Using eDNA Metabarcoding as a Monitoring Mechanism for Invasive Species: A Novel Tool for Preventing the Proliferation of the Rusty Crayfish (Faxonius Rusticus) and the Round Goby (Neogobius Melanostomus) in Northern Illinois Waterways.

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Received April 02, 2025
Accepted July 17, 2025
Electronic access July 31, 2025

Background/Objective: This study investigates the efficacy of environmental DNA (eDNA) metabarcoding to detect invasive aquatic species in Northern Illinois waterways, and will educate others about the dangers these species pose and suggest solutions to reduce or prevent their spread. eDNA is an innovative tool which allows researchers to detect organisms and identify their species using traces of DNA left behind as they interact with the environment, and it is especially effective in detecting marine organisms.
Methods: Water samples from four rivers (Des Plaines River, Skokie River, North Branch of the Chicago River and West Fork of the Chicago River) were collected using sterile filtration protocols, processed with PowerWater Sterivex^{TM} DNA Isolation Kits, and analyzed via Illumina MiSeq sequencing. Contamination prevention included bleach sterilization and negative controls.
Results: Rusty Crayfish, Round Goby, Invasive Carp, Goldfish, and White Perch were detected, but Zebra and Quagga mussels were not. eDNA identified invasive common carp (6/7 sampling sites), crucian carp/goldfish (4/7 sites), rusty crayfish (1/7 site), and round goby (1/7 site). The Des Plaines River showed the highest biodiversity (24 species) and contained both target invasives.
Conclusion: In this study, we found that eDNA is a highly effective tool, and that urgent mitigation strategies are needed to protect the environment against invasive species. These strategies must consist of education, legislation, and location-based interventions. It is essential to act on this information now, as species spread quickly.

Keywords: eDNA metabarcoding, invasive species, rusty crayfish, round goby, freshwater ecosystems

Introduction

Background and Context

Invasive species are a pressing issue on the world scale, costing the United States alone over $120 billion dollars annually[1]. The impacts of invasive species on the essential waterways of Northern Illinois remain understudied by the scientific community. In particular, the Des Plaines River, an important conduit connecting the Great Lakes to the Mississippi River Basin and therefore a huge influence on the freshwater ecosystems of the United States, is underresearched[2]. This study uses eDNA surveying to identify invasive species that are harming the waterways of Northern Illinois, and therefore fill a gap in existing research with methods that are less negatively impactful on local ecologies. eDNA surveying uses trace genetic material left behind by species moving through an environment, making it an efficient tool for surveys that may otherwise be labor-intensive. In particular, eDNA is good at detecting species that are elusive or have small populations[3].

Problem Statement and Rationale

Currently, invasive species detection in Northern Illinois is completed through traditional survey methods like visual surveys or catch-and-release surveys, which often lead to a species being detected after it has already established a foothold and done some damage to the environment. This is particularly problematic for species such as Rusty Crayfish and Round Goby, who are known for being highly aggressive species that reproduce rapidly.

Such rapid reproduction causes mitigation efforts to be highly ineffective as it is difficult to remove an invasive species once it has already gained a foothold in the environment. Without improved surveying techniques, invasive species will continue to proliferate in the environment, causing untold damage[2]. The study will attempt to detect Rusty Crayfish and Round Goby in the Des Plaines River and confirm that those species have not spread to the local branches of the Chicago River or the Skokie River.

Significance and Purpose

This study will utilize eDNA to identify invasive species in the waterways of Northern Illinois. This study therefore indicates the efficacy of eDNA as a survey method and shows which invasive species are potentially doing harm to the environment, hopefully leading to education, policy change, and conservation strategy improvement. In particular, using eDNA as a survey method would strengthen the conservation strategies of both local and governmental organizations to protect the environment.

Additionally, increasing the knowledge of the public regarding invasive species is essential for conservation efforts, as even those who care most about the aquatic world are often unaware of invasive species. According to Golebie et al., more than half of aquarium owners in the Great Lakes region remain unaware of invasive species risks[4]. eDNA is a highly effective surveying method, and the harms that invasive species pose are significant. The information in this study can help to fill existing knowledge gaps and improve the health of these important waterways.

Objectives

1. Quantify detection rates of Rusty Crayfish and Round Goby across the Des Plaines River, Skokie River, North Branch of the Chicago River and West Fork of the Chicago River.
2. Compare eDNA metabarcoding results with 2016 - 2022 trawling surveys from the Midwest Biodiversity Institute[2].
3. Evaluate contamination control measures in field sampling protocols.
4. Develop framework recommendations for integrating eDNA into the Illinois Aquatic Nuisance Species Management Plan[5].

Scope and Limitations

This study investigates the use of eDNA in detecting aquatic invasive species within the waterways of Northern Illinois. In particular, it aims to identify the proliferation of Rusty Crayfish and Round Goby, two notably aggressive and harmful invasive species. Noninvasive species, plants, and non-aquatic species lie outside the scope of this study.

Theoretical Framework

The theoretical framework that this study fits within are the principles of aquatic biodiversity, ecosystem protection, and invasive species monitoring. This study uses eDNA as a novel tool to replace the existing methods of species monitoring, such as visual surveys, but still has a similar (but more effective) approach to finding organisms. The data that has been found through this method can then be used to encourage action that protects the aquatic ecosystems that are most harmed by invasive species.

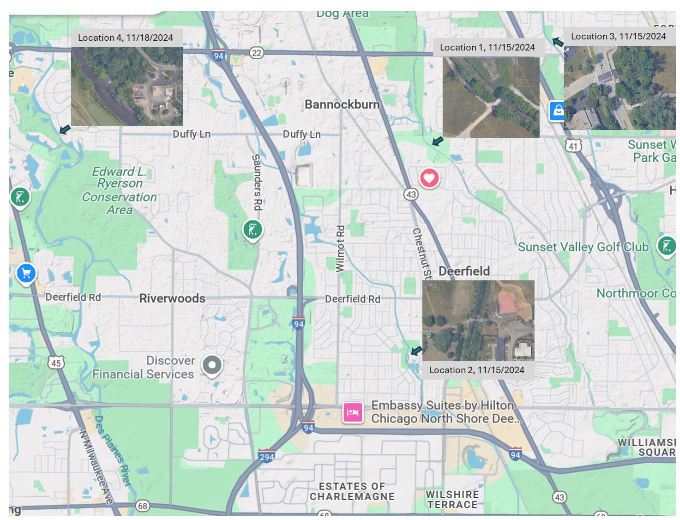
Methodology Overview

Location

The four locations were chosen because they are essential rivers in Northern Illinois. Additionally, they are relatively close in location to one another, meaning that samples from the same river system could be taken within hours of each other in similar weather conditions to reduce unnecessary error. The samples also had to be kept cold so quick sampling was essential. The number of samples taken was constrained by budget and resourcing factors. Of the eight filters, one was required for the control sample. The decision to identify the Des Plaines River as the single sample location was based on the following factors. First, extensive evidence of invasive species from traditional survey methods existed for this river making comparison against an eDNA study readily available. Second, the conditions of the Des Plaines River are far better for eDNA sampling. This is due to the combination of lower levels of organic compounds such as decaying plant matter[6], higher flow rate, and greater water volume[7], that makes the Des Plaines River a more ideal candidate for a single sample than the Chicago or Skokie Rivers.

SampleID*	Volume* (ml)	Date*	Location Description*	Control* (Y/N)	Notes/Observations
20241114 - CT	500	11/14/2024	Negative Control Home Water	Y	Clear Filter, Clear water
20241115#1A	100	11/15/2024	Behind School - North Branch Chicago River	N	Behind Deerfield High School
20241115#1B	150	11/15/2024	Behind School - North Branch Chicago River	N	Behind Deerfield High School
20241115#2A	150	11/15/2024	Behind SMS - West Fork North Branch Chicago River	N	Behind Shepard Middle School
20241115#2B	150	11/15/2024	Behind SMS - West Fork North Branch Chicago River	N	Behind Shepard Middle School
20241115#3A	150	11/15/2024	Skokie River	N	Sleepy Hollow Park
20241115#3B	100	11/15/2024	Skokie River	N	Sleepy Hollow Park
20241118#4	100	11/18/2024	Des Plaines River	N	Rivershire Des Plaines River Access

Table 1: Sampling information including dates, locations, volumes and type



Map 1: Visual depiction of sampling locations

Methods

Research Design

The study was observational, as the information found was used to observe existing patterns in nature.

Sample/Data Collection/Variables and Measurements

The scientist took a control sample from home tap water to make sure there was no contamination. No species were detected in the control sample confirming that the methodology and sampling approach were sound. Water samples were collected from multiple sites along the North Branch of the Chicago River, Skokie River, Des Plaines River, and West Fork of the Chicago River. eDNA sampling used the Genidaqs procedures protocol based on Bergman et al.[8]. The Genidaqs protocol is designed to reduce the possibility of contamination. It uses a filter that shields its filtration material within an external housing to ensure that it is never handled by users. Water samples were collected from either the bank of the river or a bridge traversing it using an extension pole to reach the center of the channel. A cordless drill-powered Cole Parmer Peristaltic Pump MasterFlex Easy Loader II (Model #77200-52) was used to draw water from a depth of 2-6 inches through Masterflex spooled peroxide-cured silicone tubing (L/S 15, internal diameter 4.8 mm).

Water was filtered at each sampling site through Millipore Sterivex^{TM}-GP 0.45 m sterile filter units (EPA# 90260-ITA-001). Water continued to be pumped through the filter until the filter was noticeably clogged with sediment and sample (see volumes and locations in Table 1). Residual water was removed from the filter by reversing the pump direction.

Procedure

Sample Preservation

Once sampling was complete for each filter, it was sealed on both ends, labeled with the scientist's name, location ID, retrieval date, retrieval time and filtered volume, and placed into a sterilized plastic bag. Samples were immediately stored on ice in a cooler for the rest of the sampling day then transferred to a -20C freezer and shipped in dry ice overnight to the Genidaqs laboratory. Due to the extreme cost and lack of availability of eDNA PCR equipment despite the scientist's extensive efforts, the scientist had to send the samples to Genidaqs for sequencing.

Contamination Prevention

The following steps were taken to minimize contamination:

1. Fresh nitrile gloves used at each sampling site,
2. Used materials disposed in sealed bags,
3. Negative field control sample taken prior to sampling day,
4. Sample taken upstream, and
5. Equipment sterilized using a 20% household bleach solution between sample gathering.

DNA Extraction

In the Genidaqs laboratory, DNA was extracted from the Sterivex^{TM} filters using the PowerWater Sterivex^{TM} DNA Isolation Kit (Mo Bio Laboratories, Inc.). PCR amplification of target DNA used Applied Biosystems QuantStudio 3 Real-Time PCR System. Platinum^{TM} Taq DNA Polymerase (Thermo Fisher Scientific) was used as a high quality amplification primer for metabarcoding. After PCR amplification, the Illumina MiSeq platform was used for high-throughput sequencing which can sequence multiple samples simultaneously to provide comprehensive data on the species present in the environmental samples.

Finally, the Artemis R-package was used to model the eDNA concentration data in the Genidaqs eDNA metabarcoding process to enhance the analysis and interpretation of eDNA data to deliver an accurate analysis of the species present in the samples. Genidaqs utilizes the Artemis R package as a specialized statistical tool within their eDNA metabarcoding workflow to model eDNA concentration data. By implementing Bayesian latent variable models that properly account for censored qPCR data, Genidaqs enhances the accuracy and reliability of their species detection analyses. This approach represents a significant advancement over traditional statistical methods for analyzing eDNA survey data, allowing for more precise environmental monitoring and biodiversity assessment[9]. The lab tested each sample three times, creating three technical replicates per sample. The results from each technical replicate were aggregated to ensure that the species read were accurate and consistent within each sample. Specifically, the samples were tested for both vertebrates and invertebrates using the following primers:

- MiFish Universal Vertebrates: 12S rRNA gene (163 - 185bp)
- GIQHerp Frogs/Toads Vertebrates (modified version of MiFish-U-F designed to enhance amplification of amphibian 12S sequences when paired with the standard MiFish-U reverse primer): 12S rRNA gene (170bp)
- Crust16S Universal Invertebrates: 16S rRNA gene (170bp)
- PrieVen_Mussel Universal Invertebrates: 16S rRNA gene (200-310bp)
- Rigid criteria were followed when classifying detections:
- Detected DNA sequences that were 95% identical to reference sequences were considered a positive (+) detection.

- A detected sequence that was 97% identical to a single reference sequence was considered a positive (+) match to that species.
- Field, DNA extraction, and PCR no-template controls were evaluated following Djurhuus et al. protocols[10].
- Results were combined into one table documenting eDNA detections.

Data Analysis, Background Research and Results

Background Research

eDNA is a novel tool used to survey populations of organisms without having to see or capture individual specimens. It uses DNA left behind by organisms as they move through the environment to identify species and their general location. eDNA is especially useful for large population studies, as the traditional method of trying to capture and/or see each species in a certain area is highly time-consuming[11].

Invasive species are nonnative species that do some form of damage to the environment[12]. It is important to find these species and mitigate their harms to protect the environment, as it is usually very hard to reverse the damage after an invasive species has gained a foothold. The best way to prevent an invasive species from doing damage is to prevent them from entering the environment in the first place, and it is essential that people are aware of the damage that invasive species can cause[13].

Analysis

Results at Location 1, the North Branch of the Chicago River

Sample 1A displayed only three organisms, as shown in Table 2. Sample 1B contained 15 species, as shown in Table 2. There were two, potentially three, invasive species in this location: species of the Carp genus, species of the Crucian Carp/Goldfish genus, and potentially White Perch. However, White Perch DNA in the location surveyed is nearly identical to Yellow Bass, a native species. Additionally, White Perch have been known to hybridize with Yellow Bass[14].

The eDNA analysis is unable to identify the difference between Crucian Carp and Goldfish. However, it is highly likely that the identified species is Goldfish, rather than Crucian Carp, as Crucian Carp have not been detected in Illinois since 1910[15].

Results at Location 2, the West Fork of the North Branch of the Chicago River

Sample 2A displayed 14 species, as shown below. Sample 2B displayed 16 species, as shown below. In sample 2A, there were two invasive/nonnative species, Atlantic Salmon and Carp. In sample 2B, there were Common Carp and Crucian Carp/Goldfish. Atlantic Salmon are not truly invasive, as they are stocked each year in Lake Michigan and cannot naturally reproduce there, but they are still nonnative and it is essential to make sure they do not harm the environment[4].

Results at Location 3, the Skokie River

Sample 3A displayed 17 species, as shown in Table 2. Sample 3B also displayed 17 species, as shown in Table 2. In both sample 3A and 3B, there were two invasive species present, the Crucian Carp/Goldfish and the Common Carp genus.

Results at Location 4, the Des Plaines River

Sample 4 displayed 24 species, as shown in Table 2. There were three invasive species at this location, the Rusty Crayfish, the Round Goby, and the Common Carp.

Data

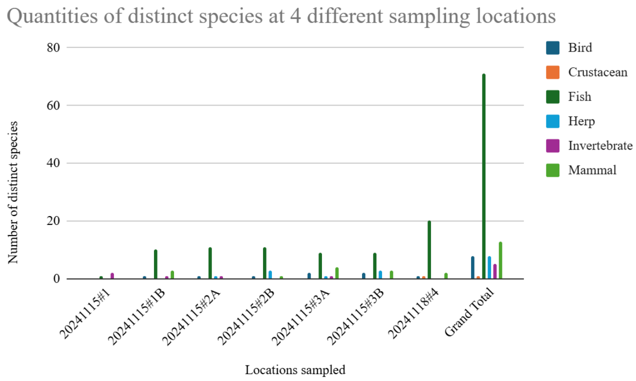
	Sample Name							
Common Name		20241115#1	20241115#1B	20241115#2A	20241115#2B	20241115#3A	20241115#3B	20241118#4	Grand Total
									0
American Bullfrog				1	1	1	1		4
American Robin						1			1
American Toad					1		1		2
Atlantic Salmon				1					1
Black Crappie					1				1
Black/Brown Bullhead							1	1	2
Bluegill Sunfish		1	1	1	1	1	1	1	7
Bluntnose Minnow								1	1
Common Carp			1	1	1	1	1	1	6
Creek Chub			1					1	2
Crucian Carp (Goldfish)			1		1	1	1		4
Eastern Chipmunk						1			1
Eastern Cottontail						1		1	2
Eastern Gray Squirrel							1		1
Fathead Minnow								1	1
Flatheaded Mayfly		1							1
Gizzard Shad			1	1	1	1			4
Golden Shiner				1		1			2
Green Frog					1		1		2
Green Sunfish			1	1	1	1	1	1	6
Hornyhead Chub								1	1
Johnny Darter								1	1
Largemouth Bass			1	1	1	1	1	1	6
Mallard			1	1	1	1	1	1	6
Mayfly (Caenis genus)		1	1						2
Mosquitofish				1	1				2
Muskrat			1		1	1	1	1	5
Non-biting Midge (Chironomus genus)				1					1
Northern Pike								1	1
Rock Bass								1	1
Round Goby								1	1
Rusty Crayfish								1	1
Smallmouth Bass							1	1	2
Spotfin Shiner								1	1
Spotted Sucker								1	1
Springtail						1			1
Tadpole Madtom								1	1
Topminnow/Killifish				1	1			1	3
Voles			1						1
White Sucker			1	1	1	1	1	1	6
White-Tailed Deer			1			1	1		3
Wood Duck							1		1
Yellow Bass/White Perch			1						1
Yellow Bullhead			1	1	1	1	1	1	6
Grand Total	**0**	**3**	**15**	**14**	**16**	**17**	**17**	**24**	**106**

This table, Table 2, depicts the distribution of different organisms between the different locations. Sample 1A and Sample 1B were sampled at the North Branch of the Chicago River, Sample 2A and Sample 2B were sampled at the West Fork of the North Branch of the Chicago River, Sample 3A and Sample 3B were sampled at the Skokie River, Sample 4 was sampled at the Des Plaines River.

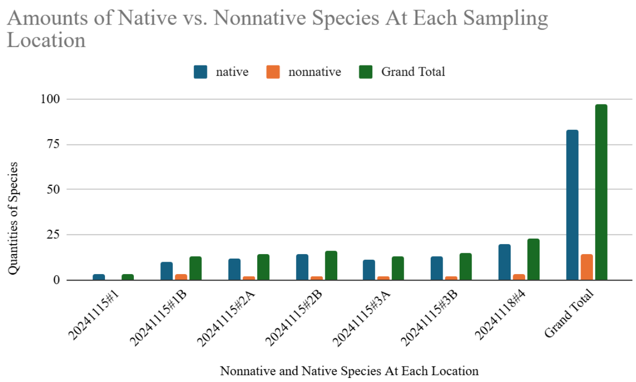
Discussion

Key Findings

Overview of Data



This graph, Figure 1, depicts the distribution of species at different locations by their animal class.



This graph, Figure 2, depicts the quantity of native and nonnative species at each location.

The most prevalent invasive species detected was the Common Carp, which showed up at six of the seven sampling locations, closely followed by the Crucian Carp/Goldfish, which showed up at four of the seven sampling locations. Other invasive species that showed up were Round Goby, Rusty Crayfish, and potentially White Perch. The Atlantic Salmon are nonnative, but not invasive, as they are stocked in the river each year and cannot establish a breeding population[4]. All of these species are incredibly harmful to the environments that they invade, with their impacts varying depending on their aggression level and the ease at which they establish a population.

Common Carp

Common Carp came to North America in the early 1830s, stocked in the Great Lakes to supply commercial fisheries[16]. At this time, the damage done by invasive species was not well known, and Carp were able to effectively invade waterways without any opposition. Common Carp are harmful because they are aggressive feeders, meaning that they often uproot the plants they are feeding on and cloud the water with their movements by churning up silt from the riverbed. This destroys the ability for other organisms to hide from predators in plants, and makes it far more difficult for native species to find food within the clouded water. Additionally, they are extremely hardy, so once they establish a population it is very difficult to remove them[16]. Efforts are in place to reduce the populations of Common Carp in the waterways of North America, including through continued commercial fishing, trapping juveniles, and helping increase predation[17]. However, as the research shows, it is very difficult to remove an invasive species that has already established its population in a specific location. Carp are still prevalent in all four of the waterways that were surveyed in this study, therefore showing the need to improve current efforts to remove existing Carp populations.

Crucian Carp/Goldfish

The Goldfish is a common household pet, usually sold as a first pet for children or won at fairs. Though they start out small, they can grow up to 16 inches long[13]. This often causes overwhelmed and unprepared owners to try to give up their oversized pets, some of whom dispose of them in local rivers. This is a huge problem, as goldfish are highly effective eaters, like most carp, and can decimate the plant life of a vulnerable ecosystem[1]. Rather than throwing their pets into lakes or rivers when they get too large to handle, individuals should try to give them to others who are willing to take in large fish or to their local fish store.

Goldfish may also enter ecosystems as bait for fishing or by being used in fisheries and then escaping or being released. Due to their hardy nature, these fish can easily establish populations and damage the surrounding ecosystem[16].

The Crucian Carp is nearly identical to Goldfish, though distinct because it is larger with more rounded fins. Both the Crucian Carp and Goldfish are part of the Carassius genus, and can hybridize with one another and Common Carp[18].

Both of these species are incredibly damaging to the ecosystems they invade, and more efforts must be put in place to educate people about the damage that releasing a nonnative aquatic pet can cause. Additionally, more must be done to reduce these populations and prevent their spread, as the only true way to stop an invasive species from doing harm is to prevent them from invading in the first place.

Round Goby

The Round Goby are a species of small benthic fish that are initially native to the Black and Caspian Seas. They were initially introduced into the Great Lakes region through irresponsible dumping of ballast water from ships, causing eggs or juvenile individuals to enter the waterways[19]. Round Gobies have a variety of advantages that make them very successful invasive species. They are generally very hardy, reproduce rapidly, and will feed on invertebrates as well as small fish. Their hardiness allows them to easily spread through many different ecosystems and have been found in all five of the Great Lakes. In addition, they have spread to rivers feeding into or out of the Great Lakes, including the Des Plaines River. Their rapid reproduction rates allow for rapid population establishment and make it quite difficult to remove a population of Round Goby once they establish themselves. Their feeding patterns also make them a very effective invasive species, as their finding food sources is not difficult. Along with feeding on invertebrates, they also eat the eggs and young of local fish species, diminishing their populations as the Round Goby population grows. Ultimately, Round Goby are an exceptionally effective invasive species, and it is important to stop their spread. They greatly impact the population of native fish by eating their eggs and young, and can rapidly spread through many different ecosystems.

The existence of Round Goby specifically in the Des Plaines river, as shown in this study's data, is supported by a study conducted by the Midwest Biodiversity Institute (MBI) between 2016 and 2022[2]. This study demonstrates the efficacy of eDNA analysis, as it has taken accurate measurements of which species are located in a specific area, as well as demonstrating that more must be done to reduce the populations of Round Goby and mitigate their environmental harm. It is particularly important to focus on the Round Goby, as the public is not as aware of their harmful effects compared to other fish.

Rusty Crayfish

Rusty Crayfish are a species of freshwater crustacean native to the Ohio River Basin. They have spread to various parts of North America, including the Great Lakes region, through human activities such as bait bucket releases and the aquarium trade[20]. Though Rusty Crayfish are not particularly adept at surviving in a wide variety of environments, preferring bodies of water with many hiding places to evade predators and requiring clear water replete with oxygen, they can survive in a wide range of temperatures, and are opportunistic omnivores, eating anything from aquatic plants to the eggs of other fish. They are exceptionally effective at establishing populations, as the females can carry sperm from an earlier encounter with a male and then lay up to 575 eggs (though many young do not survive). However, the primary reason that Rusty Crayfish are effective invasive species is their aggression. They are very territorial, and are able to rapidly colonize an entire body of water to as deep as 12 meters. Through doing this, they can outcompete native crayfish, leading to population damage. This also means that it is exceptionally difficult to remove already-established populations, as the Rusty Crayfish have likely rapidly colonized a large portion of the body of water in which they were introduced[20,21]. Rusty Crayfish are highly aggressive and can outcompete native crayfish species, leading to significant ecological disruptions. Their presence in new water bodies can result in the destruction of aquatic plant beds, displacement of native crayfish, and changes in fish populations[20].

The lack of evidence for Rusty Crayfish in the other three sample locations suggests that there may still be time to prevent their invasion into other waterways in Northern Illinois, preventing them from doing damage. There may also still be time to prevent them from moving farther north in the Des Plaines River. It is essential to raise public awareness about this issue and to implement strategies to prevent Rusty Crayfish from spreading.

White Perch

White Perch are a small, gray fish that entered the Great Lakes in the early 1950s due to unusually warm weather during that period of time[19]. They are opportunistic feeders, and are particularly harmful because they often eat the eggs of other fish. They are also known to hybridize with Yellow Bass, which are native to Northern Illinois.

It is essential to prevent their spread, because their competition with other fish, as well as their eating of fish eggs, harms local fish populations. Effective methods of doing this would be to raise public awareness and attempt to implement regulations to help prevent their spread.

Error Analysis

Limitations of eDNA

eDNA is an excellent tool for identifying species that are difficult to detect using traditional methods, especially those in small numbers that may have an outsized negative invasive impact[11]. eDNA detection, however, relies on quality water sampling, a minimum of 100ml collected at a depth that captures enough sediment to represent river-wide biodiversity. If the sample is taken improperly, as was the case for Sample 1A, as discussed in the Filtration Error section below, it will display an inaccurate survey of the rivers population.

Additionally, eDNA is unable to actually quantify the volume of each species that exists, as the quantity of trace DNA in the water may not be commensurate with the quantity of organisms located there. For example, a school of a specific type of fish may have just passed through, leaving more trace DNA than an equally prevalent species.

Filtration Errors

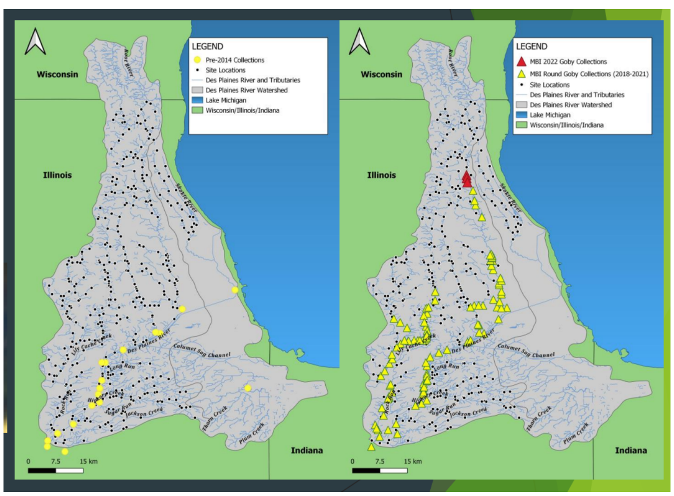
The first non-control sample, Sample 1A, was not filtered for the correct duration of time or volume. The scientist stopped filtering before enough sediment was gathered. This error caused less DNA to be caught in the filter, resulting in fewer species detected during eDNA sequencing. This error was corrected for samples 1B - 4 by filtering a larger volume of water and visually monitoring the sediment levels in each filter.

Implications of Findings

The fact that Common Carp were detected at almost all of the sampling locations, despite there being many efforts in place to mitigate their damage, indicates that a new mitigation approach is necessary. Theoretically, if these efforts had been successful, the eDNA survey would not have found Common Carp in every location. Therefore, it is clear that organizations that intend to stop Common Carp must redouble their efforts and continue to innovate on their approach.

Regarding species detection, eDNA surveying may be the new strategy that local and governmental organizations need. The alignment of eDNA results with 2016 - 2022 trawling data for Round Goby from the Midwest Biodiversity Institute[22] strengthens its credibility, while also indicating that it can be an improvement to earlier survey methods. It also does not impact organisms in any way, meaning that its efficacy is paired with the fact that it has no known negative effects on the ecosystems sampled.

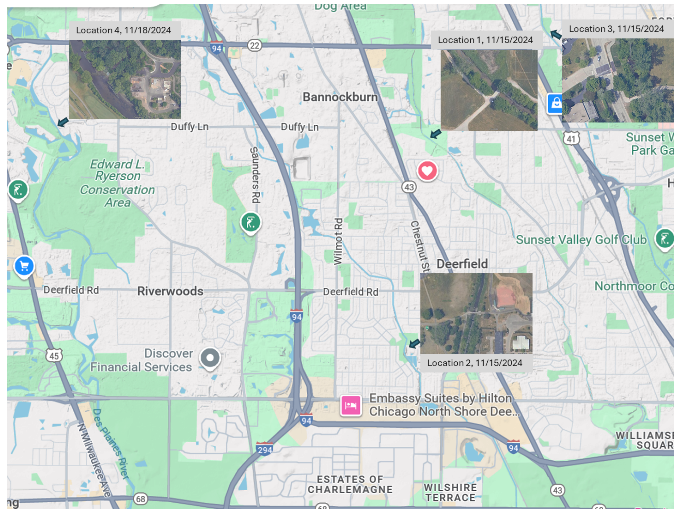
The Midwest Biodiversity Institute has tracked Round Goby spread since 2016. The below map, Map 2, shows that Round Gobies have spread from Central Illinois, Northeast towards Wisconsin in the Des Plaines River, but have not yet spread into the Chicago or Skokie Rivers. The red triangles depict the most recent Goby sample collections by the MBI from 2022 near to where the Sample 4 samples were taken.



Map 2: 2018 - 2022 results from the MBI study, showing the spread of Round Goby with the red triangles representing its most recent Round Goby collections.[2].

eDNA is a less invasive, time consuming, resource intensive, and more effective, method than traditional methods such as trawling, electrofishing and line sampling to identify invasive species in Northern Illinois waterways. It can be used to both identify when new invasive species have entered the environment (so their harmful behavior can be quickly mitigated) and determine whether efforts to remove an invasive species from a certain location have been successful.

It is also clear that rapid intervention is necessary regarding the Round Goby and the Rusty Crayfish. Both of these species



Map 1: eDNA sampling locations coinciding with the MBI trawling and electrofishing efforts. While MBI personnel spent days at each site trawling using backpacks, tote barges (wading), and boats, as well as raft electrofishing that damaged ecosystems and harmed creatures with electricity, my eDNA sampling using a water pump and Sterivex filter had nearly zero impact on the environment both in and outside of the water, yet still returned the same results.

Image 1: 2022 MBI surveys on the Des Plaines River.[2].

are known to be highly aggressive, destabilizing the populations of native wildlife while they spread. Additionally, they are able to rapidly reproduce, allowing them to quickly gain a foothold and grow their populations. However, according to this study, these species only exist in the Des Plaines River. This means that it may be possible to either stop their spread completely or reduce their potential harm, by using modern strategies such as targeted trapping and disruption of their spawning grounds[23].

The prevalence of goldfish, likely originating from either aquarium releases or use as bait, emphasizes the need for increased public education. Over 68% of Illinois aquarium owners remain unaware of invasion risks[4], signaling the need for outreach campaigns to curb pet disposal in waterways.

Similarly, their presence, as well as the presence of Rusty Crayfish, indicates the necessity for legal regulation of what can and cannot be used as bait. Both of these organisms are commonly used as fishing bait, fishermen may release them into waterways after they are done fishing, or even accidentally allow their bait to escape. These individuals do not know the damage that they cause, and education alone may not be enough to prevent this type of ecological invasion from happening. Therefore, policy change is necessary.

Methodologically, the successful implementation of Genidaqs' contamination protocols including bleach sterilization and negative controls provides an approach that is easily replicable for large-scale eDNA monitoring. However, limitations in quantifying populations or distinguishing life stages necessitate hybrid approaches combining eDNA with targeted trapping for comprehensive assessments.

Conclusion

This study used eDNA to identify different invasive species in the waterways of Northern Illinois, and its findings underscored the need to prevent the spread of invasive species through social awareness, regulatory guidelines, and control measures. The invasive species found in this survey are harmful to the environment, and it is essential that their damage be quickly mitigated.

In particular, Round Goby and Rusty Crayfish are a major threat to the waterways of Northern Illinois, though they have not yet proliferated the area in the same numbers as Carp. This means that, with rapid and strategic action, these species could be prevented from moving further and doing more damage to local populations of organisms.

This study also demonstrated the usefulness of eDNA as a survey method, allowing for quicker and more accurate results than a study conducted through visual or species-collection means. Specifically, eDNA can identify rare, or elusive, organisms (or those who are highly skittish) better than other types of studies[11]. Overall, this study was able to find invasive species in four waterways in Northern Illinois. It is essential to use this information to take action to prevent their spread, and to mitigate the damage caused by species that have already invaded these waterways.

Closing Thoughts

These results were found through one eDNA survey of four locations, and were nevertheless highly informative towards efforts to protect the environment against invasive species. If this survey was implemented across a much larger sampling location, then it would certainly provide far more information on the biodiversity of the environment, and hopefully help make environmental protection efforts far more effective.

Acknowledgements

I would like to express my sincere thanks to the following people for their invaluable support and contributions:

Deerfield High School

Principal Kathryn Anderson, for fostering an environment that encourages scientific inquiry, and the DHS Science Department including Ms. Jennifer Boller, Ms. Stephanie Chambers, and Mr. John Motzko.

Mentors

Dr. April Blakeslee, for providing connections throughout this entire process, and Dr. Mercer Brugler for teaching me about eDNA sampling and Metabarcoding in the University of South Carolina Beaufort Marine Sciences Lab and the entire Tidal Marsh Task Force.

Research Partner

GENIDAQS Laboratory, for providing access to essential equipment and resources.

Gregg Schumer, Vice President at GENIDAQS Laboratory for his technical assistance, and valuable input.

All of my Invasive Insights Podcast Guests for inspiring me to pursue my curiosity

`www.invasiveinsights.com`

Family

My parents, for their unwavering support and encouragement of my scientific pursuits; my grandparents, for instilling in me a deep curiosity about the natural world; Sloane, my sister, who inspires me every day with her own positivity and creativity.

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