41.437745	-87.565926
17	10	I&M	21	41.732676	-87.879999
12	11	Des Plaines	4	41.675838	-88.027109
7	12	9th Street	3	41.590414	-88.066954

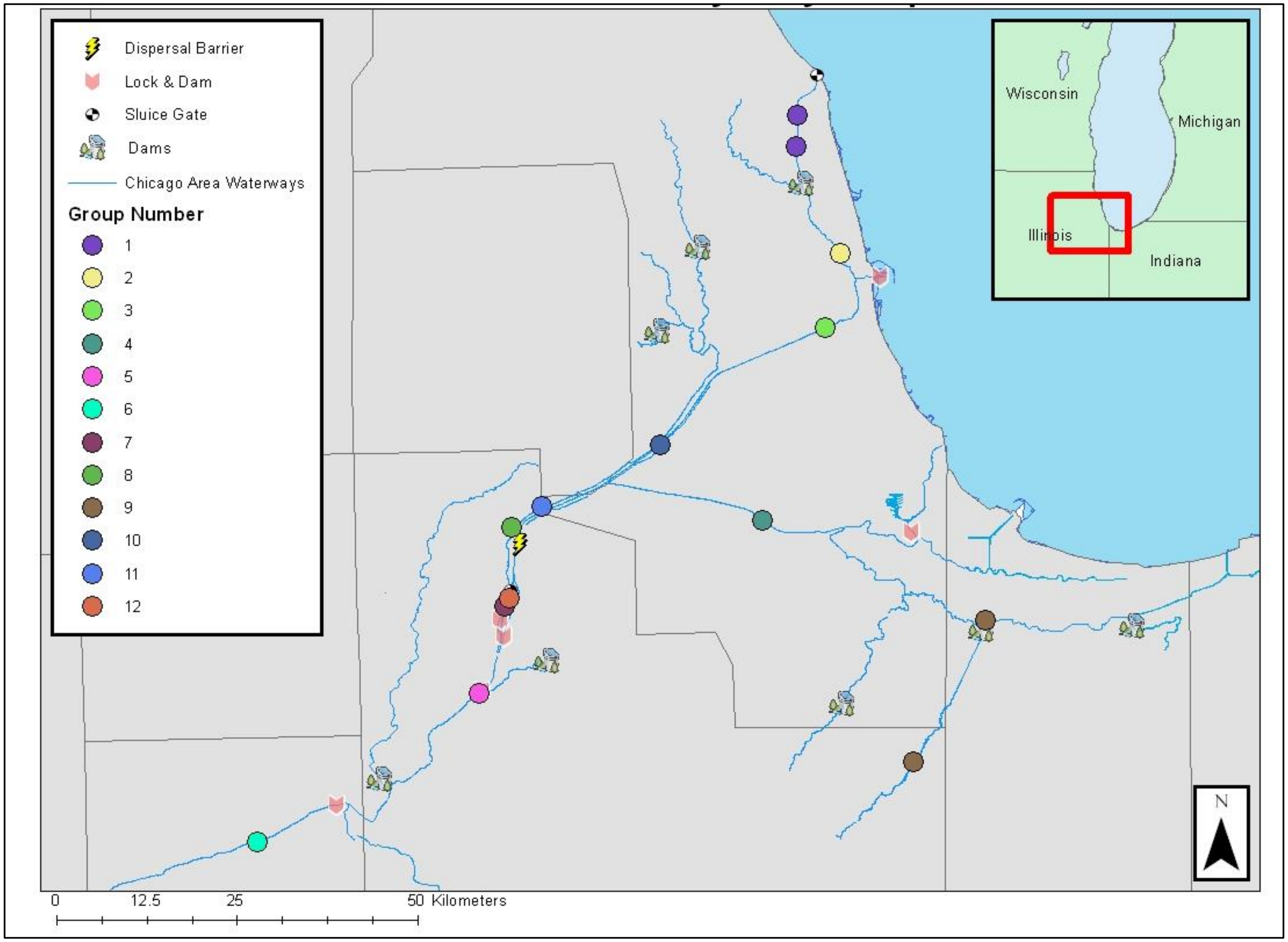

Figure 18. Group designation of weatherfish used in this study indicated by color.

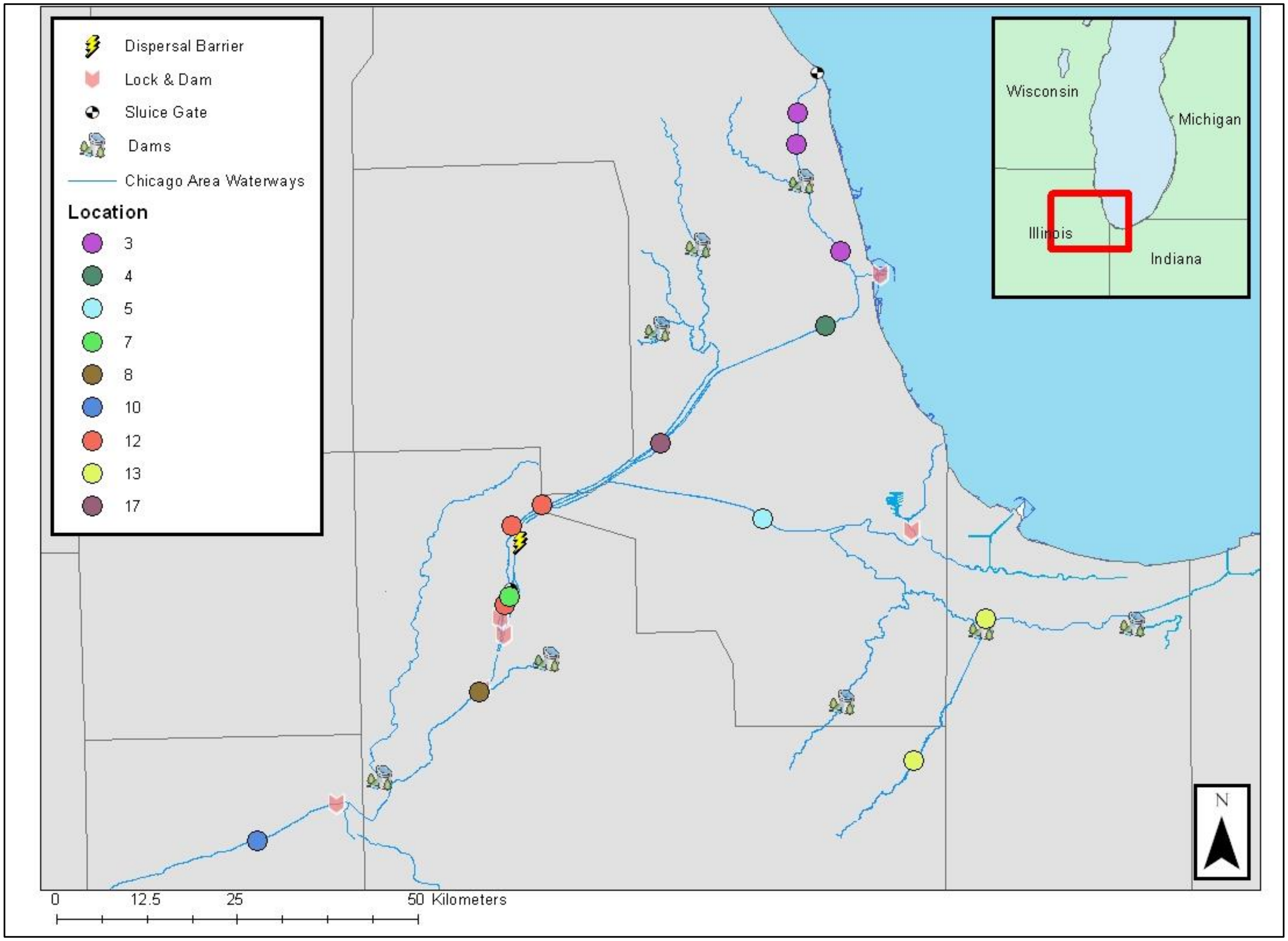

Figure 19.Collection sites of weatherfish in each location used in this study indicated by color.

Individual haplotypes for each locus were determined using the online FaBox (v 1.41) software (Villesen 2007). A haplotype is a particular arrangement of nucleotides that can be found within a sequence of basepairs for a given locus. Sequences for each individual were also combined and a haplotype list for these combined sequences was created using the same software. A median joining network of haplotypes was created for each gene and for the combined sequence with PopArt v 1.7 (http://popart.otago.ac.nz). Number of variable sites, polymorphic sites, haplotype diversity, and nucleotide diversity was calculated in PopArt 1.7 and DnaSP v5, the number of sites was confirmed manually.

Chicagoland haplotypes were compared to haplotypes of the same gene regions available for *Misgurnus anguillicaudatus* on GenBank. These sequences were aligned using Geneious software (v 8.1.8) for construction of a phylogenetic tree. The six different haplotypes of the amplified Chicagoland weatherfish COI sequences were combined with those available from GenBank (accession numbers found in Appendix D). In total 46 samples were analyzed. They include six haplotypes from Chicagoland (derived from 59 samples) and 40 haplotypes from other locations ranging from 607 to 890 base pairs in length. The amplified Chicagoland weatherfish D-Loop sequences were also combined with those available from GenBank (accession numbers found in Appendix D). In total 313 samples were analyzed, 6 haplotypes from Chicagoland (51 individuals) and 307 unique sequences from other locations ranging between 350 – 938 base pairs in length. The shorter sequences were typically from a highly variable region located at the end of the region that was amplified for the IL/IN population.

The evolutionary model for the COI sequence was determined using jModeltest v2.1.3. The model determined for COI using AIC criteria was GTR + G and for D-Loop GTR + I + G. Models were used for each locus to compare sequences using Maximum Likelihood and

Bayesian statistics, with trees being created in RaxML v1.5 and MrBayes v3.2.2 respectively with 1000 bootstrap replicates and 1.5 million generations with a burn-in rate of 25% respectively. Duplicate sequences were removed for analysis and are indicated in the figures as a single branch. The trees created for both loci were rooted using the COI and D-Loop regions of a closely related weatherfish species, *Misgurnus nikolskyi* (GenBank accession number AB242171) as the outgroup. This weatherfish species is the only Cobitidae species in Western Siberia. It shares a similar karyotype structure with *M. anguillicaudatus* and is likely to have been derived from the Oriental Weatherfish (Vasil'ev & Vasil'eva 2008).

Low sample numbers at several locations prevented F statistics and AMOVA from being employed to determine the degree of similarity between IL and IN groups.

Results

We collected 59 weatherfish samples from 14 sites across 9 locations. These locations were treated as 12 different possible groups based on the natural and manmade divisions between sampling locations. The size of the amplified COI region was 1039 bp, while the D-Loop amplicon was 931-932 bp. Each sequence was compared to sequences available in GenBank via a BLAST search to confirm field identification of the target species. COI and D-Loop sequences were most similar (93% and 96%, respectively) to others cataloged as *Misgurnus anguillicaudatus*.

There were 6 unique sequences found at the COI locus for fish in the IL/IN waterways system out of 59 sampled sequences. These 6 unique sequences were each designated as a haplotype. D-Loop had 6 haplotypes in the system from 51 samples that were amplified. Haplotypes were deposited in GenBank under the accession numbers KY780064 - KY780069 for COI and KY780070 - KY780075 for D-Loop. The number of variable sites for COI and D-

Loop was determined to be 52 and 36, respectively. D-Loop also had 3 deletion/insertion sites. These variable sites comprised 5% of the total COI and 3.4% of the D-Loop sequences. Of those sites, there were 38 (3.7%) and 29 (3.2%) informative sites for COI and D-Loop respectively. Haplotypes, variable sites, and informative sites are shown in Table 11 and Table 12. The COI variability consisted of 3 (0.4%) transversion substitutions and 49 (4.7%) transition substitutions. The D-Loop variability consisted of 6 (0.6%) transversions, 27 (2.9%) transitions, 3 (0.3%) insertion/deletion sites, and 1 site that was both a transition and insertion/deletion site. The overall nucleotide diversity (π) of COI was determined to be 0.0196 and 0.0139 for D-Loop. Mean population nucleotide diversity was 0.0139 and 0.0114 for COI and D-loop, respectively. The overall haplotype diversity (h) was calculated to be 0.679±0.05 and 0.719±0.049 for COI and D-loop, respectively. Mean population haplotype diversity were 0.5398±0.185 and 0.6013±0.239 for COI and D-Loop, respectively. Table 8, Table 9, and Table 10 show diversities (π and h) for each location for the individual loci and for the combined sequences.

Individuals that displayed a particular haplotype at one locus consistently shared the same haplotype for the other locus. For example, those that displayed the h01 haplotype for COI also displayed the h01 haplotype for D-Loop. There was only one instance where this pattern was not followed.

Table 8. Genetic diversity of COI locus of *Misgurnus anguillicaudatus* populations in Illinois and Indiana. Samples separated by group name (group number).

COI	***NSC (1)***	***Goose Island (2)***	***CSSC (3)***	***Cal Sag (4)***	***Des Plaines (11)***	***Brandon Rd (5)***	***Illinois River (6)***	***Lockport Prairie (7)***	***Romeoville (8)***	***Little Cal (9)***	***I&M (10)***	***9th Street (12)***	***Total***
No. of Samples (n)	7	3	2	1	3	3	1	8	4	2	21	4	59
No. of Variable sites (PS)	51	8	0	0	41	41	0	41	41	0	49	38	52
Number of Hap	4	3	1	1	3	3	1	3	3	1	4	2	6
Haplotype Diversity (h)	0.810 ±0.130	1 ±0.2720	0	0	1 ±0.2720	1 ±0.2720	0	0.714 ±0.1230	0.833 ±0.2220	0	0.481 ±0.1210	0.5 ±0.2650	0.679 ±0.05
Nucleotide Diversity (π)	0.0256	0.0051	0	0	0.0263	0.0264	0	0.0216	0.0258	0	0.0178	0.0183	0.0196

Table 9.Genetic diversity of D-Loop locus of *Misgurnus anguillicaudatus* populations in Illinois and Indiana. Samples separated by group name (group number).

D-Loop	***NSC (1)***	***Goose Island (2)***	***CSSC (3)***	***Cal Sag (4)***	***Des Plaines (11)***	***Brandon Rd (5)***	***Illinois River (6)***	***Lockport Prairie (7)***	***Romeoville (8)***	***Little Cal (9)***	***I&M (10)***	***9th Street (12)***	***Total***
No. of Samples (n)	7	2	2	1	2	3	0	8	4	1	21	0	51
No. of Variable sites (PS)	32	2	0	0	28	29	0	29	24	0	30	0	36
Number of Hap	4	2	1	1	2	3	0	3	3	1	4	0	6
Haplotype Diversity (h)	0.8100 ±0.130	1 ±0.5000	0	0	1 ±0.5000	1 ±0.272	0	0.7140 ±0.1230	0.8330 ±0.2220	0	0.5380 ±0.1130	0	0.7190 ±0.049
Nucleotide Diversity (π)	0.0158	0.0022	0	0	0.03	0.0208	0	0.0169	0.0167	0	0.0116	0	0.0139

Table 10; Combined genetic diversity of *Misgurnus anguillicaudatus* populations in Illinois and Indiana. Samples separated by group name (group number).

Combined	***NSC (1)***	***Goose Island (2)***	***CSSC (3)***	***Cal Sag (4)***	***Des Plaines (11)***	***Brandon Rd (5)***	***Illinois River (6)***	***Lockport Prairie (7)***	***Romeoville (8)***	***Little Cal (9)***	***I&M (10)***	***9th Street (12)***	***Total***
No. of Samples (n)	7	2	2	1	2	3	0	8	4	1	21	0	51
No. of Variable sites (PS)	83	3	0	0	65	70	0	70	65	0	79	0	88
Number of Hap	4	2	1	1	2	3	0	3	3	1	5	0	7
Haplotype Diversity (h)	0.810	1			1	1		0.714	0.833		0.548		0.722
	±0.130	±0.5	0	0	±0.50	±0.272	0	±0.1230	±0.222	0	±0.119	0	±0.050
Nucleotide Diversity (π)	0.0209	0.0015	0	0	0.033	0.0237	0	0.0194	0.0215	0	0.0148	0	0.0171

Table 11. Variable sites for COI locus haplotypes for *Misgurnus anguillicaudatus* collected in Illinois and Indiana. Numbers indicate basepair position, n is number of individuals with that haplotype, and Frequency is percentage of total collected with haplotype.

COI Haplotype	**60**	**135**	**147**	**165**	**201**	**219**	**258**	**273**	**313**	**339**	**348**	**351**	**357**	**367**	**373**	**390**	**393**	**429**	**459**	**468**	**474**	**504**	**519**	**522**	**591**	**636**	**648**	**661**	**699**	**702**	**726**	**732**
h01	C	G	T	T	C	G	A	C	C	A	T	T	T	C	T	T	T	C	A	G	A	C	C	T	A	C	T	C	C	C	A	G
h02	C	G	T	T	C	G	A	T	T	A	T	T	T	C	T	T	T	C	A	G	A	C	T	T	A	C	T	C	C	C	G	G
h03	C	A	T	C	T	A	A	T	C	T	T	C	C	T	C	T	C	C	G	A	G	T	T	T	C	T	C	T	T	T	A	A
h04	T	A	C	T	T	A	G	T	C	T	C	C	C	T	C	C	C	C	G	A	G	T	T	C	C	T	C	T	T	T	G	A
h05	C	A	T	C	T	A	A	T	C	T	T	C	C	T	C	T	C	T	G	A	G	T	T	C	C	T	C	T	T	T	G	A
h06	C	G	T	T	C	G	A	T	T	A	T	T	T	C	T	T	T	C	A	G	A	C	T	T	A	C	T	C	C	C	G	G

Haplotype	**738**	**762**	**774**	**846**	**888**	**891**	**900**	**903**	**918**	**924**	**930**	**933**	**936**	**954**	**972**	**984**	**1011**	**1014**	**1020**	**1029**	n	Frequency
h01	A	C	C	A	G	A	T	C	A	A	C	A	G	C	A	A	T	T	T	A	30	0.51
h02	A	C	C	A	G	A	T	C	A	A	C	A	G	C	A	A	C	T	C	C	5	0.08
h03	G	T	T	A	A	G	T	C	G	G	T	A	A	T	G	G	C	C	C	A	14	0.24
h04	G	T	T	G	G	G	C	C	G	G	C	G	A	T	G	G	C	C	C	A	4	0.07
h05	G	T	T	A	A	G	T	C	G	G	T	A	A	T	G	G	C	C	C	A	4	0.07
h06	A	C	C	A	G	A	T	T	A	A	C	A	G	C	A	A	C	T	C	C	2	0.03

Table 12. Variable sites for the D-Loop control region haplotypes for *Misgurnus anguillicaudatus* collected in Illinois and Indiana Numbers indicate basepair position, n is number of individuals with that haplotype, and frequency is percentage of total collected with that haplotype.

D-Loop Haplotype	32	42	54	64	72	74	81	89	90	100	229	231	253	265	343	355	415	513	545	554	587	612	645	650	695	708	737	746	747	750	752
h01	C	A	G	C	C	A	A	T	A	T	T	A	T	C	A	C	A	C	T	A	G	C	T	G	T	G	T	-	G	T	T
h02	C	A	G	C	C	A	A	T	A	T	T	A	T	C	A	C	A	T	T	G	G	C	T	G	T	A	C	-	A	T	T
h03	T	G	A	G	C	G	G	C	G	A	C	G	C	G	-	C	T	T	C	A	A	T	C	A	A	G	T	A	G	A	T
h04	T	G	G	G	T	G	G	C	G	T	T	A	C	G	A	T	T	T	C	G	G	C	T	G	A	G	T	A	A	A	-
h05	T	G	G	G	C	G	G	C	G	A	C	G	C	G	-	C	T	T	C	A	A	C	C	A	A	G	T	A	G	A	-
h06	C	A	G	C	C	A	A	T	A	T	T	A	T	C	A	C	A	T	T	A	G	C	T	G	T	A	T	-	A	T	T

Haplotype	773	782	784	789	885	*n*	*Frequency*
h01	A	T	G	G	C	24	0.47
h02	A	C	G	G	C	5	0.10
h03	A	T	A	A	C	11	0.22
h04	G	T	A	G	T	5	0.10
h05	A	T	A	G	C	4	0.08
h06	A	C	G	G	C	2	0.04

Networks

There were 6 COI haplotypes in my study region. Haplotype h01 has the highest number of individuals with 30 followed by haplotype h03 with 14 individuals. Both haplotypes are found in the highest number of groups as well, 9 and 10 respectively. The Median-Joining Network for COI can be seen in Figure 20 and the breakdown of haplotype by group is given in Table 13. The COI network shows two haplotypes that are at least 7 mutational steps from their closest neighbor (h01 & h04). Two clusters of two (h02 and h06; h03 and h05) are 1-3 steps from each other within the cluster, but greater than seven steps from other haplotypes.

D-Loop exhibits 6 haplotypes in the system. Like COI, haplotype h01 of D-Loop is seen in the most individuals with 24; the next is haplotype h03 with 11 individuals. These haplotypes are seen in the most groups, 6 and 8 respectively. With one exception, individuals sharing a COI haplotype share their D-Loop haplotype. There is only one instance where a fish does not follow this haplotype pattern for both loci. The Median-Joining Network for D-Loop can be seen in Figure 21 and the breakdown of haplotype by group is displayed in Table 14. Similar to COI, patterns of differences exist for the D-Loop loci in that several haplotypes are separated by 10 or more differences from each other, but others show only 2-4 differences.

The combined Median-Joining Network is shown in Figure 22. There are some instances where only one of the two loci could be amplified, and as such were excluded from the combined network. When combining these two loci and creating a network, many of the individuals found within a haplotype in the previous networks are found with those same individuals. As expected the number of changes has increased between haplotypes. The pattern of a cluster of closely related haplotypes being separated from the next cluster by many differences continues in this network. One additional haplotype has been created for this combined network. It consists of one individual that had h01 for COI and h04 for D-Loop rather than h01 or h04 for both loci. It is a possible case of mitochondrial recombination or mitochondrial heteroplasmy (multiple, different mitochondrial genomes in an individual) as it is the only instance of a combination of the COI and D-Loop loci that differs from the other samples (Brown et al. 1992). The other scenario is that there was a mislabeling of samples, though this cannot be confirmed. The locations of the combined haplotypes are displayed in Table 15 along with the number of individuals displaying that combination. A full listing of individuals, their corresponding haplotypes for both loci, and the location they were collected can be found in Appendix D.

Table 13. Number of individuals in each group (group number) that were found to have a given haplotype for the COI locus.

COI Haplotype	NSC (1)	Goose Island (2)	CSSC (3)	Cal Sag (4)	Des Plaines (11)	Brandon Rd (5)	Illinois River (6)	Lockport Prairie (7)	Romeoville (8)	Little Cal (9)	I&M (10)	9th Street (12)	Total
h01	3	1	2	0	1	1	0	2	2	0	15	3	30
h02	0	1	0	0	1	1	0	2	0	0	0	0	5
h03	1	0	0	1	1	1	1	4	1	2	1	1	14
h04	2	0	0	0	0	0	0	0	0	0	2	0	4
h05	0	0	0	0	0	0	0	0	1	0	3	0	4
h06	1	1	0	0	0	0	0	0	0	0	0	0	2

Table 14. Number of individuals in each group (group number) that were found to have a given haplotype for the D-Loop region.

D-Loop Haplotype	NSC (1)	Goose Island (2)	CSSC (3)	Cal Sag (4)	Des Plaines (11)	Brandon Rd (5)	Illinois River (6)	Lockport Prairie (7)	Romeoville (8)	Little Cal (9)	I&M (10)	9th Street (12)	Total
h01	3	0	2	0	0	1	0	2	2	0	14	0	24
h02	0	1	0	0	1	1	0	2	0	0	0	0	5
h03	1	0	0	1	1	1	0	4	1	1	1	0	11
h04	2	0	0	0	0	0	0	0	0	0	3	0	5
h05	0	0	0	0	0	0	0	0	1	0	3	0	4
h06	1	1	0	0	0	0	0	0	0	0	0	0	2

Table 15. Number of individuals in each group (group number) that were found to have a given combined haplotype.

Combined Haplotype	NSC (1)	Goose Island (2)	CSSC (3)	Cal Sag (4)	Des Plaines (11)	Brandon Rd (5)	Illinois River (6)	Lockport Prairie (7)	Romeoville (8)	Little Cal (9)	I&M (10)	9th Street (12)	Total
h01	3	0	2	0	0	1	0	2	2	0	14	0	24
h02	0	1	0	0	1	1	0	2	0	0	0	0	5
h03	1	0	0	1	1	1	0	4	1	1	1	0	11
h04	2	0	0	0	0	0	0	0	0	0	2	0	4
h05	0	0	0	0	0	0	0	0	1	0	3	0	4
h06	1	1	0	0	0	0	0	0	0	0	0	0	2
h07	0	0	0	0	0	0	0	0	0	0	1	0	1

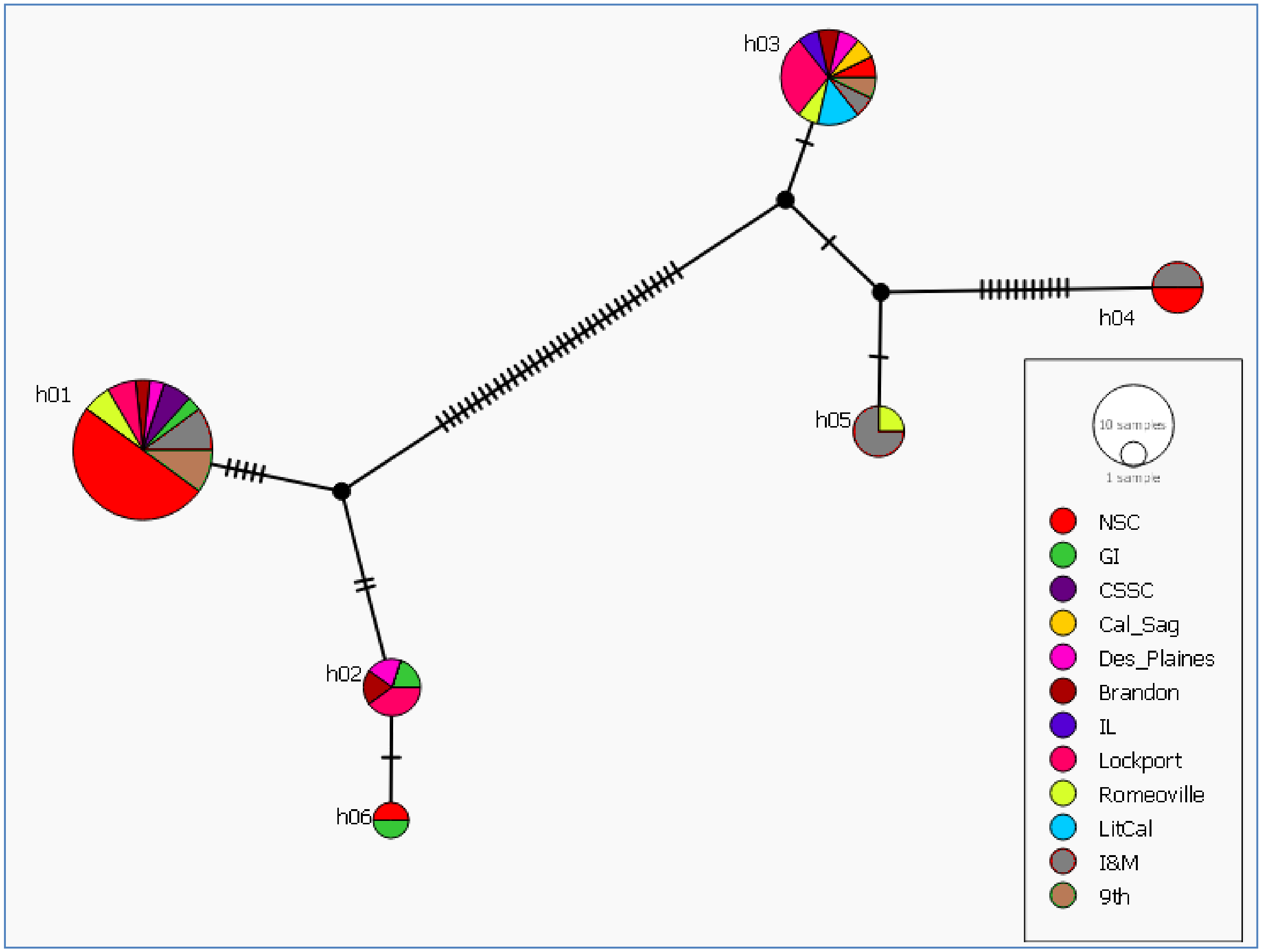

Figure 20. Haplotype Median Joining Network for Illinois /Indiana weatherfish samples created for the COI gene fragment (1039 bp). A haplotype is represented by a circle. Hash marks on the line represent the number of nucleotide changes between each haplotype. Circle size indicates number of individuals; larger circles indicate more individuals. Colors indicate the group and sizes of wedges indicate number of fish in that group with that haplotype.

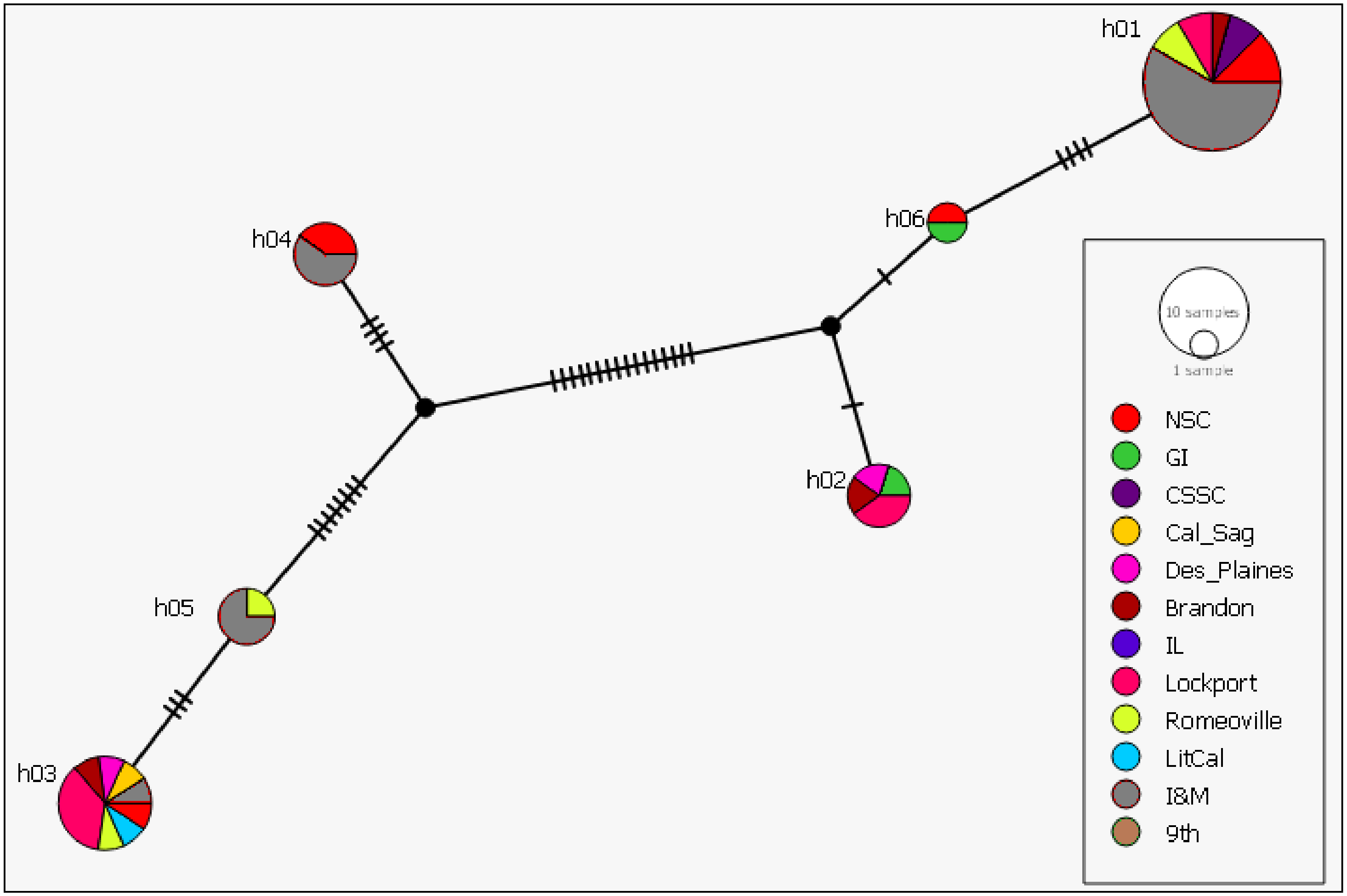

Figure 21. Haplotype Median Joining Network for Illinois/Indiana weatherfish samples created for the D-Loop region fragment (931-932 bp). A haplotype is represented by a circle. Hash marks on the line represent the number of nucleotide changes between each haplotype. Circle size indicates number of individuals; larger circles indicate more individuals. Colors indicate the group and sizes of wedges indicate number of fish in that group with that haplotype.

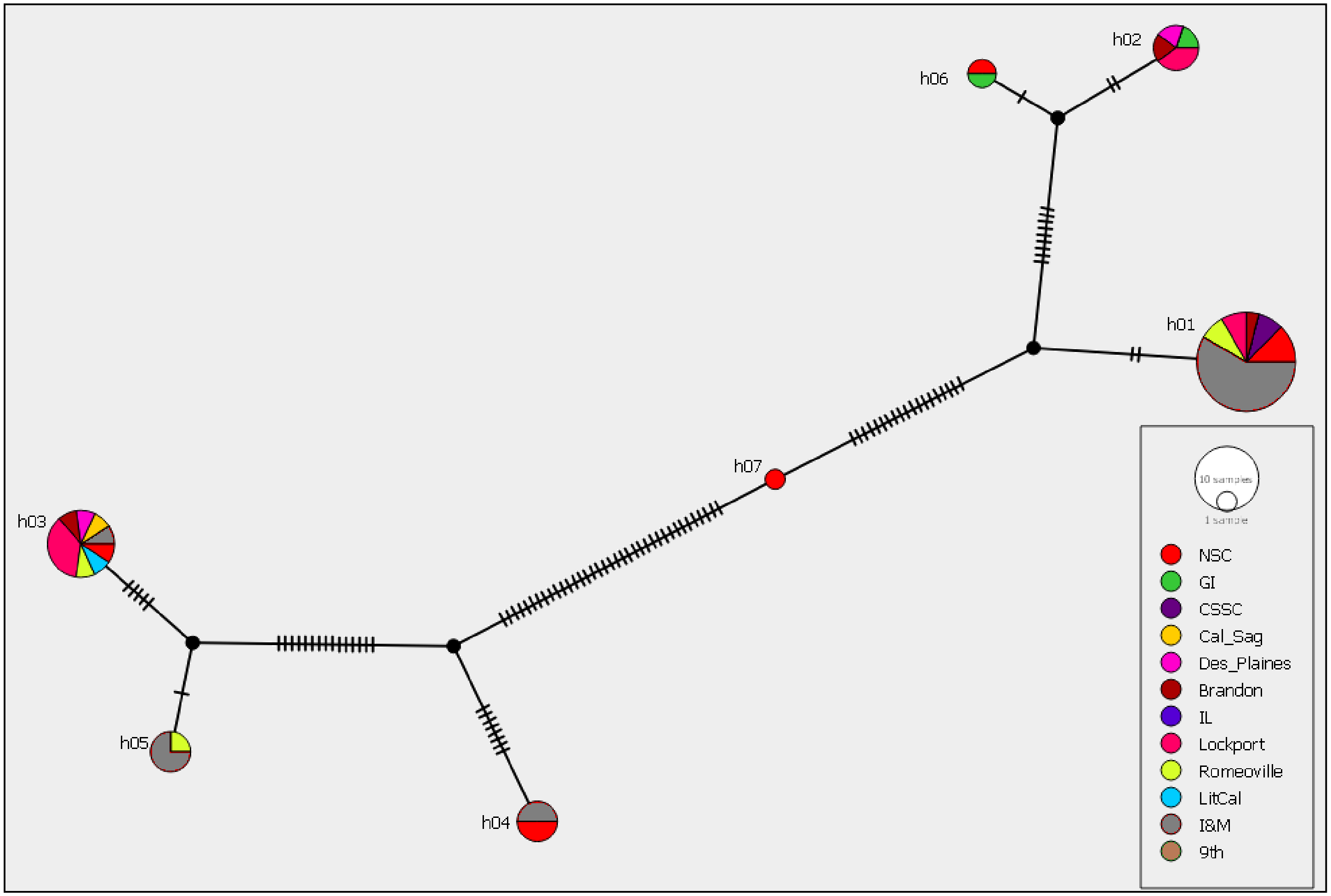

Figure 22. Haplotype Median Joining Network for Illinois/Indiana weatherfish samples created for the combined sequence (1971-1972 bp). A haplotype is represented by a circle. Hash marks on the line represent the number of nucleotide changes. Circle size indicates number of individuals; larger circles indicate more individuals. Colors indicate the group and sizes of wedges indicate number of fish in that group with that haplotype.

Haplotype Locations

Several haplotypes occur in multiple groups. For the COI locus, seven of the 12 groups had three or more haplotypes. In the remaining five groups, one had two haplotypes and four had a single haplotype. The North Shore Channel (NSC) and I&M Canal had the most haplotypes present with four. Both of those groups were dominated by the presence of individuals with the h01 haplotype. Three of seven (42.9%) fish collected in the NSC and 15 of 21(71.4%) fish in the I&M groups had that haplotype. Seven other groups had the h01 haplotype. Together, this haplotype follows a path of dispersal where it is found at all sampling locations along the NSC and CSS between the first introduction point and Joliet, IL, then upstream from Lockport, IL to a site in Lemont, IL on the Des Plaines River. In total, this dispersal covers approximately 100 miles (161km) of river. This haplotype was not, however, found in the eastern portion of the weatherfish's range through the Cal Sag Channel into the Little Calumet River.

A similar pattern can be seen with haplotype h03, as it was found in 10 of the 12 groups. This haplotype is found throughout the southern portion of the range and in one instance in the NSC at the original introduction site. It is missing from the two sampling points to the north and south of downtown Chicago. This forms a break in the distribution of the northern h03 and the southern.

The greatest haplotype diversity was found in two groups, the group in the Des Plaines River (11) and the one just above Brandon Rd (5). Each group had three haplotypes from three individuals. These sites also had the highest nucleotide diversity of any of the 12 sampling groups. The lowest haplotype diversity was seen in the Little Calumet River, the CSSC, the Cal Sag Channel. All individuals captured in these places carried the same COI haplotype.

The groups with only one or two haplotypes could be an artifact of low sample size and not actually a true representation of the haplotype diversity in those areas. All but one of those groups had 1 or 2 fish collected, the remaining had four fish collected. The full distribution of the COI haplotypes is shown in Figure 23 and Table 13 and the COI diversity is shown in Table 8.

Two of the twelve COI groupings were not included in the D-Loop data set because the samples failed to amplify. For the distribution of D-Loop haplotypes, the same patterns can be seen for many of the sites. Haplotype h01 is seen in six of the 10 sites followed by h03 being found in eight sites. As with COI, the NSC and I&M had the most haplotypes present at four, with h01 dominating the samples in three of seven and 14 of 21 fish respectively. Four other sites also had h01 present, these were the same locations that the COI h01 haplotype was present. There is also a break in the sites where h03 is present. It is seen in the northern most groups and is not detected again until several kilometers downstream at the site on the I&M canal where fish carrying this haplotype would have to travel upstream from the Cal Sag Channel to reach that site.

Three of the four sites with the lowest COI haplotype diversity, also show low haplotype diversity for D-loop. The fourth site did not have an amplified sequence but would be expected to give the same result. Likewise, many of the same patterns for both nucleotide and haplotype diversity, can be seen in D-Loop as compared to COI. The locations of the D-Loop haplotypes are shown in Figure 24 and Table 14 and Table 9 shows the diversity.

For those individuals where both COI and D-Loop were amplified, their sequences were concatenated. Like individual loci, the h03 haplotype is present in the majority of groups, eight, followed by h01 being found in six groups. Also similar to previous results, h01 was found in the majority of the samples at 24 and h03 was found in 11. There was one instance of a unique haplotype combination, designated h07. It is a combination of a fish with COI h01 and D-Loop h04. This haplotype is only found in the I&M canal. This puts the number of haplotypes sampled from the I&M Canal at five, as opposed to four as seen in the individual loci analysis. The NSC still has four haplotypes present and is the next most diverse group. All other groups have the same number of haplotypes seen in D-Loop analysis. This diversity almost mirrors that of the D-Loop locus, as is expected, and Table 10 shows those values. The distribution of the combined haplotypes can be seen in Figure 25 and Table 15.

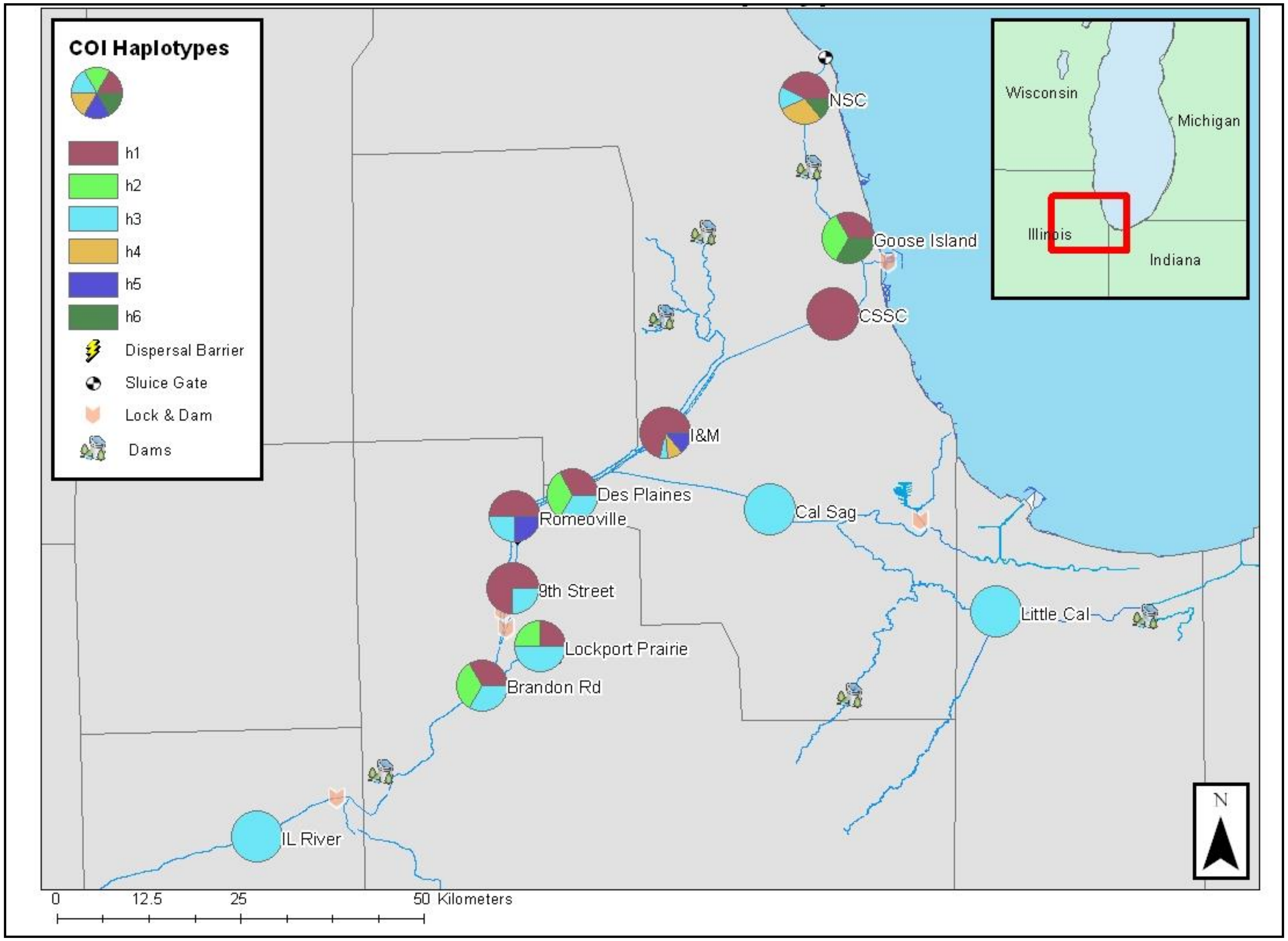

Figure 23: Distribution of weatherfish COI haplotypes in the Chicagoland Region. Circles represent the group, color the haplotype, and wedges represent the number of fish with a specific haplotype.

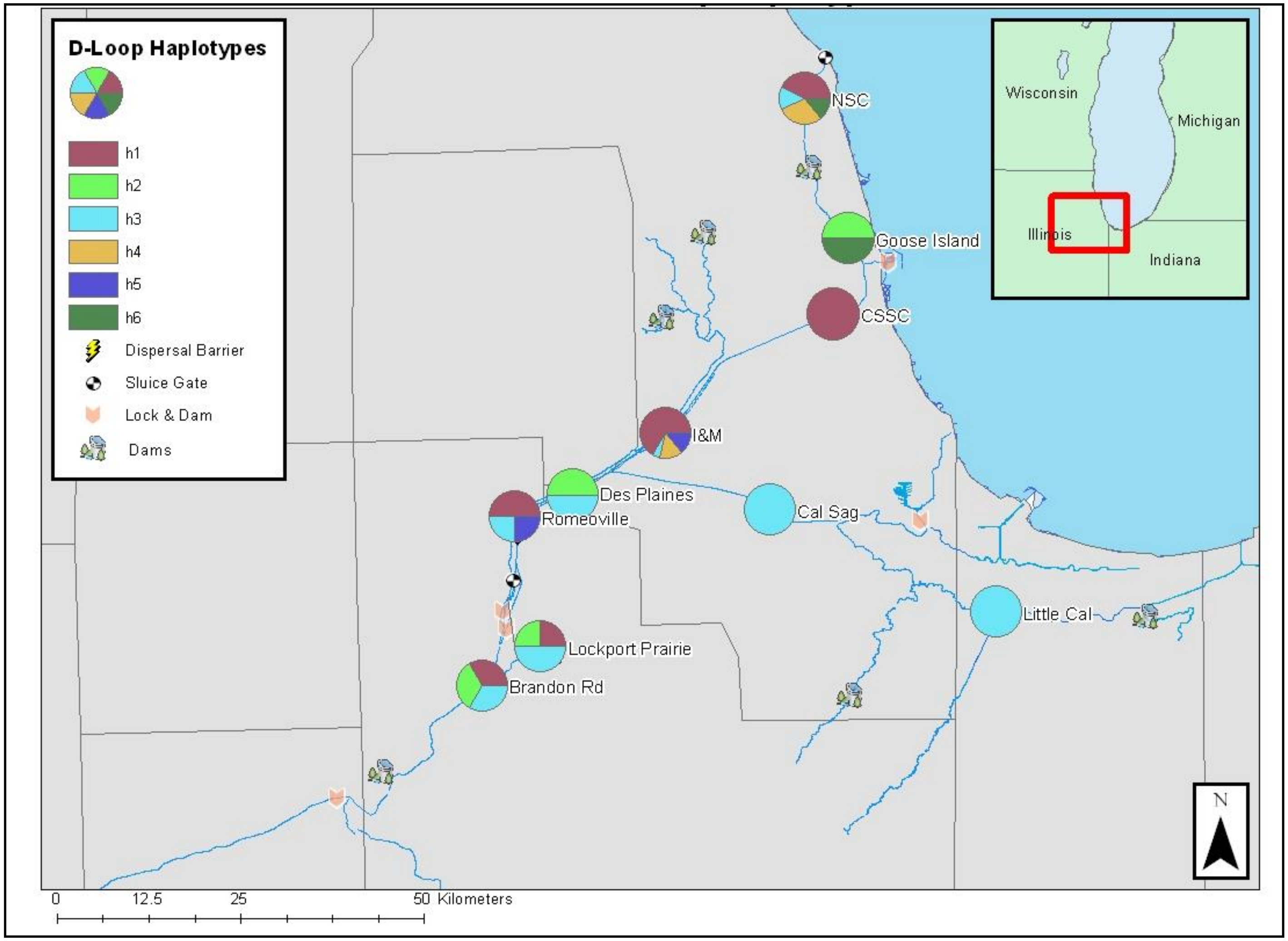

Figure 24. Distribution of the weatherfish D-Loop haplotypes in the Chicagoland Region. Circles represent the group, color the haplotype, and wedges represent the number of fish with a specific haplotype.

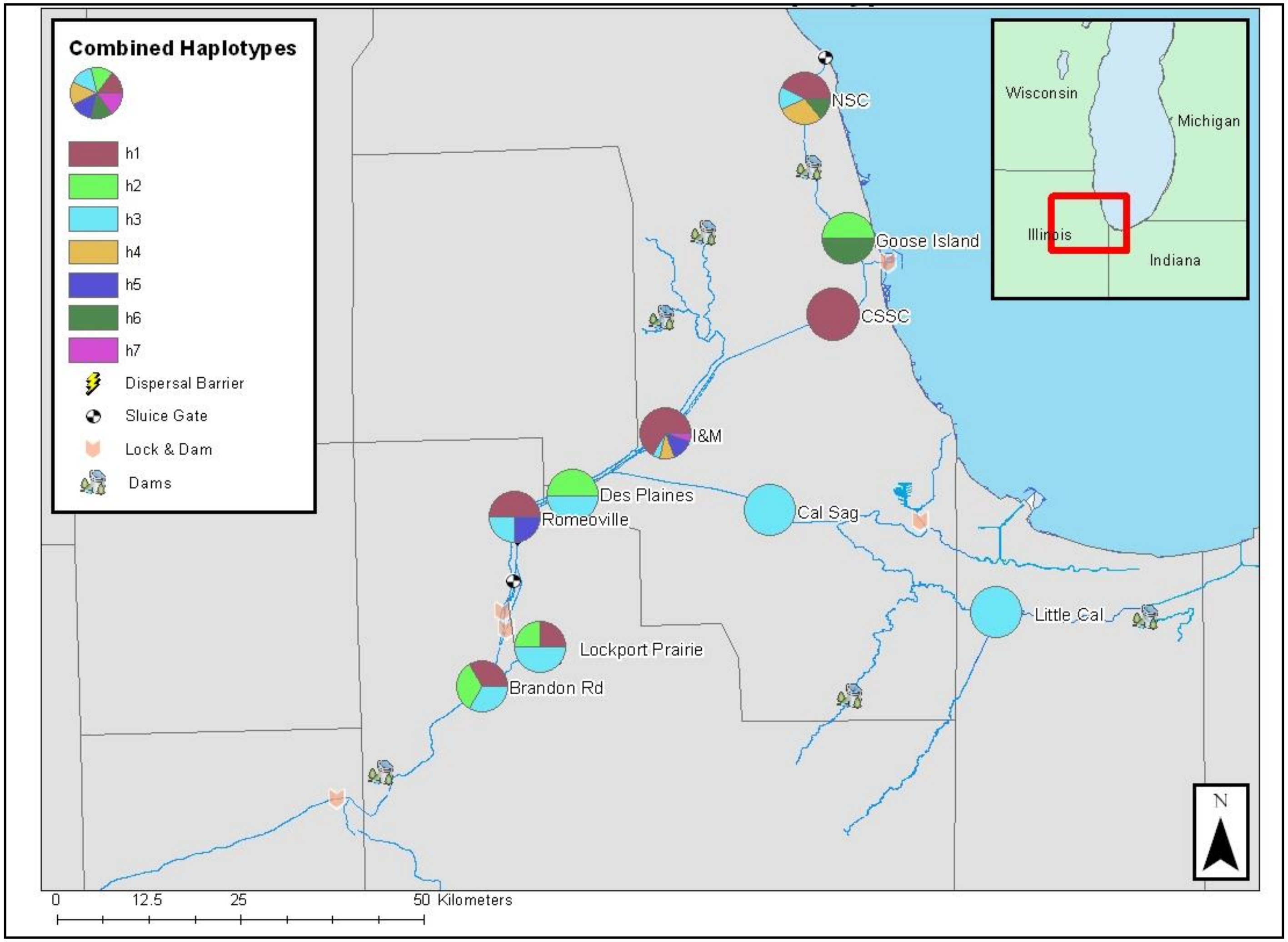

Figure 25. Distribution of the combined weatherfish D-Loop and COI loci haplotypes in the Chicagoland Region. Circles represent the group, color the haplotype, and wedges represent the number of fish with a specific haplotype.

Illinois/Indiana and Global Population Comparisons

The Bayesian and ML generated trees are shown in Figure 26 and Figure 27 respectively with country of origin indicated. The Chicagoland haplotypes are grouped into two different clades each with three haplotypes (h01, h02, and h06; h03, h05, and h04). There is high support in the Bayesian analyses for the arrangement of the six Chicagoland haplotypes, at 82%. For the ML analysis, the IL/IN clades are also separated from the rest of the sequences. However, they are also separated from each other with h01, h02, and h06 being an ancestral group. There is 100% support for this clade's arrangement and 34% for the placement of h03, h04, and h05. This separation can be attributed to the approximately 4%-5% sequence divergence seen between the two groups. The Chicagoland haplotypes are shown to be divergent from the all of the native samples that were used in this analysis, but are not separate from all non-native populations.

Five of the six IL/IN haplotypes were identical to at least one individual from another global population, outside of the United States and were represented on the same branch as the IL/IN haplotypes. These other individuals do not represent members of a native population and are instead sampled from the Australian invasive population. These samples are from four river basins in Australia. They are the Murrumbidgee River and Ginninderra Creek, which empty into an area around Lake Alexandrina, and the Yarra River Basin and Maribyrnong River Basin which empty into an area around Melbourne Australia. Two GenBank sequences were grouped with h01 (JQ011426: Murrumbidgee and KJ669524: Ginninderra), one with h03 (JQ011428: Yarra), and three with h04 (JQ011422: Yarra, JQ011434: Maribyrnong, and KJ669523: Murrumbidgee). The sample JQ011416 from the Yarra River shares an identical sequence with both the h02 and h06 haplotypes. Three other Australian sequences on this tree group more closely with sequences from China and Korea. This grouping pattern remained consistent across both Bayesian and ML tests.

These seven GenBank sequences only partially overlap with the IL/IN sequences in terms of sequence length. Those from GenBank are 607 bp long while the local sequences are 1039 bp long. In the overlapping area however, their sequences are identical. If the Chicagoland sequences are pared down to 607bp, matching the length and DNA region of those from GenBank, the Chicagoland and the corresponding GenBank haplotypes are identical (i.e., those GenBank sequences related to haplotype h01, display the same sequence as h01). This is why they are represented on the same branch and also why even though h02 and h06 are different haplotypes they are both identical to a sample from the Yarra River. The differences between h02 and h06 appear outside of the overlapping GenBank region.

Several other sequences included in this analysis were from the home range of the weatherfish (locations within China, North Korea, South Korea, and Vietnam). Other sequences were from invaded areas including Italy, a different river in Australia (Bunyip River), an Alabama pet shop, and a Florida river. None of these other sequences were closely related to weatherfish sequences found in IL and IN and form their own clade, separate from the IL/IN population. Members of this clade predominantly came from areas in China, but are intermixed with samples from invaded areas such as Italy (KJ553659) and the Alafia River in Florida (JQ011420 & JQ11421).

There is a native range sequence that appears to be ancestral to all sequences used in these analyses. These sequences are from an area in China, but a similar sequence was collected from a pet store in Alabama, likely imported from China. The full Bayesian and ML trees can be seen in Figure 26 and Figure 27 and the order of individual as they appear on the trees and their accession numbers can be found in Appendix D.

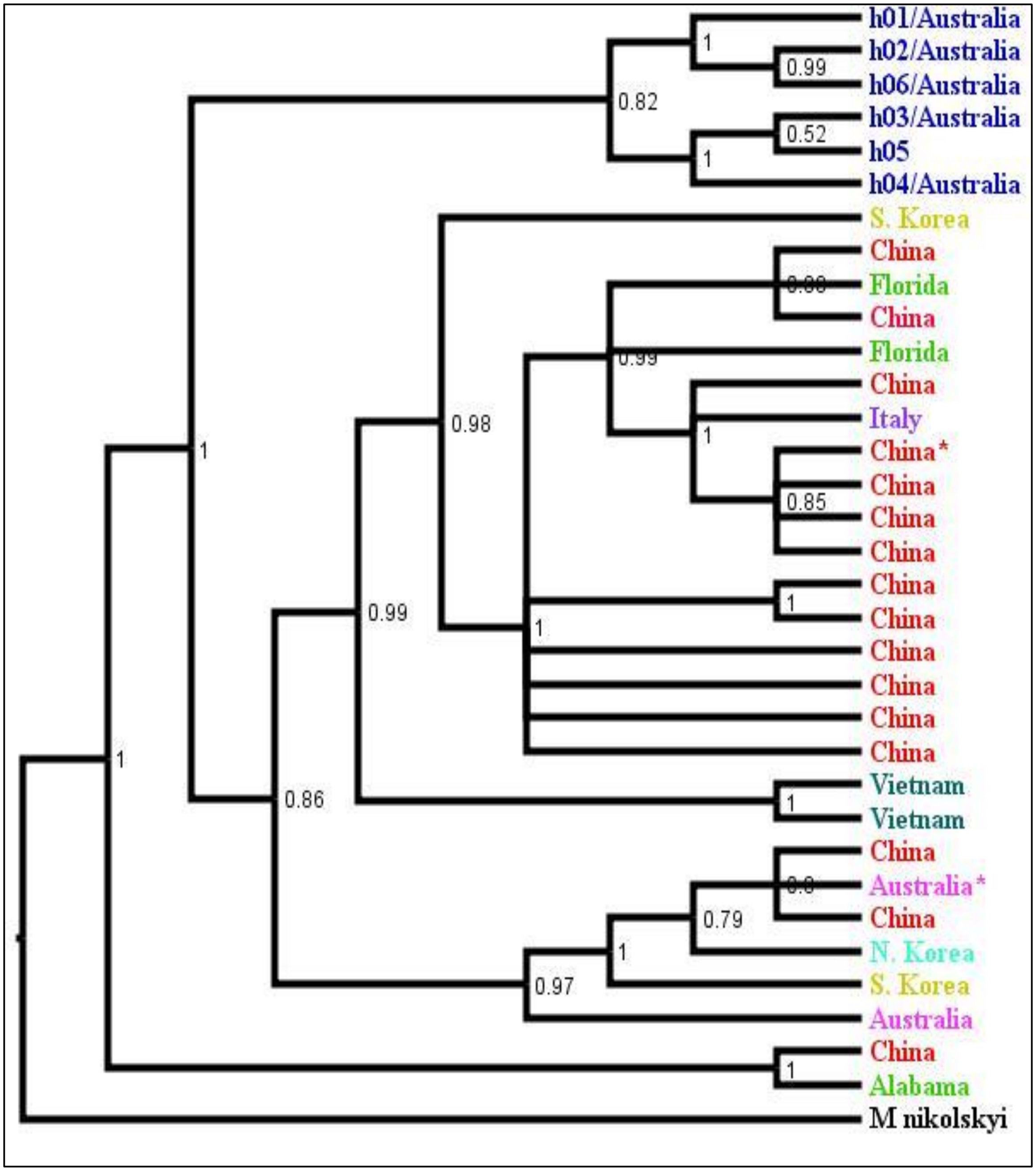

Figure 26. Phylogenetic tree created for COI locus using Bayesian analysis. Countries of origin indicated at branch tips, h01 – h06 collected from Illinois and Indiana. Numbers at branch nodes indicate support values. * indicates two or more identical haplotypes being represented at branch tip.

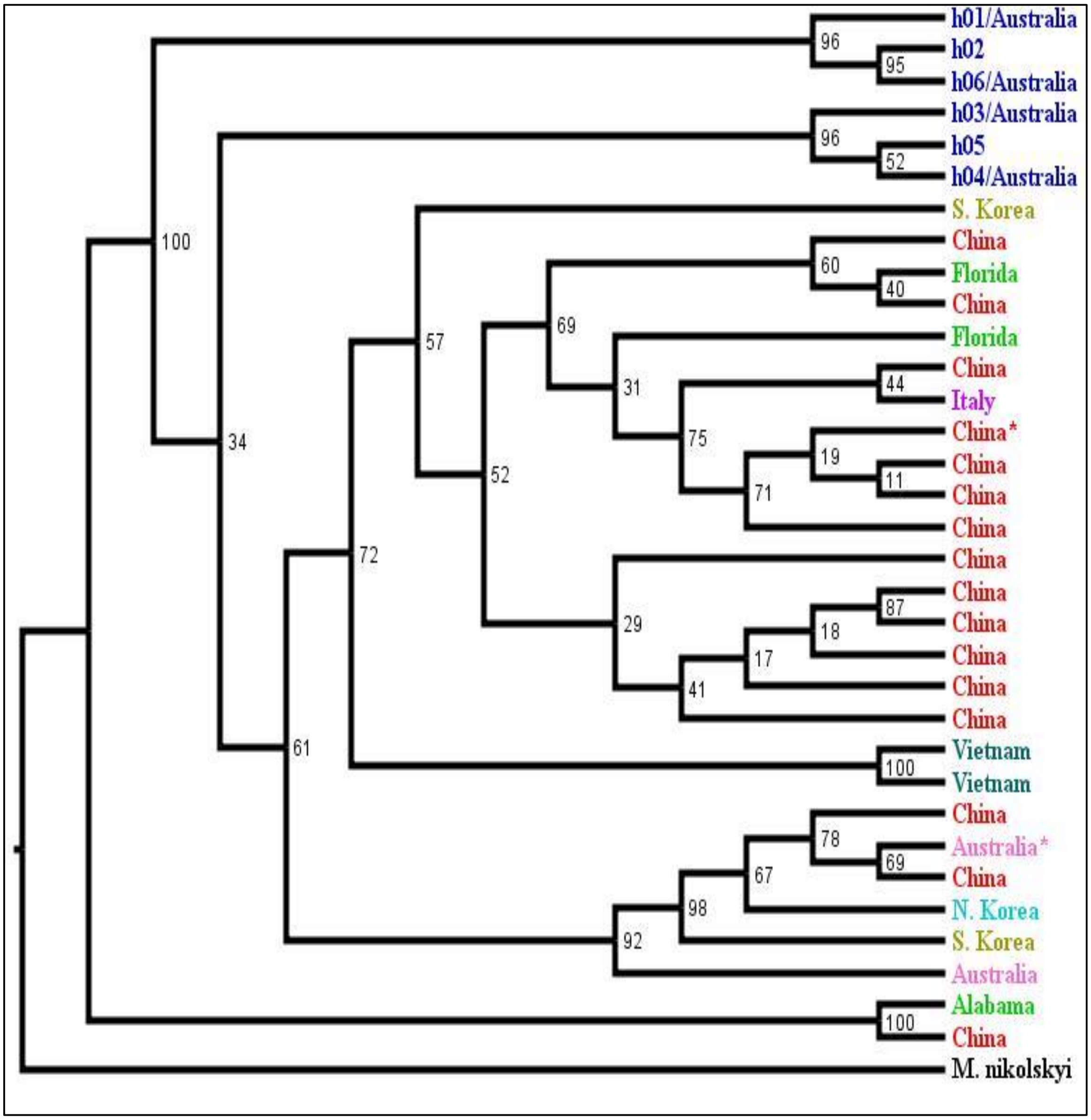

Figure 27. Phylogenetic tree created for COI locus using Maximum Likelihood analysis. Countries of origin indicated at branch tips, h01 – h06 collected from Illinois and Indiana. Numbers at branch nodes indicate support values. * indicates two or more identical haplotypes being represented at branch tip.

Like the COI analysis, these Chicagoland D-Loop haplotypes were grouped into two different clades, separate from other sequences in the analysis. Each clade contained the same three haplotypes from the sampled region as was shown in the previous COI analysis (h01, h02, and h06; h03, h05, and h04). There was 92% support for this arrangement using ML analysis. For Bayesian analysis the two clades had different support values for their relation to other sequences. There was 100% support for the placement of h01, h02, and h06 being ancestral to h03, h04, and h05 which had 69% support for their placement. Abbreviated Bayesian and ML generated trees are shown in Figure 28 and Figure 29 respectively with blue colored branch tips indicating the local, Chicagoland haplotypes the other branch tips are color coded by their country of origin. These other areas predominantly include the island of Japan, as it has approximately 236 unique haplotypes for D-Loop. Twenty sequences were sampled from areas in China and one each from North and South Korea. The remaining 49 sequences had no locational information available in GenBank. All except one of the sequences were from areas that were not sampled in the COI analysis. The only area that had a sequence come from approximately the same area was from Pyongyang North Korea (COI: KF732665, D-Loop: KF732667). The sequences from Japan are spread throughout the entire tree in both analyses with the sequences from China and Korea as well as the unknown locations being intermixed among the Japanese samples.

The native range sequences are grouped apart from the IL/IN sequences and characterized by several polytomies of individuals in the Bayesian analysis or several poorly supported branching arrangements using ML. No GenBank D-Loop sequences shared an identical sequence with the IL/IN population. Due to this, no relationship between IL/IN weatherfish and those from the native range could be concluded definitively using the D-Loop loci. The full trees for Bayesian and ML analyses of the D-Loop loci can be found in Appendix D along with a table showing the sequence accession numbers displayed in the order they appear in those trees.

The ML generated tree contains many more bifurcated branches than the Bayesian tree. Though some of the ML relationships are well supported, many of the individual taxa relationships are poorly supported throughout the tree (47, 14, 6, etc.) (Figure 29, and Appendix D) compared with their Bayesian counterparts. This is the result of how the trees are calculated by each program and not a result of errors in the dataset as described above. Overall, the taxa are grouped with the same individuals within the clades and the Chicagoland sequences are shown to be separate clades from all other sequences for both analyses.

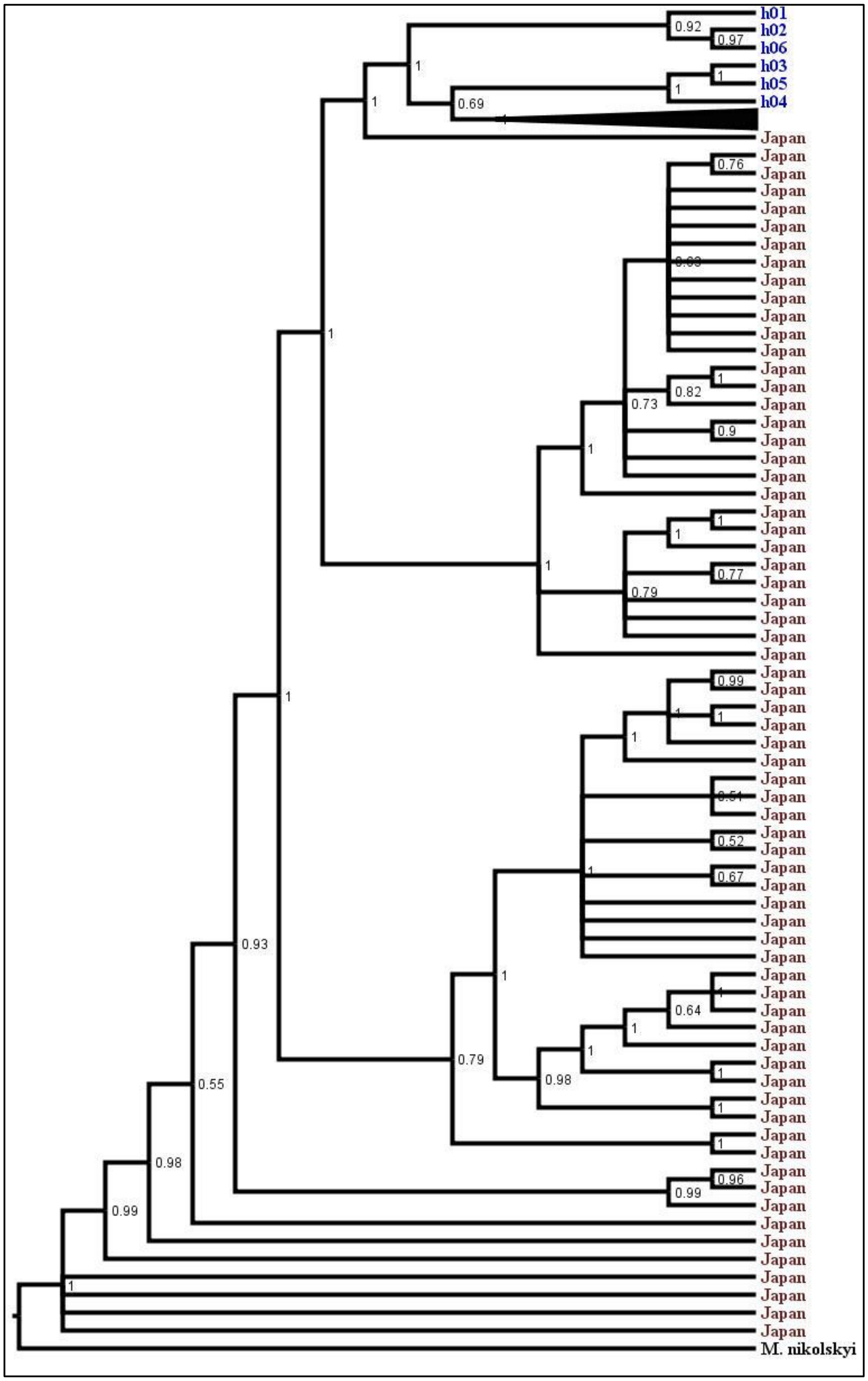

Figure 28. Phylogenetic tree created for D-Loop locus using Bayesian analysis. Countries of origin indicated at branch tips, h01 – h06 collected from Illinois and Indiana. Numbers at branch nodes indicate support values. * indicates collapsed clade of greater than 100 sequences.

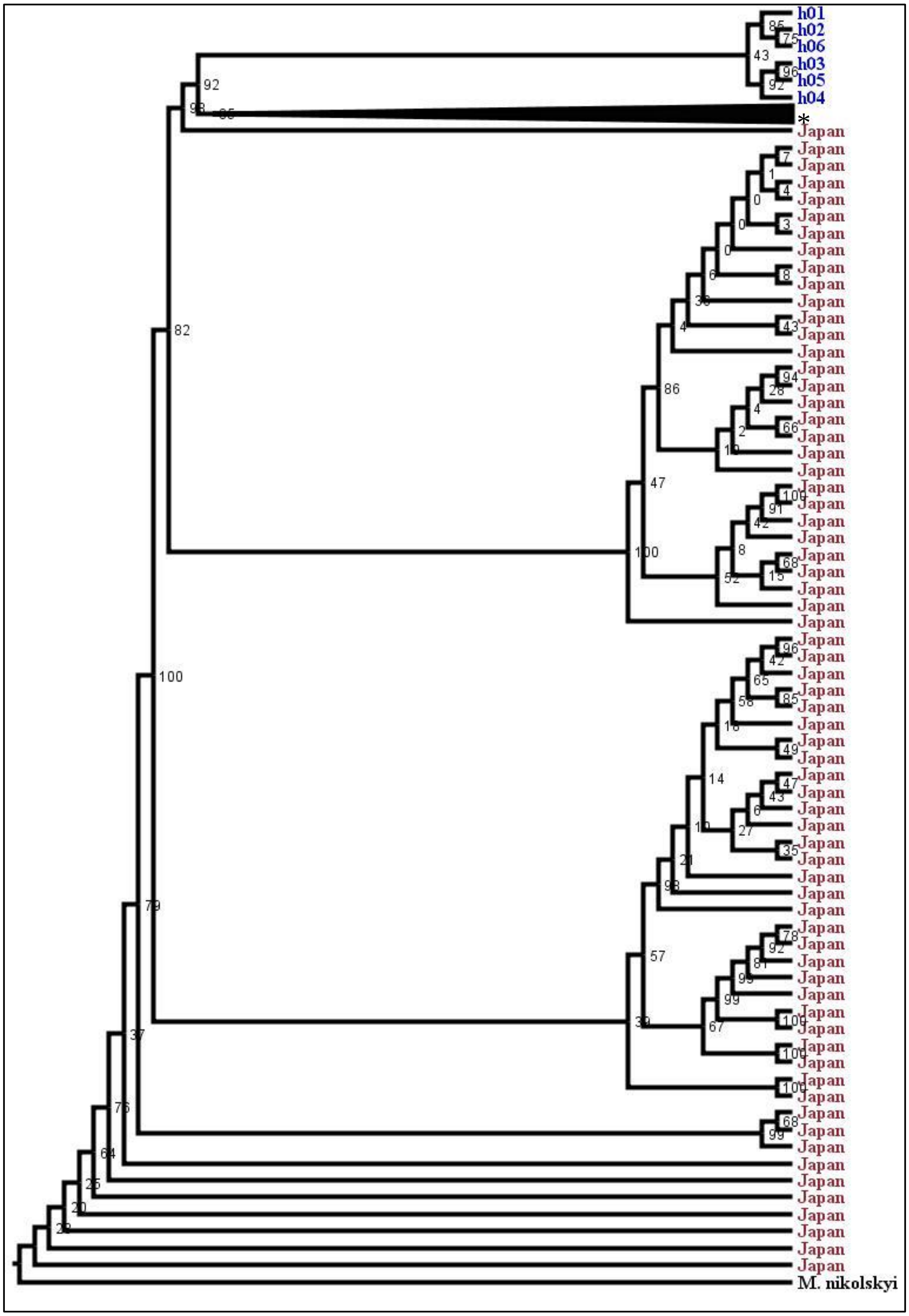

Figure 29. Phylogenetic tree created for D-Loop locus using Maximum Likelihood analysis. Countries of origin indicated at branch tips, h01 – h06 collected from Illinois and Indiana. Numbers at branch nodes indicate support values. * indicates collapsed clade of greater than 100 sequences.

Discussion

There is a growing number of species being transported and released into areas where they are not native. This poses a risk to the native species as non-natives can be in direct competition with or be predated upon by non-native organisms (Richter et al 1997). Various efforts have been put forward in an attempt to stop further spread of non-natives from areas where they are already established into areas where they are not yet established. In areas where they are already established it will be important to monitor their movements using tools such as telemetry or genetic sampling.

Chicagoland Weatherfish

Weatherfish were first introduced to North America during the 1930s in the state of Michigan (Brock 1960). They have since been found in several other states including the Midwestern States of IL and IN. Their introduction to the Midwest and elsewhere is viewed as problematic for many waterways and native species. Specifically, in IL, MI, and WI they are believed to pose such a great threat that there are ordinances prohibiting the possession or transport of live weatherfish. In other states, including those with established populations, *Misgurnus* sp. are not directly outlawed and can be found readily available for sale, both alive and dead, marketed as pets or as food in some instances. In these cases, it would be possible for individuals to buy these organisms and release them into area waterways where they could become established.

Many non-natives come from unknown sources and it is difficult to identify their point of original introduction, dispersal over time, or source population. Genetic tools have become increasingly useful when trying to determine the source(s) or origins of an invasive species. In the case of weatherfish found in IL and IN, it was not previously known where these fish came from and how populations in the rivers within and around Chicago were related.

In this study, six COI and D-Loop haplotypes (h01 – h06) were present in the weatherfish population in Chicago Area Waterways (CAWS) and Illinois Waterways (IWW). These haplotypes were found consistently at many of the sampling locations, which were many kilometers apart in some instances. Specifically, two haplotypes (h01 & h03) were found to be present at almost all of the sampled locations. This suggests that individuals carrying those haplotypes have spread through the entire system. This scenario is supported by the dates weatherfish were found in many of the locations throughout the system. As shown in chapter 2 of this thesis, the progression of weatherfish was documented to have occurred moving in a north to south and west to east progression over a distance and time that roughly matched their estimated natural dispersal rate of between 0.5 and 4.5 miles (0.8 and 7.25km) per year (Lintermans et al 1990; Shultz 1960). These haplotypes were at the northern most sampling location, a possible indication that they have spread from that area early in their establishment period to be present in most if not all of the system. Maps of the haplotype distributions for each gene individually as well as combined haplotypes can be seen in Figure 23, Figure 24, and Figure 25.

There are four locations where only one haplotype was found. This could be due to a variety of factors. The most likely is that these areas have low densities of weatherfish (seen by low sampling numbers) and rarer haplotypes are less likely to be collected in those areas versus more common ones such as h01 and h03. These two haplotypes make up approximately two-thirds of the sampled sequences and are likely the two most abundant haplotypes in the entire system. Haplotype h03 was the only haplotype found at three of four outer range locations and haplotype h01 was at the fourth. These sites where characterized by having only one or two fish collected. If more fish were collected in the future, additional haplotypes around these locations might be collected, but the majority of those fish would likely carry h01 and h03.

One other possible scenario is that genetic drift is occurring in this population, reducing the number of haplotypes present in areas at the outer edges of the range. This could either be occurring through founder effects where only a few haplotypes expand into an area or because of small population sizes. Small populations would be more likely to see large changes in haplotype frequencies over time than a larger population. The mtDNA is maternally inherited and the father's mtDNA is not represented in the next generation, potentially reducing the mitochondrial genetic variability of a population in the next generation. As a result, the founder effect would have greater effects on mtDNA than it would on nuclear DNA which is inherited through maternal and paternal lines. Previous studies have shown that reduced mitochondrial variability can be attributed to genetic drift (Ashley & Wills 1987; Khedkar et al. 2014) and rarer haplotypes would be more likely to be lost over time. In the case of h04 and potentially h05, rare haplotypes found to be in 4 fish in two of the 12 groups, it is possible that they were rare in the original invading population and spread with the other haplotypes, but over time have drifted out of most of the area and replaced by the more frequent haplotypes. This could result in them being found in fewer or isolated areas and give the appearance of separate invasions having taken place. If this drift continues, it could eventually lead to a decrease in the genetic diversity of the IL/IN population of weatherfish and future studies may find fewer haplotypes present.

A single fish carried a combined mitochondrial haplotype, a combination of haplotype h01 and h04 of the COI and D-Loop regions. Other fish sampled had combinations of h01 and h01, h02 and h02, etc. for COI and D-Loop respectively, not a mixing of different sequences. This could be an instance of a separate introduction of a unique haplotype, at this site. However, this scenario seems unlikely especially because weatherfish were abundant at this site, indicating they have likely been established here for many years. Another possible explanation could be random mutation in one of the loci, changing it from h01 to h04 or visa-versa. The h01 and h04 haplotypes vary by several basepairs (Figure 20 and Figure 21), requiring several point mutations to occur to go from one to the other, this seems unlikely when considering established mutation rates (Horne & Herwerden 2013; Brown et al. 1993; McMillan & Palumbi 1997). A final explanation would be mitochondrial recombination, which has been reported under some conditions in various taxa and recombination in the control region (D-Loop) is reported to be more likely than in the rest of the mtDNA genome (Lunt & Hyman 1997; Ladoukakis & Zouros; Hoarau et al. 2002; Rokas et al 2003). Evidence of recombination has been shown in at least five animal species: humans (Kraytsberg et al. 2004), nematode (*Meloidogyne javanica*) (Lunt & Hyman 1997), mussel (*Mytilus galloprovincialis*) (Ladoukakis & Zouros 2001), Flatfish (*Platichthys flesus*) (Hoarau et al. 2002), and Crucian Carp (*Carassius auratus*) (Guo et al. 2005).

There also remains the possibility of an error occurring during the extraction, amplification, or analysis stage. This could range from contamination of samples to incorrect labeling and cannot be ruled out.

Lastly, haplotype h06 is only present in the two northernmost sampling locations. This could indicate that individuals carrying this haplotype have not dispersed as far as those carrying other haplotypes such as h01 and h03. It could also indicate that this is a relatively new addition to the region and it has not had time to disperse through the system. Alternatively, it could be that this is a similar scenario to other infrequent haplotypes. Haplotype h06 is one of the rarest haplotypes represented in the system, as it was found in only two individuals. Therefore, it would be more susceptible to the effects of genetic drift or be so rare throughout the system that it appears to be nonexistent except in a few places. Future testing with a larger sample size would be required to detect it in other locations or monitor its progress through the system.

It is likely that the first reported occurrence of weatherfish in 1987 in the North Shore Channel (NSC) is close to the original introduction point of the species in this area. The near ubiquity of at least some of the haplotypes across the whole area suggests these haplotypes have been present since early on in the weatherfish's colonization of Chicagoland waterways. They would have spread from this point to the rest of the system through natural expansion such as larval/egg drift. The weatherfish's apparent linear progression through the system since 1987 shown in the historical range map of chapter 2 also supports a linear progression through the CAWS rather than several separate introductions. There have likely been few to no additional secondary introductions of weatherfish into this system. The evidence presented indicates that the Chicagoland population is largely the product of a single introduction in the NSC in the early to mid-1980s. This introduction would have included all of the haplotypes present today. They would have then spread through natural processes to their current range and in the process have undergone events such as the founder effect, giving them their current genetic distribution. Additional tests using more fish and/or nuclear DNA markers would be needed to test for gene flow patterns and habitat connectivity. These would provide further insight into the connectivity of the various pockets of weatherfish found in the Chicagoland waterways.

Global Weatherfish Comparisons

Weatherfish populations have been present in Michigan since the 1930s, and in the western hemisphere since the turn of the 20th century (Mills et al. 1993; Brock 1960). Since then, they have been introduced to 15 other states (Nico et al. 2016). Weatherfish have also become established in at least 10 countries outside of their native East Asia range (Nico et al. 2016). As with most invasive species, it is hypothesized that many of these populations are the product of aquarium or food trade releases. However, it is unknown where many of the populations have originated or where the ancestral stocks can be located.

This study used the COI and D-Loop sequences amplified from the Chicagoland population and compared them with those available on GenBank. Those sequences on GenBank encompass several countries from the home range of the weatherfish as well as places where they have been introduced. The native countries represented include China, Japan, North and South Korea, and Vietnam. Non-native samples came from Italy, Florida, an Alabama pet store, and five river basins in Australia. A detailed list of the accession numbers and the associated locations can be found in Appendix D. These sequences were aligned and analyzed using Bayesian and maximum likelihood (ML) phylogenetic tools in an attempt to determine the origins of Chicagoland weatherfish.

The weatherfish haplotypes found in the CAWS were more closely related to each other than to those from their ancestral home range using the D-Loop region. For COI, the CAWS sequences were again more similar to each other than to any samples from the home range. However, as was shown in Figure 26 and Figure 27**,** samples found in four of the five populations of weatherfish from Australia share the same haplotypes as to those found in the CAWS and IWW. This suggests that both these introduced stocks came from the same, but as of yet, unidentified region. The founding populations in Australia and Chicago are thus a representative subset of an unknown native population.

The reduced haplotype diversity of the Chicagoland population relative to that reported in its native range is expected for an invasive species. There are 24 COI haplotypes reported from the native range of weatherfish and over 200 D-loop haplotypes reported, mostly from Japan (Kano et al. 2011), but also from other areas. However, neither loci were sampled across the entire native range and many of the native locations with available sequences only had one of the two loci sequenced. Somewhere there is an unsampled population that contains some or all of the haplotypes seen in IL/IN.

Even though the Chicagoland haplotypes are more closely related to each other than the available native range, there is upwards of 5% divergence between the six haplotypes. This could be an indication that the source of the introduction likely came from more than one region or watershed in the native range that were brought together and released around the same time. Accounting for the near uniformity of haplotypes through the system and the high level of sequence divergence. In an instance of introductions from different sources at different times, we would expect to see multiple different relationships between different native range populations and the invasive population. While there is little evidence of multiple introductions and unconfirmed evidence of multiple sources in the IL/IN weatherfish there is evidence of it in Australia. In the Australian population there are at least two other haplotypes present that are distantly related to those of the IL/IN haplotypes. One haplotype is closely related to a population of weatherfish sampled from the Middle Yangtze River Basin in China, whereas the other appears to be from a population in South Korea. This suggests that Australia has had at least three sources of weatherfish that have contributed to its invasive population, one from South Korea, another from China, and a third from an unknown source.

Given the current dataset, the origins of the Chicagoland population of weatherfish remain unknown. It can be said that this invasive population is likely the result of a single introduction from a yet unsampled population in their native range. As more new and different locations are sampled, it will be possible to determine a likely ancestral location for this population of fish. It is plausible that weatherfish found in parts of Australia and Chicago are from similar locations or populations that are highly genetically related. Unfortunately, only two sequences were available from other United States locations so we cannot speculate about human mediated movement from another invasive U.S. population. Additional samples are needed to determine the relationships between the disjunct American populations as well as their relationships to ancestral locations.

CHAPTER IV

CONCLUSIONS AND IMPLICATIONS

The movement of species across and between habitats is not a new phenomenon, as it naturally occurs as species migrate and expand their range. However, a growing concern is the number of species that have been brought to new areas by humans. These species would likely never have reached the new areas under unassisted expansion conditions. This movement of non-native species has become exacerbated as international travel and shipping has become faster and more commonplace. This allows organisms to bypass obstacles that once acted as barriers to their natural spread. By some estimates about 50,000 non-native species have been introduced to the United States alone (Pimentel et al. 2005), and it is not expected to end any time soon. These species cause billions of dollars in damage to crops, human infrastructure, and the environment each year and can be responsible for major declines in the biodiversity found in a system (USDA 2001; Pimentel et al. 2005).

The release of non-native species can be accidental or purposeful. Accidental releases happen through means such as dumping of ballast water (i.e. Round Goby in the Great Lakes), cargo stowaways (i.e. black rat), or containment breaches (i.e. Asian carp in the Mississippi River) (Jude et al. 1992; Engels 1999; Kolar et al. 2005). Purposeful releases are not always sinister in nature and are usually attributed to events such as the release of organisms that were once pets (i.e. Goldfish), were intended to create economicgains, or to control other invasive species (i.e. Salmon in the Great Lakes to establish a fishery and control the invasive alewife)

(Nico et al. 2016a; Fenichel et al. 2010). Although some interactions are understood, consequences are often unknown or unintended when species are introduced to a foreign region. Some species are studied extensively as they are thought to pose great risks to the area, but many are understudied and their impacts are poorly understood or unknown entirely.

The Oriental Weatherfish is native to eastern Asia with a range from Siberia to Northern Vietnam, including the island of Japan. Over the past several decades they have been introduced to many other countries where they are not native. These include the United States, Australia, and parts of Europe (Berg 1965; Allen 1984; Razzetti et al. 2001). The means of introduction to these places is unknown, but it is speculated that such populations were the result of aquaria releases or were intended to be a stock population to be used in traditional Asian food dishes (Maciolek 1984; Devick 1991). These introduced populations are thought to be contracting, expanding, or stable depending on the area, but few studies have been conducted to determine their condition, and if expanding, the range extension of the population (Laird & Page 1996). Each population's relation to each other is also largely unknown. For instance, in the United States several disjunct populations of weatherfish occur in at least 15 states (Nico et al. 2016b; Laird & Page 1996) and their origins are largely speculative. No tests have been conducted to determine if individuals across populations share common ancestry or if a given population is the result of multiple introductions. The goals of this thesis were to: 1) establish an up-to-date range map of the weatherfish in and around the Chicagoland region, 2) determine a reliable survey method for weatherfish, and 3) to determine the number of introductions of weatherfish in IL and IN and their possible origin(s).

Updated Range

Initial weatherfish proliferation was likely the result of the species filling an open niche in the Chicago Area Waterways (CAWS). Currently, the CAWS is home to over 70 different native and invasive species of fish. The total number is up from around 10 in 1974 (MWRD 2016). When the weatherfish was introduced in the late 1980s there were approximately 41 species in all of the Chicago and Calumet river systems (greatriverschicago.com). Few species were present that weatherfish had to compete with directly as only one benthic dwelling species was consistently collected around the same time, the Black Bullhead (*Ameiurus melas*) (MWRD 1991). It's possible that due to the lack of multiple benthic species the weatherfish colonized an available niche relatively easily which lead to its proliferation and the current extent of its established range. If a niche was not open, we would expect there to be a decline in a species that has similar habitat requirements to the weatherfish as their population increases. Currently, benthic species such as Yellow Bullhead (*Ameiurus natalis*), Round Goby (*Neogobius melanostomus*) and Channel Catfish (*Ictalurus punctatus*) are consistently caught in the system and could be competing with the weatherfish (Yellin 2014; Zeigler et al. 2014). Such was the case when Round Goby was introduced to the Great Lakes. The goby directly competed with Mottled Sculpin (*Cottus bairdii*) for resources and spawning nests and subsequently eliminated it from many parts of the Great Lakes (Janssen & Jude 2001; Lauer et al. 2004,). There however have been no studies that indicate that these or other species are impacting weatherfish population or vice versa.

Weatherfish have expanded their range in some areas of the CAWS and Illinois Waterways (IWW), but remain stationary in others. This could be an indication that some areas are better suited for weatherfish. For instance, weatherfish have not been found in Lake

Michigan since their introduction into the North Shore Channel (NSC) despite their close proximity. It is likely that they will continue this trend because of highly variable nature of the lake (e.g. strong currents) and its less than favorable conditions (e.g. sandy bottom with minimal cover) for weatherfish. These and other factors would work against weatherfish movement into Lake Michigan, forming an effective barrier of entry against these fish. However, there is still some expansion being exhibited by these fish in the river system.

Weatherfish have expanded into the Little Calumet River and into at least one of its tributaries, increasing the known range several miles to the east. The Southernmost range of the weatherfish population has expanded slightly, but appears to be slowing. Few weatherfish were found in the southern most sites during this study and after this study there was only one weatherfish found to be present in the same location in the Illinois River in 2015 (Widloe et al. 2015). It is unknown why they have not been found in as great abundances as they are in more northern locations. One factor could be a lower abundance of food sources present in this section of river. Weatherfish have a diet that, at certain times of the year, is dominated by fingernail clams (Norris 2015), but few fingernail clams are present in areas to the south of the weatherfish's range (USGS 1999). This lack of a primary food source could be influencing the expansion of weatherfish in that it is preventing them from establishing a permanent population in those areas where fingernail clams are scarce.

This is not to say that the lack of clams could be the only cause of a slowing weatherfish population front to the south. One alternative could be that the two Asian Carp species (*Hypophthalmichthys nobilis* and *H. molitrix*) are interfering with the weatherfish expansion (competition, trophic cascades) as the carp are expanding their own range northward from the Mississippi River toward Lake Michigan. The population fronts of both the weatherfish and the

Asian carp currently overlap and 2015 is the first year that Asian carp larvae have been found in the same area as the weatherfish (Butler et al. 2015). There could be some unknown direct or indirect interaction occurring between these three species, or times when their life history stages compete for resources, thus slowing the progression of weatherfish through this portion of the system. Additional studies would be needed to examine the species' interactions, which could entail direct competition, trophic cascade effects, or some unknown factor yet to be determined.

Weatherfish are also coming into contact, and potentially competing, with native species that are not present in Northern areas of the CAWS. As seen in this study, weatherfish have recently been found in the Illinois River and in some upstream sections of the Des Plaines River. These are systems where dozens of species have been continuously found for many decades. The Des Plaines River has had at least 30 fish species found in Cook County alone in the years leading up to the first weatherfish detection (www.inhs.illinois.edu). This abundance of species would mean that it is less likely for there to be an open niche that weatherfish could fill. Over the two study years, 11 and 23 of the 269 weatherfish captured were from the IL and Des Plaines rivers respectively. The low capture number of weatherfish in the areas might be indicative of more intense competition or predation by these species than those in the CAWS. For example, in both systems various *Moxostoma* spp. (redhorse) are found. These species are not only benthic, but also have diets that consist of larval insects, small mollusks, detritus, and algae, all components of the weatherfish diet. This overlap could limit weatherfish access to food sources and thus prevent establishment of a weatherfish population. However, no studies examining the competition exhibited or experienced by weatherfish in these local waterways have been done to confirm these hypotheses.

Direct effects of man-made barriers on the movements of weatherfish are present when traveling in the upstream direction. Larger structures provide the greatest barrier to upstream weatherfish movements. However, those same large structures do not hinder their downstream movement. There is some evidence suggested by this study to indicate that small water diversion structures do not offer a barrier to upstream movements cases, particularly if increased flow events can submerge said structure, allowing fish to swim over the top. Studies would need to be conducted to understand the upper limits of weatherfish swimming speed and potential for upstream movement in relation to various flow speeds. To prevent further spread of these fish into the side channels and feeder streams of the CAWS and IWW, it would be necessary to build structures at least of similar size to the one that separates the North Shore Channel (NSC) and the North Branch of the Chicago River, around six feet (two meters). This structure is taller than the known upper limit of the weatherfish's vertical migration distance around 3 feet (1 meter) (Fujimoto et al. 2008) and to date no weatherfish have been found on its upstream side while being continually present on its downstream side since the early 1990s.

It is truly unknown what is causing and has caused the successes and hindrances of the weatherfish throughout the CAWS. It could be that predatory pressures, food supply (as indicated above), or habitat types are contributing to the success or hindrance of weatherfish depending on the area. To better understand the weatherfish's impact on flora and fauna and vice versa it would be necessary to conduct studies of competition, predation, habitat preference, etc. for this species and compare it to other species in the system.

Gear Effectiveness

The active and passive gear types (electrofishing and minnow traps/fyke nets respectively) used in this study have different rates of catch depending on the site in which they were used. Overall, active sampling caught more fish per hour of effort, but the total catch numbers of both gear types were almost identical as found by this study (i.e., after many more hours of sampling, the passive gear types caught the same number of weatherfish as active sampling). Both gear types were used at many of the same sites in this study as a way to determine if habitat type played a role in catch rates.

In total, 21 locations were sampled at various sites, of which weatherfish were present in ten. Those sites displayed many different habitat types that could have influenced the rate of fish capture for a specific gear. This would explain both the varying capture rates and the interaction shown in the ANOVA between gear type and location seen in Chapter 2**.** Overall, the active gear type collected weatherfish at a faster rate than passive gear, but both gears' rates were highly dependent on a site's features. In some instances, when used together, one gear type would outperform the other at a site, but at a different site within the same locations, the opposite gear would obtain more fish. For example, much of the CAWS and IWW is used for shipping so the main channel depth is 9 feet deep (2.7 meters) or greater. In these areas, it is beneficial to use more passive techniques that can be placed on the bottom, where the weatherfish are found. The active gear's electrical current may penetrate to the bottom of the water column in these areas. However, as noted by Keller & Lake (2007) and through field observations, weatherfish rarely reach the water surface when shocked, resulting in inaccurate assessments in deep areas using this method. In shallower areas containing weedy vegetation, active gear can cover more area and drive weatherfish from thick vegetation instead of relying on them leaving cover to enter traps. This variation necessitated that many different locations be sampled in each site using multiple gears. This unevenness in catch size and number is not an indication of poor sampling of the system, but rather of habitat conditions being more conducive to one gear type over another or to weatherfish establishment. This study optimized sampling techniques at many locations and if weatherfish were caught in low numbers or not at all in an area, it was a reflection of their densities or lack of establishment in the area, not of poor sampling coverage.

For this study we used habitat information such as this to evaluate a specific gear type's potential for catching weatherfish and focused efforts to maximize the weatherfish catch at an area during study sampling. An example would be in instances of deep water, study efforts were focused more on passive gears, with active gears being used around the shores if possible. In shallower areas, more active gear effort was used with supplemental passive gears. The study's sample effort was also complemented with other efforts by state, federal, and local institutions that used active and passive sampling across many habitat types regardless of features in many of the same areas as the study (Stewart 2013; IDNR et al 2013; IDNR & USFWS 2013). This provided additional comparisons of gear catch rates across locations and habitat types and ensured an area of more complete coverage. Overall, this study examined the effect of over 800 hours of active sampling and 1,250 net-nights (24hr periods) of passive sampling on the catch rate of weatherfish (Stewart 2013; IDNR 2012; Price & Robertson 2005).

This knowledge is valuable because it was not only used to maximize the number of weatherfish caught in this study, but it can be used to both accurately and consistently survey areas by taking habitat features into account in the future. This would in turn maximize the number of weatherfish caught while minimizing survey effort hours if the species were to be selectively targeted. A similar approach has already been taken by state and federal agencies that survey for Asian Carp. These efforts target specific areas and size classes/life stages with gear that was previously determined to provide the best methods for detection and collection of those fish (Stewart 2013; IDNR et al 2013; IDNR & USFWS 2013). If targeted sampling were to be implemented, each site's features would need to be evaluated to choose an optimal sample technique and it is recommended that both gear types be an option for use with crews alternating gear type based on specific site or location conditions.

Local Genetic Survey

As shown in Chapter 2, weatherfish ranges have expanded in the CAWS and IWW waterways every year since 1987. The years of first occurrences indicated a natural expansion in this system. The distances and timeframe between various first occurrences also matched up with previously established expansion rates of 0.5 - 4.5 miles, (0.8 – 7.2 km) per year (Shultz 1960; Lintermans et al. 1990). If the haplotypes present in IL/IN were introduced around the time of the first occurrence and underwent a natural expansion processes, we would expect several haplotypes to be present across much of the established range. We also expect there not to be any isolated locations that have their own unique haplotype set. If isolated pockets of weatherfish did not share haplotypes, it could be reasoned that they were not expanding naturally and instead were the product of separate introductions. The resulting dates of first occurrence and their locations would be a coincidence that only gave the appearance of natural expansion.

This study supports the hypothesis that the IL/IN population of weatherfish is the result of one introduction. These fish were introduced in the early to mid-1980s around the site of the first detection. They subsequently spread through natural means to occupy their current distribution. All six current haplotypes were present in that original introduction. In their subsequent spread to other areas, the population was subjected to events, such as the founder effect, which reduced the genetic variation in some locations to give the current haplotype distribution.

This study examined the Displacement Loop (D-Loop) and Cytochrome C Oxidase Subunit I (COI), two mitochondrial loci, of weatherfish collected along the distribution range determined by this study. The sequences determined here represent the entire Chicagoland weatherfish population, a subset of a population found in their native, Asian range. Results from this study determined that many of the haplotypes are found in several locations, termed groups, throughout the Chicagoland range and at least two of the haplotypes (h01 and h03) are found throughout the entire area, including the site of first occurrence and are also in the most individuals. No haplotype is found in only one area and likewise there exists no area that has its own, unique set of haplotypes, separate from the rest of the range. This is a strong indication that this population as a whole is the product of a natural dispersal and this study supports the hypothesis of natural expansion.

Three of the haplotypes are found in two of the 12 sampling groups. This could be an indication that these haplotypes are not expanding at the same rate as the others. However, these haplotypes were identified in approximately one quarter of the total individuals that were tested and individually, the haplotypes occurred in five or fewer of the 60 fish tested. Given this low occurrence rate, they are likely rare in the system and thus are less likely to be encountered than the more predominant haplotypes while sampling. This is especially prevalent in sites where few fish were collected. To detect these rare haplotypes, it would be necessary to collect several times the number of fish used in this study to increase the encounter rate for these haplotypes. Given that haplotypes h01 and h03 are nearly ubiquitous in this population it is expected that other haplotypes would show a similar pattern if enough samples are tested.

If more fish were tested and the rare haplotypes are not seen in an area that we would expect them to be, it could be that these sequences have been bred out of locations by the more prevalent haplotypes. This could take place via genetic drift through events such as bottle neck or the founder effect. The mtDNA is maternally inherited and the father's mtDNA is not represented in the next generation, potentially reducing the mitochondrial genetic variability of a population in the next generation. As a result, the founder effect would have greater effects on mtDNA than it would on nuclear DNA which is inherited through maternal and paternal lines. Previous studies have shown that reduced mitochondrial variability can be attributed to genetic drift events such as the founder effect (Ashley and Wills 1987; Khedkar et al. 2014). Over a few breeding seasons, this results in rare haplotypes becoming rarer and potentially being removed from a location. Given enough time, this would create the appearance of multiple introductions if it leads to distinct pockets of rarer or somewhat unique haplotypes.

Global Genetic Comparisons

The COI and D-Loop haplotypes of the Chicagoland weatherfish were compared to the same genetic regions deposited in GenBank. These GenBank sequences were of specimens from multiple locations within the weatherfish native home range (e.g., China, Japan, North Korea, South Korea, and Vietnam), as well as non-native areas (e.g., Italy, Australia and Florida). A listing of all GenBank sequences used can be found in Appendix D.

Results show that haplotypes found in IL/IN form their own clade that is separate from all other native range haplotypes. As such this population is not closely related to those found in any portion of their Asian home range. They are however, closely related to those found in several Australian Rivers, another area where they are not native (Figure 26 and Figure 27**)**. Part of the Australian population has identical haplotypes to those in IL and IN. The Australian population contains at least six different haplotypes with four of them matching five IL/IN haplotypes exactly. This is an indication that both populations have similar ancestral locations or stocking sources. Though this is an indication that these two regions share a common history, there is no closely related native population that has been sampled despite several previous studies (Kano et al. 2011; Morishima et al. 2008; Jakovlic et al. 2013). As such, it cannot be said where these two populations have come from although this study utilized over 300 unique sequences between the two loci from several locations within 6 native and 4 non-native countries. It might be possible that since both of these populations are invasive and thought to be the result of pet trade releases, that they are from the same fish supplier or farm. There is however, no way to determine which one, as weatherfish were imported to Australia between the 1960s and 1986 at a rate of 50,000 fish per year (Keller & Lake 2007) and are still imported in the United States via multiple suppliers.

Weatherfish Potentials

The weatherfish population in the CAWS and IWW has been expanding since it was first recorded in 1987. It has made it through 150 miles of waterway and is repeatedly caught during waterway surveys, making weatherfish an established IL fauna. Given its previous history in the area, this species is likely to expand into many of the smaller feeder streams of this system. There is potential for this species to make it through the Illinois River toward the Mississippi River as it can currently be found in the main stem of the Illinois River. However, as indicated in this study, their expansion in this system has slowed dramatically due to an unknown factor. If this trend continues, the weatherfish will likely take many more years to advance through each pool of the Illinois River before it reaches the Mississippi River. If weatherfish were to be introduced into the Mississippi River, there is also no guarantee that it will establish in the main stem of the river, as this river is much larger, deeper, and subject to stronger currents than the weatherfish typically prefers (www.nps.gov; Koetsler & Urquhart, 2012). These are also thought to be one of the two driving factors behind why these fish have not invaded Lake Michigan. The lake is subject to vastly different flow regimes than the river and has different habitat types that have so far deterred weatherfish from colonization.

Rivers such as the DuPage and North Branch of the Chicago River that are separated by a large water diversion structure are likely to not see weatherfish establishment in those area through natural dispersal. As shown in chapter 2, weatherfish were not found to have moved upstream into areas where larger water diversion structures separate an area populated by weatherfish downstream from areas where weatherfish were not previously recorded upstream. This pertains to the weatherfish’s inability to make the large vertical migrations necessary to move over those structures (Fujimoto et al. 2008). This is thought to be the other factor responsible for the lack of a weatherfish presence in Lake Michigan. These fish cannot make the migration over the lock sills to establish a presence on the lake side. There still remains a possibility that these fish can be moved through other means, such as human transport, and released into new waterways. Currently, there are no known biological or physical means, other than water diversion structures, that can prevent the movement of this species. It is suggested that areas lacking a weatherfish presence, but are connected to one that has them, be subjected to sampling efforts specifically designed to target these fish to monitor their movements.

Weatherfish have the potential to be successful invaders of most waterways where they are introduced. This species possesses many unique traits such as low oxygen tolerance and a temperature range between 2° - 38°C (Logan et al. 1996) that allow it to survive in many types of environments that other, more sensitive species cannot. Their tolerance for low oxygen environments comes from their ability to take gulps of air into their gut and diffuse oxygen through their intestinal wall (McMahon & Burggren 1987). This species is also primarily nocturnal, which, in conjunction with their burrowing behavior, is believed to lower their predation risk (Logan et al. 1996).

Weatherfish can also significantly disturb habitats through their actions. Though the CAWS and IWW are not oxygen poor as a whole, there is potential for the stagnant backwaters weatherfish are found in to become oxygen poor though their disturbances. These fish will feed by moving sediment into their mouths to filter out food items (Watanabe & Hidaka 1983). In the process, they constantly agitate the bottom sediment. Additional habitat disruption can come from them burrowing into the soft substrates. This activity could increase the turbidity of an area, especially in stagnant backwaters where they are typically found. Weatherfish have even been described as having a similar impact on water quality as Common Carp (*Cyprinus carpio*) (Keller & Lake 2007). This has the potential to disrupt fish communities that survive best in less turbid environments or rely on vegetation at various life stages as the weatherfish's activity can uproot plants and alter vegetated areas.

As shown in previous studies (Norris 2015; Watanabe & Hidaka 1983), weatherfish do not have a specialized diet and instead are more opportunistic feeders. This means that in most areas, there will likely be a food source these fish can utilize and reduce competition that a more specialized invader would experience. Components of their diet have the potential to disrupt native fishes through direct consumption of prey items. Weatherfish have the potential to reduce macroinvertebrate population sizes in areas where they are present (Keller & Lake 2007). Their diet could include fish eggs (Norris 2015; Simon et al. 2006) and the occasional *Gambusia* fry as shown in laboratory studies (Logan et al. 1996). Though there is speculation that *Gambusia* are present in the CAWS, it is unknown if weatherfish consume fry of other, more common fish species. There was one case of fish egg ingestion by a weatherfish in the CAWS and IWW

(Norris 2015). Though there are no other data suggesting the frequency of ingestion these fish eggs could come from native species, potentially impacting the number of offspring in future generations. While speculated here, there is little evidence to suggest that there are substantial direct impacts of weatherfish on native fauna.

This study provides the foundation for future studies monitoring Chicagoland weatherfish by establishing an up-to-date range assessment and the baseline genetic composition for this population. If this population is to be monitored, results of this thesis show the optimal method for effective sampling of this species. The genetic work also provides an opportunity to identify additional invasion sites if new haplotypes are identified in the CAWS or IWW in the future. It also allows for a comparison with other populations to establish possible relationships or origins.

The IL/IN weatherfish population shares an origin with some members of the Australian population, but no direct relationship can be established to any native range populations. Several other mitochondrial genes are available for this species (Zhongjie et al. 1997; Perdices et al. 2012) as well as several microsatellite markers (Morishima et al. 2001; Arias-Rodrigues et al. 2007; Morishima et al. 2002) that can be utilized for additional genetic analysis. These markers have been sampled from other geographic areas that differ from those sampled with the COI and D-Loop loci. Future studies could utilize these markers to relate the Chicagoland weatherfish population to a region in the fish's native range. Using new markers or those from this study, studies can also be conducted to compare populations of weatherfish found across the United States, both those in the environment and sold in stores for food or pet trade. This study did include genetic material from a specimen from a pet store in Alabama and one from a wild population in Florida. No relationship was found to the Chicagoland fish, but a larger sample size from pet stores might yield a match to an invasive population, including those in IL and IN.

Additionally, if future studies were genetic similarities or structure of the Chicagoland weatherfish, nuclear loci could better model and elaborate the connectedness of weatherfish populations. This would help to understand gene flow within and (potentially) across populations. This study emphasizes the need for future research into possible expansion and ecological impacts of this non-native species and how native and non-native populations are related.

APPENDIX A: ABBREVIATIONS

- BP – Backpack Electroshocker
- BS – Boat mounted Electroshocker
- CAWS - Chicago Area Waterways
- CPUE – Catch per Unit Effort
- CSSC - Chicago Sanitary and Shipping Canal
- I & M Canal – Illinois and Michigan Canal
- ILDNR – Illinois Department of Natural Resources
- INDNR – Indiana Department of Natural Resources
- INHS - Illinois Natural History Survey
- IWW - Illinois Waterway
- MF – Mini-Fyke Net
- MT – Minnow Trap
- NB – North Branch of the Chicago River
- NSC - North Shore Channel
- USACE – United States Army Corps of Engineers
- USFWS – United States Fish and Wildlife Service
- USGS – United States Geological Survey

APPENDIX B: LOCATION MAPS

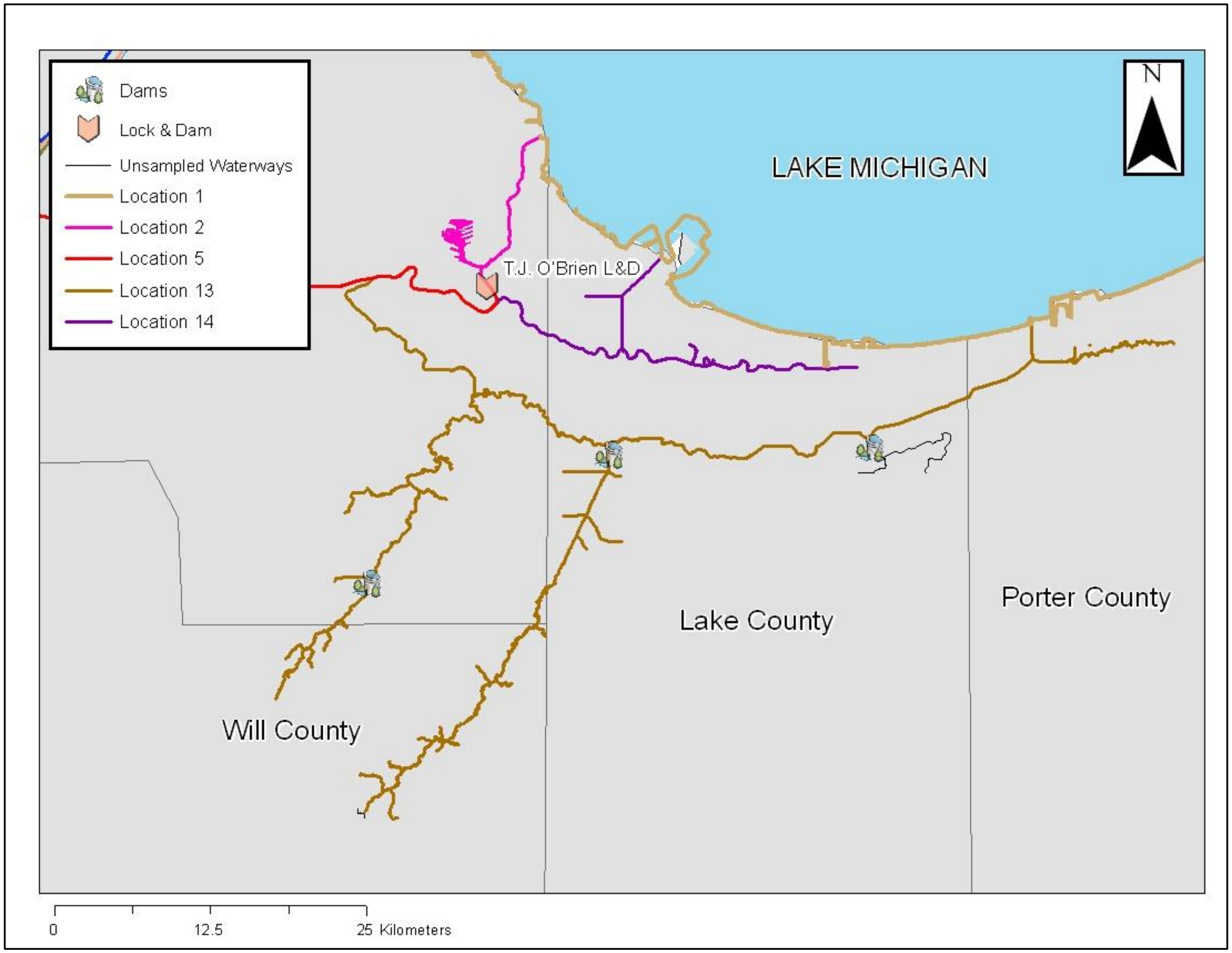

Figure 30. Map of Illinois and Indiana counties showing main stem of locations sampled during 2013 and 2014 sampling seasons. Locations include the Little and Grand Calumet Rivers, Lake Calumet, Lake Michigan, Cal Sag Channel, Plum and Thorn creeks.

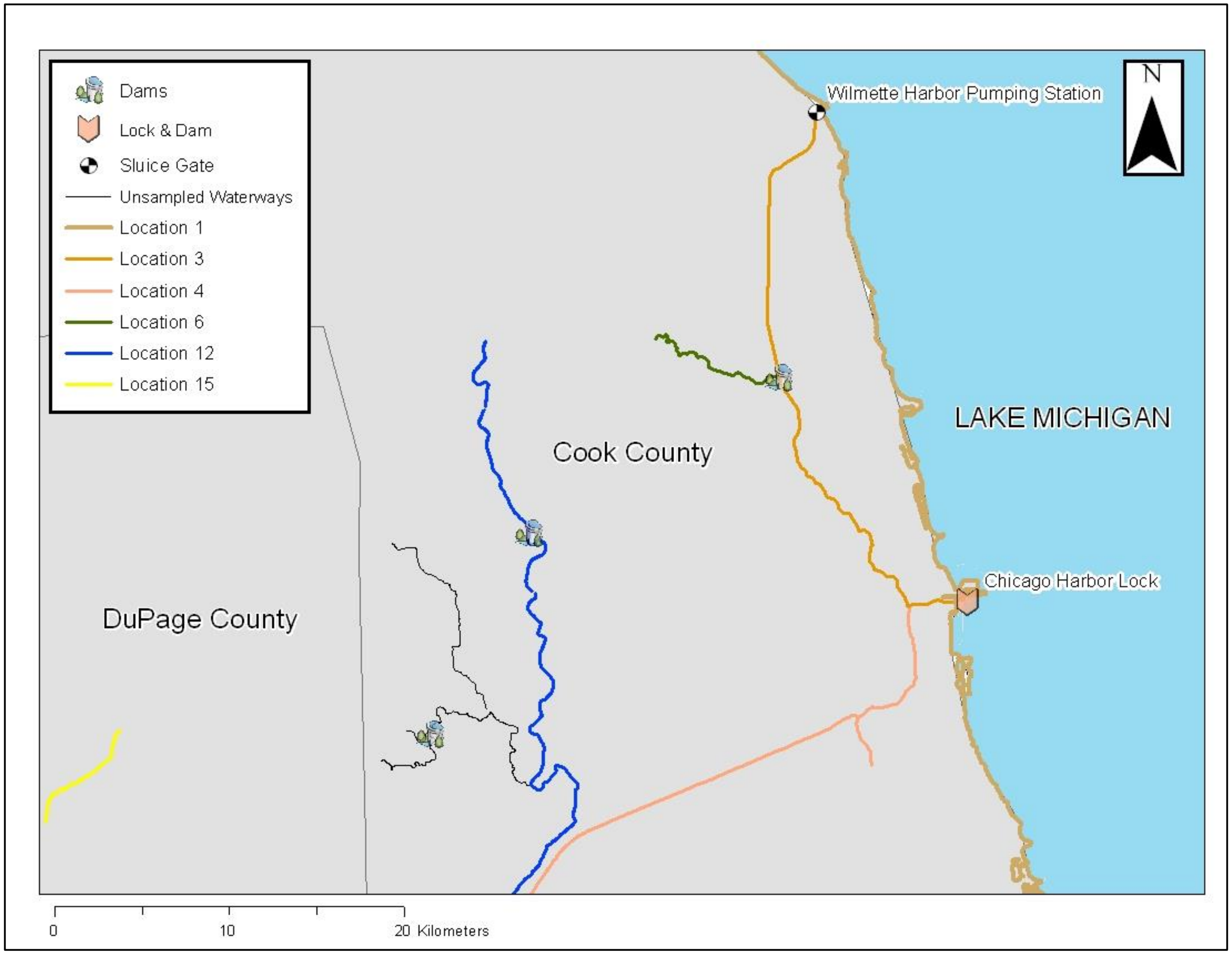

Figure 31. Map of Northern Chicagoland counties showing main stem of Des Plaines River, North Shore Channel, Chicago River, and Chicago Sanitary and Ship Canal sampled during 2013 and 2014 sampling seasons.

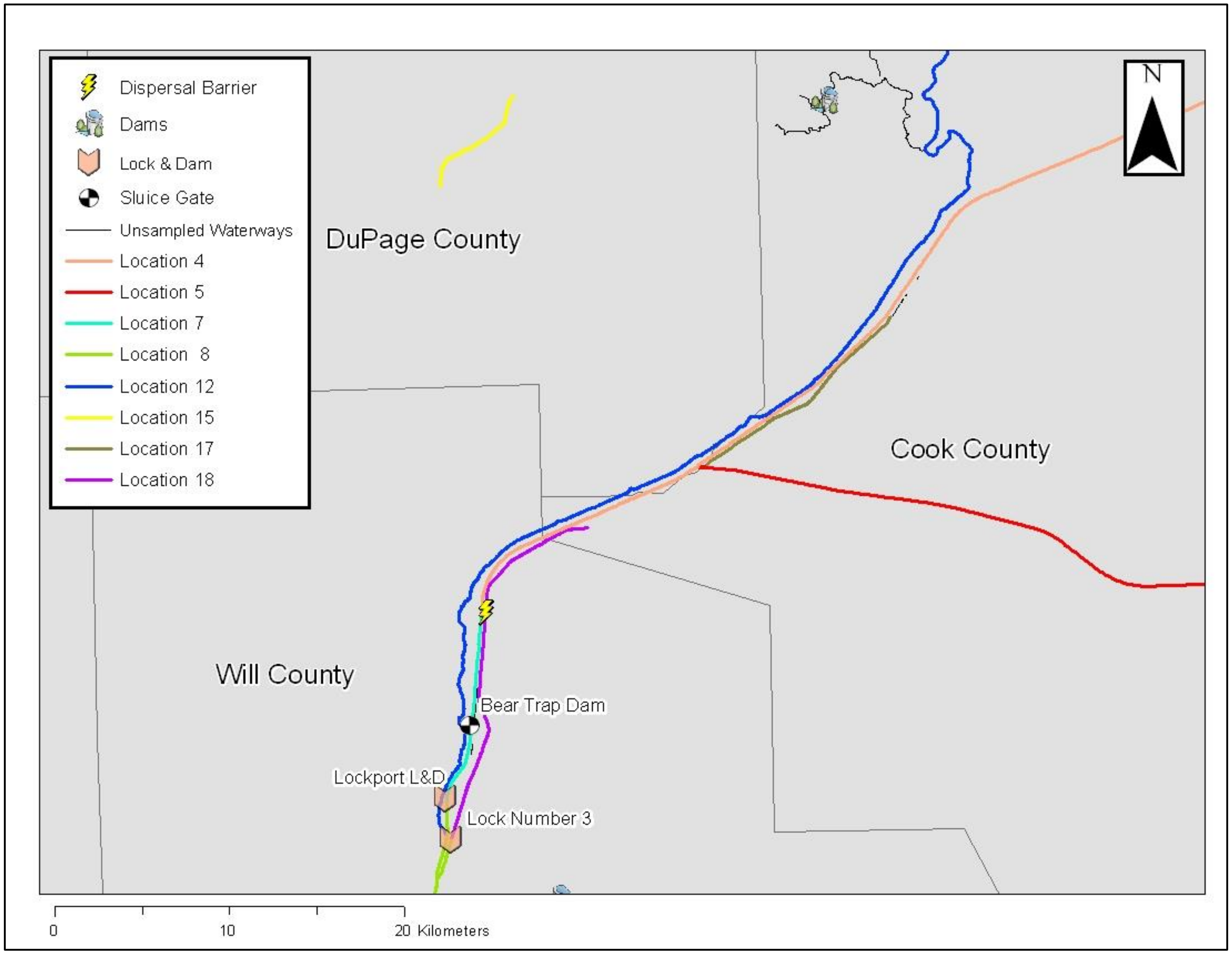

Figure 32. Map of Illinois displaying central portion of locations sampled during 2013 and 2014 sampling seasons. Locations include the Chicago Sanitary and Ship Canal, Cal Sag Channel, Des Plaines River, DuPage River, and the Illinois & Michigan Canal.

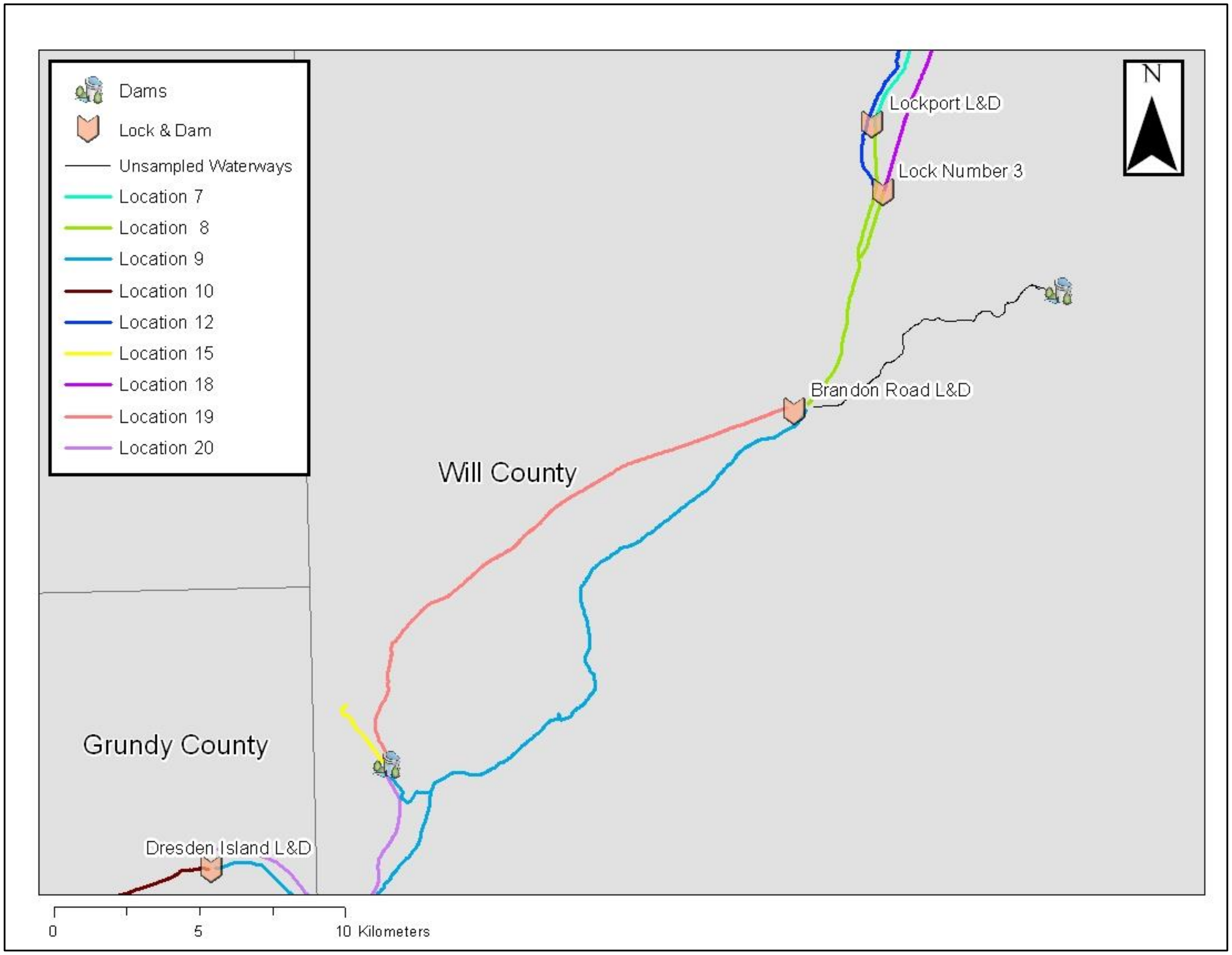

Figure 33. Continuation of central portion of locations sampled in Illinois during 2013 and 2014 sampling seasons. Locations shown include Des Plaines River, Illinois & Michigan Canal, DuPage River, and Chicago Sanitary and Ship Canal.

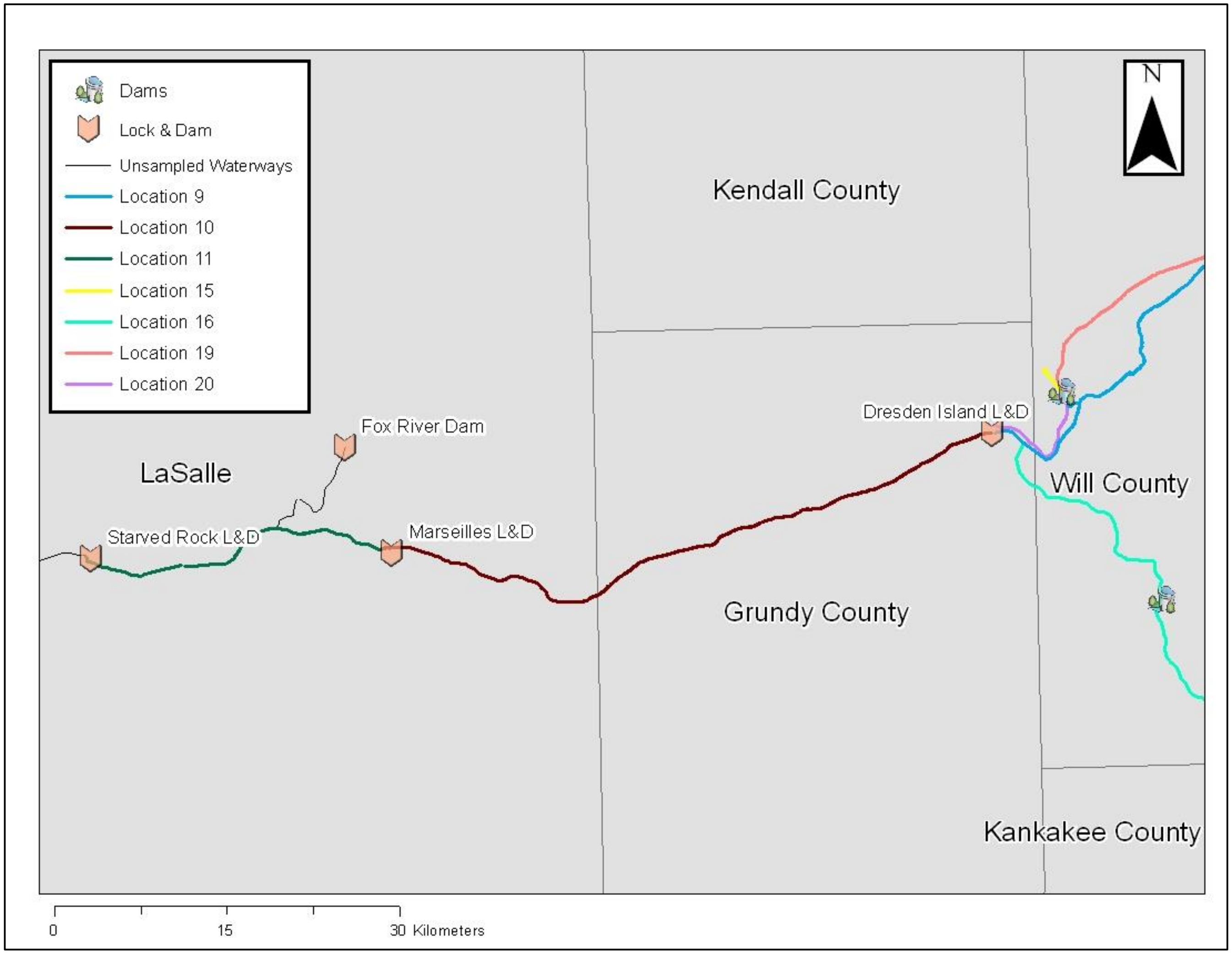

Figure 34. Western most Illinois locations sampled during 2013 and 2014 sampling season. Locations include Illinois River, Des Plaines River, Kankakee River, DuPage River, and Illinois & Michigan Canal.

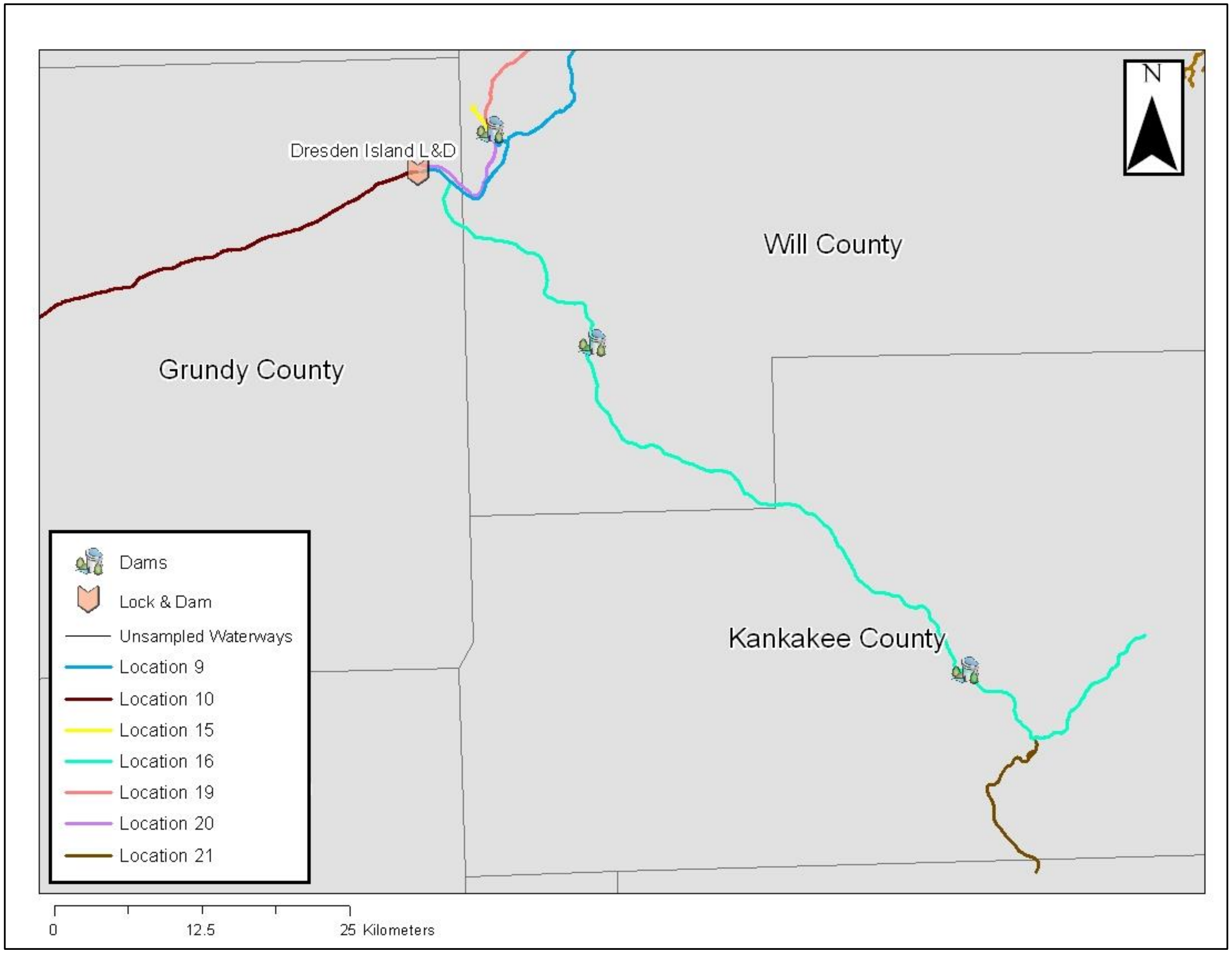

Figure 35. Southernmost portion of Illinois locations sampled during 2013 and 2014 sampling seasons. Locations include Kankakee River, Des Plaines River, DuPage River, Illinois River, and the Illinois & Michigan Canal.

APPENDIX C: SAMPLING EFFORT AND CATCH NUMBER

Table 16. Sampling sites and method type conducted for study. Does not include supplementary sampling efforts conducted as part of the Asian Carp Monitroing Program.

Month	Year	Latitude	Longitude	Waterbody	Method	Number of Traps	Net Nights (24 hrs.)	Button Time (Sec)
5	2013	42.03739	-87.710069	North Shore Channel	Trap	5	3	
5	2013	41.640503	-88.058169	I&M Canal	Trap	2	2	
5	2013	41.582368	-88.073778	Des Plaines River Flood area	Trap	4	2	
5	2013	41.583977	-88.072318	Des Plaines River	Trap	2	2	
5	2013	41.656257	-88.064938	Des Plaines River	Trap	2	2	
6	2013	41.942842	-87.695572	Chicago River	Trap	2	1	
6	2013	41.942348	-87.695355	Chicago River	Trap	3	1	
6	2013	41.836989	-87.686874	Chicago Sanitary and Ship Canal	Trap	5	1	
6	2013	42.03739	-87.710069	North Shore Channel	Trap	4	1	
6	2013	42.03739	-87.710069	North Shore Channel	Electroshocker			514
6	2013	42.07377	-87.685974	North Shore Channel	Trap	5	1	
6	2013	41.623968	-87.516823	Grand Calumet River	Trap	3	2	
6	2013	41.613036	-87.432228	Grand Calumet River	Trap	3	2	
6	2013	41.582368	-88.073778	Des Plaines River Flood area	Trap	6	1	
6	2013	41.662661	-87.75275	Calumet Sag Channel	Trap	5	1	
7	2013	41.731095	-87.888561	Des Plaines River	Trap	4	6	
7	2013	41.384478	-88.239648	I&M Canal	Trap	4	1	
7	2013	41.621349	-87.056226	Little Calumet River	Electroshocker			900
7	2013	41.841357	-87.675464	Chicago Sanitary and Ship Canal	Trap	5	1	
7	2013	42.009557	-87.710479	North Shore Channel	Trap	4	8	
8	2013	41.732676	-87.879999	I&M Canal	Electroshocker			1800
8	2013	41.73276	-87.879811	I&M Canal	Trap	4	1	
8	2013	41.73276	-87.879811	I&M Canal	Electroshocker			440
8	2013	41.734505	-87.879679	Chicago Sanitary and Ship Canal	Trap	5	1	
8	2013	41.65188	-87.660005	Midlothian Creek	Trap	3	3	

8	2013	41.617862	-87.489518	Grand Calumet River	Trap	5	3	
9	2013	41.501691	-88.104828	Des Plaines	Trap	3	1	
9	2013	41.841357	-87.675464	Chicago Sanitary and Ship Canal	Trap	3	1	
9	2013	41.648064	-87.120295	Lake Michigan	Mini Fyke	8	2	
9	2013	41.647583	-87.128148	Lake Michigan	Fyke	8	2	
9	2013	41.647683	-87.147288	Lake Michigan	Trap	25	1	
9	2013	41.634401	-87.174926	Lake Michigan	Mini Fyke	8	2	
9	2013	41.635749	-87.163167	Lake Michigan	Fyke	8	2	
9	2013	41.638635	-87.161708	Lake Michigan	Trap	25	1	
9	2013	41.500615	-88.105075	Des Plaines River	Trap	2	13	
9	2013	41.425272	-88.204833	Des Plaines River	Trap	4	13	
9	2013	41.973307	-87.704075	North Branch Chicago River	Trap	2	2	
10	2013	41.504182	-88.105826	I&M Canal	Electroshocker			1625
10	2013	41.500293	-88.1056	Des Plaines River	Electroshocker			2150
10	2013	41.507549	-88.08437	Hickory Creek	Electroshocker			1800
10	2013	41.507838	-88.098489	Des Plaines River	Trap	4	2	
5	2014	41.582368	-88.073778	Des Plaines River Flood area	Trap	3	1	
5	2014	41.583977	-88.072318	Des Plaines River	Trap	2	1	
5	2014	41.377753	-88.221759	Grant Creek	Trap	2	3	
5	2014	41.411408	-88.222917	Des Plaines River	Trap	2	3	
5	2014	41.501691	-88.104828	Des Plaines River	Trap	3	3	
5	2014	41.569185	-87.475909	Little Calumet River	Trap	3	2	
5	2014	41.613036	-87.432228	Grand Calumet River	Trap	3	2	
5	2014	41.615815	-87.493149	Grand Calumet River	Trap	3	2	
5	2014	41.662576	-87.752913	Cal Sag Channel	Trap	3	2	
6	2014	41.456372	-87.699442	Thorn Creek	Electroshocker			900
6	2014	41.479903	-87.664992	Thorn Creek	Electroshocker			900
6	2014	41.518904	-87.628104	Thorn Creek	Electroshocker			900
6	2014	41.55046	-87.619718	Thorn Creek	Electroshocker			900
6	2014	41.391499	-87.62802	Plum Creek	Electroshocker			900

6	2014	41.369574	-87.655109		Electroshocker			900
6	2014	41.437745	-87.565926	Plum Creek	Electroshocker			900
6	2014	41.656257	-88.064938	Des Plaines River	Trap	4	1	
6	2014	41.71356	-87.912	I&M Canal	Electroshocker			1500
6	2014	41.732782	-87.879799	I&M Canal	Electroshocker			1500
6	2014	41.582368	-88.073778	Des Plaines River	Electroshocker			1500
6	2014	41.681294	-88.002369	Des Plaines River	Trap	4	1	
6	2014	41.675838	-88.027109	Des Plaines River	Electroshocker			1500
6	2014	41.731339	-87.886935	Des Plaines River	Trap	4	7	
7	2014	41.974337	-87.706246	North Branch	Trap	3	22	
7	2014	41.408311	-88.222663	Des Plaines River	Trap	3	20	
7	2014	41.427436	-88.236344	DuPage River	Trap	3	20	
7	2014	41.50817	-88.098222	Des Plaines River	Trap	3	20	
7	2014	42.164024	-87.766192	Lake Michigan	Electroshocker			900
7	2014	41.859519	-87.610727	Lake Michigan	Trap	2	0.5	
7	2014	41.930161	-87.633658	Lake Michigan	Trap	2	0.5	
10	2014	41.854558	-87.608236	Lake Michigan	Electroshocker			900
10	2014	41.854558	-87.608236	Lake Michigan	Seine			
10	2014	41.856344	-87.608064	Lake Michigan	Electroshocker			900
10	2014	41.856344	-87.608064	Lake Michigan	Seine			
10	2014	41.856344	-87.608064	Lake Michigan	Electroshocker			1200
10	2014	41.856344	-87.608064	Lake Michigan	Seine			

Table 17. Sampling hours in 2013 shown for each gear type. Blank spaces indicate no sampling effort. BS – Boat mounted electroshocker, BP – Backpack electroshocker, MF – mini-fyke nets, MT – minnow trap

	Mar-13				*Apr-13*				*May-13*				*Jun-13*			
	Active		Passive		Active		Passive		Active		Passive		Active		Passive	
	BS	BP	MF	MT	BS	BP	MF	MT	BS	BP	MF	MT	BS	BP	MF	MT
Location 1																
Location 2	2.5				2.5				3.75				17.3			120
Location 3	3				3				2.5			360	3.5	0.14		336
Location 4	3				3				4.25				6.75			72
Location 5	3				3				2.5				3.5			120
Location 6																
Location 7	2				6		192		8		192		3			
Location 8	2				6		192		8		192		2		96	
Location 9	2				1		192		12		192		2		96	
Location 10	2				1		192		12		192		2		96	
Location 11													1		480	
Location 12					4.57							384	2.83			144
Location 13																
Location 14																288
Location 15																
Location 16													3		384	
Location 17																
Location 18												96				
Location 19																
Location 20																
Location 21													2			
Device Total	19.5				30.07		768		53		768	840	48.88	0.143	1152	1080
Method Total	19.5		0		30.07		768		53		1608		49.023		2232	
Monthly Total	19.5				798.07				1661				2281.02			

Table 17 cont.

	Jul-13				*Aug-13*				*Sep-13*				*Oct-13*			
	Active		Passive		Active		Passive		Active		Passive		Active		Passive	
	BS	BP	MF	MT	BS	BP	MF	MT	BS	BP	MF	MT	BS	BP	MF	MT
Location 1									1.67		768	1200				
Location 2	2.5				2.5				2.5				2.5			
Location 3	3			768	3			144	3			96	8.75			
Location 4	7.25			120	6.25			120	3			72	18.45			
Location 5	3				3				3				18.8			
Location 6																
Location 7	12		288		8		192		8		192		4		192	
Location 8	8		192		8		192		8		192		4		192	192
Location 9	8		192		8		192		8		192	1944	4	1.097	192	
Location 10	8		192		8		192		8		192		4		192	
Location 11	1				1				1							
Location 12				576												
Location 13		0.25						216								
Location 14								360								
Location 15																
Location 16	3		384		3		384		3		384		3		384	
Location 17						0.6222		96								
Location 18																
Location 19														0.451		
Location 20				96												
Location 21	2				2				1				1			
Device Total	57.75	0.25	1248	1560	52.75	0.6222	1152	936	50.17		1920	3312	68.5	1.549	1152	192
Method Total	58		2808		53.37		2088		50.17		5232		70.049		1344	
Monthly Total	2866				2141.37				5282.17				1414.05			

Table 17 cont.

	Nov-13				*Yearly Total*				*All Methods Total*
	Active		Passive		Active		Passive		
	BS	BP	MF	MT	BS	BP	MF	MT	
Location 1					1.67		768	1200	1969.67
Location 2	2.5				38.55			120	158.55
Location 3	3				32.75	0.14		1704	1736.89
Location 4	4				55.95			384	439.95
Location 5	3				42.8			120	162.8
Location 6									
Location 7	8				59		1248		1307
Location 8	8				54		1248	192	1494
Location 9	8				53	1.10	1248	1944	3246.10
Location 10	8				53		1248		1301
Location 11					4		480		484
Location 12					7.4			1104	1111.4
Location 13						0.25		216	216.25
Location 14								648	648
Location 15									
Location 16					15		1920		1935
Location 17						0.62		96	96.62
Location 18								96	96
Location 19						0.45			0.45
Location 20								96.00	96
Location 21					8.00				8
Device Total	44.5				425.12	2.56	8160	7920	
Method Total	44.5		0		427.68		16080		16507.68
Monthly Total	44.5								

Table 18. Weatherfish captured in 2013. Numbers shown for gears and Locations that experienced sampling effort. Blank spaces

indicate no sampling effort conducted. BS – Boat electroshocker, BP – Backpack electroshocker, MF – mini-fyke nets, MT – minnow trap. Zeros represent fish events where effort was applied, but no fish were caught.

	Mar-13				*Apr-13*				*May-13*				*Jun-13*				*Jul-13*			
	Active		Passive		Active		Passive		Active		Passive		Active		Passive		Active		Passive	
	BS	BP	MF	MT	BS	BP	MF	MT	BS	BP	MF	MT	BS	BP	MF	MT	BS	BP	MF	MT
Location 1																				
Location 2	0				0				0				0			0	0			
Location 3	0				0				0			5	1	1		1	0			1
Location 4	5				0				1				1			0	7			2
Location 5	0				0				0				1			0	3			
Location 6																				
Location 7	0				0		3		5		14		3				0		4	
Location 8	0				1		8		2		3		0		1		0		0	
Location 9	0				0		0		0		2		0		0		0		0	
Location 10	0				0		0		0		2		0		0		0		0	
Location 11													0				0			
Location 12					1							6	0			3				0
Location 13																		0		
Location 14																0				
Location 15																				
Location 16													0		0		0		0	
Location 17																				
Location 18												0								
Location 19																				
Location 20																				0
Location 21													0				0			
Device Total	5				2		11		8		21	11	6	1	1	4	10	0	4	3
Method Total	5		0		2		11		8		32		7		5		10		7	
Monthly Total	5				13				40				12				17			

Table 18 cont.

	Aug-13				*Sep-13*				*Oct-13*				*Nov-13*				*Yearly Total*				*All Methods Total*
	Active		Passive		Active		Passive		Active		Passive		Active		Passive		Active		Passive		
	BS	BP	MF	MT	BS	BP	MF	MT	BS	BP	MF	MT	BS	BP	MF	MT	BS	BP	MF	MT	
Location 1					0		0	0									0		0	0	0
Location 2	0				0				0				0				0			0	0
Location 3	0			3	1			0	2				0				4	1		10	15
Location 4	4			0	7			0	0				0				25			2	27
Location 5	0				0				0				0				4			0	4
Location 6																					
Location 7	0		2		1		0		0		0		0				9		23		32
Location 8	0		0		0		0		0		0		0				3		12	0	15
Location 9	0		0		0		0	0	0	0	0		0				0	0	2	0	2
Location 10	0		0		0		0		0		0		0				0		2		2
Location 11	0				0												0		0		0
Location 12																	1			9	10
Location 13				0														0		0	0
Location 14				0																0	0
Location 15																					
Location 16	0		0		0		0		0		0						0		0		0
Location 17		12		10														12		10	22
Location 18																				0	0
Location 19										0								0		0	0
Location 20																				0	0
Location 21	0				0				0								0	0	0	0	0
Device Total	4	12	2	13	9		0	0	2	0	0	0	0				46	13	39	31	129
Method Total	16		15		9		0		2		0		0		0		59		70		
Monthly Total	31				9				2				0								

Table 19. Sampling hours in 2014 shown for each gear type. Blank spaced indicate no sampling effort. BS – Boat mounted electroshocker, BP – Backpack electroshocker, MF – mini-fyke nets, MT – minnow trap

	Mar-14				*Apr-14*				*May-14*				*Jun-14*			
	Active		Passive		Active		Passive		Active		Passive		Active		Passive	
	BS	BP	MF	MT	BS	BP	MF	MT	BS	BP	MF	MT	BS	BP	MF	MT
Location 1									0.7				7.92			
Location 2													36.5			
Location 3													5			
Location 4													5			
Location 5												144	3			
Location 6																696
Location 7	3				6		96		6		96		6		96	
Location 8	3				6		96		6		96		6		96	
Location 9	3				6		96		6		96	504	9.25		336	168
Location 10	3				6		96		6		96		9.75		336	
Location 11							240				624		3.25		69	
Location 12									5.69			120	2.46	0.83		864
Location 13												144		2		
Location 14												288				
Location 15																
Location 16													3			
Location 17														0.83		
Location 18																
Location 19																
Location 20																
Location 21													2			
Device Total	12				24		624		30.39		1008	1200	99.13	3.67	933	1728
Method Total	12		0		24		624		30.39		2208		102.80		2661	
Monthly Total	12				648				2238.39				2763.80			

Table 19 cont.

	Jul-14				*Aug-14*				*Sep-14*				*Oct-14*			
	Active		Passive		Active		Passive		Active		Passive		Active		Passive	
	BS	BP	MF	MT	BS	BP	MF	MT	BS	BP	MF	MT	BS	BP	MF	MT
Location 1	5.73	0.25		48	1.82				10.08			2808	10.08	0.833		
Location 2									4.25							
Location 3									15							
Location 4									11.75							
Location 5									4.5							
Location 6																
Location 7	6		96		5.75		96		6		96		6		96	
Location 8	6		96	648	6		96		6		96		6		96	
Location 9	9.25		336	1440	3.25		96		4		96		6.75		96	
Location 10	11.75		720		5.5		480		7.25		96		7.25		96	
Location 11	5.25				3.75											
Location 12																
Location 13																
Location 14																
Location 15				1440						1						
Location 16	3				3				3				3			
Location 17																
Location 18																
Location 19																
Location 20																
Location 21	2				2				1				1			
Device Total	48.98	0.25	1248	3576	31.07	0	768	0	72.83		384	2808	40.08	0.833	384	0
Method Total	49.23		4824		31.07		768		72.83		3192		40.91		384	
Monthly Total	4873.23				799.07				3264.83				424.91			

Table 19 cont.

	Oct-14				*Nov-14*				*Yearly Total*				*All Methods Total*
	Active		Passive		Active		Passive		Active		Passive		
	BS	BP	MF	MT	BS	BP	MF	MT	BS	BP	MF	MT	
Location 1	10.08	0.833			6.9				43.23	1.08		2856	2900.31
Location 2									40.75				40.75
Location 3									20				20
Location 4									16.75				16.75
Location 5									7.5			144	151.5
Location 6												696	696
Location 7	6		96		6				50.75		672		722.75
Location 8	6		96		6				51		672	648	1371
Location 9	6.75		96		5.5		96		53		1248	2112	3413
Location 10	7.25		96		6		96		62.5		2016		2078.5
Location 11									12.25		933		945.25
Location 12									8.15	0.83		984	992.98
Location 13										2		144	146
Location 14												288	288
Location 15										1		1440	1441
Location 16	3								15				15
Location 17										0.83			0.83
Location 18													
Location 19													
Location 20													
Location 21	1								8				8
Device Total	40.08	0.833	384	0	30.4				388.88	4.75	5349	9312	15054.6
Method Total	40.91		384		30.4		0		393.63		14661		
Monthly Total	424.91				30.4								

Table 20. Weatherfish captured in 2013. Numbers shown for gears and Locations that experienced sampling effort. BS – Boat electroshocker, BP – Backpack electroshocker, MF – mini-fyke nets, MT – minnow trap. Zeros represent fish events where effort was applied, but no fish were caught.

	Mar-14				*Apr-14*				*May-14*				*Jun-14*				*Jul-14*			
	Active		Passive		Active		Passive		Active		Passive		Active		Passive		Active		Passive	
	BS	BP	MF	MT	BS	BP	MF	MT	BS	BP	MF	MT	BS	BP	MF	MT	BS	BP	MF	MT
Location 1									0				0				0	0		0
Location 2													0							
Location 3													2							
Location 4													3							
Location 5												1	0							
Location 6																0				
Location 7	0				2		7		8		5		0		8		2		1	
Location 8	0				0		1		1		0		0		9		0		0	2
Location 9	0				0		0		0		2	0	0		0	3	0		0	0
Location 10	0				0		0		0		1		0		0		0		0	
Location 11							0				0		0		0		0			
Location 12									0			0	1	3		9				
Location 13												1		1						
Location 14												0								
Location 15																				0
Location 16													0				0			
Location 17														17						
Location 18																				
Location 19																				
Location 20																				
Location 21													0				0			
Device Total	0				2		8		9		8	2	6	21	17	12	2	0	1	2
Method Total	0		0		2		8		9		10		27		29		2		3	
Monthly Total	0				10				19				56				5			

Table 20 cont.

	Aug-14				*Sep-14*				*Oct-14*				*Nov-14*				*Yearly Total*				*All Methods Total*
	Active		Passive		Active		Passive		Active		Passive		Active		Passive		Active		Passive		
	BS	BP	MF	MT	BS	BP	MF	MT	BS	BP	MF	MT	BS	BP	MF	MT	BS	BP	MF	MT	
Location 1	0				0				0	0			0				0	0		0	0
Location 2					0												0				0
Location 3					7												9				9
Location 4					4												7				7
Location 5					0												0			1	1
Location 6																				0	0
Location 7	5		1		6		3		3		10		0				26		35		61
Location 8	0		6		4		0		0		0		0				5		16	2	23
Location 9	1		0		0		0		0		0		0		0		1		2	3	6
Location 10	0		0		0		0		0		0		0		0		0		1		1
Location 11	0																0		0		0
Location 12																	1	3		9	13
Location 13																		1		1	2
Location 14																				0	0
Location 15						0												0		0	0
Location 16	0				0				0								0				0
Location 17																		17			17
Location 18																					
Location 19																					
Location 20																					
Location 21	0				0				0								0				0
Device Total	6	0	7	0	21		3	0	3	0	10	0	0				49	21	54	16	
Method Total	6		7		21		3		3		10		0		0		70		70		140
Monthly Total	13				24				13				0								

APPENDIX D: COI & D-LOOP HAPLOTYPES AND ACCESSION NUMBERS

Table 21. Nucleotide composition and basepair length of the Chicagoland COI haplotypes**.**

COI Haplotype	A	T	G	C	Length
h01	25.5%	31.7%	18.2%	24.6%	1039
h02	25.3%	31.8%	18.3%	24.6%	1039
h03	25.1%	32.1%	18.4%	24.4%	1039
h04	24.6%	31.8%	18.9%	24.7%	1039
h05	25.0%	32.1%	18.5%	24.4%	1039
h06	25.3%	31.9%	18.3%	24.5%	1039

Table 22. Nucleotide composition and basepair length of the Chicagoland D-Loop haplotypes.

D-Loop Haplotype	A	T	G	C	Length
h01	30.8%	34.5%	20.3%	14.4%	932
h02	30.9%	34.4%	20.2%	14.5%	932
h03	31.0%	34.1%	20.5%	14.4%	932
h04	30.6%	34.5%	20.9%	13.9%	932
h05	30.8%	33.9%	20.7%	14.5%	931
h06	31.0%	34.5%	20.1%	14.4%	932

Table 23. Chicagoland sample ID and haplotype shown for the COI locus, D-Loop region, and combined sequences.

Sequence Identity	**COI Haplotype**	**D-Loop Haplotype**	**Combined**	**Collection Location**
1	1	1	1	NSC
2	1	1	1	NSC
4	1	1	1	Lockport Prairie
5	2	2	2	Lockport Prairie
6	3	3	3	Lockport Prairie
7	4	4	4	NSC
8	4	4	4	NSC
9	1	1	1	NSC
10	3	3	3	Lockport Prairie
11	2	2	2	Lockport Prairie
12	1	1	1	CSSC
13	1	1	1	I & M
14	1	1	1	I & M
15	1	4	7	I & M
16	1	1	1	I & M
17	3	3	3	Lockport Prairie
18	3	3	3	Lockport Prairie
19	1	1	1	Lockport Prairie
20	1	1	1	CSSC
21	1	1	1	I & M
22	5	5	5	I & M
23	1	1	1	I & M
24	1	1	1	I & M
25	1	1	1	I & M
26	4	4	4	I & M
27	1	1	1	I & M
28	4	4	4	I & M
29	6	6	6	NSC
30	3	3	3	NSC
31	1	1	1	I & M
32	1	1	1	I & M
33	1	1	1	I & M
34	5	5	5	I & M
35	3	3	3	I & M

36	1	1	1	I & M
37	5	5	5	I & M
38	1	1	1	I & M
39	1	1	1	I & M
41	6	6	6	Goose Island
42	1			Goose Island
43	2	2	2	Goose Island
44	1	1	1	Brandon Road
45	1			9th
46	1			9th
47	3			Little Calumet
48	3	3	3	Cal Sag Channel
49	3	3	3	Little Calumet
50	1	1	1	Romeoville Forest Preserve
51	3	3	3	Romeoville Forest Preserve
52	1	1	1	Romeoville Forest Preserve
53	5	5	5	Romeoville Forest Preserve
58	1			Des Plaines River
66	2	2	2	Des Plaines River
69	3	3	3	Des Plaines River
70	3			Illinois River
75	2	2	2	Brandon Road
76	3	3	3	Brandon Road
78	3			9th
92	1			9th

Table 24. COI haplotypes of samples amplified from Chicagoland weatherfish and from those found on GenBank. Samples displayed in the order they appear on the COI Bayesian phylogenetic tree shown in the results section of chapter III of this thesis.

Taxon ID	**Shared Tip**	**Watershed**	**Country**	**Species**
h01		IL/IN	USA	M. anguillicaudatus
	JQ011426	Murrumbidgee River Basin	Australia	M. sp
	KJ669524	Ginninderra Creek	Australia	M. anguillicaudatus
h02		IL/IN	USA	M. anguillicaudatus
h06		IL/IN	USA	M. anguillicaudatus
	JQ011416	Yarra River Basin	Australia	M. sp
h03		IL/IN	USA	M. anguillicaudatus
	JQ011428	Yarra River Basin	Australia	M. sp
h05		IL/IN	USA	M. anguillicaudatus
h04		IL/IN	USA	M. anguillicaudatus
	JQ011422	Yarra River Basin	Australia	M. sp
	JQ011434	Maribyrnong River Basin	Australia	M. sp
	KJ669523	Murrumbidgee River Basin	Australia	M. anguillicaudatus
EU670785.1		Okcheon-myeon	South Korea	M. anguillicaudatus
HM446336.1		Nan Si Lake	China	M. anguillicaudatus
JQ011420		Alafia River Basin	Florida	M. sp
KP112318		Wuhan Market	China	M. anguillicaudatus
JQ011421		Alafia River Basin	Florida	M. sp
JQ011424		Middle Yangtze River Basin	China	M. sp
KJ553659		Po Drainage	Italy	M. anguillicaudatus
KM610758		Nujiang River	China	M. anguillicaudatus
	KM610759	Nujiang River	China	M. anguillicaudatus
	KM610764	Nujiang River	China	M. anguillicaudatus
	KM610773	Nujiang River	China	M. anguillicaudatus
	KM610774	Nujiang River	China	M. anguillicaudatus
KM610767		Nujiang River	China	M. anguillicaudatus
KM610768		Nujiang River	China	M. anguillicaudatus
KM610772		Nujiang River	China	M. anguillicaudatus
HM446337.1		Nan Si Lake	China	M. anguillicaudatus
KP112322		Wuhan Market	China	M. anguillicaudatus
HM446341.1		Nan Si Lake	China	M. anguillicaudatus
JQ011425		Middle Yangtze River Basin	China	M. sp
KP112319		Wuhan Market	China	M. anguillicaudatus
KP112320		Wuhan Market	China	M. anguillicaudatus
JQ011432		Hue	Vietnam	M. sp

JQ011433		Hue	Vietnam	M. sp
HM446338.1		Nan Si Lake	China	M. anguillicaudatus
JQ011419		Bunyip River Basin	Australia	M. sp
	JQ011435	Bunyip River Basin	Australia	M. sp
JQ011423		Middle Yangtze River Basin	China	M. sp
KF732665		Pyongyang	North Korea	M. anguillicaudatus
JQ011418			South Korea	M. sp
JQ011417		Yarra River Basin	Australia	M. sp
JN027250.1		Spiller Pet Store	USA	M. anguillicaudatus
JN177217.1			China	M. anguillicaudatus
AB242171		Amur Basin	China	M. nikolskyi

Table 25**.** COI haplotypes of samples amplified from Chicagoland weatherfish and from those found on GenBank. Samples displayed in the order they appear on the COI Maximum Likelihood phylogenetic tree shown in the results section of Chapter III of this thesis.

Taxon ID	**Shared Tip**	**Watershed**	**Country**	**Species**
h01		IL/IN	USA	M. anguillicaudatus
	JQ011426	Murrumbidgee River Basin	Australia	M. sp
	KJ669524	Ginninderra Creek	Australia	M. anguillicaudatus
h02		IL/IN	USA	M. anguillicaudatus
h06		IL/IN	USA	M. anguillicaudatus
	JQ011416	Yarra River Basin	Australia	M. sp
h03		IL/IN	USA	M. anguillicaudatus
	JQ011428	Yarra River Basin	Australia	M. sp
h05		IL/IN	USA	M. anguillicaudatus
h04		IL/IN	USA	M. anguillicaudatus
	JQ011422	Yarra River Basin	Australia	M. sp
	JQ011434	Maribyrnong River Basin	Australia	M. sp
	KJ669523	Murrumbidgee River Basin	Australia	M. anguillicaudatus
EU670785.1		Okcheon-myeon	South Korea	M. anguillicaudatus
HM446336.1		Nan Si Lake	China	M. anguillicaudatus
JQ011420		Alafia River Basin	Florida	M. sp
KP112318		Wuhan Market	China	M. anguillicaudatus
JQ011421		Alafia River Basin	Florida	M. sp
JQ011424		Middle Yangtze River Basin	China	M. sp
KJ553659		Po Drainage	Italy	M. anguillicaudatus
KM610758		Nujiang River	China	M. anguillicaudatus
	KM610759	Nujiang River	China	M. anguillicaudatus
	KM610764	Nujiang River	China	M. anguillicaudatus
	KM610774	Nujiang River	China	M. anguillicaudatus
	KM610773	Nujiang River	China	M. anguillicaudatus
KM610767		Nujiang River	China	M. anguillicaudatus
KM610772		Nujiang River	China	M. anguillicaudatus
KM610768		Nujiang River	China	M. anguillicaudatus
HM446341.1		Nan Si Lake	China	M. anguillicaudatus
HM446337.1		Nan Si Lake	China	M. anguillicaudatus
KP112322		Wuhan Market	China	M. anguillicaudatus
KP112319		Wuhan Market	China	M. anguillicaudatus
JQ011425		Middle Yangtze River Basin	China	M. sp
KP112320		Wuhan Market	China	M. anguillicaudatus
JQ011432		Hue	Vietnam	M. sp

JQ011433		Hue	Vietnam	M. sp
HM446338.1		Nan Si Lake	China	M. anguillicaudatus
JQ011419		Bunyip River Basin	Australia	M. sp
	JQ011435	Bunyip River Basin	Australia	M. sp
JQ011423		Middle Yangtze River Basin	China	M. sp
KF732665		Pyongyang	North Korea	M. anguillicaudatus
JQ011418			South Korea	M. sp
JQ011417		Yarra River Basin	Australia	M. sp
JN027250.1		Spiller Pet Store	USA	M. anguillicaudatus
JN177217.1			China	M. anguillicaudatus
AB242171		Amur Basin	China	M. nikolskyi

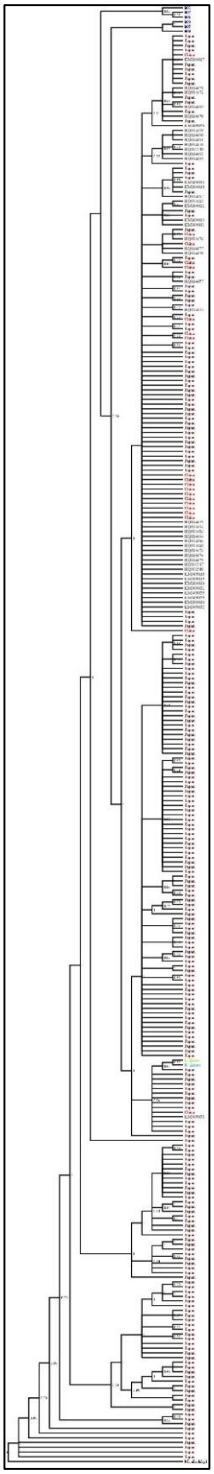

Figure 36.Full Bayesian phylogenetic tree of 313 D-Loop samples using Chicagoland sequences and sequences from GenBank. Colors indicate country of origin. Red – China, blue – Chicagoland, brown – Japan, Gold – South Korea, light blue – North Korea, grey – unknown origin, black – outgroup. Taxon presented in Table 26 in the order they appear in this tree.

Table 26. D-Loop haplotypes amplified from Chicagoland weatherfish and gathered from GenBank used in Bayesian analysis. Samples displayed in the order they appear in the D-Loop Bayesian phylogenetic tree in Figure 36.

Taxon ID	**Watershed**	**Country**	**Species**
h01	IL/IN	USA	M. anguillicaudatus
h02	IL/IN	USA	M. anguillicaudatus
h06	IL/IN	USA	M. anguillicaudatus
h03	IL/IN	USA	M. anguillicaudatus
h05	IL/IN	USA	M. anguillicaudatus
h04	IL/IN	USA	M. anguillicaudatus
AB306765		Japan	M. anguillicaudatus
AB306770.1		Japan	M. anguillicaudatus
AB469218.1		Japan	M. anguillicaudatus
AB543968.1	Sado Island	Japan	M. anguillicaudatus
EU380222.1	Shaoguan	China	M. anguillicaudatus
KM609027			M. anguillicaudatus
LC011084		Japan	M. anguillicaudatus
LC011087		Japan	M. anguillicaudatus
LC011097		Japan	M. anguillicaudatus
LC011098		Japan	M. anguillicaudatus
LC011099		Japan	M. anguillicaudatus
HQ014471			M. anguillicaudatus
HQ014472			M. anguillicaudatus
LC011095		Japan	M. anguillicaudatus
AB469233.1		Japan	M. anguillicaudatus
HQ014469			M. anguillicaudatus
AB469219.1		Japan	M. anguillicaudatus
HQ014470			M. anguillicaudatus
LC011086		Japan	M. anguillicaudatus
KM609023			M. anguillicaudatus
HQ014459			M. anguillicaudatus
HQ014460			M. anguillicaudatus
HQ014461			M. anguillicaudatus
HQ014458			M. anguillicaudatus
HQ891549			M. anguillicaudatus
HQ014462			M. anguillicaudatus
HQ014463			M. anguillicaudatus
LC011100		Japan	M. anguillicaudatus
AB306775		Japan	M. anguillicaudatus
AB469226.1		Japan	M. anguillicaudatus

AB543966.1	Sado Island	Japan	M. anguillicaudatus
KM609026			M. anguillicaudatus
KM609028			M. anguillicaudatus
LC011094		Japan	M. anguillicaudatus
HQ014467			M. anguillicaudatus
HQ014465			M. anguillicaudatus
KM609022			M. anguillicaudatus
AB469227.1		Japan	M. anguillicaudatus
AB469228.1		Japan	M. anguillicaudatus
KM609024			M. anguillicaudatus
KM609031			M. anguillicaudatus
AB469252.1		Japan	M. anguillicaudatus
GU583699.1	Hubei	China	M. anguillicaudatus
HQ014476			M. anguillicaudatus
GU583697.1	Hubei	China	M. anguillicaudatus
HQ014477			M. anguillicaudatus
HQ014478			M. anguillicaudatus
AB469210.1		Japan	M. anguillicaudatus
DQ105312.1	Xianning, Hubei	China	M. anguillicaudatus
GU583703.1	Hubei	China	M. anguillicaudatus
AB469211.1		Japan	M. anguillicaudatus
AB469212.1		Japan	M. anguillicaudatus
HQ014457			M. anguillicaudatus
AB306760.1		Japan	M. anguillicaudatus
LC011088		Japan	M. anguillicaudatus
AB306764.1		Japan	M. anguillicaudatus
AB543971.1	Sado Island	Japan	M. anguillicaudatus
AB469241.1		Japan	M. anguillicaudatus
HQ014455			M. anguillicaudatus
AB469242.1		Japan	M. anguillicaudatus
GU583696.1	Hubei	China	M. anguillicaudatus
AB469280.1		Japan	M. anguillicaudatus
LC011092		Japan	M. anguillicaudatus
GU583700.1	Hubei	China	M. anguillicaudatus
GU583705.1	Hubei	China	M. anguillicaudatus
LC011090		Japan	M. anguillicaudatus
LC011093		Japan	M. anguillicaudatus
AB306761		Japan	M. anguillicaudatus
AB306762.1		Japan	M. anguillicaudatus
AB306773		Japan	M. anguillicaudatus

AB469203.1		Japan	M. anguillicaudatus
AB469204.1		Japan	M. anguillicaudatus
AB469205.1		Japan	M. anguillicaudatus
AB469206.1		Japan	M. anguillicaudatus
AB469207.1		Japan	M. anguillicaudatus
AB469208.1		Japan	M. anguillicaudatus
AB469209.1		Japan	M. anguillicaudatus
AB469213.1		Japan	M. anguillicaudatus
AB469225.1		Japan	M. anguillicaudatus
AB469230.1		Japan	M. anguillicaudatus
AB469235.1		Japan	M. anguillicaudatus
AB469236.1		Japan	M. anguillicaudatus
AB469237.1		Japan	M. anguillicaudatus
AB469238.1		Japan	M. anguillicaudatus
AB469239.1		Japan	M. anguillicaudatus
AB469240.1		Japan	M. anguillicaudatus
AB469260.1		Japan	M. anguillicaudatus
AB469279.1		Japan	M. anguillicaudatus
AB469281.1		Japan	M. anguillicaudatus
AB469282.1		Japan	M. anguillicaudatus
AB543967.1	Sado Island	Japan	M. anguillicaudatus
AB543969.1	Sado Island	Japan	M. anguillicaudatus
AB543970.1	Sado Island	Japan	M. anguillicaudatus
AB645739.1	East Tiaoxi River	China	M. anguillicaudatus
AB645740.1	East Tiaoxi River	China	M. anguillicaudatus
AB645741.1	East Tiaoxi River	China	M. anguillicaudatus
AB645742.1	East Tiaoxi River	China	M. anguillicaudatus
EU697121.1		China	M. anguillicaudatus
EU697122.1		China	M. anguillicaudatus
GU583698.1	Hubei	China	M. anguillicaudatus
GU583701.1	Hubei	China	M. anguillicaudatus
GU583702.1	Hubei	China	M. anguillicaudatus
GU583704.1	Hubei	China	M. anguillicaudatus
HQ014453			M. anguillicaudatus
HQ014454			M. anguillicaudatus
HQ014456			M. anguillicaudatus
HQ014464			M. anguillicaudatus
HQ014466			M. anguillicaudatus
HQ014468			M. anguillicaudatus
HQ014473			M. anguillicaudatus

HQ014474			M. anguillicaudatus
HQ014475			M. anguillicaudatus
HQ891547			M. anguillicaudatus
HQ891548			M. anguillicaudatus
KM609018			M. anguillicaudatus
KM609019			M. anguillicaudatus
KM609020			M. anguillicaudatus
KM609021			M. anguillicaudatus
KM609025			M. anguillicaudatus
KM609029			M. anguillicaudatus
KM609030			M. anguillicaudatus
KM609032			M. anguillicaudatus
LC011085		Japan	M. anguillicaudatus
LC011089		Japan	M. anguillicaudatus
LC011091		Japan	M. anguillicaudatus
LC011096		Japan	M. anguillicaudatus
AY600879.1	Guangxi	China	M. anguillicaudatus
AB306742		Japan	M. anguillicaudatus
AB306748		Japan	M. anguillicaudatus
AB543951.1	Sado Island	Japan	M. anguillicaudatus
LC011079		Japan	M. anguillicaudatus
AB306754.1		Japan	M. anguillicaudatus
AB543950.1	Sado Island	Japan	M. anguillicaudatus
LC011064		Japan	M. anguillicaudatus
AB306734		Japan	M. anguillicaudatus
AB306737		Japan	M. anguillicaudatus
AB306738		Japan	M. anguillicaudatus
AB306740		Japan	M. anguillicaudatus
AB306747		Japan	M. anguillicaudatus
AB306752.1		Japan	M. anguillicaudatus
AB306753		Japan	M. anguillicaudatus
AB306756.1		Japan	M. anguillicaudatus
AB306759		Japan	M. anguillicaudatus
AB306778.1		Japan	M. anguillicaudatus
AB543944.1	Sado Island	Japan	M. anguillicaudatus
AB543945.1	Sado Island	Japan	M. anguillicaudatus
AB543946.1	Sado Island	Japan	M. anguillicaudatus
AB543947.1	Sado Island	Japan	M. anguillicaudatus
AB543948.1	Sado Island	Japan	M. anguillicaudatus
AB543949.1	Sado Island	Japan	M. anguillicaudatus

LC011058		Japan	M. anguillicaudatus
LC011077		Japan	M. anguillicaudatus
LC011080		Japan	M. anguillicaudatus
AB306743		Japan	M. anguillicaudatus
LC011063		Japan	M. anguillicaudatus
LC011055		Japan	M. anguillicaudatus
LC011056		Japan	M. anguillicaudatus
AB306733		Japan	M. anguillicaudatus
AB306735		Japan	M. anguillicaudatus
AB306736		Japan	M. anguillicaudatus
AB306739		Japan	M. anguillicaudatus
AB306744		Japan	M. anguillicaudatus
AB306749		Japan	M. anguillicaudatus
AB543959.1	Sado Island	Japan	M. anguillicaudatus
AB543960.1	Sado Island	Japan	M. anguillicaudatus
AB543961.1	Sado Island	Japan	M. anguillicaudatus
AB543962.1	Sado Island	Japan	M. anguillicaudatus
AB543963.1	Sado Island	Japan	M. anguillicaudatus
AB543964.1	Sado Island	Japan	M. anguillicaudatus
AB543965.1	Sado Island	Japan	M. anguillicaudatus
LC011050		Japan	M. anguillicaudatus
LC011051		Japan	M. anguillicaudatus
LC011053		Japan	M. anguillicaudatus
LC011054		Japan	M. anguillicaudatus
LC011057		Japan	M. anguillicaudatus
LC011060		Japan	M. anguillicaudatus
LC011061		Japan	M. anguillicaudatus
LC011069		Japan	M. anguillicaudatus
AB306774.1		Japan	M. anguillicaudatus
LC011062		Japan	M. anguillicaudatus
LC011074		Japan	M. anguillicaudatus
AB306763		Japan	M. anguillicaudatus
LC011071		Japan	M. anguillicaudatus
AB306772.1		Japan	M. anguillicaudatus
LC011073		Japan	M. anguillicaudatus
LC011072		Japan	M. anguillicaudatus
AB306767		Japan	M. anguillicaudatus
AB306769		Japan	M. anguillicaudatus
AB543952.1	Sado Island	Japan	M. anguillicaudatus
AB543953.1	Sado Island	Japan	M. anguillicaudatus

LC011078		Japan	M. anguillicaudatus
AB306755		Japan	M. anguillicaudatus
AB543957.1	Sado Island	Japan	M. anguillicaudatus
AB543958.1	Sado Island	Japan	M. anguillicaudatus
AB306768.1		Japan	M. anguillicaudatus
AB306780.1		Japan	M. anguillicaudatus
AB306777		Japan	M. anguillicaudatus
AB306741		Japan	M. anguillicaudatus
LC011076		Japan	M. anguillicaudatus
AB306771		Japan	M. anguillicaudatus
AB306779		Japan	M. anguillicaudatus
AB306745		Japan	M. anguillicaudatus
AB306746		Japan	M. anguillicaudatus
AB306750.1		Japan	M. anguillicaudatus
AB306751		Japan	M. anguillicaudatus
AB306757		Japan	M. anguillicaudatus
AB306758.1		Japan	M. anguillicaudatus
AB543954.1	Sado Island	Japan	M. anguillicaudatus
AB543955.1	Sado Island	Japan	M. anguillicaudatus
AB543956.1	Sado Island	Japan	M. anguillicaudatus
LC011059		Japan	M. anguillicaudatus
LC011066		Japan	M. anguillicaudatus
LC011067		Japan	M. anguillicaudatus
LC011068		Japan	M. anguillicaudatus
LC011070		Japan	M. anguillicaudatus
LC011075		Japan	M. anguillicaudatus
LC011081		Japan	M. anguillicaudatus
EU670823.1	Okcheon-myeon	South Korea	M. anguillicaudatus
KF732667	Pyongyang	North Korea	M. anguillicaudatus
AB469216.1		Japan	M. anguillicaudatus
AB306781		Japan	M. anguillicaudatus
AB306782.1		Japan	M. anguillicaudatus
AB469217.1		Japan	M. anguillicaudatus
AB469220.1		Japan	M. anguillicaudatus
AB469231.1		Japan	M. anguillicaudatus
AB469232.1		Japan	M. anguillicaudatus
AB469234.1		Japan	M. anguillicaudatus
AB469277.1		Japan	M. anguillicaudatus
DQ105310.1	Luonan, Shanxi	China	M. anguillicaudatus
KM609033			M. anguillicaudatus

LC011065		Japan	M. anguillicaudatus
LC011082		Japan	M. anguillicaudatus
LC011083		Japan	M. anguillicaudatus
AB469221.1		Japan	M. anguillicaudatus
AB645738.1	Iriomote Island	Japan	M. anguillicaudatus
AB469257.1		Japan	M. anguillicaudatus
AB469262.1		Japan	M. anguillicaudatus
AB469248.1		Japan	M. anguillicaudatus
AB469250.1		Japan	M. anguillicaudatus
AB469253.1		Japan	M. anguillicaudatus
AB469254.1		Japan	M. anguillicaudatus
AB469258.1		Japan	M. anguillicaudatus
AB469261.1		Japan	M. anguillicaudatus
AB469263.1		Japan	M. anguillicaudatus
AB469264.1		Japan	M. anguillicaudatus
AB469265.1		Japan	M. anguillicaudatus
AB469268.1		Japan	M. anguillicaudatus
AB469247.1		Japan	M. anguillicaudatus
AB469251.1		Japan	M. anguillicaudatus
AB469256.1		Japan	M. anguillicaudatus
AB469249.1		Japan	M. anguillicaudatus
AB469259.1		Japan	M. anguillicaudatus
AB469255.1		Japan	M. anguillicaudatus
AB469267.1		Japan	M. anguillicaudatus
AB469266.1		Japan	M. anguillicaudatus
AB469244.1		Japan	M. anguillicaudatus
AB469245.1		Japan	M. anguillicaudatus
AB469243.1		Japan	M. anguillicaudatus
AB469214.1		Japan	M. anguillicaudatus
AB469215.1		Japan	M. anguillicaudatus
AB469222.1		Japan	M. anguillicaudatus
AB469223.1		Japan	M. anguillicaudatus
AB469229.1		Japan	M. anguillicaudatus
AB469224.1		Japan	M. anguillicaudatus
AB306725		Japan	M. anguillicaudatus
AB306726		Japan	M. anguillicaudatus
AB306727		Japan	M. anguillicaudatus
AB306728		Japan	M. anguillicaudatus
AB543942.1	Sado Island	Japan	M. anguillicaudatus
AB306724		Japan	M. anguillicaudatus

AB306731		Japan	M. anguillicaudatus
AB306732		Japan	M. anguillicaudatus
AB543940.1	Sado Island	Japan	M. anguillicaudatus
AB306717		Japan	M. anguillicaudatus
AB306723		Japan	M. anguillicaudatus
AB306718		Japan	M. anguillicaudatus
AB306721		Japan	M. anguillicaudatus
AB306719		Japan	M. anguillicaudatus
AB306720		Japan	M. anguillicaudatus
AB543941.1	Sado Island	Japan	M. anguillicaudatus
AB543943.1	Sado Island	Japan	M. anguillicaudatus
AB306788.1		Japan	M. anguillicaudatus
AB306789		Japan	M. anguillicaudatus
AB306790.1		Japan	M. anguillicaudatus
AB306791		Japan	M. anguillicaudatus
AB306722		Japan	M. anguillicaudatus
AB306786.1		Japan	M. anguillicaudatus
AB306787		Japan	M. anguillicaudatus
AB306729		Japan	M. anguillicaudatus
AB306730		Japan	M. anguillicaudatus
AB306783		Japan	M. anguillicaudatus
AB306784.1		Japan	M. anguillicaudatus
AB469246.1		Japan	M. anguillicaudatus
AB469274.1		Japan	M. anguillicaudatus
AB469273.1		Japan	M. anguillicaudatus
AB469275.1		Japan	M. anguillicaudatus
AB469276.1		Japan	M. anguillicaudatus
AB469278.1		Japan	M. anguillicaudatus
AB469269.1		Japan	M. anguillicaudatus
AB469270.1		Japan	M. anguillicaudatus
AB469271.1		Japan	M. anguillicaudatus
AB469272.1		Japan	M. anguillicaudatus
AB3242171			M. nikolskyi

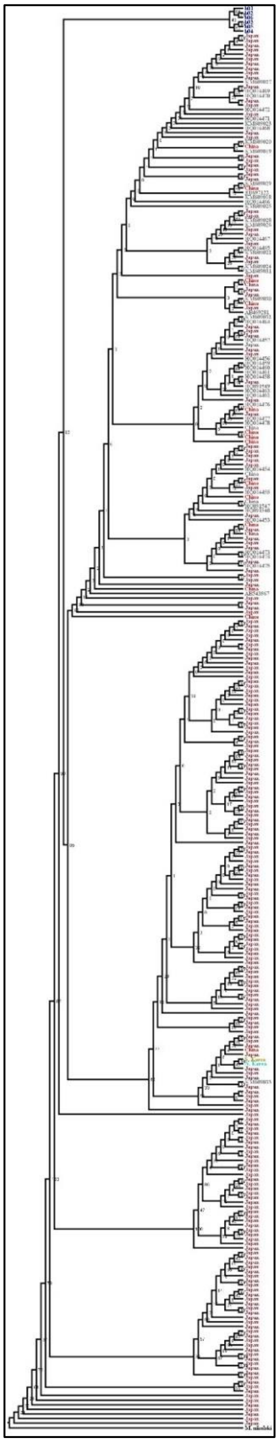

Figure 37. Full Maximum Likelihood phylogenetic tree of 313 D-Loop samples using Chicagoland sequences and sequences from GenBank. Colors indicate country of origin. Red – China, blue – Chicagoland, brown – Japan, Gold – South Korea, light blue – North Korea, grey – unknown origin, black – outgroup. Taxon presented in Table 27 in the order they appear in this tree.

Table 27**.** D-Loop haplotypes amplified from Chicagoland weatherfish and gathered from GenBank used in Maximum Likelihood (ML) analysis. Samples displayed in the order they appear in the D-Loop ML phylogenetic tree in Figure 37.

Taxon ID	**Watershed**	**Country**	**Species**
h01	IL/IN	USA	M. anguillicaudatus
h02	IL/IN	USA	M. anguillicaudatus
h06	IL/IN	USA	M. anguillicaudatus
h03	IL/IN	USA	M. anguillicaudatus
h05	IL/IN	USA	M. anguillicaudatus
h04	IL/IN	USA	M. anguillicaudatus
AB306770.1		Japan	M. anguillicaudatus
EU380222.1	Shaoguan	China	M. anguillicaudatus
LC011097		Japan	M. anguillicaudatus
LC011087		Japan	M. anguillicaudatus
LC011098		Japan	M. anguillicaudatus
LC011099		Japan	M. anguillicaudatus
LC011084		Japan	M. anguillicaudatus
AB469218.1		Japan	M. anguillicaudatus
AB543968.1	Sado Island	Japan	M. anguillicaudatus
AB306765		Japan	M. anguillicaudatus
KM609027			M. anguillicaudatus
AB469233.1		Japan	M. anguillicaudatus
HQ014469			M. anguillicaudatus
HQ014470			M. anguillicaudatus
AB469219.1		Japan	M. anguillicaudatus
LC011086		Japan	M. anguillicaudatus
HQ014472			M. anguillicaudatus
LC011095		Japan	M. anguillicaudatus
HQ014471			M. anguillicaudatus
KM609023			M. anguillicaudatus
HQ014468			M. anguillicaudatus
AB543970.1	Sado Island	Japan	M. anguillicaudatus
AB306773		Japan	M. anguillicaudatus
KM609020			M. anguillicaudatus
EU697121.1		China	M. anguillicaudatus
KM609019			M. anguillicaudatus
AB543971.1	Sado Island	Japan	M. anguillicaudatus
AB306764.1		Japan	M. anguillicaudatus
AB543969.1	Sado Island	Japan	M. anguillicaudatus

AB306762.1		Japan	M. anguillicaudatus
LC011090		Japan	M. anguillicaudatus
LC011093		Japan	M. anguillicaudatus
KM609029			M. anguillicaudatus
KM609021			M. anguillicaudatus
EU697122.1		China	M. anguillicaudatus
KM609018			M. anguillicaudatus
HQ014466			M. anguillicaudatus
KM609025			M. anguillicaudatus
AB469226.1		Japan	M. anguillicaudatus
LC011094		Japan	M. anguillicaudatus
KM609028			M. anguillicaudatus
KM609026			M. anguillicaudatus
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AB3242171			M. nikolskyi

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VITA

John Belcik was born and raised in Chicago, Illinois. Before attending Loyola University Chicago, he attended Dominican University in River Forest, IL, where he earned a Bachelor of Science in Biology, with Great Honor, in 2012. As an undergraduate he studied Marine Island Ecology abroad in the Bahamas through the John G. Shedd Aquarium.

John began his master of science in Biology at Loyola University Chicago in fall of 2012, focusing on invasive fish biology in the Midwest. This thesis was presented in part at the Joint Meetings of Ichthyologists and Herpetologists (JMIH 2013). While at Loyola, he was also elected Vice President of Biology Graduate Student Association and enjoyed teaching General Biology Laboratory (BIO 111 & 112). He also was employed with the United States Army Corps of Engineers as a fish biologist from June of 2014 to August 2015. While with the Corps he worked on the Asian Carp Monitoring project, surveying Chicago Waterways and tracking movements of the invasive Asian Carps. Currently, Belcik is attending University of Illinois at Chicago where he is enrolled in the doctoral program in Ecology and Evolution, where he continues to study fishes.