

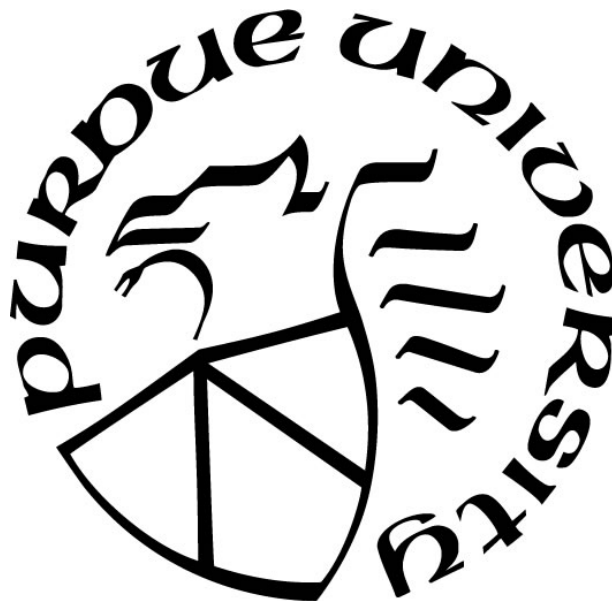
**APPLICATION AND VALIDATION OF THE EDNA-METABARCODED
MIFISH/MITOFISH PIPELINE FOR ASSESSMENT OF NATIVE AND
NON-NATIVE FISH COMMUNITIES OF LAKE MICHIGAN**

by
Samantha Jurecki

A Thesis

*Submitted to the Faculty of Purdue University
In Partial Fulfillment of the Requirements for the degree of*

Master of Science



Department of Biological Sciences
Hammond, Indiana
May 2020

THE PURDUE UNIVERSITY GRADUATE SCHOOL
STATEMENT OF COMMITTEE APPROVAL

Dr. Scott Thomas Bates, Chair

Department of Biological Sciences

Dr. Robin W. Scribailo

Department of Biological Sciences

Dr. Lindsay Gielda

Department of Biological Sciences

Dr. Muruleedhara Byappanahalli

U.S. Geological Survey Great Lakes Science Center

Meredith Nevers

U.S. Geological Survey Great Lakes Science Center

Approved by:

Dr. Scott Thomas Bates

ACKNOWLEDGMENTS

I thank Scott Bates, Meredith Nevers, Murulee Byappanahalli, Ashley Spoljaric, Wendy Banta-Long, Crystal Hall, Kasia Kelly, Dawn Shively, Rex Blackwell, and various other USGS personnel for their assistance with field sampling and laboratory processing. I also thank P. Ryan Jackson (USGS), Matt Shanks (US Army Corps of Engineers), and Robin Scribailo (Purdue University Northwest), for their assistance with field sampling and access to electrofishing and seining data. The Bass Pro Shops of Portage, Indiana provided access to their on-site freshwater fish tank, with Assistant General Manager Chris Tackace helping arrange that visit. I appreciate Leslie Dorworth's guidance and her role in facilitating funding through the Illinois-Indiana Sea Grant (grant no. NA14OAR4170095). The USGS and Purdue University Northwest's Department of Biological Sciences provided additional funding. Finally, I thank Aaron Aunins at the USGS Leetown Science Center for technical consultations and DNA sequencing of samples with the MiFish primers. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

TABLE OF CONTENTS

LIST OF TABLES	6
LIST OF FIGURES	7
ABSTRACT	9
CHAPTER 1. INTRODUCTION	10
1.1 The Jeorse Park Beach Study Site	10
1.2 Invasive Species	12
1.2.1 The Round Goby	13
1.3 Environmental DNA	13
1.4 eDNA Paired with High-Throughput Sequencing	14
1.5 Efficient Sampling and eDNA Extraction	15
1.6 MiFish Primers	16
1.7 Study Objectives	17
CHAPTER 2. MATERIALS AND METHODS	18
2.1 Baseline Biomonitoring Study at Jeorse Park Beach	18
2.2 Validation Study for the Traditional Survey at Jeorse Park Beach	19
2.3 Validation Study for the Native Fish Tank at Bass Pro Shop	20
2.4 eDNA Extraction Efficiency	21
2.5 Water Sample Collection and Processing for the <u>Baseling Biomonitoring Study</u>	22
2.6 Water Sample Collection and Processing for the <u>Validation Studies</u>	23
2.7 eDNA Extraction from Samples	23
2.8 MiFish Primer Testing on eDNA from Samples	26
2.9 Mitochondrial 12S Gene Metabarcoded Sequencing	27
2.10 Bioinformatic Processing and Statistical Analyses	28
2.11 Angler's Survey	29
CHAPTER 3. RESULTS	31
3.1 eDNA Extraction Efficiency	31
3.2 MiFish Primer Testing	33
3.3 eDNA-Metabarcoding and Taxonomic Assignments	35
3.4 Validation Studies	36

3.4.1 Bass Pro Native Species Tank Comparison.....	36
3.4.2 Traditional Surveys Comparison	38
3.5 Baseline Biomonitoring Study at Jeorse Park Beach.....	39
3.6 Angler’s Survey	43
CHAPTER 4. DISCUSSION.....	45
4.1 Protocol Development	45
4.1.1 Validation of eDNA-Metabarcoding for Biomonitoring.....	45
4.1.1.1 Closed Freshwater Tank	45
4.1.1.2 Traditional Monitoring Methods	47
4.2 Baseling Biomonitoring at Jeorse Park Beach using eDNA-Metabarcoding	48
CHAPTER 5. CONCLUSION.....	50
REFERENCES	52
APPENDIX.....	61

LIST OF TABLES

Table 1. Fish species found within 1 mile of Jeorse Park Beach from 2000–2014.....	12
Table 2. Jeorse Park Beach artificial reef coordinates	12
Table 3. The eDNA concentrations (ng/μL) and purity [i.e., the ratio of absorbance at 260 nm (DNA) and 280 nm (protein)] of all 74 study samples	24
Table 4. DNA concentrations and purity for samples extracted in the Extraction Efficiency study.	31
Table 5. Set up and results of an electrophoretic gel to confirm efficiency results, with symbols indicating mitochondrial 12S rRNA gene PCR amplicons were (+) or were not (-) visualized (and see Figure 6 below).	31
Table 6. Set up and results of an electrophoretic gel to confirm PCR amplification of Bass Pro eDNA samples with the MiFish primers, with symbols indicating mitochondrial 12S rRNA gene amplicons were (+) or were not (-) visualized (and see Figure 8 below).	34
Table 7. The community composition of fish species that where native to Lake Michigan in the Bass Pro freshwater fish tank at the time of sampling (12 August 2018).....	37
Table 8. Validation study conducted at Jeorse Park Beach in the comparison with traditional surveys using seining (left) and electrofishing (right). For each methods the results shown reflect the actual number species detected in the traditional survey “Counts” and the number of OTU sequences representing fish species detected by our eDNA “Metabarcoding” approach. Areas of the table shaded in gray highlight fish species that were detected under both methods.....	39
Table 9. Baseline biomonitoring study conducted at Jeorse Park Beach. The number of OTU sequences representing fish species detected by our eDNA “Metabarcoding” approach across all sites over the entire summer 2018 sampling period. Native versus non-native status is indicated as is the historic record showing fish previously documented at Jeorse Park Beach or in the southern Lake Michigan area, follow data from United States Army Corps of Engineers (2016).	40
Table 10. Average site alpha diversity values across the entire summer 2018 sampling period for richness and Shannon Index, with standard deviation (SD) indicated.....	40
Table 11. The compiled responses of the Angler's survey given on July 6 and July 20.	44

LIST OF FIGURES

Figure 1. An outline of protocol for an eDNA-metabarcoding approach to monitoring native and invasive fish species in Lake Michigan water samples. High-Throughput sequences from sample eDNA were processed using a modified MiFish/MitoFish metabarcode bioinformatic pipeline to provide data for subsequent analyses.....	17
Figure 2. Lake Michigan freshwater sampling sites at Jearse Park Beach used in our studies. Sampling sites 1 and 2 represent the nearshore “Beach” locations; site 3 represents the modified “Breakwall” location; and sites 4 and 5 represent open waters above the installed artificial reef location (“Reef Surface” and “Reef Depth”, respectively).....	19
Figure 3. Electrofishing surveying methods employed nets used to catch the fish that are momentarily stunned by the electrical current (electroshock). After capture each fish collected was identified, counted, and then released back into the water.	20
Figure 4. Sampling to validate our eDNA-metabarcoding approach. A) Gathering samples from the freshwater tank for eDNA extraction; B) Store location housing the enormous tank; C) The known native Lake Michigan fish species inhabiting the tank.	21
Figure 5. Angler's Survey created to better understand the awareness of invasive species, such as the round goby, within the local fishing community.	30
Figure 6. Bands in wells 2–7 indicate PCR amplification of the mitochondrial 12S rRNA gene from eDNA samples treated with proteinase K in extraction relative to samples in wells 8–13 that were not treated with proteinase K during extraction.	33
Figure 7. Electrophoreses gel indicate PCR amplification of the mitochondrial 12S rRNA gene using the MiFish primers in in 58 out of 74 eDNA samples from the study site, and suggesting that a DNA cleanup process might be required for all eDNA samples prior to HTS.....	34
Figure 8. The MiFish primers testing results, showing amplification of Bass Pro eDNA samples.	35
Figure 9. Rarefaction curves for OTUs recovered from Sites 1–5 in the Baseline Biomonitoring study across the sampling months (indicated June, July, and August for e.g., Site 1 labeled as 1.1, 1.2, and 1.3, respectively) and the Validation study for comparison with the traditional survey methods employed at the Jearse Park Beach as well (labeled as “Electro” 1–3).	36
Figure 10. Bar graph showing relative abundances of fish genera detected in the validation studies. A) Genera of known native Lake Michigan fish inhabiting the freshwater tank; B) Genera of fish detected in the freshwater tank using our eDNA-metabarcoding methods.	37
Figure 11. Boxplot showing Shannon Index value means A) across sites for the different months sampled, and B) across month for the different sites sampled. Boxes sharing the same letter are not significantly different according to THSD test.....	41
Figure 12. Bar graph showing relative abundances of fish genera detected in the Biomonitoring Study at Jearse Park Beach over the course of the summer 2018 sampling period.....	42

Figure 13. Nonmetric multidimensional scaling ordination plots showing similarity of fish communities detected using eDNA-metabarcoding methods in the Biomonitoring Study at Jeorse Park Beach. A) No significant clustering according to sampling month (ANOSIM $p = 0.24$); B) Highly significant clustering according to general area where samples were taken (ANOSIM $p = 0.001$). 43

ABSTRACT

Environmental DNA (eDNA) is being used increasingly for biomonitoring of communities (e.g., microbes, macroinvertebrates, fish species) across terrestrial and aquatic ecosystems. Developing methods that combine eDNA approaches with metagenomic barcoded amplicon sequencing (eDNA-metabarcoding) are now providing a powerful noninvasive and cost-effective means for comprehensively surveying biodiversity in a wide range of habitats. Invasive species have a substantial impact on the ecology and economics of the Great Lakes region, and eDNA-metabarcoding methods have recently been applied in monitoring non-native, as well as native, fish populations in the freshwater systems there. In this research, we validated an eDNA-metabarcoding approach that uses established platforms, the MiFish/MitoFish pipeline, for fish community monitoring on Lake Michigan. For validation, we compared survey results from our eDNA-metabarcoding approach to those obtained using traditional surveys (e.g., electrofishing and seining). We also sampled a closed 180,000-gallon freshwater fish tank system to see how well our methods characterized a known native fish population that resided in the tank. Finally, we applied the approach to monitoring invasive and native fish populations in southern Lake Michigan at a site that is currently undergoing restoration to improve the aquatic habitats.. We were able to reliably capture the fish community structure of the native fish tank as well as those of open waters on the lake using our methods. Diversity patterns detected at the restoration site using our eDNA-metabarcoding approach accurately reflected those of the historical record, which have taken many years to establish by conventional means. Overall, this study suggests eDNA-metabarcoding is an efficient, credible, and powerful approach to biomonitoring.

CHAPTER 1. INTRODUCTION

In this study we validate a modern DNA-based approach for use in surveying fish of the Great Lakes, testing the potential of the method to detect known native populations in a closed tank system as well as in detecting native and non-native fish species that were also recovered in traditional surveying methods at our study site, Jearse Park Beach on Lake Michigan. After validation, we then applied the approach to establish baselines of the freshwater fish community composition at Jearse Park Beach, a site that has been targeted for restoration. This site includes the installation of a breakwall and artificial reef to influence the local fish populations, and we were especially interested in the impact these feature have on reestablishing native fish communities in relation to the non-native round goby populations that were known to inhabit the waters of Jearse Park Beach. Additionally, we also developed methods for more efficiently collecting water samples and extract environmental DNA (eDNA) for use in an eDNA-metabarcoding surveying approach to monitor fish populations as a standalone tool and/or in conjunction with traditional with traditional surveys. Overall, the methods developed here can be applied in future biomonitoring in southern Lake Michigan, as well as more generally for natural resource monitoring and management programs on the Great Lakes and other freshwater systems.

1.1 The Jearse Park Beach Study Site

Jearse Park Beach is located on the southern shores of Lake Michigan near the city of East Chicago, IN in the Midwestern United States. In the Jearse Park Beach Restoration Study (U.S. Army Corps of Engineers, 2015), the Chicago District of the US Army Corp of Engineers (USACE) assessed the ecological conditions of the site and developed plans to restore important migratory bird, fish and wildlife habitat within Jearse Park Beach, in coordination with other ongoing efforts at Jearse Park Beach and adjacent beaches to restore lost beneficial uses of these shorelines (e.g., bathing water quality) initiated under the Grand Calumet River Area of Concern restoration programs (Byappanahalli et al., 2015; Nevers et al., 2018). At the time of this assessment, the once-thriving natural dune habitat along the southern rim of Lake Michigan has become highly urbanized, which results in heavily modified shorelines that are populated with a mixture of industrial, commercial, and residential areas. As a result, habitat diversity in the near

shore areas of the lake has been in decline, with reduced fish spawning and foraging habitats (U.S. Army Corps of Engineers, 2015). In-lake structures in have also altered the water circulation, trapping algae (*Cladophora*) and debris, which decreases nutrient cycling and the general water quality (U.S. Army Corps of Engineers, 2015). Jeorse Park Beach also experienced high levels of *Escherichia coli*, due to unnatural geomorphology, which created a depositional zone that trapped algal mats, bird feces, and human generated debris as well as bacterial contributions from nearshore sand/sediment (U.S. Army Corps of Engineers, 2015; Byappanahalli et al., 2015).

A total of 55 species of fish were recorded at the Jeorse Park Beach site in monitoring between 1878 and 2005 (U.S. Army Corps of Engineers, 2015). Recent surveys, from 2000 to 2014 within 1 mile of the project area, showed only 24 native species and 10 non-native species (Table 1). Reduced species richness is indicative of poor habitat resources, and the overall conditions that had developed at Jeorse Park Beach appeared to have negatively impacted the local fishery habitats and local fish population there. Also of concern was the occurrence of the non-native round goby (*Neogobius melanostomus*) in the local waters (see Table 1). Because historical records indicated the area was once dominated by reef and bedrock shoals, the city of East Chicago requested that the USACE Chicago District restore both the aquatic and coastal habitats at Jeorse Park Beach. The restoration project aimed to re-create the once diverse underwater landscape by creating rock reefs along the lakeshore with natural cobble. Accordingly, rock reef structures of 4–5 feet in height were installed in four cobble mound pockets, located 200–300 feet from the breakwater and at an approximate depth of 16 feet. The goal was to provide spawning, protective and foraging habitat for native species (U.S. Army Corps of Engineers, 2015). The coordinates of this artificial reef, which was installed at Jeorse Park Beach on July 19, 2017 can be found below in Table 2.

Table 1. Fish species found within 1 mile of Jeorse Park Beach from 2000–2014
(Source: U.S. Army Corps of Engineers 2015).

Common Name	Scientific Name
Rock Bass	<i>Ambloplites rupestris</i>
White Sucker	<i>Catostomus commersonii</i>
Common Carp	<i>Cyprinus carpio</i> *
Gizzard Shad	<i>Dorosoma cepedianum</i>
Banded Killifish	<i>Fundulus diaphanus</i>
Pumpkinseed	<i>Lepomis gibbosus</i>
Bluegill	<i>Lepomis macrochirus</i>
Smallmouth Bass	<i>Micropterus dolomieu</i>
Largemouth Bass	<i>Micropterus salmoides</i>
Round Goby	<i>Neogobius melanostomus</i> *
Rainbow Trout	<i>Oncorhynchus mykiss</i> *
Bluntnose Minnow	<i>Pimephales notatus</i>
Brown Trout	<i>Salmo trutta</i> *
Lake Trout	<i>Salvelinus namaycush</i>
Walleye	<i>Sander vitreus</i>

*non-native species

Table 2. Jeorse Park Beach artificial reef coordinates

Coordinates	Cobble Pile 1 (North)	Cobble Pile 2 (South)
Latitude (°)	41.650943	41.650503
Longitude (°)	-87.431566	-87.431275

1.2 Invasive Species

Invasive species substantially impact the ecology and economics of the United States each year, disrupting native communities and imperiling those that are already at risk (Pimentel et al., 2005). In the U.S. Great Lakes region, numerous invasive species are becoming established in the area, each with varying potentials for environmental and socio-economic impacts (Davidson et al., 2016). Non-native fish species of the Great Lakes, such as the aggressive round goby (*Neogobius melanostomus*), likewise negatively affect the freshwater habitat by competing for resources (e.g., space and food) (Kornis et al., 2012).

1.2.1 The Round Goby

The round goby (*N. melanostomus*) is an invasive fish originating from the Black Sea that has spread to occupy all five Great Lakes and many tributaries, being responsible for declines in native populations as well as outcompeting and preying upon eggs of native fish (French et al., 2001; Corkum et al., 2004; Bergstrom & Mensinger, 2011; Lauer et al., 2011). This invasive fish was first found in 1990 in the St. Clair River and had dispersed to all the Great Lakes and some of their surrounding waters by 1998. The round goby was likely introduced to these waters from the ballast water of ocean-going ships (Kornis et al., 2012). The round goby spawns multiple times per year, roughly every 3–4 weeks between the months of April and September (Charlebois et al., 1997). During this time of year spawning is cued by warmer water temperatures (9–26°C), and during prolonged periods of warm waters, gravid females and mating-colored males have been captured as late as November (Kornis et al., 2012). The round goby feed, hide, and spawn in hard substrata and are generally most abundant in rock habitats (Kornis et al., 2012). They compete with the native fish species, such as sculpin and darter, for food and habitat (Kornis et al., 2012). The round goby can alter local food webs and may even serve as a vector for avian botulism (Cooper et al., 2009; Madenjian et al., 2011). The diet of the round goby mainly consists of zebra mussels, smaller fish, and eggs (Charlebois, et al., 2001). Declines in native fish populations have been seen where round goby have established due to competition for resources (Kornis et al., 2012).

1.3 Environmental DNA

Nucleic acids have been recognized as a tool to detect organisms in the habitats where they live (Pace, 1997). DNA fragments shed by life forms into habitats, termed environmental DNA (eDNA, see Tsuji et al., 2019), have been used in polymerase chain reaction (PCR) and quantitative PCR (qPCR) approaches to provide data on the presence-absence (PCR) and presence-abundance (qPCR) of single fish species in different sample types, such as water (e.g., Balasingham et al., 2016) or sediments (e.g., Turner et al., 2015). The use of eDNA in natural resource monitoring may be preferable to traditional monitoring methods, such as seining, trawling, and electrofishing, considering these methods disrupt the natural community and can even lead to the death and destruction of individuals being monitored (Deiner et al., 2017). In addition to minimizing impacts,

integrating eDNA methods into monitoring programs can enhance their effectiveness as species can be detected at low densities (Ficetola et al., 2008; Spear et al., 2015), which may otherwise be missed using traditional detection methods. The method can also expand monitoring programs by conserving resources (e.g., personnel and financial) and through use in areas that are difficult to access using traditional methods. Moreover, DNA-based methods can remedy other problems associated with traditional techniques, such as the labor-intensive nature of surveys (Lim et al., 2016) and morphology-based identifications that can be error-prone (Bortolus, 2008).

1.4 eDNA Paired with High-Throughput Sequencing

High-throughput sequencing technology has accelerated research in many areas of biology, and metagenomic amplicon sequencing methods of “barcode” genes (metabarcoding) are now frequently used to survey biodiversity in aquatic (e.g., Shaw et al., 2016; Günther et al., 2018; Rivera et al., 2018) and terrestrial environments (e.g., Schmidt et al., 2013; Ramirez et al., 2014). As qPCR-based eDNA methods are generally focused on individual species or groups, which can be useful for detection of invasive or threatened species of interest, they do not comprehensively address biodiversity or whole-community dynamics. The use of eDNA-metabarcoding is an alternative way to survey macroorganisms over traditional and eDNA-qPCR approaches because eDNA-metabarcoding is noninvasive, cost-effective, robust, and more comprehensive (Deiner et al., 2017). Accordingly, eDNA-metabarcoding has now been applied in biomonitoring for a number of different groups of macroorganisms across a wide array of environments, including plant pollen in air (Kraaijeveld et al., 2015), arthropods visiting flowers (Thomsen & Sigsgaard, 2019), amphibians, invertebrates, and mammals in natural waterways (Thomsen et al., 2012a), as well as fish of marine (Yamamoto et al., 2017) and freshwater (Thomsen et al., 2012b) systems. While eDNA-qPCR methods have been developed to rapidly detect aquatic invasive species (Adrian-Kalchhauser et al., 2016; Nevers et al., 2018a; Thomas et al., 2019), eDNA-metabarcoding is just beginning to be used in monitoring species in and around important lacustrine systems in the United States, such as the Great Lakes (Klymus et al., 2017; Balasingham et al., 2017).

Metabarcoding is being proposed as a potential efficient method for resource managers to monitoring fish communities. A previous study found that eDNA-metabarcoding using only 1 L of water sample offered high detection rate (85%) of fish taxa when multiple capture methods

(minnow traps, long-line fishing, gill nets, cast nets, dip nets, drag nets, and electro-fishing) and eDNA-metabarcoding were compared to evaluate fish communities in backwaters (Fujii et al., 2019). In the same study, between 1 and 11 individual fish species caught using the seven capture methods were taxa that were not detected using eDNA. This could have been a result of low eDNA abundance. One such example, *Lethenteron*, is a species that inhabits the sediment or sand bed of the lakes, and an adequate amount of eDNA may not be available around the surface water (Fujii et al., 2019) resulting in a lack of detection by eDNA-metabarcoding. Overall, however, eDNA-metabarcoding was seen as an efficient method for biomonitoring (Fujii et al., 2019).

1.5 Efficient Sampling and eDNA Extraction

Filtration and extraction methods can significantly affect the overall yield of eDNA recovered from freshwater samples, with some approaches only recovering small amounts of highly degraded eDNA. DNA binds differently to filter papers dependent on the type of material used in the manufacturing process (Hinlo et al., 2017). Cellulose filters have a high DNA and protein binding capacity when compared to other filters, such as glass fiber (Towbin et al., 1979; Thornton et al., 1996). Different types of extraction kits used can also affect the eDNA yield, and they can use different methodologies in the extraction protocol. For example, the DNeasy extraction kit (Qiagen, Hilden, Germany) relies on a biochemical method to lyse cells, whereas the PowerWater extraction kit (Qiagen, Hilden, Germany) uses a mechanical bead beating method (Deiner et al., 2015). A previous study compared DNA yields resulting from different combinations of filter papers and extraction kits and showed that using a cellulose nitrate filter paper paired with the DNeasy extraction kit (Qiagen, Hilden, Germany) yielded the highest amount of eDNA (Hinlo et al., 2017). The DNeasy kit (Qiagen, Hilden, Germany) and PowerWater kit (Qiagen, Hilden, Germany) paired with glass fiber filters combination gave the lowest DNA yield and had the highest cost per sample, which is the most widely used extraction-filter paper combination in eDNA studies (Jerde et al., 2011; Olson et al., 2012). As eDNA yield is an important consideration for metabarcoding studies, we examined different filter paper and extraction kit combinations to determine which of these resulted in the highest eDNA yields. As there are no set protocols for extraction of eDNA from water samples using the kits we tested, we also worked with a Qiagen technical support representative to design two different methods used to test which approach yielded the highest eDNA concentration per sample.

1.6 MiFish Primers

Miya and colleagues (2015) developed the universal fish primers (MiFish-U/E), where a hypervariable region of the mitochondrial 12S rRNA gene can be amplified. Using a high-throughput next generation platform (Illumina MiSeq) in a eDNA-metabarcoding approach for detecting fish species, the study confirmed detection of 232 fish species distributed across 70 families and 152 genera from four aquarium tanks and coral reefs in saltwater communities (Miya et al., 2015). There was a higher detection rate for species (>93%) in aquaria (Miya et al., 2015). Using the MiFish primers and high-throughput sequencing, Yamamoto and colleagues (2016) investigated marine fish communities in Maizuru Bay, Japan, and they detected a total of 128 fish species in their water samples. Only 80 fish species were detected by 140 underwater visual censuses over a period of 14 years in the same study site (Yamamoto et al., 2016). These studies indicate the great potential of using eDNA with the MiFish primers in targeted mitochondrial 12S rRNA gene metabarcoding as a useful tool for biodiversity assessment compared to traditional methods; however, the MiFish primers had been primarily used in assessing saltwater fish communities.

We set out to validate this method for application in monitoring native and non-native fish species in southern Lake Michigan. In order to do this, we first used the MiFish primers in traditional PCR to see if we could recover amplicons, putatively representing fish species, from eDNA extracted using the protocol we developed on freshwater samples collected at the Jearse Park Beach study site. After confirming 12S amplification with the MiFish primers in our samples, we then worked with colleagues at the United States Geological Survey (USGS) Great Lakes Science Center and the Chicago District USACE to develop and test a MiFish eDNA-metabarcoding approach for surveying fish species. Our methods including detection of the invasive species, such as the round goby, in the waters of southern Lake Michigan so that they would be deemed appropriate for biomonitoring purposes at the Jearse Park Beach study site. Validation was carried out in both closed and open freshwater systems. In this work for comparison with the eDNA-metabarcoding approach, samples were also collected from a large freshwater fish tank at Bass Pro Shops in Portage, Indiana with a known population of fish species that were native to Lake Michigan (the closed freshwater system). To represent open freshwater systems for comparative purposes, samples were also taken at the Jearse Park Beach site in conjunction with traditional surveying that consisted of seining and electrofishing.

1.7 Study Objectives

Numerous studies have used eDNA-metabarcoding approaches to monitor fish communities in various water systems, including the Great Lakes (Klymus et al., 2017); however, there are no studies in the literature, to the best of our knowledge, that have applied this approach to monitor fish communities in Lake Michigan. The objectives of this study were to (1) develop an eDNA-metabarcoding approach (Figure 1) that can be used to effectively biomonitor the abundances of individual fish species in freshwater aquatic systems; (2) validate our methods by comparing eDNA-metabarcoding results with species populations recovered from samples taken at the same time and location as surveys using traditional monitoring methods (i.e., electrofishing, seining, and trawling) in open waterways of Lake Michigan as well as with samples taken from known populations of native Lake Michigan fish species in a freshwater closed system; and (3) use our methods for baseline monitoring of invasive and native fish communities at Jeorse Park Beach in Lake Michigan.



Figure 1. An outline of protocol for an eDNA-metabarcoding approach to monitoring native and invasive fish species in Lake Michigan water samples. High-Throughput sequences from sample eDNA were processed using a modified MiFish/MitoFish metabarcoding bioinformatic pipeline to provide data for subsequent analyses

CHAPTER 2. MATERIALS AND METHODS

2.1 Baseline Biomonitoring Study at Jeorse Park Beach

Jeorse Park Beach is positioned along the southern shoreline of Lake Michigan, within the U.S. Environmental Protection Agency (EPA) designated Grand Calumet River Area of Concern (United States Army Corps of Engineers, 2016). The beach is located southeast of Indiana Harbor and Ship Canal in East Chicago, IN, and is bounded to the north and south by shoreline casinos. Restoration efforts of approximately 12 hectares were initiated in 2013 to improve the aquatic and coastal habitats, and the project included plans to modify the existing artificial rock breakwall and install four offshore artificial rock reefs (United States Army Corps of Engineers, 2016). The work on these structures was carried out by the U.S. Army Corps of Engineers and completed in 2017, with the aim that they serve as improved aquatic habitat for the local native fish populations.

Five sampling sites from Jeorse Park Beach (Figure 2) were used in this study to establish fish community baselines and seasonal/spatial patterns for use in future biomonitoring. Sites 1 and 2 (beach sampling sites) were established by the Indiana Department of Environmental Management as bacteria sampling sites that USGS has used in the past. Site 1 (“Beach 1”; 41°38’53.38”N, 87°25’54.84”W) and Site 2 (“Beach 2”; 41°39’1.80”N, 87°26’0.64”W) are located in nearshore waters at an approximate depth of ~0.5 m. The remaining sites were newly established for our studies. Site 3 (“Breakwall”; 41°39’11.2”N, 87°25’55.76”W) is located on the harbor side of the installed breakwall. Site 4 (“Reef-Surface”; 41°39’3.39”N, 87°25’53.64”W) and Sites 4 and 5 (“Reef”; 41°39’3.39”N, 87°25’53.64W) are located above the installed artificial rock reef structures. Site 4 (“Reef Surface”) samples were gathered from the surface water and Site 5 (“Reef Depth”) samples were collected at an approximate depth of ~3 m, near the top of the submerged reefs. For biomonitoring, three sampling events were carried out at sites 1–5 in the Jeorse Park Beach study site (“Baseline Biomonitoring Study”) over the course of the 2018 summer: June 25, July 27, and August 27.

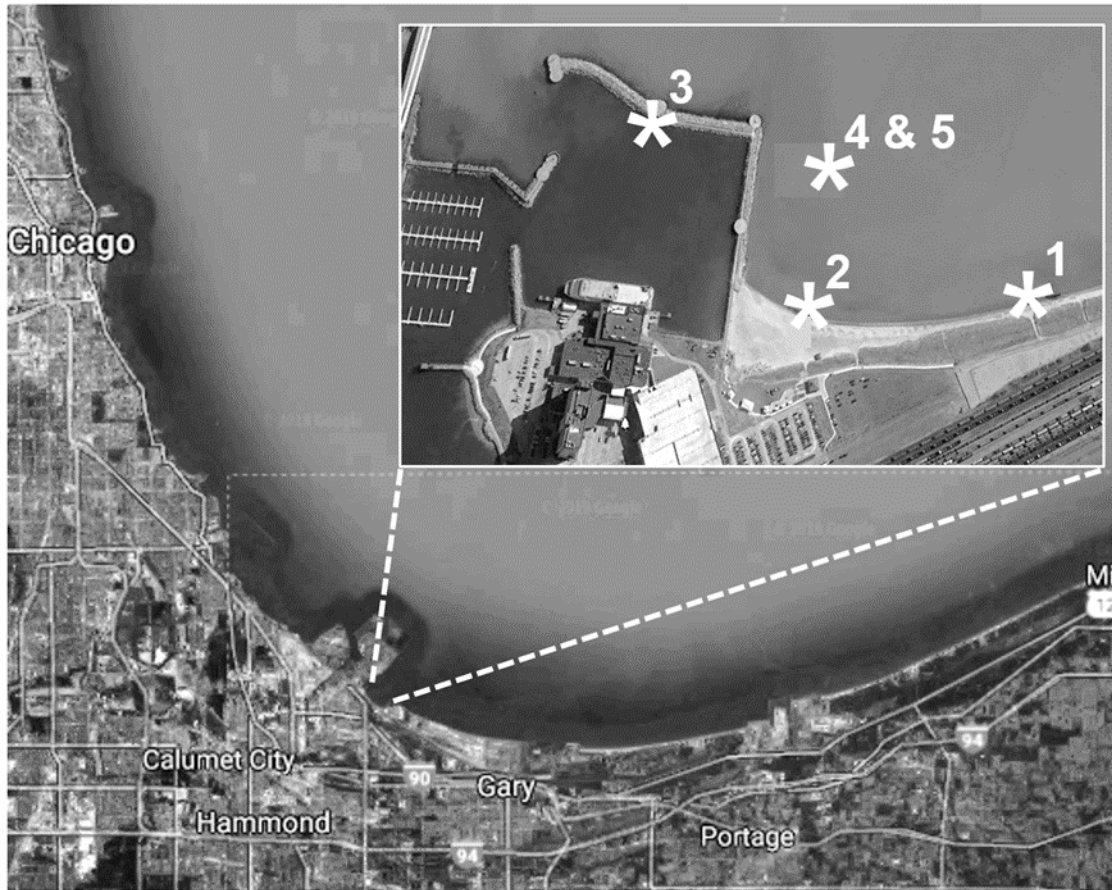


Figure 2. Lake Michigan freshwater sampling sites at Jearse Park Beach used in our studies. Sampling sites 1 and 2 represent the nearshore “Beach” locations; site 3 represents the modified “Breakwall” location; and sites 4 and 5 represent open waters above the installed artificial reef location (“Reef Surface” and “Reef Depth”, respectively).

2.2 Validation Study for the Traditional Survey at Jearse Park Beach

In order to validate our methods (“Validation Studies”), eDNA-metabarcoding results obtained using our methods were compared with data captured from traditional surveys at Jearse Park Beach and on the open waters of Lake Michigan. These data were collected over the course of the 2018 summer, with water samples for eDNA extraction taken concurrently (in triplicate) with the traditional survey. The traditional survey methods included fish seining, electrofishing (Figure 3), and trawling. The seining survey was comprised of four seine netting events from the shore at Site 2. The electrofishing survey at Jearse Park Beach was comprised of two 15 min. electrofishing events; the first being three linear passes as a triangle within the confines of the casino harbor just to the south of Site 3 and the second being three consecutive linear passes along

the breakwall just to the north of Site 3. During this survey the samples collected for eDNA extraction were taken at four different locations in total on the waters of Lake Michigan (at Site 1, 41°38'53.38"N, 87°25'54.84"W; at Site 2, 41°39'1.80"N, 87°26'0.64"; and slightly to the north and south of Site 3, 41°39'11.2"N, 87°25'55.76"W).



Figure 3. Electrofishing surveying methods employed nets used to catch the fish that are momentarily stunned by the electrical current (electroshock). After capture each fish collected was identified, counted, and then released back into the water.

2.3 Validation Study for the Native Fish Tank at Bass Pro Shop

In order to validate our methods, samples were taken for comparison between the known composition of fish in a freshwater tank at Bass Pro Shop in Portage, IN (Figure 4) and eDNA-metabarcoding results obtained using our methods. These Bass Pro Shop samples were taken in triplicate from a 180,000-gallon closed freshwater fish tank system with a known population of species that are native to Lake Michigan.



Figure 4. Sampling to validate our eDNA-metabarcoding approach. A) Gathering samples from the freshwater tank for eDNA extraction; B) Store location housing the enormous tank; C) The known native Lake Michigan fish species inhabiting the tank.

2.4 eDNA Extraction Efficiency

In order to test the efficacy of using commercially available DNA extraction kits, the efficiency eDNA extraction was examined using various modified kit protocol. Water samples (1000 mL) for the eDNA extraction efficiency study were collected on July 6, 2018 at two beach (Sites 1 and 2) locations on Lake Michigan: Jeorse Park Beach in East Chicago, IN (described above) and Washington Park Beach on Lake Michigan in Michigan City, IN (41° 43' 41.33" N, 86° 54' 14.58" W). Six water samples were collected at each location in 1 L-sized bleach-sterilized polypropylene bottles. The samples were taken offshore approximately 25 cm below the water's surface where the water depth approached 0.5 m. The samples were then placed in coolers containing ice until arrival at the laboratory. Each sample (1000 mL) was then filtered through a 1.2 μ m Whatman cellulose nitrate filter (GE Healthcare Life Sciences, Buckinghamshire, UK) using a disposable Nalgene™ Analytical Test Filter Funnels (Nalgene Nunc International, Rochester, NY). After filtration, the filter papers were removed from the funnels using sterile disposable forceps (TWD Scientific LLC, Pleasant Prairie, WI) and placed into sterile test tubes and held at -80°C until extraction.

The manufacturer's protocol was followed for the DNeasy Blood and Tissue® DNA extraction kit (Qiagen, Hilden, Germany) with several following modifications that were suggested by Qiagen technical support. Briefly, filters were placed in 3X the amount of ATL buffer with ~1.5 g of 0.5 mm bleached glass beads (BioSpec Products, Bartlesville, OK). Two different modification procedures were then used in testing for eDNA extraction efficiency. These were

each tested on six samples (three samples at each pilot study location), and included: 1) using a 3X amount of Proteinase K added to the test tube along with the ATL buffer; 2) without Proteinase K being added to the test tube along with the ATL buffer. All samples were then vortexed for 5 min, for bead beating, and AL buffer (with Ethanol mixture) was also used in tripled kit volumes. The rest of the extraction procedure followed the kit protocol, with the final DNA elution step including two sequential rinses of the spin column with 100 μ L each of EB buffer, for a final volume of 200 μ L. Following extraction, DNA concentrations for all samples (12 in total) were measured in fluorometric quantification using the Qubit® High Sensitivity dsDNA HS Assay (Thermo Fisher Scientific, Waltham, MA), with DNA quality being measured using the 260/280 ratio in a Nanophotometer Pearl (Implen Inc., Westlake Village, CA). To validate polymerase chain reaction (PCR) amplification of mitochondrial 12S rRNA gene, the extracted sample eDNA was then used in PCR along with primers specific for the round goby (*N. melanostomus*; Nevers et al., 2018a), with amplicons then visually checked for quantity, quality, and general target gene bp size using gel electrophoresis.

2.5 Water Sample Collection and Processing for the Baseline Biomonitoring Study

All water samples were collected in triplicate using a peristaltic pump (Geotech Environmental Equipment, Denver, CO). For the nearshore (Sites 1 and 2) and the breakwall (Site 3) sampling, water was filtered using a modified ~3 m telescopic sampling pole with a sterile, disposable filtration cup fitted with 1.2 μ m; 47 mm diameter Whatman cellulose nitrate filters (GE Healthcare Life Sciences, Buckinghamshire, UK) affixed to the end. Water was pumped through the tubing into a collection receptacle until 1 L was gathered. Filtered water was emptied into graded bucket to keep track of volume filtered. For the reef sampling on open water, the surface samples (Site 4) were collected by dipping (with latex gloved hands) a 1 L-sized bleach-sterilized polypropylene bottle approximately 15 cm below the surface of the water and the depth samples (Site 5) were collected by dropping a weighted sterile hose to depth (~3 m) and pumping to fill the 1 L-sized bleach-sterilized polypropylene bottle on the boat. All reef site samples were taken back to shore and filtered on site using the process described above, though without the telescopic sampling pole. Between each onsite sampling event, the tubing was flushed with 1 L of sterile water, and then an additional 1 L of sterile water was filtered using the same process described above to serve as method blank samples (i.e., sampling blank), these taken in order to ensure there

was no carryover contamination. Filtration cup and filter paper were replaced between every replicate, and the end of the sampling pole was bleached between every sampling site.

2.6 Water Sample Collection and Processing for the Validation Studies

All water collected for the traditional survey validation study was taken in triplicate 1 L samples at Jeorse Park Beach sites concurrently at the time of the traditional surveying. For the seining survey, water samples were collected at Site 2 in bleached/sterile bottles approximately 1 min. after the seining event. Water was collected approximately 25 cm below the water's surface at a point where the water was at a depth of ~ 0.5 m. For the electrofishing the water samples were collected at Site 3 at the start of each standardized 15 min. electroshock event using the methods described above for Site 3 (see Section 2.5 above). All samples for the traditional survey validation study were taken back to shore and filtered on site using the methods described above (see Section 2.5). After filtration, these filters were placed into centrifuge vials and stored in a cooler containing ice at ~20°C until reaching the USGS laboratory. For the traditional trawling survey validation study, duplicate water samples were collected on the open waters of Lake Michigan. Samples were collected in 2 L bleached/sterile bottles and stored at -20°C until arrival at the laboratory. For the closed tank validation study, triplicate 1 L water samples were gathered with a peristaltic pump directly from the freshwater tank and filtered onsite as Bass Pro Shops (Figure 4) using the water processing procedure outlined above (see Section 2.5). After filtration, all filters were placed into centrifuge vials and stored in a cooler containing ice at ~20°C until reaching the USGS laboratory. At laboratory, each sample (2000 mL) was filtered using the methods described above (see Section 2.5). In the laboratory, all filters were stores at -80°C until the time of extraction.

2.7 eDNA Extraction from Samples

Environmental DNA was extracted directly from filters using a commercial DNeasy Blood and Tissue® DNA extraction kit (Qiagen, Hilden, Germany). The manufacturer's protocol was followed with modifications found to be effective in the testing described above (see Section 2.4). The final eDNA extraction protocol follows. Briefly, filters were placed in 3X the amount of ATL buffer with sterile glass beads added and were then vortexed for 10 min. for bead beating. After beating, Proteinase K and AL buffer (with Ethanol mixture) were also added in tripled kit volumes.

The rest of the extraction procedure followed the kit protocol, with the final DNA elution step including two sequential rinses of the spin column with 100 μ L each of EB buffer, for a final volume of 200 μ L. To check for cross-contamination during eDNA extraction, the above protocol was simultaneously used on triplicate filters from a sterile deionized water filtration (i.e., extraction blank). Overall, eDNA was extracted from 74 study samples (see Section 2.1 above), along with the 14 sampling blanks and three extraction blanks. Following extraction, DNA concentrations for all samples (91 in total) were measured in fluorometric quantification using the Qubit® High Sensitivity dsDNA HS Assay (Thermo Fisher Scientific, Waltham, MA), with DNA quality being measured using the 260/280 ratio in a Nanophotometer Pearl (Implen Inc., Westlake Village, CA).

Table 3. The eDNA concentrations (ng/ μ L) and purity [i.e., the ratio of absorbance at 260 nm (DNA) and 280 nm (protein)] of all 74 study samples

DNA ID	Date	Event	Site	Replicate	Qubit (ng/uL)	Nano 260/280
1	6/25/2018	Biomonitoring	1	A	19.40	1.86
2	6/25/2018	Biomonitoring	1	B	16.00	1.93
3	6/25/2018	Biomonitoring	1	C	16.20	1.90
4	6/25/2018	Biomonitoring	2	A	13.50	1.77
5	6/25/2018	Biomonitoring	2	B	10.70	1.72
6	6/25/2018	Biomonitoring	2	C	11.20	1.90
7	6/25/2018	Biomonitoring	3	A	0.43	2.00
8	6/25/2018	Biomonitoring	3	B	14.90	1.96
9	6/25/2018	Biomonitoring	3	C	7.99	2.11
10	6/25/2018	Biomonitoring	4	A	18.2	2.00
11	6/25/2018	Biomonitoring	4	B	15.90	1.968
12	6/25/2018	Biomonitoring	4	C	0.79	2.05
13	6/25/2018	Biomonitoring	5	A	16.00	1.92
14	6/25/2018	Biomonitoring	5	B	7.88	2.00
15	6/25/2018	Biomonitoring	5	C	1.71	2.00
16	7/23/2018	Biomonitoring	1	A	9.46	1.93
17	7/23/2018	Biomonitoring	1	B	10.60	1.96
18	7/23/2018	Biomonitoring	1	C	7.96	1.86
19	7/23/2018	Biomonitoring	2	A	12.10	1.94
20	7/23/2018	Biomonitoring	2	B	9.44	1.89
21	7/23/2018	Biomonitoring	2	C	12.1	1.89
22	7/23/2018	Biomonitoring	3	A	7.15	1.86
23	7/23/2018	Biomonitoring	3	B	4.34	2.00

Table 3 continued'

24	7/23/2018	Biomonitoring	3	C	6.60	1.91
25	7/23/2018	Biomonitoring	4	A	9.92	1.92
26	7/23/2018	Biomonitoring	4	B	9.49	1.92
27	7/23/2018	Biomonitoring	4	C	10.80	1.89
28	7/23/2018	Biomonitoring	5	A	8.05	2.00
29	7/23/2018	Biomonitoring	5	B	14.00	1.94
30	7/23/2018	Biomonitoring	5	C	8.55	2.00
31	8/27/2018	Biomonitoring	1	A	54.00	1.91
32	8/27/2018	Biomonitoring	1	B	55.00	1.93
33	8/27/2018	Biomonitoring	1	C	56.00	1.91
34	8/27/2018	Biomonitoring	2	A	51.00	1.93
35	8/27/2018	Biomonitoring	2	B	57.00	1.93
36	8/27/2018	Biomonitoring	2	C	58.00	1.93
37	8/27/2018	Biomonitoring	3	A	5.92	2.06
38	8/27/2018	Biomonitoring	3	B	3.81	2.09
39	8/27/2018	Biomonitoring	3	C	1.71	2.14
40	8/27/2018	Biomonitoring	4	A	49.60	1.93
41	8/27/2018	Biomonitoring	4	B	34.20	1.98
42	8/27/2018	Biomonitoring	4	C	9.12	2.00
43	8/27/2018	Biomonitoring	5	A	0.56	2.17
44	8/27/2018	Biomonitoring	5	B	45.50	1.95
45	8/27/2018	Biomonitoring	5	C	43.10	1.94
46	6/28/2018	Electrofishing	1	A	51.00	1.95
47	6/28/2018	Electrofishing	1	B	38.40	1.95
48	6/28/2018	Electrofishing	1	C	9.36	2.05
49	6/28/2018	Electrofishing	2	A	3.08	1.66
50	6/28/2018	Electrofishing	2	B	6.46	1.72
51	6/28/2018	Electrofishing	2	C	11.50	1.80
52	6/28/2018	Electrofishing	3	A	12.30	1.65
53	6/28/2018	Electrofishing	3	B	17.00	1.79
54	8/13/2018	Bass Pro	1	A	0.29	1.70
55	8/13/2018	Bass Pro	1	B	0.10	2.75
56	8/13/2018	Bass Pro	1	C	0.19	1.88
57	9/18/2018	Deep water survey	30m	A	16.20	1.97
58	9/18/2018	Deep water survey	30m	B	11.80	2.07
59	9/18/2018	Deep water survey	60m	A	13.40	2.00
60	9/18/2018	Deep water survey	60m	B	12.80	2.04
61	9/18/2018	Deep water survey	90m	A	9.27	2.11
62	9/18/2018	Deep water survey	90m	B	10.70	1.26
63	7/23/2018	Onshore beach PW	1	A	18.60	1.78
34	7/23/2018	Onshore beach PW	1	B	17.00	1.76
65	7/23/2018	Onshore beach PW	1	C	16.70	1.79

Table 3 Continued'

66	7/23/2018	Onshore beach PW	2	A	12.80	1.77
67	7/23/2018	Onshore beach PW	2	B	17.10	1.79
68	7/23/2018	Onshore beach PW	2	C	19.90	1.75
69	7/25/2017	Onshore beach PW	1	A	40.80	1.83
70	7/25/2017	Onshore beach PW	1	B	38.20	1.88
71	7/25/2017	Onshore beach PW	1	C	54.00	1.75
72	7/25/2017	Onshore beach PW	2	A	too high	1.74
73	7/25/2017	Onshore beach PW	2	B	37.20	1.81
74	7/25/2017	Onshore beach PW	2	C	30.50	1.81

2.8 MiFish Primer Testing on eDNA from Samples

Prior to sequencing, we tested the primer we had selected for metabarcoding, MiFish (Miya et al., 2015), to determine if amplicons could be obtained from our samples. The “MiFish” primers (MiFish-U-F 5'- GTCGGTAAACTCGTGCCAGC -3' and MiFish-U-R 5'- CATAGTGGGGTATCTAATCCCAGTTTG -3') targeted mitochondrial ribosomal genes, and this primer pair is known to be suitable for use in high-throughput amplicon sequencing (Miya et al., 2015). The MiFish primers amplify hypervariable regions of fish mitochondrial 12S rRNA gene (163–185 bp) from the extracted eDNA, and the primer set was initially designed to amplify 12S from over 800 fish species, generally providing enough information for taxonomic identification of fish to the species level (Miya et al., 2015). All 74 of the eDNA study samples collected from water used in PCR with the MiFish primers. Universal reactions for PCR preparation included the following: 12.5 µl KAPA HiFi HotStart ReadyMix (Roche Holding AG, Basel, Switzerland), 0.75 µL of each forward and reverse tailed MiFish primer, 2 µl template DNA, and 9 µl PCR grade water in a final volume of 25 µl. Thermal cycling was performed as follows: 95°C denaturation for 3 min., followed by 35 cycles of 98°C for 20 sec., 65°C for 15 sec., and 72°C for 15 sec., followed by a final extension at 72°C for five min. Amplicons were visually checked for quantity, quality, and general target gene bp size using gel electrophoresis, with a 1.5% agarose gel ran at 150 V for 2 hr. After validating amplicons, all extracted DNA from water samples were sent to Leetown Science Center (Kearneysville, WV) for high-throughput sequencing.

2.9 Mitochondrial 12S Gene Metabarcoded Sequencing

Fish mitochondrial ribosomal genes were targeted in high-throughput amplicon sequencing using the MiFish primers and protocol of Miya et al. (2015) for generation of Illumina MiSeq compatible amplicons. Reactions for library preparation included the following: 6 μ l KAPA HiFi HotStart ReadyMix (Roche Holding AG, Basel, Switzerland), 0.3 μ M of each forward and reverse Illumina compatible adapter-tailed MiFish primer, 2 μ l template DNA, and 2.56 μ l PCR grade water in a final volume of 12 μ l. Thermal cycling was performed as follows: 95°C denaturation for 3 min., followed by 35 cycles of 98°C for 20 sec., 66°C for 15 sec., and 72°C for 15 sec., followed by a final extension at 72°C for five min. Each DNA sample was amplified in three separate replicate PCRs, then pooled after amplification into a composite 36 μ l sample. Initial PCR resulted in little or no amplification for some samples despite sufficient amounts of template DNA in the reactions.

To assess the possible presence of inhibitory compounds in the template DNA, a subset of the template DNA samples were processed with the DNeasy Powerclean Pro Cleanup Kit (Qiagen) following the manufacturer's instructions. Amplification of this processed DNA was successful, indicating the presence of inhibitors in the extracted DNA. Therefore, all extracted DNA samples were additionally processed through the DNeasy Cleanup kit prior to PCR amplification with the MiFish primers and eluted in a volume of 100 μ l. After successful PCR re-amplification, 25 μ l of each 36 μ l amplicon product was purified from residual primers and primer dimer species and dual indexed according to the available Illumina protocol:

See:

https://support.illumina.com/downloads/16s_metagenomic_sequencing_library_preparation.html

To ensure even representation of each sample in the sequencing run, each sample was quantified and diluted to a standardized concentration. To accomplish this, each dual-indexed purified PCR product was quantified using the Qubit dsDNA HS Assay kit (Thermo Fisher Scientific, Waltham, MA) following the manufacturer's instructions and utilizing 2 μ l of template for each quantification. Each concentration in ng/ μ l was converted to nMs utilizing the equation provided in the Illumina documentation. This equation requires the size of the amplicon. To approximate this size across all 74 sequencing sample libraries, a subset of samples were run on a

BioAnalyzer DNA High Sensitivity assay chip (Agilent Technologies, Santa Clara, CA, USA), which resulted in an average amplicon size of 416 bp (min 378 bp, max 436 bp). Therefore, the value of 416 bp was used to calculate each sample's concentration in nM. A portion of each sample was individually diluted to 4 nM in separate wells of a 96-well plate for final pooling. Dilutions were calculated to require at least 2 µl of template to reduce the impact of pipetting low sample volumes. A final 4 nM pool was made in a 1.5 ml microcentrifuge tube by combining 5 µl of each 4 nM library. Final dilution and loading of the library followed the Illumina documentation. The concentration of the library for loading on the flowcell was 6 pM, as well as a 6 pM PhiX library. The samples were paired end 250 bp X 2 sequenced on Illumina MiSeq version 2 flowcell.

2.10 Bioinformatic Processing and Statistical Analyses

Illumina sequencing produced an output of paired-end reads for each sample as FASTQ files. Residual Nextera adapter sequences were trimmed from the amplicons using the bbdut.sh script from the BBMap version 36.49 software package (<https://sourceforge.net/projects/bbmap>). Overlapping paired-end reads were merged using bbmerge.sh (Bushnell et al., 2017), where the average number of merged read pairs across samples was 88%. These merged reads were then used for generation of an OTU (Operational Taxonomic Unit) table using SWARM clustering (Mahé et al., 2015) and the OTU building pipeline described by Mahé (available at: <https://github.com/frederic-mahe/swarm/wiki/Fred's-metabarcoding-pipeline>). Briefly, primers were trimmed from amplicons using cutadapt (Martin, 2011), and all sequences were globally dereplicated and the expected error rate calculated using vsearch version 2.0.3 (Rognes et al., 2016). Representative sequences from the vSearch clustering were subjected to SWARM clustering using a distance value of 1, and the fastidious option. These SWARM cluster representative sequences were screened for the presence of chimeras using UCHIME (Edgar et al., 2011) implemented in vSearch.

For taxonomic assignment to the SWARM cluster representative sequences, we generated a reference database (see Supplemental materials below) by first downloading all complete and partial fish mitogenomic sequences (on April 23, 2018) from the MitoFish online database (Iwasaki et al., 2013). An in-silico PCR using the MiFish primers and the bbsuk.sh script from BBMap was used to reduce the number of sequences to 8,240 trimmed 12S reference “amplicon” sequences. At the time of download from MitoFish, a 12S sequence for the round goby (*N.*

melanostomus) was not included but was present at GenBank (Accession # VHKM000000000.1), thus the 12S sequence was manually added to the reference database. Taxonomy was assigned to OTUs using a percent sequence similarity threshold of 98%. In cases where an OTU matched more than one species with 98% or greater similarity, the LCA algorithm (lowest-common ancestor; Huson et al., 2007) was used to assign taxonomy. This process was automated using the STAMPA software (<https://github.com/frederic-mahe/stampa>). Finally, the OTU sequences and taxonomic assignments were arranged into an OTU table, where the rows corresponded to OTUs and taxonomic assignments, and the columns corresponded to each sample examined. The entries within the table represent the abundance of sequences in that sample assigned to that specific OTU. We filtered the OTU table to include those OTUs that were not identified as chimeric, had an expected error rate of less than 0.25, and had an OTU representative sequence composed of at least three sequences (no singletons). At this stage of processing, we did not impose any threshold for a minimum number of reads to be assigned to an OTU to keep it in the table. Sequences have been deposited in the Sequence Read Archive database of NCBI. All downstream processing of the initial OTU table and associated analyses were carried out using the QIIME (Caporaso et al., 2010) bioinformatic and statistical R (www.r-project.org) software.

2.11 Angler's Survey

In order to better understand the awareness of invasive species, such as the round goby, within the local fishing community, we devised an “Angler's Survey” (Figure 5) that was part of an outreach effort. The survey was administered to fishermen between July 6 and July 20, at the Portage Lakefront and Washington Park Beach; 22 fishermen in total were surveyed. Before each survey was given, we asked fishermen if they would be willing to voluntarily take part in the surveying effort. Prior to the survey we sought guidance with the Purdue University Institutional Review Board as the survey involved human subjects.



ANGLER'S SURVEY



Date: _____

FUN FACT: Lake Michigan Trout or Salmon with a missing adipose fin (between the dorsal fin and caudal (tail) fin) contain a small micro wire tag in the head of the fish with information important to DNR research. Please save the head from your tagged trout and salmon and call the Division of Fish & Wildlife at (219) 874-6824 for instructions on drop-off locations. Only trout and salmon with a missing adipose fin have micro tags.

GENERAL INFORMATION

How many times **per year** do you go fishing?

1-20 20-50 More than 50

How would you rate your fishing skills?

1 2 3 4 5 6 7 8 9 10
Beginner Expert

How many years have you been fishing in the **Southern Lake Michigan** area? _____

YOUR FISHING EXPERIENCE TODAY

Where were you fishing in the Southern Lake Michigan area?

Hammond Port Authority Jearse Park Portage Lakefront Michigan City Other _____

Where/What were you fishing from?

Shore Dock Boat Breakwall Other (please specify) _____

If you were fishing from a **boat**, approximately how far off shore were you? _____

How many hours were you fishing? _____ How many lines did you have in the water? _____

What type of hook did you use? (circle, bait, treble, etc.) _____

What kind of bait did you use? (live bait, lure, etc.) _____

What did you catch? (please list each fish in format: Fish species: ____; Approx. Length: ____; Approx. Weight: ____)

Most Commonly Caught: Trout, Salmon, Rainbow Smelt, Sunfish (Small/Largemouth bass, crappies, sunfish), Panfish (Bluegill, pumpkinseed), Rock Bass, Yellow Perch, Skamania Steelhead, ETC.



Native Sculpin

Of the fish you caught **today**, did you:

Keep (Number): Catch and Release (Number):

Did you **[CATCH / SEE]** (circle) any **round goby** (see photos on right)?



Invasive Round Goby

COMMENTS

Figure 5. Angler's Survey created to better understand the awareness of invasive species, such as the round goby, within the local fishing community.

CHAPTER 3. RESULTS

3.1 eDNA Extraction Efficiency

In the comparison of samples examining the eDNA extracted process, using proteinase K vs. not using proteinase K, resulted in higher eDNA concentration in samples which proteinase K was used. Table 4 shows that all of the samples using proteinase K generally resulted in a higher yield of DNA when measured by qubit for each set of samples taken at both sampling locations.

Table 4. DNA concentrations and purity for samples extracted in the Extraction Efficiency study.

ID	Date	Site	Replicate	Proteinase K (Yes/No)	Qubit (ng/uL)	Nano 260/280
1	7/6/2018	Jeorse Park	A	Yes	19.40	1.85
2	7/6/2018	Jeorse Park	B	Yes	18.10	1.88
3	7/6/2018	Jeorse Park	C	Yes	18.50	1.88
4	7/6/2018	Jeorse Park	D	No	15.00	1.85
5	7/6/2018	Jeorse Park	E	No	15.80	1.89
6	7/6/2018	Jeorse Park	F	No	15.50	1.71
7	7/6/2018	Washington Park	A	Yes	20.20	1.82
8	7/6/2018	Washington Park	B	Yes	18.30	1.79
9	7/6/2018	Washington Park	C	Yes	19.20	1.82
10	7/6/2018	Washington Park	D	No	0.41	1.88
11	7/6/2018	Washington Park	E	No	14.40	1.83
12	7/6/2018	Washington Park	F	No	14.20	1.96

Table 5. Set up and results of an electrophoretic gel to confirm efficiency results, with symbols indicating mitochondrial 12S rRNA gene PCR amplicons were (+) or were not (-) visualized (and see Figure 6 below).

Well #	DNA ID	Proteinase K (Yes/No)	Results
1	(-) control	n/a	-
2	1	Yes	-
3	2	Yes	+
4	3	Yes	+
5	7	Yes	+
6	8	Yes	+
7	9	Yes	+
8	4	No	-
9	5	No	-
10	6	No	-
11	10	No	-
12	11	No	-
13	12	No	+
14	(+) control	n/a	+
15	(+) control	n/a	+
16	(+) control	n/a	+
17	Ladder	n/a	n/a
18	(+) control	n/a	+

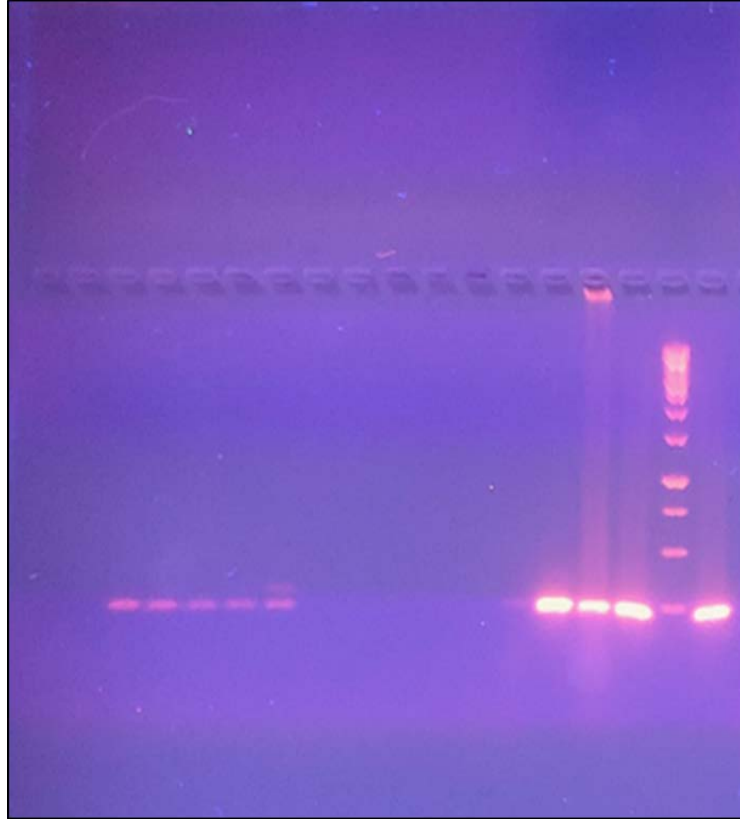


Figure 6. Bands in wells 2–7 indicate PCR amplification of the mitochondrial 12S rRNA gene from eDNA samples treated with proteinase K in extraction relative to samples in wells 8–13 that were not treated with proteinase K during extraction.

Figure 6 shows the mitochondrial 12S rRNA gene products PCR amplified from eDNA samples. Overall, the mitochondrial 12S rRNA gene was PCR amplified in five out of six samples where proteinase K was used in the eDNA extraction protocol; whereas, only one out of six of the samples saw amplification when proteinase K was not used.

3.2 MiFish Primer Testing

Figure 7 shows that over 78% (58 out of 74) of our samples showed PCR amplification of the mitochondrial 12S rRNA gene using the MiFish primer (Miya et al., 2015). Further testing suggested extraction cleanup was warranted for all samples prior to Illumina sequencing (see Section 2.9 above). Figure 8 shows the mitochondrial 12S rRNA gene was also amplified in eDNA samples from the Bass Pro closed system tank samples using the MiFish primers (Miya et al., 2015), despite them having the lowest DNA concentrations of all samples collected (see Table 6).

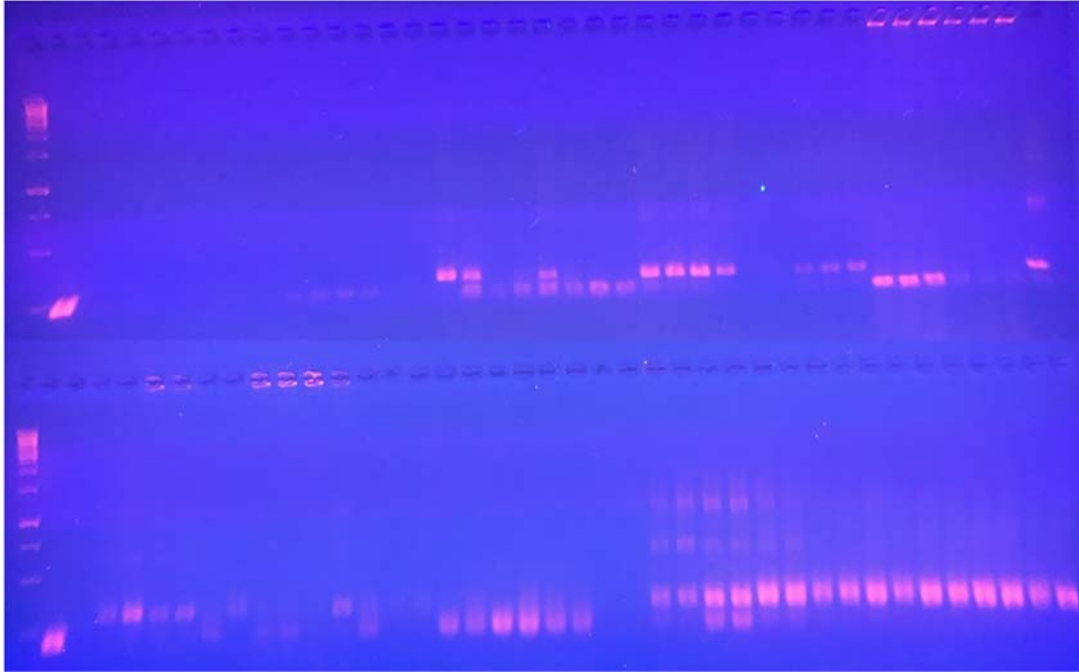


Figure 7. Electrophoreses gel indicate PCR amplification of the mitochondrial 12S rRNA gene using the MiFish primers in in 58 out of 74 eDNA samples from the study site, and suggesting that a DNA cleanup process might be required for all eDNA samples prior to HTS.

Table 6. Set up and results of an electrophoretic gel to confirm PCR amplification of Bass Pro eDNA samples with the MiFish primers, with symbols indicating mitochondrial 12S rRNA gene amplicons were (+) or were not (-) visualized (and see Figure 8 below).

Well#	DNA ID	Qubit (ng/uL)
1	Ladder	n/a
2	(+) control	n/a
3	(-) control	n/a
4	Bass Pro	0.287
5	Bass Pro	0.102
6	Bass Pro	0.188

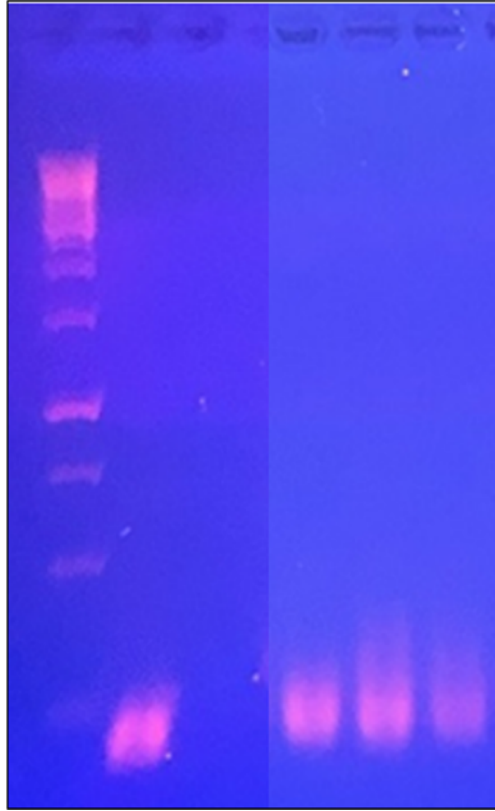


Figure 8. The MiFish primers testing results, showing amplification of Bass Pro eDNA samples.

3.3 eDNA-Metabarcoding and Taxonomic Assignments

Sequencing yielded 2,000,829 quality reads across the 74 study samples, with individual samples yielded ~24,000 sequences on average. Representative sequences for some recovered OTUs required specialized BLAST searches (Altschul et al., 1990) as those taxa (i.e., *Carpiodes cyprinus*, *Fundulus diaphanus*, and *Moxostoma lepidotum*) were initially lacking in the taxonomic database. The triplicate samples did not vary significantly and were computationally pooled into a single representative sample for each sampling site/type/timepoint using the methods of Song and colleagues (2015). Finally, the OTU table was rarefied in QIIME to 14,500 sequences per sample. Rarefaction analysis (Figure 9) showed that collector's curves trended toward asymptote starting at a sequencing depth of ~1,200, suggesting that eDNA-metabarcoding allowed us to capture a good proportion of the fish species diversity in samples at the level of sample rarefaction used. Overall, OTUs representing fish species recovered included taxa from 14 families (*Amiidae*, *Catostomidae*, *Centrarchidae*, *Clupeidae*, *Cyprinidae*, *Esocidae*, *Gobiidae*, *Ictaluridae*, *Lepisosteidae*, *Moronidae*, *Osmeridae*, *Percidae*, *Salmonidae*, *Sciaenidae*) in 9 orders

(Amiiformes, Clupeiformes, Cypriniformes, Gobiiformes, Lepisosteiformes, Osmeriformes, Perciformes, Salmoniformes, Siluriformes).

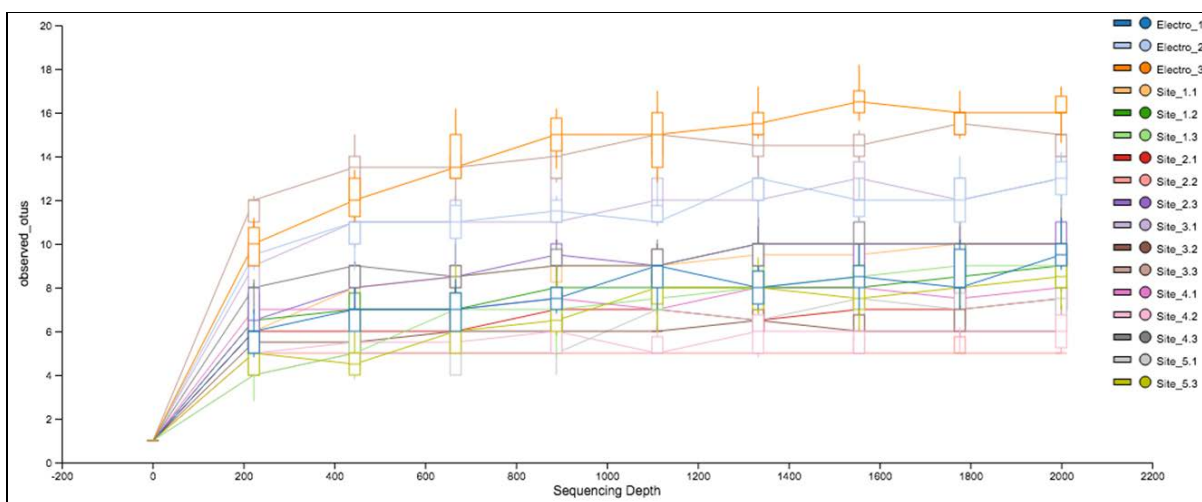


Figure 9. Rarefaction curves for OTUs recovered from Sites 1–5 in the Baseline Biomonitoring study across the sampling months (indicated June, July, and August for e.g., Site 1 labeled as 1.1, 1.2, and 1.3, respectively) and the Validation study for comparison with the traditional survey methods employed at the Jeorse Park Beach as well (labeled as “Electro” 1–3).

3.4 Validation Studies

3.4.1 Bass Pro Native Species Tank Comparison

In the closed system, validation showed that the majority of fish that are native to Lake Michigan (Table 7) were detectable in the freshwater tank using our methods (Figure 10), and they were, for the most part, close to their relative abundances when corrected to the log scale. Overall, our method was able to detect 8 of 10 (80%) of the known genera in the tank, with some of the lower abundance fish (catfish and quillback) not being represented using our methods. We also recovered sequences representing two taxa (alewife and shiner) that were not present in the tank at the time of sampling.

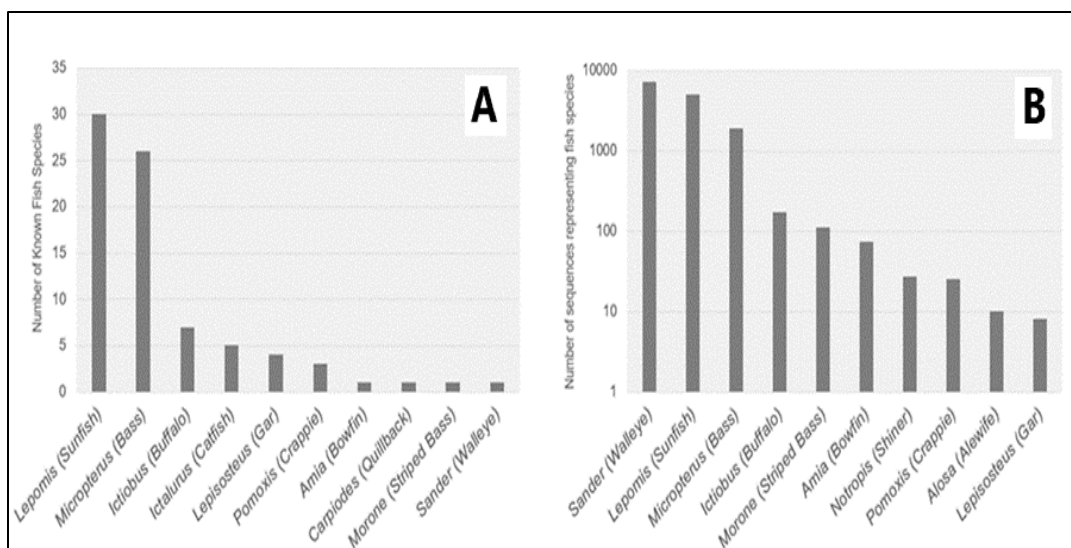


Figure 10. Bar graph showing relative abundances of fish genera detected in the validation studies. A) Genera of known native Lake Michigan fish inhabiting the freshwater tank; B) Genera of fish detected in the freshwater tank using our eDNA-metabarcoding methods.

Table 7. The community composition of fish species that were native to Lake Michigan in the Bass Pro freshwater fish tank at the time of sampling (12 August 2018).

Scientific Name	Common name	Number of Fish in Tank
<i>Acipenser fulvescens</i>	Lake sturgeon	0
<i>Amia calva</i>	Bowfin	1
<i>Aplodinotus grunniens</i>	Freshwater drum	0
<i>Carpiodes cyprinus</i>	Quillback	1
<i>Ictalurus furcatus</i>	Blue Catfish	2
<i>Ictalurus punctatus</i>	Channel catfish	3
<i>Ictiobus bubalus</i>	Smallmouth buffalo	6
<i>Ictiobus cyprinellus</i>	Bigmouth buffalo	1
<i>Lepisosteus oculatus</i>	Spotted Garfish	1
<i>Lepisosteus osseus</i>	Longnose Gar	1
<i>Lepisosteus platostomus</i>	Shortnose Gar	2
<i>Lepomis sp.</i>	Sunfish	30
<i>Micropterus dolomieu</i>	Smallmouth bass	0
<i>Micropterus salmoides</i>	Largemouth bass	26

Table 7. Continued'

<i>Morone chrysops</i>	White bass	0
<i>Morone saxatilis</i>	Striped bass	0
<i>Morone chrysops x saxatilis</i>	Hybrid striped bass	1
<i>Pomoxis annularis</i>	White crappie	0
<i>Pomoxis nigromaculatus</i>	Black crappie	3
<i>Sander vitreus</i>	Walleye	1

3.4.2 Traditional Surveys Comparison

The traditional surveys at Jeorse Park Beach captured a number of fish, yielding 330 individuals by seining and 33 by electrofishing (Table 8). After four seining runs at the beach survey site (Site 2), the total number of species captured numbered five, and after eight electrofishing passes to the north and the south of the Breakwall (Site 3), the species captured numbered six. The numbers of species detected using our eDNA-metabarcoding approach were much higher (Table 8), with 16 taxa detected in the eDNA samples collected at Site 2 during the seining survey and 12 taxa detected in the eDNA samples collected at the Site 3 during the electrofishing survey.

A comparison of the fish species detected in the traditional versus eDNA-metabarcoding surveys is provided in Table 8. While eDNA-metabarcoding generally detected much higher numbers of fish species at the survey sites, the overlap with the traditional survey was much lower than what was seen in the freshwater tank study. Overall, only two out of five (40%) fish species were detected in both the seining survey and by our eDNA-metabarcoding approach, while three out of six (50%) species were detected in both the electrofishing survey and by molecular means. Traditional surveys and eDNA-metabarcoding, however, both reliably detected native and invasive fish species (data not shown).

Table 8. Validation study conducted at Jearse Park Beach in the comparison with traditional surveys using seining (left) and electrofishing (right). For each methods the results shown reflect the actual number species detected in the traditional survey “Counts” and the number of OTU sequences representing fish species detected by our eDNA “Metabarcoding” approach. Areas of the table shaded in gray highlight fish species that were detected under both methods.

Common Name	Species	Seining		Electrofishing	
		Counts	Metabarcoding	Counts	Metabarcoding
Alewife	<i>Alosa pseudoharengus</i>	0	1130	0	9093
American Gizzard Shad	<i>Dorosoma cepedianum</i>	0	1315	0	1897
Banded Killfish	<i>Fundulus diaphanus</i>	49	0	0	0
Blacktip Jumprock	<i>Moxostoma cervinum</i>	0	1	0	0
Common Carp	<i>Cyprinus carpio</i>	0	875	0	8
Creek Chub	<i>Semotilus atromaculatus</i>	0	41	0	0
Emerald Shiner	<i>Notropis atherinoides</i>	0	206	0	0
Freshwater Drum	<i>Aplodinotus grunniens</i>	0	0	0	6
Green Sunfish	<i>Lepomis cyanellus</i>	0	3	0	3
Largemouth Bass	<i>Micropterus salmoides</i>	0	1	0	0
Longnose Dace	<i>Rhinichthys cataractae</i>	0	72	0	0
Northern Pike	<i>Esox lucius</i>	0	0	0	0
Pumpkinseed	<i>Lepomis gibbosus</i>	0	0	1	0
Rainbow Smelt	<i>Osmerus mordax</i>	0	0	0	0
Rainbow Trout	<i>Oncorhynchus mykiss</i>	0	0	0	0
River Carpsucker	<i>Carpionodes carpio</i>	0	0	0	868
Rock Bass	<i>Ambloplites rupestris</i>	0	546	9	2
Round Goby	<i>Neogobius melanostomus</i>	16	90	3	87
Sand Shiner	<i>Notropis stramineus</i>	127	0	0	0
Shorthead Redhorse	<i>Moxostoma macrolepidotum</i>	0	0	4	0
Silver Redhorse	<i>Moxostoma anisurum</i>	0	42	0	0
Smallmouth Bass	<i>Micropterus dolomieu</i>	0	8	15	2
Spottail Shiner	<i>Notropis hudsonius</i>	137	10,113	0	2484
Walleye	<i>Sander vitreus</i>	0	8	0	4
White Sucker	<i>Catostomus commersonii</i>	0	46	0	0
Yellow Bullhead	<i>Ameiurus natalis</i>	1	0	1	0
Yellow Perch	<i>Perca flavescens</i>	0	0	0	45
Total Species Detected:		5	16	6	12

3.5 Baseline Biomonitoring Study at Jearse Park Beach

A high diversity of fish species were detected over the course of our summer sampling period at the Jearse Park Beach study site, with OTUs recovered corresponding to 27 fish species (Table 9). Table 9 also indicates the native versus non-native status of each species along with the historical record of detection at the study site. Eight of the 27 species (30%) were non-native species, while 19 (70%) were native species, and the majority of these species were known to occur in Lake Michigan. While 10 of the 27 species (37%) had been recorded previously at Jearse Park Beach, another 14 (52%) were known to occur in southern Lake Michigan; thus, leaving 3 (11%) that had not been recorded in these waters previously. The average sample alpha diversity for all of the sites is reported in Table 10.

Table 9. Baseline biomonitoring study conducted at Jearse Park Beach. The number of OTU sequences representing fish species detected by our eDNA “Metabarcoding” approach across all sites over the entire summer 2018 sampling period. Native versus non-native status is indicated as is the historic record showing fish previously documented at Jearse Park Beach or in the southern Lake Michigan area, follow data from United States Army Corps of Engineers (2016).

Common Name	Species	Metabarcoding	Status	Historic Record
Alewife	<i>Alosa pseudoharengus</i>	58,555	Non-Native	Southern Lake Michigan
American Gizzard Shad	<i>Dorosoma cepedianum</i>	27,016	Native	Jearse Park
Blacktip Jumprock	<i>Moxostoma cervinum</i>	51	Non-Native	No Record
Bluegill	<i>Lepomis macrochirus</i>	952	Native	Jearse Park
Chinook Salmon	<i>Oncorhynchus tshawytscha</i>	2108	Non-Native	No Record
Coho Salmon	<i>Oncorhynchus kisutch</i>	66	Non-Native	Southern Lake Michigan
Common Carp	<i>Cyprinus carpio</i>	32,140	Non-Native	Southern Lake Michigan
Emerald Shiner	<i>Notropis atherinoides</i>	102	Native	Southern Lake Michigan
Freshwater Drum	<i>Aplodinotus grunniens</i>	216	Native	Southern Lake Michigan
Green Sunfish	<i>Lepomis cyanellus</i>	15	Native	Southern Lake Michigan
Lake Herring	<i>Coregonus artedii</i>	31	Native	Southern Lake Michigan
Largemouth Bass	<i>Micropterus salmoides</i>	6	Native	Jearse Park
Longnose Dace	<i>Rhinichthys cataractae</i>	185	Native	Southern Lake Michigan
Northern Pike	<i>Esox lucius</i>	523	Native	Southern Lake Michigan
Pumpkinseed	<i>Lepomis gibbosus</i>	3040	Native	Jearse Park
Rainbow Smelt	<i>Osmerus mordax</i>	1	Native	Southern Lake Michigan
Rainbow Trout	<i>Oncorhynchus mykiss</i>	337	Non-Native	Jearse Park
River Carpsucker	<i>Carpodacus carpio</i>	1734	Non-Native	No Record
Rock Bass	<i>Ambloplites rupestris</i>	3411	Native	Jearse Park
Round Goby	<i>Neogobius melanostomus</i>	1775	Non-Native	Jearse Park
Silver Redhorse	<i>Moxostoma anisurum</i>	142	Native	Southern Lake Michigan
Smallmouth Bass	<i>Micropterus dolomieu</i>	4918	Native	Jearse Park
Spottail Shiner	<i>Notropis hudsonius</i>	77,078	Native	Southern Lake Michigan
Walleye	<i>Sander vitreus</i>	327	Native	Jearse Park
White Sucker	<i>Catostomus commersonii</i>	232	Native	Jearse Park
Yellow Bullhead	<i>Ameiurus natalis</i>	67	Native	Southern Lake Michigan
Yellow Perch	<i>Perca flavescens</i>	2469	Native	Southern Lake Michigan

Table 10. Average site alpha diversity values across the entire summer 2018 sampling period for richness and Shannon Index, with standard deviation (SD) indicated.

Site	Ave. Richness	SD	Ave. Shannon	SD
Beach 1	11.00	0.00	0.49	0.28
Beach 2	10.67	1.25	1.09	0.42
Breakwall	13.00	2.16	1.43	0.28
Reef (Surface)	10.33	1.70	1.19	0.28
Reef (Depth)	13.33	1.70	0.91	0.31

Two-way ANOVA analysis found no significant effect for Richness values for both months ($p = 0.30$) and sites ($p = 0.32$) sampled. A significant effect was found, however, for Shannon Index values for both months ($p = 0.015$) and sites ($p = 0.010$) sampled. Post-hoc Tukey Honest Significant Differences (THSD) testing indicating significant variance among the month means with THSD indicating means for diversity measures between June and July (Figure 11A) varied significantly (adjusted $p = 0.01$); further, significant variance was also determined among the site means (Figure 11B) for Site 1 and Site 3 (adjusted $p = 0.007$) as well as Site 1 and Site 4 (adjusted $p = 0.04$).

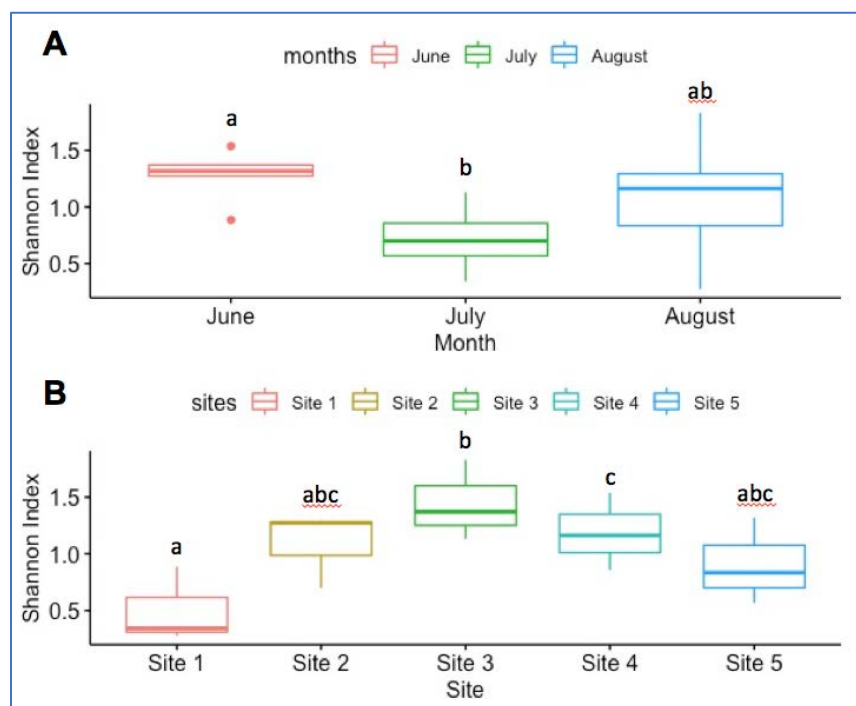


Figure 11. Boxplot showing Shannon Index value means A) across sites for the different months sampled, and B) across month for the different sites sampled. Boxes sharing the same letter are not significantly different according to THSD test.

The most abundant fish species detected over the course of the Biomonitoring Study included the spottail shiner (35% of all sequences recovered in the study), alewife (27%; invasive), common carp (15%; invasive), and American gizzard shad (12%), with all other taxa representing 2% or less. Of these four, two (alewife and common carp) are invasive and were also frequently encountered, being detected in all samples; however, in many instances the abundances of each of

these species fell below the 5% level within individual samples. Another important invasive (round goby) was detected in all but two samples, with its abundance varying from 1-594 sequences in individual samples. Overall, the 27 species represented 21 different genera of fish and their relative abundances across the different sampling sites and times are indicated in Figure 12.

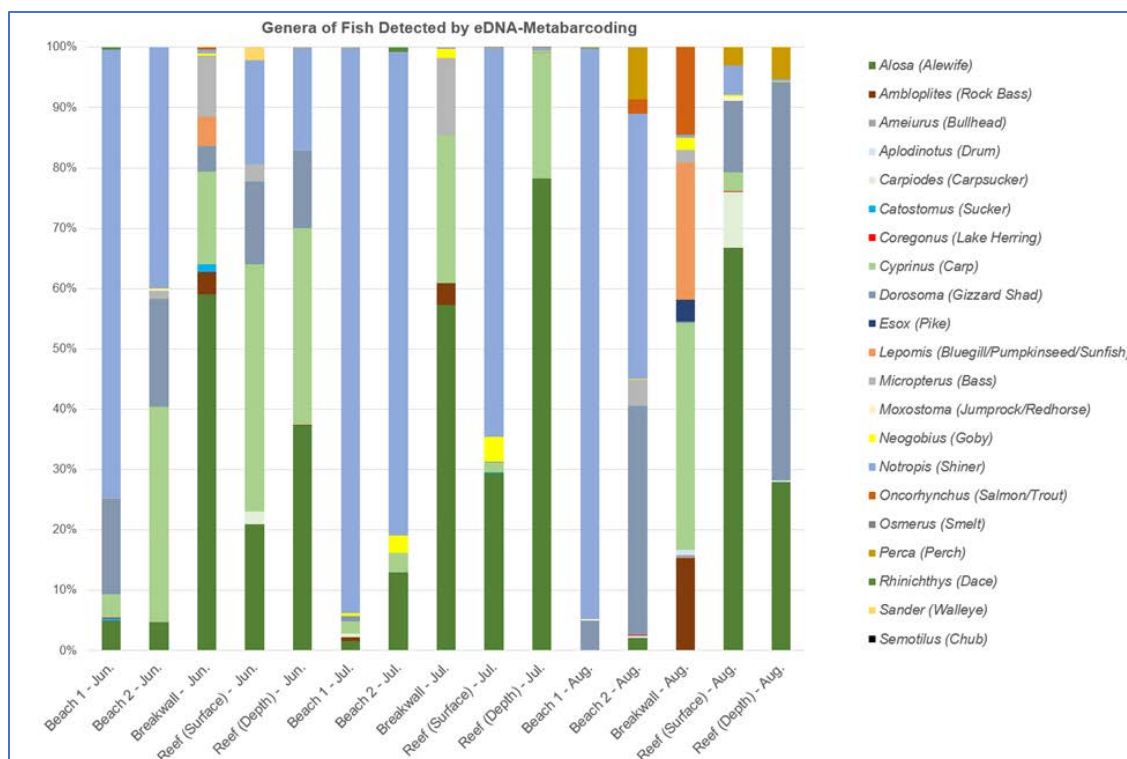


Figure 12. Bar graph showing relative abundances of fish genera detected in the Biomonitoring Study at Jeorse Park Beach over the course of the summer 2018 sampling period.

While relative abundances within each sample site appear to be somewhat similar, across the sampling season, there was a noticeable shift that occurred late in the season, especially in the breakwall samples (Figure 12). Ordination plots (Figure 13) reflected this trend, showing these fish communities are primarily structured according to the general area sampled (near to the shore vs. near the breakwall vs. open water over the reef, both surface and depth) rather than by time of sampling in the season. Thus, samples did not show significant clustering according to the sampling month (Figure 13A, ANOSIM $p = 0.24$), but were significantly clustered according to sample types (Figure 13B, ANOSIM $p = 0.001$).

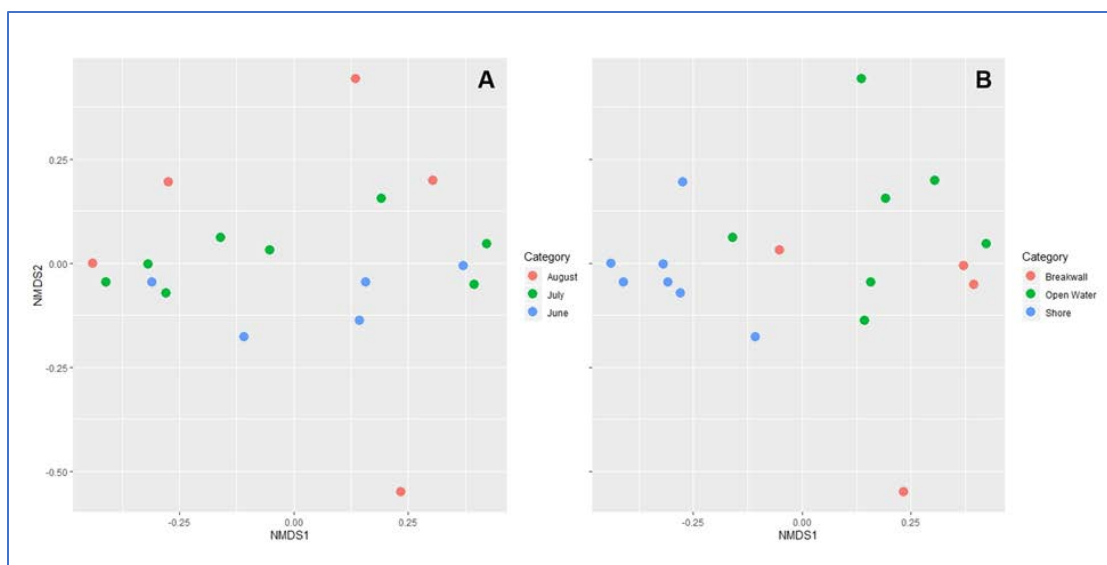


Figure 13. Nonmetric multidimensional scaling ordination plots showing similarity of fish communities detected using eDNA-metabarcoding methods in the Biomonitoring Study at Jeorse Park Beach. A) No significant clustering according to sampling month (ANOSIM $p = 0.24$); B) Highly significant clustering according to general area where samples were taken (ANOSIM $p = 0.001$).

3.6 Angler's Survey

Over the course of the survey there were 22 anglers in total that were surveyed Portage Lakefront and Washington Park Beach. The results of the survey are indicated in Table 11. Questions were asked about their skill level, frequency of fishing the area, what equipment was used and what they caught. From the surveys we were able to see that there are many skilled anglers fishing, who have been fishing the area for several years. Anglers spent most of the day fishing, starting in the early morning, and some traveled over 100 miles just to fish a certain area. Most anglers surveyed had the max number of lines in the water allotted (3 lines) and were fishing for and catching Steelhead. Their equipment was tailored towards what they found worked best. Most anglers were aware of the invasive round goby in the area, but only 22% had caught or seen any on days I surveyed.

Table 11. The compiled responses of the Angler's survey given on July 6 and July 20.

Date of your fishing trip?	Where were you fishing in the Southern Lake Michigan area?	Where/What were you fishing from?	What did you catch?	Did you [CATCH / SEE] round goby
7/6/2018	Michigan City	Breakwall	Nothing	Yes
7/6/2018	Michigan City	Breakwall	Nothing	No
7/6/2018	Michigan City	Breakwall	Nothing	Yes
7/6/2018	Michigan City	Breakwall	Steelhead	No
7/6/2018	Michigan City	Breakwall	Nothing	No
7/8/2018	Michigan City	Boat	Trout, Salmon	No
7/18/2018	Michigan City	Breakwall	12 Steelhead	n/a
7/19/2018	Michigan City	Breakwall	Nothing	No
7/19/2018	Michigan City	Breakwall	Nothing	No
7/19/2018	Michigan City	Breakwall	4 Trout (steelhead)	Caught
7/19/2018	Michigan City	Breakwall	Steelhead	No
7/19/2018	Michigan City	Boat	Trout	n/a
7/19/2018	Michigan City	Breakwall	2 Steelhead	Yes
7/19/2018	Michigan City	Breakwall	10 Skamania steelhead	No
7/19/2018	Michigan City	Breakwall	Nothing	No
7/20/2018	Michigan City	Breakwall	Nothing	No
7/20/2018	Portage Lakefront	Breakwall	Nothing	Yes
7/20/2018	Michigan City	Breakwall	Nothing	n/a
7/20/2018	Michigan City	Breakwall	Steelhead	No
7/20/2018	Michigan City	Breakwall	Fish, didn't specify species	n/a
7/20/2018	Michigan City	Breakwall	Nothing	No
7/20/2018	Michigan City	Breakwall	Nothing	No

CHAPTER 4. DISCUSSION

4.1 Protocol Development

It is important to choose the proper extraction method when working with environmental DNA because as the eDNA quantities of the target organism is often low, which is a common problem in many environmental samples (e.g., water, sediments etc.). The Power Water kit combined with the glass fiber filter paper is the most widely used extraction-filter paper combination in eDNA studies (Jerde et al., 2011; Olson et al., 2012). In Hinlo et al. (2017) the PowerWater kit and glass Fiber combination gave the lowest DNA yield and had the highest cost per sample. Cellulose filters have a high DNA and protein binding capacity when compared to other filters, such as glass fiber (Towbin et al., 1979; Thornton et al., 1996), which is why we ultimately chose to use the cellulose filter paper. Miya et al. (2015) developed the universal fish primers (MiFish-U/E) and used the DNeasy kit for eDNA extraction in their study. Likewise, our protocol testing confirm that Qiagen's DNeasy Blood and Tissue kit is a good choice for use with the MiFish primer, and suggests that, when used in combination with proteinase K in the extracted process, it is a cost effective and high-yielding approach for eDNA-extraction in studies implementing eDNA-metabarcoding for fish biomonitoring projects.

4.1.1 Validation of eDNA-Metabarcoding for Biomonitoring

4.1.1.1 Closed Freshwater Tank

Considering that traditional macro-organismal surveying techniques are a labor-intensive undertaking and can be harmful to the species being monitored, natural resource monitoring using eDNA methods are increasingly seen as a more desirable option, with eDNA-metabarcoding being a powerful choice (Taberlet et al., 2012; Valentini et al., 2016; Deiner et al., 2017). We set out to validate and apply eDNA-metabarcoding for use in biomonitoring and invasive species detection on Lake Michigan in the Great Lakes region, building on the success of established platforms (Iwasaki et al., 2013; Miya et al., 2015). In validating our eDNA-metabarcoding methods we were able to recover, when filtering just one liter of tank water, the majority of a fish population in a closed tank system with similar proportional abundances to the known community. Previous

mesocosm studies (Evans et al., 2015) have shown that eDNA-metabarcoding can reliably recover known populations of freshwater macrofauna, including fish as well as amphibian species, and our results reflected similar findings for fish species that are native to Lake Michigan. While Evans et al. (2015) found only a ‘modest’ correspondence between known communities in 340-L tanks, relative abundances detected by eDNA-metabarcoding in the closed tank of our validation studies mirrored those of the known community fairly precisely, in a tank nearly 2,000 times the size. The only exception was the high numbers of Walleye mitochondrial 12S gene copies found in our study, which were detected at the highest abundance level by our eDNA-metabarcoding methods. Anecdotally, while sampling the tank a walleye was seen biting at the end of the sampling cup, potentially leaving additional biological material (e.g., cells/tissue materials) for DNA extraction and likely leading to the high abundance of walleye 12S genes detected. Thus, it is likely that under certain conditions of the true community structure can be skewed when individual fish move in close proximity to sampling the apparatus. Further, we were not able to capture the same degree of the community as the former study, with our methods missing catfish and quillback. As Evans and colleagues (2015) suggest, this result could reflect the phenomenon of ‘species masking’, whereby the presence of highly abundant species may prevent the detection of species found in low abundance or may be the result of primer biases. Considering we were able to detect other low-abundance fish species (e.g., those with just one individual in the tank) and that these ‘missing’ species were not detected at the study site, the former explanation seems more plausible. Finally, fish detected in the eDNA-metabarcoding that were not known to be in the tank, were species found in high abundance in samples from the study site. As we ran extensive sample and extraction blanks, we were able to determine that these species were found in very low abundances (1-6 sequences) in a few of our blanks, suggesting that some cross contamination may have occurred and highlighting the extreme sensitivity of metabarcoding methods as well as the importance of incorporating blank controls into the study design.

4.1.1.2 Traditional Monitoring Methods

Traditional survey methods, such as netting, have also been previously compared with eDNA-metabarcoding results from open-river (Shaw et al., 2016) and lake (Fujii et al., 2019) systems. While these studies report slightly higher overlap percentages between the methods, both applied much more rigor in their traditional survey efforts; however, they also demonstrate discrepancies can occur, especially with eDNA-metabarcoding methods missing species detected by traditional means (Shaw et al., 2016; Fujii et al., 2019). Simple primer biases (Nichols et al., 2017) against particular fish species may also be at play here; however, open systems, such as rivers and lakes, are known to have complex hydrodynamics at large (Csanady, 1967) and small scales (Wüest & Lorke, 2003) that may randomly or systematically (Shogren et al., 2017) prevent eDNA of particular species from entering the sampling apparatus, especially when they are in low abundance and small amounts of water are being filtered. Additional, controlled experiments with fish species at different abundances will be helpful to better understand these factors. Other factors, such as eDNA dispersal and degradation (Li et al., 2019) and fish population size and behavior (Jerde et al., 2019), influence eDNA studies in open waterways as well. Gathering multiple samples, in a broader area, at varying depths, and filtering large volumes of water, as well as increasing sample replicates could ameliorate the problem of missing species in a surveying effort, all which become more feasible with the decreasing costs of sequencing (Deiner et al., 2017). Notably, our validation studies comparing traditional survey methods demonstrated that concurrent detection of invasives (e.g., round goby) and much higher species capture, two to four times that of traditional method (with additional non-natives detected), was possible with our eDNA-metabarcoding approach. The fact that the species were primarily known from regional waters or Jeorse Park Beach from the historical records further lends credence to the method and demonstrates the capacity of the eDNA-metabarcoding to capture in a period of short time with very little filtered water volume datasets that have taken long periods of time to develop with conventional methods, as has been noted in other studies (Civade et al., 2016; Hänfling et al., 2016). Additionally, the fact that some species, such as creek chub (*Semotilus atromaculatus*), were detected that are not known from the historical record but are known from Lake Michigan, suggests that the method is highly sensitive and may be useful for obtaining new or updating inventory records, although further sampling would be required to confirm these records.

4.2 Baseline Biomonitoring at Jearse Park Beach using eDNA-Metabarcoding

We applied our eDNA-metabarcoding approach in monitoring the native and non-native fish populations of Jearse Park Beach, a site in the EPA's Grand Calumet River Area of Concern, where several restoration initiatives have been implemented to improve water quality and restore native species under the direction of the United States Army Corps of Engineers (USACE) and other local agencies (see Nevers et al., 2018b). At this site, restoration activities have included modifying an existing rock breakwall structure and constructing submerged rock reef structures (United States Army Corps of Engineers, 2016). The intention for these structures is to provide improved aquatic habitat for native fish populations. As with the traditional survey comparison results in the validation studies, eDNA-metabarcoding detected numerous fish species across the Jearse Park Beach sampling sites that have been historically recorded from the southern Lake Michigan region or that had been recorded previously from this specific site, and also included a few probable new records. The high degree of overlap with the historical records from these waters again suggests our eDNA-metabarcoding approach is reliably reflecting the natural fish populations in these waters. Further, the ordination analysis showed fish community composition at the various sampling sites at Jearse Park Beach were stable during the sampling period, with little seasonal influence discernable. This suggests that monitoring via eDNA-metabarcoding is highly feasible across time within specific areas, including nearshore, breakwall, and open waters above and near the reef sites, these corresponding to particular habitats. Other similar studies in large lentic and lotic systems have likewise noted distinct spatial structuring of fish communities, though at larger spatial scales, and note this pattern is particularly relevant for warmer months, which has relevance for monitoring study designs and planning (Civade et al., 2016; Handley et al., 2019).

While we detected several invasive species (e.g., alewife, common carp, river carpsucker, and round goby) and other non-native fish species (e.g., coho salmon and rainbow trout) that are stocked in Lake Michigan for sport, a flourishing population of native species (e.g., bluegill, largemouth bass, and rock bass) was also discernable at the Jearse Park Beach site. Overall, the native fish species outnumbered those of the non-native by slightly more than a factor of two. Further, the break wall and reef structures showed the highest average levels of species diversity, suggesting the improved habitat provided by these structures promote robust communities; however, caveats must be noted, such as the Shannon Index showed sensitivity to both site and

seasonal factor. While these signs may be positive overall, other patterns were worth noting, especially the very high abundances of alewife and common carp, both these having the potential to further impact native community interactions (Brandt et al., 1987; Lougheed et al., 2011; Madenjian et al., 2011), potentially competing for resources. These abundance patterns are, however, contrasted with the native spottail shiner being the most abundant species recovered across the sampling sites, which serves as a planktivorous member of the food web, important for energy transformations (Hartman et al., 1992). We also note that the aggressive round goby was recovered in samples with high frequency, though always at levels below 4% of the total sample population, and typically well below that. A number of natural predators (Kornis et al., 2012) of the round goby (e.g., freshwater drum, rock bass, smallmouth bass, walleye, and yellow perch) were also detected with some frequency across the sample sites.

CHAPTER 5. CONCLUSIONS

Overall, this study suggests eDNA-metabarcoding is an efficient, credible, and powerful tool to biomonitoring. In addition to being able to detect higher numbers of species than traditional survey techniques, the method was able to capture patterns of diversity that previously would have taken years to establish by conventional means. While the MiFish/MitoFish pipeline worked effectively for our purposes, there were a number of fish species from our study site that are not currently in the MitoFish database, which suggests that it will need to be further augmented with sequences representing fish, native as well as non-native species, from the Great Lakes before the pipeline can be effectively used within these waters. The study also points to the need for modifications to sampling protocol and caveats relevant to planning for future programs, such as the potential need to sample more broadly or filter more water at sample sites, which can to improve the quality of future monitoring.

Finally, our study suggests the reef installation at Jeorse Park Beach likely does not favor round goby over other native fish species, and that the break wall modification appears to foster species that are desirable to anglers (e.g., bass, salmon, and trout). Further monitoring will be required, however, to evaluate change in the fish community structure over time as reef and breakwall structures become fully inhabited. Overall, the patterns observed in this study provide benchmarks and baseline data for future natural resource monitoring projects in southern Lake Michigan, as well as across the Great Lakes, and especially for such future biomonitoring efforts using eDNA-metabarcoding at the Jeorse Park Beach site. While we did detect several invasive species (e.g., alewife, common carp, river carpsucker, and round goby) and other non-native fish species (e.g., coho salmon and rainbow trout) that are stocked in Lake Michigan for sport, a flourishing population of native species (e.g., bluegill, largemouth bass, and rock bass) was also discernable at the Jeorse Park Beach site. Overall, the native fish species outnumbered those of the non-native by slightly more than a factor of two. Further, the breakwall and reef structures showed the highest average levels of diversity, suggesting the improved habitat provided by these structures promote robust communities. While these signs may be positive, other patterns were worth noting, especially the very high abundances of alewife and common carp, both these having the potential to further impact native community interactions (Brandt et al., 1987; Loughheed et al., 2011; Madenjian et al., 2011). These abundance patterns are, however, contrasted with the native

spottail shiner being the most abundant species recovered across the sampling sites, which serves as a planktivorous member of the food web, important for energy transformations (Hartman et al., 1992). Finally, our study suggests the reef installation at Jeorse Park Beach likely does not favor round goby over other native fish species, and that the break wall modification appears to foster species that are desirable to anglers (e.g., bass, salmon, and trout). Future monitoring will be required, however, to evaluate change in the fish community structure over time as reef and breakwall structures become fully inhabited.

REFERENCES

- Adrian-Kalchhauser, I., & Burkhardt-Holm, P. 2016. An eDNA Assay to Monitor a Globally Invasive Fish Species from Flowing Freshwater. *PLoS ONE*, 11(1), e0147558. <https://doi.org/10.1371/journal.pone.0147558>
- Altschul, S. 1990. Basic Local Alignment Search Tool. *Journal of Molecular Biology*, 215(3), 403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
- Balasingham, K. D., Walter, R. P., & Heath, D. D. 2016. Residual eDNA Detection Sensitivity Assessed by Quantitative Real-Time PCR in a River Ecosystem. *Molecular Ecology Resources*, 17(3), 523–532. <http://doi.org/10.1111/1755-0998.12598>
- Balasingham, K. D., Walter, R. P., Mandrak, N. E., & Heath, D. 2017. Environmental DNA Detection of Rare and Invasive Fish Species in Two Great Lakes Tributaries. *Molecular Ecology*, 27(1), 112–127. <https://doi.org/10.1111/mec.14395>
- Bergstrom, M. A., & Mensinger, A. F. 2009. Interspecific Resource Competition Between the Invasive Round Goby and Three Native Species: Log Perch, Slimy Sculpin, and Spoonhead Sculpin. *Transactions of the American Fisheries Society*, 138(5), 1009–1017. <https://doi.org/10.1577/T08-095.1>
- Bortolus, A. 2008. Error Cascades in the Biological Sciences: The Unwanted Consequences of Using Bad Taxonomy in Ecology. *AMBIO: A Journal of the Human Environment*, 37(2), 114–118. [https://doi.org/10.1579/0044-7447\(2008\)37\[114:ECITBS\]2.0.CO;2](https://doi.org/10.1579/0044-7447(2008)37[114:ECITBS]2.0.CO;2)
- Brandt, S. B., Mason, D. M., Macneill, D. B., Coates, T., & Gannon, J. E. 1987. Predation by Alewives on Larvae of Yellow Perch in Lake Ontario. *Transactions of the American Fisheries Society*, 116(4), 641–645. [https://doi.org/10.1577/1548-8659\(1987\)1162.0.CO;2](https://doi.org/10.1577/1548-8659(1987)1162.0.CO;2)
- Bushnell, B., Rood, J., & Singer, E. 2017. BBMerge – Accurate Paired Shotgun Read Merging via Overlap. *PLoS ONE* 12(10), e0185056. <https://doi.org/10.1371/journal.pone.0185056>
- Byappanahalli, M. N., Nevers, M. B., Whitman, R. L., Ge, Z., Shively, D., Spoljaric, A., & Przybyla-Kelly, K. 2015. Wildlife, Urban Inputs, and Landscape Configuration are Responsible for Degraded Swimming Water Quality at an Embayed Beach. *Journal of Great Lakes Research* 41, 156–163. <https://doi.org/10.1016/j.jglr.2014.11.027>

- Caporaso, J., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F., Costello, E., ... Knight, R. n.d. QIIME Allows Analysis of High-Throughput Community Sequencing Data. *Nature Methods*, 7(5), 335–336. <https://doi.org/10.1038/nmeth.f.303>
- Charlebois, P. M., Corkum, L. D., Jude, D. J., & Knight, C. 2001. The Round Goby (*Neogobius melanostomus*) Invasion: Current Research and Future Needs. *Journal of Great Lakes Research*, 27(3), 263-266. [https://doi.org/10.1016/S0380-1330\(01\)70641-7](https://doi.org/10.1016/S0380-1330(01)70641-7)
- Charlebois, P. M., Marsden, J. E., Goettel, R. G., Wolfe, R. K., Jude, D. J. & Rudnika, S. 1997. The Round Goby (*Neogobius melanostomus*): A Review of European and North American Literature. Illinois Natural History Survey Special Publication 20. Retrieved from https://www.ideals.illinois.edu/bitstream/handle/2142/10174/inhscaev01996i00010_opt.pdf?sequence=2 (accessed on 15 April, 2020)
- Civade, R., Dejean, T., Valentini, A., Roset, N., Raymond, J., Bonin, A., ... Pont, D. 2016. Spatial Representativeness of Environmental DNA Metabarcoding Signal for Fish Biodiversity Assessment in a Natural Freshwater System. *PLoS ONE*, 11(6), e0157366. <https://doi.org/10.1371/journal.pone.0157366>
- Cooper, M. J., Ruetz, C. R., Uzarski, D. G., & Shafer, B. M. 2009. Habitat Use and Diet of the Round Goby (*Neogobius melanostomus*) in Coastal Areas of Lake Michigan and Lake Huron. *The Journal of Freshwater Ecology*, 24(3), 477–488. <https://doi.org/10.1080/02705060.2009.9664321>
- Corkum, L. D., Sapota, M. R., & Skora, K. E. 2004. The Round Goby, *Neogobius melanostomus*, a Fish Invader on Both Sides of the Atlantic Ocean. *Biological Invasions*, 6(2), 173–181. <http://doi.org/10.1023/b:binv.0000022136.43502.db>
- Csanady, G. T. 1967. Large-Scale Motion in the Great Lakes. *Journal of Geophysical Research*, 72(16), 4151–4162. <https://doi.org/10.1029/JZ072i016p04151>
- Davidson, A., Fusaro, A., Sturtevant, R., & Kashian, D. 2017. Development of a Risk Assessment Framework to Predict Invasive Species Establishment for Multiple Taxonomic Groups and Vectors of Introduction. *Management of Biological Invasions*, 8(1), 25–36. <https://doi.org/10.3391/mbi.2017.8.1.03>

- Deiner, K., Bik, H. M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., ... Bernatchez, L. 2017. Environmental DNA Metabarcoding: Transforming How We Survey Animal and Plant Communities. *Molecular Ecology*, 26(21), 5872–5895. <https://doi.org/10.1111/mec.14350>
- Deiner, K., Walser, J., Mächler, E., & Altermatt, F. 2015. Choice of Capture and Extraction Methods Affect Detection of Freshwater Biodiversity from Environmental DNA. *Biological Conservation*, 183(C), 53–63. <https://doi.org/10.1016/j.biocon.2014.11.018>
- Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., & Knight, R. 2011. UCHIME Improves Sensitivity and Speed of Chimera Detection. *Bioinformatics* 27(16), 2194–200. <https://doi.org/10.1093/bioinformatics/btr381>
- Evans, N. T., Olds, B. P., Renshaw, M. A., Turner, C. R., Li, Y., Jerde, C. L., ... Lodge, D. M. 2015. Quantification of Mesocosm Fish and Amphibian Species Diversity via Environmental DNA Metabarcoding. *Molecular Ecology Resources*, 16(1), 29–41. <https://doi.org/10.1111/1755-0998.12433>
- Ficetola, G. F., Miaud, C., Pompanon, F., & Taberlet, P. 2008. Species Detection Using Environmental DNA from Water Samples. *Biology Letters*, 4(4), 423–425. <https://doi.org/10.1098/rsbl.2008.0118>
- French, J. R., & Jude, D. J. 2001. Diets and Diet Overlap of Nonindigenous Gobies and Small Benthic Native Fishes Co-Inhabiting the St. Clair River. Michigan. *Journal of Great Lakes Research*, 27(3), 300–311. [https://doi.org/10.1016/S0380-1330\(01\)70645-4](https://doi.org/10.1016/S0380-1330(01)70645-4)
- Fujii, K., Doi, H., Matsuoka, S., Nagano, M., Sato, H., & Yamanaka, H. 2019. Environmental DNA Metabarcoding for Fish Community Analysis in Backwater Lakes: A Comparison of Capture Methods. *PLoS ONE*, 14(1), e0210357. <https://doi.org/10.1371/journal.pone.0210357>
- Günther, B., Knebelsberger, T., Neumann, H., Laakmann, S., & Arbizu, P. M. 2018. Metabarcoding of Marine Environmental DNA Based on Mitochondrial and Nuclear Genes. *Scientific Reports*, 8(1), e14822. <http://doi.org/10.1038/s41598-018-32917-x>
- Handley, L. L., Read, D. S., Winfield, I. J., Kimbell, H., Johnson, H., Li, J., ... Hänfling, B. 2019. Temporal and Spatial Variation in Distribution of Fish Environmental DNA in England's Largest Lake. *Environmental DNA*, 1(1), 26–39. <https://doi.org/10.1002/edn3.5>

- Hartman, K. J., Vondracek, B., Parrish, D. L., & Muth, K. M. 1992. Diets of Emerald and Spottail Shiners and Potential Interactions with Other Western Lake Erie pPlanktivorous Fishes. *Journal of Great Lakes Research*, 18(1), 43–50. [https://doi.org/10.1016/S0380-1330\(92\)71273-8](https://doi.org/10.1016/S0380-1330(92)71273-8)
- Hassan, M. 2018. Environmental DNA (eDNA) 18S metabarcoding Illumina MiSeq NGS PCR Protocol v1 (protocols.io.ud9es96). <http://doi.org/10.17504/protocols.io.ud9es96>
- Hänfling, B., Handley, L. L., Read, D. S., Hahn, C., Li, J., Nichols, P., ... Winfield, I. J. 2016. Environmental DNA Metabarcoding of Lake Fish Communities Reflects Long-Term Data from Established Survey Methods. *Molecular Ecology*, 25(13), 3101–3119. <https://doi.org/10.1111/mec.13660>
- Hinlo, R., Gleeson, D., Lintermans, M., & Furlan, E. 2017. Methods to Maximize Recovery of Environmental DNA from Water Samples. *PLoS ONE*, 12(6), e0179251. <https://doi.org/10.1371/journal.pone.0179251>
- Huson, D. H., Auch, A. F., Qi, J., & Schuster, S. C. 2007. MEGAN Analysis of Metagenomic Data. *Genome Research* 17, 377–386. <http://doi.org/10.1101/gr.5969107>
- Iwasaki, W., Fukunaga, T., Isagozawa, R., Yamada, K., Maeda, Y., Satoh, T. P., ... Nishida, M. 2013. MitoFish and MitoAnnotator: A Mitochondrial Genome Database of Fish with an Accurate and Automatic Annotation Pipeline. *Molecular Biology and Evolution*, 30(11), 2531–2540. <https://doi.org/10.1093/molbev/mst141>
- Jerde, C., Mahon, A., Chadderton, L., Lodge, D. 2011, “Sight Unseen” Detection of Rare Aquatic Species Using Environmental DNA. *Conservation Letters*, 4 (2), 150–157. <https://doi.org/10.1111/j.1755-263X.2010.00158.x>
- Jerde, C. L., Wilson, E. A., & Dressler, T. L. 2019. Measuring Global Fish Species Richness with eDNA Metabarcoding. *Molecular Ecology Resources*, 19(1), 19–22. <https://doi.org/10.1111/1755-0998.12929>
- Klymus, K. E., Marshall, N. T., & Stepien, C. A. 2017. Environmental DNA (eDNA) Metabarcoding Assays to Detect Invasive Invertebrate Species in the Great Lakes. *PLoS ONE*, 12(5), e0177643. <https://doi.org/10.1371/journal.pone.0177643>

- Kornis, M. S., Mercado-Silva, N., & Zanden, M. J. 2012. Twenty Years of Invasion: A Review of Round Goby *Neogobius melanostomus* Biology, Spread and Ecological Implications. *Journal of Fish Biology*, 80(2), 235–285. <http://doi.org/10.1111/j.1095-8649.2011.03157.x>
- Kraaijeveld, K., Weger, L. A., García, M. V., Buermans, H., Frank, J., Hiemstra, P. S., & Dunnen, J. T. 2014. Efficient and Sensitive Identification and Quantification of Airborne Pollen Using Next-Generation DNA Sequencing. *Molecular Ecology Resources*, 15(1), 8–16. <https://doi.org/10.1111/1755-0998.12288>
- Lauer, T. E., Allen, P. J., & McComish, T. S. 2004. Changes in Mottled Sculpin and Johnny Darter Trawl Catches After the Appearance of Round Gobies in the Indiana Waters of Lake Michigan. *Transactions of the American Fisheries Society*, 133(1), 185–189. <https://doi.org/10.1577/T02-123>
- Li, J., Handley, L. L., Harper, L. R., Brys, R., Watson, H. V., & Hänfling, B. 2018. Limited Dispersion and Quick Degradation of Environmental DNA in Fish Ponds Inferred by Metabarcoding. *Environmental DNA*, 1(3), 238–250. <https://doi.org/10.1101/459321>
- Lim, N. K., Tay, Y. C., Srivathsan, A., Tan, J. W., Kwik, J. T., Baloglu, B., ... Yeo, D. C. 2016. Next-Generation Freshwater Bioassessment: EDNA Metabarcoding with a Conserved Metazoan Primer Reveals Species-Rich and Reservoir-Specific Communities. *Royal Society Open Science*, 3(11), 160635. <https://doi.org/10.1098/rsos.160635>
- Lougheed, V. L., Crosbie, B., & Chow-Fraser, P. 1998. Predictions on the Effect of Common Carp (*Cyprinus carpio*) Exclusion on Water Quality, Zooplankton, and Submergent Macrophytes in a Great Lakes Wetland. *Canadian Journal of Fisheries and Aquatic Sciences*, 55(5), 1189–1197. <https://doi.org/10.1139/f97-315>
- Madenjian, C. P., O'gorman, R., Bunnell, D. B., Argyle, R. L., Roseman, E. F., Warner, D. M., ... Stapanian, M. A. 2008. Adverse Effects of Alewives on Laurentian Great Lakes Fish Communities. *North American Journal of Fisheries Management*, 28(1), 263–282. <https://doi.org/10.1577/M07-012.1>
- Madenjian, C. P., Stapanian, M. A., Witzel, L. D., Einhouse, D. W., Pothoven, S. A., & Whitford, H. L. 2010. Evidence for Predatory Control of the Invasive Round Goby. *Biological Invasions*, 13(4), 987–1002. <http://doi.org/10.1007/s10530-010-9884-7>

- Mahé, F., Rognes, T., Quince, C., Vargas, C. D., & Dunthorn, M. 2015. Swarm v2: Highly-Scalable and High-Resolution Amplicon Clustering. PeerJ. <https://doi.org/10.7717/peerj.1420>
- Martin, M. 2011. Cutadapt Removes Adapter Sequences from High-Throughput Sequencing Reads. EMBnet Journal 17(1), e10. <https://doi.org/10.14806/ej.17.1.200>
- Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J. Y., Sato, K., ... Iwasaki, W. 2015. MiFish, a Set of Universal PCR Primers for Metabarcoding Environmental DNA from Fishes: Detection of More Than 230 Subtropical Marine Species. Royal Society Open Science, 2(7), 150088. <https://doi.org/10.1098/rsos.150088>
- Nevers, M. B., Byappanahalli, M. N., Morris, C. C., Shively, D., Przybyla-Kelly, K., Spoljaric, A. M., ... Roseman, E. F. 2018a. Environmental DNA (eDNA): A Tool for Quantifying the Abundant but Elusive Round Goby (*Neogobius melanostomus*). PLoS ONE, 13(1), e0191720. <https://doi.org/10.1371/journal.pone.0191720>
- Nevers, M. B., Byappanahalli, M. N., Shively, D., Buszka, P. M., Jackson, P. R., & Phanikumar, M. S. 2018b. Identifying and Eliminating Sources of Recreational Water Quality Degradation Along an Urban Coast. Journal of Environmental Quality 47, 1042–1050. <https://doi.org/10.2134/jeq2017.11.0461>
- Nichols, R. V., Vollmers, C., Newsom, L. A., Wang, Y., Heintzman, P. D., Leighton, M., ... Shapiro, B. 2018. Minimizing Polymerase Biases in Metabarcoding. Molecular Ecology Resources, 18(5), 927–939. <http://doi.org/10.1111/1755-0998.12895>
- Olson, Z., Briggler, J., Williams R. 2012 An eDNA Approach to Detect Eastern Hellbenders (*Cryptobranchus alleganiensis*) Using Samples of Water. Wildlife Research 39, 629–636. <https://doi.org/10.1071/WR12114>
- Pace, N. R. 1997. A Molecular View of Microbial Diversity and the Biosphere. Science, 276(5313), 734–740. <http://doi.org/10.1126/science.276.5313.734>
- Pimentel, D., Zuniga, R., & Morrison, D. 2005. Update on the Environmental and Economic Costs Associated with Alien-Invasive Species in the United States. Ecological Economics, 52(3), 273–288. <https://doi.org/10.1016/j.ecolecon.2004.10.002>

- Ramirez, K. S., Leff, J. W., Barberán, A., Bates, S. T., Betley, J., Crowther, T. W., ... Fierer, N. 2014. Biogeographic Patterns in Below-Ground Diversity in New York City's Central Park Are Similar to Those Observed Globally. *Proceedings of the Royal Society B: Biological Sciences*, 281(1795), e20141988. <http://doi.org/10.1098/rspb.2014.1988>
- Rivera, S. F., Vasselon, V., Jacquet, S., Bouchez, A., Ariztegui, D., & Rimet, F. 2017. Metabarcoding of Lake Benthic Diatoms: From Structure Assemblages to Ecological Assessment. *Hydrobiologia*, 807(1), 37–51. <http://doi.org/10.1007/s10750-017-3381-2>
- Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. 2016. vSearch: A Versatile Open Source Tool for Metagenomics. *PeerJ* 4, e2584. <https://doi.org/10.7717/peerj.2584>
- Schmidt, P., Bálint, M., Greshake, B., Bandow, C., Römcke, J., & Schmitt, I. 2013. Illumina Metabarcoding of a Soil Fungal Community. *Soil Biology and Biochemistry*, 65, 128–132. <https://doi.org/10.1016/j.soilbio.2013.05.014>
- Shaw, J. L., Clarke, L. J., Wedderburn, S. D., Barnes, T. C., Weyrich, L. S., & Cooper, A. 2016. Comparison of Environmental DNA Metabarcoding and Conventional Fish Survey Methods in a River System. *Biological Conservation*, 197, 131–138. <https://doi.org/10.1016/j.biocon.2016.03.010>
- Shogren, A. J., Tank, J. L., Andruszkiewicz, E., Olds, B., Mahon, A. R., Jerde, C. L., & Bolster, D. 2017. Controls on eDNA Movement in Streams: Transport, Retention, and Resuspension. *Scientific Reports*, 7(1), e 5065. <http://doi.org/10.1038/s41598-017-05223-1>
- Song, Z., Schlatter, D., Kennedy, P., Kinkel, L. L., Kistler, H. C., Nguyen, N., & Bates, S. T. 2015. Effort Versus Reward: Preparing Samples for Fungal Community Characterization in High-Throughput Sequencing Surveys of Soils. *PLoS ONE*, 10(5), e0127234. <http://doi.org/10.1371/journal.pone.0127234>
- Spear, S. F., Groves, J. D., Williams, L. A., & Waits, L. P. 2015. Using Environmental DNA Methods to Improve Detectability in a Hellbender (*Cryptobranchus alleganiensis*) Monitoring Program. *Biological Conservation*, 183, 38–45. <http://doi.org/10.1016/j.biocon.2014.11.016>
- Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., & Willerslev, E. 2012. Towards Next-Generation Biodiversity assessment using DNA Metabarcoding. *Molecular Ecology*, 21(8), 2045–2050. <https://doi.org/10.1111/j.1365-294X.2012.05470.x>

- Towbin, H., Staehelin, T., Gordon, J. 1979. Electrophoretic Transfer of Proteins from Polyacrylamide Gels to Nitrocellulose Sheets: Procedure and Some Applications. *Proceedings of the National Academy of Sciences*, 76 (9), 4350-4354. <https://doi.org/10.1073/pnas.76.9.4350>
- Thomas, A. C., Tank, S., Nguyen, P. L., Ponce, J., Sinnesael, M., & Goldberg, C. S. 2019. A System for Rapid eDNA Detection of Aquatic Invasive Species. *Environmental DNA* (early view). <https://doi.org/10.1002/edn3.25>
- Thomsen, P. F., & Sigsgaard, E. E. 2019. Environmental DNA Metabarcoding of Wild Flowers Reveals Diverse Communities of Terrestrial Arthropods. *Ecology and Evolution*, 9(4), 1665–1679. <https://doi.org/10.1002/ece3.4809>
- Thomsen, P. F., Kielgast, J., Iversen, L. L., Wiuf, C., Rasmussen, M., Gilbert, M. T., ... Willerslev, E. 2012. Monitoring Endangered Freshwater Biodiversity Using Environmental DNA. *Molecular Ecology*, 21(11), 2565–2573. <http://doi.org/10.1111/j.1365-294x.2011.05418.x>
- Thornton, D., Carlstedt, J., & Sheehan, I. 1996. Identification of Glycoproteins on Nitrocellulose Membranes and Gels. *Molecular Biotechnology*, 5(2), 171–176. <https://doi.org/10.1007/BF02789065>
- Tsuji, S., Takahara, T., Doi, H., Shibata, N., & Yamanaka, H. 2019. The Detection of Aquatic Macroorganisms Using Environmental DNA Analysis—A Review of Methods for Collection, Extraction, and Detection. *Environmental DNA*, 1(2), 99–108. <http://doi.org/10.1002/edn3.21>
- Turner, C. R., Uy, K. L., & Everhart, R. C. 2015. Fish Environmental DNA Is More Concentrated in Aquatic Sediments Than Surface Water. *Biological Conservation*, 183, 93–102. <http://doi.org/10.1016/j.biocon.2014.11.017>
- U.S. Army Corps of Engineers. 2015. Jeorse Park Beach section 506: Great Lakes fishery & ecosystem restoration study. Retrieved from <http://www.lrc.usace.army.mil/Portals/36/docs/projects/Jeorse%20Park/Jeorse%2030day%20NEPA%20AFB%20Doc.pdf> (accessed on 15 April, 2020)
- Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P. F., ... Dejean, T. 2016. Next-Generation Monitoring of Aquatic Biodiversity Using Environmental DNA Metabarcoding. *Molecular Ecology*, 25(4), 929–942. <https://doi.org/10.1111/mec.13428>

- Wüest, A., & Lorke, A. 2003. Small-Scale Hydrodynamics in Lakes. *Annual Review of Fluid Mechanics*, 35(1), 373–412. <https://doi.org/10.1146/annurev.fluid.35.101101.161220>
- Yamamoto, S., Masuda, R., Sato, Y., Sado, T., Araki, H., Kondoh, M., Minamoto, T., & Miya, M. 2017. Environmental DNA Metabarcoding Reveals Local Fish Communities in a Species-Rich Coastal Sea. *Scientific Reports*, 7(1), e 40368. <http://doi.org/10.1038/srep40368>

APPENDIX

This Appendix contains the updated fish mitochondrial 12S rRNA gene database that was used for taxonomic assignment in our MiFish/MitoFish pipeline. This updated database is based on an original 12S rRNA gene-specific version created from data obtained via the Mitochondrial Genome Database of Fish (MitoFish; Iwasaki et al., 2013; available at <http://mitofish.aori.u-tokyo.ac.jp/>); however, additional mitochondrial 12S rRNA gene sequences from NCBI's GenBank were added to include fish species (e.g., *N. melanostomus*) that were known to be in the waters of southern Lake Michigan and were taxa of interest in our study.

```
>KM267716      Metazoa|Chordata|<not
present>|Petromyzontiformes|Petromyzontidae|Ichthyomyzon|Ichthyomyzon fossor
CACCGCGGTTATACGAGGAGCTCAAGCTGATATATCCGGCACAAGCGTGATTAATAATTAGCTTAATTATACTATAGAAGCCAT
TATACCCACCAGTTAAATAGATATGCCTAATGTATCCCAACATCGAAAGAATCTATATTAATAAACTTACTTTGATATCACGAAAG
CAAACCCA
>KM267717      Metazoa|Chordata|<not
present>|Petromyzontiformes|Petromyzontidae|Ichthyomyzon|Ichthyomyzon unicuspis
CACCGCGGTTATACGAGGAGCTCAAGCTGATATATCCGGCACAAGCGTGATTAATAATTAGCTTAATTATACTATAGAAGCCAT
TATACCCACCAGTTAAATAGATATGCCTAATGTATCCCAACATCGAAAGAATCTATATTAATAAACTTACTTTGATATCACGAAAG
CAAACCCA
>KM267719      Metazoa|Chordata|<not
present>|Petromyzontiformes|Petromyzontidae|Lampetra|Lampetra appendix
CACCGCGGTTATACGAGGAGCTCAAGCTGATATTCTTCGGCACAAGCGTGATTAATAATTAGCTTAATTAATACTATAGAAGCC
ATCATGCCTGCTAGTTGAATAGGTATGCTTAAATATCTCAACATCGAAAGAATCTATATTAATAAACTCACTTTGACATCACGAAA
GCAAAACTCA
>U11880        Metazoa|Chordata|<not
present>|Petromyzontiformes|Petromyzontidae|Petromyzon|Petromyzon marinus
CACCGCGGTTATACGAGGAGCTCAAGCTGATATCTCCGGCACAAGCGTGATTAATAATTTAGCTTAATTAATACTATAGAAGCC
ATCATGCCTGCTAGTTAAATAGGTATGCCTAAGTATCCCAACATCGAAAGAATCTATATTAATAAGCTCACTTTGATATCACGAAA
GCAAAACTCA
>AF125595
      Metazoa|Chordata|Actinopteri|Acipenseriformes|Acipenseridae|Acipenser|Acipenser
fulvescens
CACCGCGGTTATACGAGAGGCCCAACTGATAGTCCACGGCGTAAAGCGTGATTAAGGATACCTACTACACTAGAGCCAAAAGCC
CCCTAAGCTGTCATACGCACTTGAGAGCCCGAAGCCCAACCACGAAGGTAGCTCTACCCAACAAGGACCCCTTGAACCCACGACAA
CTGAGACA
```

>KU985081

Metazoa|Chordata|Actinopteri|Acipenseriformes|Acipenseridae|Acipenser|Acipenser
fulvescens

CACCGCGTTATACGAGAGGCCCAACTGATAGTCCACGGCGTAAAGCGTGATTAAAGGATACCTACTACACTAGAGCCAAAAGCC
CCCTAAGCTGTCATACGCACTTGAGAGCCCGAAGCCCAACCACGAAGGTAGCTCTACCCAACAAGGACCCCTTGAACCCACGACAA
CTGAGACA

>KU985082

Metazoa|Chordata|Actinopteri|Acipenseriformes|Acipenseridae|Acipenser|Acipenser
fulvescens

CACCGCGTTATACGAGAGGCCCAACTGATAGTCCACGGCGTAAAGCGTGATTAAAGGATACCTACTACACTAGAGCCAAAAGCC
CCCTAAGCTGTCATACGCACTTGAGAGCCCGAAGCCCAACCACGAAGGTAGCTCTACCCAACAAGGACCCCTTGAACCCACGACAA
CTGAGACA

>KU985083

Metazoa|Chordata|Actinopteri|Acipenseriformes|Acipenseridae|Acipenser|Acipenser
fulvescens

CACCGCGTTATACGAGAGGCCCAACTGATAGTCCACGGCGTAAAGCGTGATTAAAGGATACCTACTACACTAGAGCCAAAAGCC
CCCTAAGCTGTCATACGCACTTGAGAGCCCGAAGCCCAACCACGAAGGTAGCTCTACCCAACAAGGACCCCTTGAACCCACGACAA
CTGAGACA

>KU985084

Metazoa|Chordata|Actinopteri|Acipenseriformes|Acipenseridae|Acipenser|Acipenser
fulvescens

CACCGCGTTATACGAGAGGCCCAACTGATAGTCCACGGCGTAAAGCGTGATTAAAGGATACCTACTACACTAGAGCCAAAAGCC
CCCTAAGCTGTCATACGCACTTGAGAGCCCGAAGCCCAACCACGAAGGTAGCTCTACCCAACAAGGACCCCTTGAACCCACGACAA
CTGAGACA

>KU985070

Metazoa|Chordata|Actinopteri|Acipenseriformes|Acipenseridae|Acipenser|Acipenser
fulvescens

CACCGCGTTATACGAGAGGCCCAACTGATAGTCCACGGCGTAAAGCGTGATTAAAGGATACCTACTACACTAGAGCCAAAAGCC
CCCTAAGCTGTCATACGCACTTGAGAGCCCGAAGCCCAACCACGAAGGTAGCTCTACCCAACAAGGACCCCTTGAACCCACGACAA
CTGAGACA

>AY442350

Metazoa|Chordata|Actinopteri|Semionotiformes|Lepisosteidae|Lepisosteus|Lepisost
eus oculatus

CACCGCGTTATACGAGAGGCTCCAAGTATAGTCCACGGCGTAAAGCGTGATTATAGGATGCTGCCCCAACTAAAGTCAAATCT
CCCTAAGCCGTCATACGCACTTGGGAGCATGAAGCCCTCCACGAAGGTAACCTTATAGCTACAGCCACCCTGAACTCACGATAG
CTAAGACA

>JF912028

Metazoa|Chordata|Actinopteri|Semionotiformes|Lepisosteidae|Lepisosteus|Lepisost
eus oculatus

CACCGCGTTATACGAGAGACTCTAACTGACAGCCACGGCGTAAAGCGTGATTATAGGATGCTACCCTAATTAAAGTCAAATCT
CCTTAAGCTGTCATACGCACTTGGGAGCATGAAGCCCTCCACGAAGGTAACCTTATAACCCACAGCCACCCTGAACTCACGATAG
TTAAGACA

>JF912031
Metazoa|Chordata|Actinopteri|Semionotiformes|Lepisosteidae|Lepisosteus|Lepisosteus oculatus
CACCGCGTTATACGAGAGGCTCCAACTGATAGCCACGGCGTAAAGCGTGATTATAGGATGCTGCCCCAACTAAAGTCAAATCT
CCTTAAGCCGTCATACGCACTTGGGAGCATGAAGCCCTCCACGAAGGTAACCTTATAATTACAGCCACCCTGAACTCACGATAG
TTAAGACC

>JF912034
Metazoa|Chordata|Actinopteri|Semionotiformes|Lepisosteidae|Lepisosteus|Lepisosteus oculatus
CACCGCGTTATACGAGAGGCTCCAACTGATAGCCACGGCGTAAAGCGTGATTATAGGATGCTGCCCCAACTAAAGTCAAATCT
CCCTAAGCCGTCATACGCACTTGGGAGCATGAAGCCCTCCACGAAGGTAACCTTATAGCCTACAGCCACCCTGAACTCACGATAG
CTAAGACA

>AB042861
Metazoa|Chordata|Actinopteri|Semionotiformes|Lepisosteidae|Lepisosteus|Lepisosteus oculatus
CACCGCGTTATACGAGAGGCTCCAACTGATAGCCACGGCGTAAAGCGTGATTATAGGATGCTGCCCCAACTAAAGTCAAATCT
CCCTAAGCCGTCATACGCACTTGGGAGCATGAAGCCCTCCACGAAGGTAACCTTATAGCCTACAGCCACCCTGAACTCACGATAG
CTAAGACA

>DQ536423
Metazoa|Chordata|Actinopteri|Semionotiformes|Lepisosteidae|Lepisosteus|Lepisosteus osseus
CACCGCGTTATACGAGAGGCTCCAACTGATAGCCACGGCGTAAAGCGTGATTATAGGATGCTGCCCCAACTAAAGTCAAATCT
CCCTAAGCCGTCATACGCACTTGGGAGCATGAAGCCCTCCACGAAGGTAACCTTATAGCCTACAGCCACCCTGAACTCACGATAG
CTAAGACA

>JF912030
Metazoa|Chordata|Actinopteri|Semionotiformes|Lepisosteidae|Lepisosteus|Lepisosteus osseus
CACCGCGTTATACGAGAGGCTCCAACTGATAGCCACGGCGTAAAGCGTGATTATAGGATGCTGCCCCAATTAAGTCAAATCT
CCTTAAGCCGTCATACGCACTTGGGAGCATGAAGCCCTCCACGAAGGTAACCTTATAATTTACAGCCACCCTGAACTCACGATAG
TTAAGACA

>JF912032
Metazoa|Chordata|Actinopteri|Semionotiformes|Lepisosteidae|Lepisosteus|Lepisosteus osseus
CACCGCGTTATACGAGAGGCTCCAACTGATAGCCACGGCGTAAAGCGTGATTATAGGATGCTACCCCACTAAAGTCAAATCT
CCTTAAGCCGTCATACGCACTTGGGAGCATGAAGCCCTCCAGGAAGGTAACCTTATAGCTTACAGCCACCCTGAACTCACGATAG
TTAAGACA

>JF912036
Metazoa|Chordata|Actinopteri|Semionotiformes|Lepisosteidae|Lepisosteus|Lepisosteus osseus
CACCGCGTTATACGAGAGGCTCCAACTGATAGCCACGGCGTAAAGCGTGATTATAGGATGCTGCCCCAACTAAAGTCAAATCT
CCCTAAGCCGTCATACGCACTTGGGAACATGAAGCCCTCCACGAAGGTAACCTTATAATTACAGCCACCCTGAACTCACGATAG
CTAAGACA

>AB042952 Metazoa|Chordata|Actinopteri|Amiiformes|Amiidae|Amia|Amia calva
CACCGCGTTATACGAGAGGCCCAATTGATAGACATCGGCGTAAAGCGTGATTACAGGCTGCTGCTATAAATTAAAGCGGAAATC
TCTCTCGACCGTTATACGTATATGAAAAGATGAAAATCAACCACGAAAGTAGCTTTATACAAAACAGCCACCTTGAAGTCACGACA
GCTAAGACA

>AY442347 Metazoa|Chordata|Actinopteri|Amiiformes|Amiidae|Amia|Amia calva
CACCGCGTTATACGAGAGGCCCAATTGATAGACATCGGCGTAAAGCGTGATTACAGGCTGCTGCTATAAATTAAAGCAGAAATC
TCTCTCGACCGTCATACGTATATGAAAAGATGAAAATCAACCACGAAAGTAGCTTTATGAAAACAGCCACCTTGAAGTCACGACA
GCTAAGACA

>AP009499 Metazoa|Chordata|Actinopteri|Hiodontiformes|Hiodontidae|Hiodon|Hiodon
tergisus
CACCGCGTTATACGAGAGGCCCAAGTTAACAGCTATCGGTGTAAAGCGTGATTATAGGACGCTAAACAACCTAAAGCCAAAAACCC
TCCGGGCCGTACATACGCATCCGAGGGCGCGAGGCCCCATTACGAAAGTAGCTTTAACAAAAGCACCTAGAACTCACGACAGCTGA
GAAA

>AP007249 Metazoa|Chordata|Actinopteri|Anguilliformes|Anguillidae|Anguilla|Anguilla
rostrata
CACCGCGTTATACGAGGGGCTCAAATTGATATTACACGGCGTAAAGCGTGATTAAAAAATAACAACTAAAGCCAAACACTTCC
CAAGTGTCATACGCTACCGACAAAACGAAGCCCTATAACGAAAGTAGCTTTAACACCTTTGAAGTCACGACAGTTGAGGAA

>MG570439 Metazoa|Chordata|Actinopteri|Clupeiformes|Clupeidae|Alosa|Alosa
pseudoharengus
CACCGCGTTATACGAGAGGCCCTAGTTGATTCACTCGGCGTAAAGAGTGTTATGGAGAATAAAATACTAAAGCCGAAGACCCCT
TAGGCCGTACATACGCACCTAGGGGCTCGAATTATAGACACGAAAGTAGCTTTACCCCTTCCCACCAGAACCCACGACAGCTGGGAC
A

>MG570440 Metazoa|Chordata|Actinopteri|Clupeiformes|Clupeidae|Alosa|Alosa
pseudoharengus
CACCGCGTTATACGAGAGGCCCTAGTTGATTCACTCGGCGTAAAGAGTGTTATGGAGAATAAAATACTAAAGCCGAAGACCCCT
TAGGCCGTACATACGCACCTAGGGGCTCGAATTATAGACACGAAAGTAGCTTTACCCCTTCCCACCAGAACCCACGACAGCTGGGAC
A

>MG570421 Metazoa|Chordata|Actinopteri|Clupeiformes|Clupeidae|Alosa|Alosa
pseudoharengus
CACCGCGTTATACGAGAGGCCCTAGTTGATTCACTCGGCGTAAAGAGTGTTATGGAGAATAAAATACTAAAGCCGAAGACCCCT
TAGGCCGTACATACGCACCTAGGGGCTCGAATTATAGACACGAAAGTAGCTTTACCCCTTCCCACCAGAACCCACGACAGCTGGGAC
A

>AP009132 Metazoa|Chordata|Actinopteri|Clupeiformes|Clupeidae|Alosa|Alosa
pseudoharengus
CACCGCGTTATACGAGAGGCCCTAGTTGATTCACTCGGCGTAAAGAGTGTTATGGAGAATAAAATACTAAAGCCGAAGACCCCT
TAGGCCGTACATACGCACCTAGGGGCTCGAATTATAGACACGAAAGTAGCTTTACCCCTTCCCACCAGAACCCACGACAGCTGGGAC
A

>MG570459 Metazoa|Chordata|Actinopteri|Clupeiformes|Clupeidae|Dorosoma|Dorosoma
cepedianum
CACCGCGTTATACGAGAGGCCCAAGTTGATAAGCCCGGCGTAAAGAGTGTTATGGAGAATAAAACTAAAGCTAAAGACCCCC
TAGGCTGTTATACGCACCTGGCGGCTCGAACCACCTATACGAAAGTAGCTTTACCCCTTCCCACCAGAATCCACGACAGCTGGGG
CA

>MG570429 Metazoa|Chordata|Actinopteri|Clupeiformes|Clupeidae|Dorosoma|Dorosoma
 cepedianum
 CACCGCGGTTATACGAGAGGCCCAAGTTGATAAGCCCGGCGTAAAGAGTGTTATGGAGAATAAAACACTAAAGCTAAAGACCCCC
 TAGGCTGTTATACGCACCTGGCGGCTCGAACCACCTATACGAAAGTAGCTTTACCCCCCTTCCACCAGAATCCACGACAGCTGGGG
 CA

>MG570415 Metazoa|Chordata|Actinopteri|Clupeiformes|Clupeidae|Dorosoma|Dorosoma
 cepedianum
 CACCGCGGTTATACGAGAGGCCCAAGTTGATAAGCCCGGCGTAAAGAGTGTTATGGAGAATAAAACACTAAAGCTAAAGACCCCC
 TAGGCTGTTATACGCACCTGGCGGCTCGAACCACCTATACGAAAGTAGCTTTACCCCCCTTCCACCAGAATCCACGACAGCTGGGG
 CA

>DQ536426 Metazoa|Chordata|Actinopteri|Clupeiformes|Clupeidae|Dorosoma|Dorosoma
 cepedianum
 CACCGCGGTTATACGAGAGGCCCAAGTTGATAAGCCCGGCGTAAAGAGTGTTATGGAGAATAAAACACTAAAGCTAAAGACCCCC
 TAGGCTGTTATACGCACCTGGCGGCTCGAACCACCTATACGAAAGTAGCTTTACCCCCCTTCCACCAGAATCCACGACAGCTGGGG
 CA

>KP013113
 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Campostoma|Campostoma
 anomalum
 CACCGCGGTTAGACGAGAGGCCCTAGTTGATAGTATAACGGCGTAAAGGGTGTTAAGGATACTGAGACAATAAAGCCGAATGGCC
 CTTTGCTGTCATACGCTTCTAGGAGTCCGAAGACCAATATACGAAAGTAGCTTTAAAGGAGTCCACCTGACCCACGAAAGCTGA
 GAAA

>AF023184
 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Campostoma|Campostoma
 anomalum
 CACCGCGGTTAGACGAGAGGCCCTAGTTGATAGTATAACGGCGTAAAGGGTGTTAAGGATACTGAGACAATAAAGCCGAATGGCC
 CTTTGCTGTCATACGCTTCTAGGAGTCCGAAGACCAATATACGAAAGTAGCTTTAAAGGAGTCCACCTGACCCACGAAAGCTGA
 GAAA

>DQ536421
 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Campostoma|Campostoma
 anomalum
 CACCGCGGTTAGACGAGAGGCCCTAGTTGATAATATAACGGCGTAAAGGGTGTTAAGGATACTGAGACAATAAAGCCAAATGACC
 CTTTAGCTGTCATACGCTTCTAGGAGTCCGAAGACCAATATACGAAAGTAGCTTTAAAGAGGCCACCTGACCCACGAAAGCTGA
 GAAA

>AP011280
 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Clinostomus|Clinostomus
 elongatus
 CACCGCGGTTAAACGAGAGGCCCTAGTTGATATGCAACGGCGTAAAGGGTGTTAAGGAGGGTGAAATAATAAAGCCGAATGGCCC
 TTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCAATATACGAAAGTAGCTTTAGGAAAGTCCACCTGACCCACGAAAGCTGAG
 GAA

>AF081840
 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Clinostomus|Clinostomus
 elongatus

CACCGCGGTTAAACGAGAGGCCCTAGTTGATATGCAACGGCGTAAAGGGTGGTTAAGGAGGGTGAAATAATAAAGCCGAATGGCCC
TTTGCTGTCATACGCTTCTAGGAGTCCGAAGCCCAATATACGAAAGTAGCTTTAGGAAAGTCCACCTGACCCACGAAAGCTGAG
GAA

>AP011274 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Coesius|Coesius
plumbeus

CACCGCGGTTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGAACGCATAACAATAAAGCCGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGTGTCCGAAGTCCAATATACGAAAGTAGCTTTAGAAAAGCCACCTGACCCACGAAAGCTGA
GAAA

>AF023185 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Coesius|Coesius
plumbeus

CACCGCGGTTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGAACGCATAACAATAAAGCCGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGTGTCCGAAGTCCAATATACGAAAGTAGCTTTAGAAAAGCCACCTGACCCACGAAAGCTGA
GAAA

>AF023186 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Coesius|Coesius
plumbeus

CACCGCGGTTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGAACGCATAACAATAAAGCCGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGTGTCCGAAGTCCAATATACGAAAGTAGCTTTAGAAAAGCCACCTGACCCACGAAAGCTGA
GAAA

>DQ536422

Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinella|Cyprinella
spioptera

CACCGCGGTTAAACGAGAGGCCCTAGTTGATTGAACAACGGCGTAAAGGGTGGTTAAGGACAGTGATATAATAAAGTCGAATGGCCC
TTTGCTGTCATACGCTTCTAGGAGTCTGAAGCCCACTGTACGAAAGTAGCTTTAAACATCCACCTGACCCACGAAAAGCTGAG
AAA

>AP012078 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Luxilus|Luxilus
chrysocephalus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATGTAACGGCGTAAAGGGTGGTTAAGGGATGCAAACAATAAAGTCGAATGGCCCT
TTGGCTGTCATACGCTTCTAGGTGCCTGAAGCCCAGCATACGAAAGTCACTTTAAATAGCCACCTGACCCACGAAAGCTGAGA
AA

>AY216539 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Luxilus|Luxilus
chrysocephalus

CACCGCGGTTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGATAGTAAAATAATAAAGTCGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCAATTATACGAAAGTAGCTTTAGTAAGGCCACCTGACCCACGAAAAGCTGA
GAAA

>MG570447 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Luxilus|Luxilus
cornutus

CACCGCGGTTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGATAGTAAAACAATAAAGTCGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCAATATACGAAAGTAACTTTAAACAAAGCCACCTGACCCACGAAAAGCTGA
GAAA

>MG570449 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Luxilus|Luxilus
cornutus

CACCGCGGTTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGATAGTAAAACAATAAAGTCGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCAATATACGAAAGTAACCTTAACAAAGCCCACCTGACCCACGAAAAGTGA
GAAA

>AP012090 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Luxilus|Luxilus
cornutus

CACCGCGGTTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGATAGTAAAACAATAAAGTCGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCAATATACGAAAGTAACCTTAACAAAGCCCACCTGACCCACGAAAAGTGA
GAAA

>AP012094 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Lythrurus|Lythrurus
umbratilis

CACCGCGGTTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGATAGTGAATTGATAAAGTCGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCAATATACGAAAGTAACCTTAAGAAAGTCCACCTGACCCACGAAAAGTGA
GAAA

>AY216541 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Lythrurus|Lythrurus
umbratilis

CACCGCGGTTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGGTAGTAAATTAATAAAGTCGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCAATTACGAAAGTAACCTTAAAAAGGCCACCTGACCCACGAAAAGTGA
AGAAA

>AF081852

Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Macrhybopsis|Macrhybopsis
storeriana

CACCGCGGTTAAACGAGAGGCCCTAGTTGATAGTGCAACGGCGTAAAGGGTGGTTAAGGATTAATGAGATAATAAAGTCGAATGGC
CCTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCAATATACGAAAGTAGCTTTAAGAAAGTCCACCTGACCCACGAAAAGTGA
AGAAA

>AF081853

Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Macrhybopsis|Macrhybopsis
storeriana

CACCGCGGTTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGATTAGTAAAATAATAAAGTCGAATGGC
CCTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCAATATACGAAAGTAGCTTTAAGAAAGTCCACCTGACCCACGAAAAGTGA
AGAAA

>KX139438

Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Macrhybopsis|Macrhybopsis
storeriana

CACCGCGGTTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGATAGTAAAATAATAAAGTCGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCAATATACGAAAGTAGCTTTAAGAAAGTCCACCTGACCCACGAAAAGTGA
GAAA

>AP012081 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Nocomis|Nocomis
biguttatus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGACGCACAATAATAAAGCCGAATGGCCC
TTTGGCTGTCATACGCTTCTAGGTGTCCGAAGCCCAATATACGAAAGTAGCTTTAAGAGAGCCCACCTGACCCACGAAAGCTGAG
GAA

>NC_042391 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Nocomis|Nocomis micropogon
CACC GCGGTTAGACGAGAGGCCCTAGTTAATGATGTAACGGCGTAAAGGGTGGTTAAGGATAGTAAATTAATAAAGCCGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCAACATACGAAAATAGCTTTAGAAAAGCCACCTGACCCACGAAAAGTGA
GAAA

>AY216542
Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Notemigonus|Notemigonus crysoleucas
CACC GCGGTTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGATAGCGAGTTAATAAAGTCGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGCCCGAAGCCCGATATACGAAAGTAACCTTAAGAGAGTCCACCTGAACCCACGAAAAGTGA
GAAA

>AF023194
Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Notemigonus|Notemigonus crysoleucas
CACC GCGGTTAAACGAGAGGCCCCAGTTAATAATACACGGCGTAAAGGGTGGTTAAGGAAAGCATGGTAATAAAGCCGAATGGCCC
TTTGGCTGTCATACGCTTCTAGGTGTCCGAAGCCCAACGTACGAAAGTAACCTTTAGTAAAACCCACCTGACCCACGAAAGCTGAG
AAA

>MG570425
Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Notemigonus|Notemigonus crysoleucas
CACC GCGGTTAAACGAGAGGCCCCAGTTAATAATACACGGCGTAAAGGGTGGTTAAGGAAAGCATGGTAATAAAGCCGAATGGCCC
TTTGGCTGTCATACGCTTCTAGGTGTCCGAAGCCCAACATACGAAAGTAACCTTTAGTAAAACCCACCTGACCCACGAAAGCTGAG
AAA

>MG570428
Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Notemigonus|Notemigonus crysoleucas
CACC GCGGTTAAACGAGAGGCCCCAGTTAATAATACACGGCGTAAAGGGTGGTTAAGGAAAGCATGGTAATAAAGCCGAATGGCCC
TTTGGCTGTCATACGCTTCTAGGTGTCCGAAGCCCAACATACGAAAGTAACCTTTAGTAAAACCCACCTGACCCACGAAAGCTGAG
AAA

>MG570438
Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Notemigonus|Notemigonus crysoleucas
CACC GCGGTTAAACGAGAGGCCCCAGTTAATAATACACGGCGTAAAGGGTGGTTAAGGAAAGCATGGTAATAAAGCCGAATGGCCC
TTTGGCTGTCATACGCTTCTAGGTGTCCGAAGCCCAACATACGAAAGTAACCTTTAGTAAAACCCACCTGACCCACGAAAGCTGAG
AAA

>MG570412
Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Notemigonus|Notemigonus crysoleucas
CACC GCGGTTAAACGAGAGGCCCCAGTTAATAATACACGGCGTAAAGGGTGGTTAAGGAAAGCATGGTAATAAAGCCGAATGGCCC
TTTGGCTGTCATACGCTTCTAGGTGTCCGAAGCCCAACGTACGAAAGTAACCTTTAGTAAAACCCACCTGACCCACGAAAGCTGAG
AAA

>KP013116
Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Notemigonus|Notemigonus
crysoleucas
CACCGCGTTAAACGAGAGGCCCCAGTTAATAATACACGGCGTAAAGGGTGGTTAAGGAAAGCATGGTAATAAGCCGAATGGCCC
TTTGCTGTCATACGCTTCTAGGTGTCCGAAGCCCAACATACGAAAGTAACCTTTAGTAAAACCCACCTGACCCACGAAAGCTGAG
AAA

>AB127393
Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Notemigonus|Notemigonus
crysoleucas
CACCGCGGTAAACGAGAGGCCCCAGTTAATAATACACGGCGTAAAGGGTGGTTAAGGAAAGCATGGTAATAAGCCGAATGGCCC
TTTGCTGTCATACGCTTCTAGGTGTCCGAAGCCCAACATACGAAAGTAACCTTTAGTAAAACCCACCTGACCCACGAAAGCTGAG
AAA

>AP012082 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Notropis|Notropis
atherinoides
CACCGCGTTAGACGAGAGGCCCTAGTTGATAACACAACGGCGTAAAGGGTGGTTAAGGACAGTGAAATAATAAGCCGAACGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGTCCAATATACGAAGGTAGCTTTAAGAAGCCACCTGACCCACGAAAAGTGA
GGAA

>MG570456 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Notropis|Notropis
atherinoides
CACCGCGGTAAACGAGAGGCCCTAGTTGATAGTATAACGGCGTAAAGGGTGGTTATGGATAGCGAAATAATAAGTGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCAGTATACGAAAGTAACCTTTAAGAAGCCACCTGACCCACGAAAAGTGA
GGAA

>AF023195 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Notropis|Notropis
atherinoides
CACCGCGGTAAACGAGAGGCCCTAGTTGATAGTATAACGGCGTAAAGGGTGGTTATGGATAGCGAAATAATAAGTGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCAGTATACGAAAGTAACCTTTAAGAAGCCACCTGACCCACGAAAAGTGA
GGAA

>MG570455 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Notropis|Notropis
atherinoides
CACCGCGGTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTATGGATAGCGAAATAATAAGTGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCAGTATACGAAAGTAACCTTTAAGAAGCCACCTGACCCACGAAAAGTGA
GGAA

>AY216534 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Ericymba|Ericymba
buccata
CCAGCCACCGCGTTAAACGAGAGGCCCTAGTTGATAGTATAACGGCGTAAAGGGTGGTTAAGGATAATAAACTAATAAGTCGAA
TGGCCCTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCAACGTACGAAAGTAACCTTTAGAAAGCCACCTGACCCACGAAAA
CTGAGGAA

>MG570414 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Notropis|Notropis
heterolepis
CACCGCGGTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGATAGTAAAACAATAAGTCGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCAATATACGAAAGTAACCTTTAAGAAGCCACCTGACCCACGAAAAGTGA
GGAA

>MG570413 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Notropis|Notropis heterolepis
 CACCGCGGTTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGATAGTAAAACAATAAAGTCGAATGGCC
 CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCAATATACGAAAGTAACCTTAAGAAAGCCACCTGACCCACGAAAAGTGA
 GGAA

>MG570443 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Notropis|Notropis hudsonius
 CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTATAGCGGCGTAAAGGGTGGTTAAGGATAGTGAAATAATAAGCCGAATGGCC
 CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCAATATACGAAAGTAGCTTTAAGAAAGTCCACCTGACCCACGAAAAGTGA
 GAAA

>DQ536429 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Notropis|Notropis stramineus
 CACCGCGGTTAAACGAGAGGCCCCAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGATAGCAAAACAATAAAGTCGAATGGCC
 CTTTGGCTGTCATACGCTTCTAGGAGCCCGAAGCCAGCATACGAAAGTAACCTTTAGGAAAGTCCACCTGACCCACGAAAAGTGA
 GAAA

>AY216552 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Notropis|Notropis volucellus
 CACCGCGGTTAAACGAGAGGCCCTAGTTAATAGTACAACGGCGTAAAGGGTGGTTAAGGATAGTGAAATAATAAAGTCGAATGGCC
 CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCTCAATATACGAAAGTAACCTTTAAGAACGCCACCTGACCCACGAAAAGTGA
 GAAA

>AY216553
 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Opsopoeodus|Opsopoeodus emiliae
 CACCGCGGTTAAACGAGAGGCCCTAGTTAATAGTACAACGGCGTAAAGGGTGGTTAAGGATAGTGAAATAATAAAGTCGAATGGCC
 CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCTCAACATACGAAAGTAACCTTTAAGAACGCCACCTGACCCACGAAAAGTGA
 GAAA

>AY216554
 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Opsopoeodus|Opsopoeodus emiliae
 CGCCGCGGTTAAACGAGAGGCCCTAGTTGATAAATAGCGGCGTAAAGGGTGGTTAAGGATAATAAATAATAAAGTCGCATGGCCCT
 TTGGCTGTCATACGCTTCTGGGAGCCCGAAGTCCAATATACGAAAGTAACCTTTAAGAAAGCCACCTGACCCACGAAAGCTGAGA
 CA

>AP012084
 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Opsopoeodus|Opsopoeodus emiliae
 CACCGCGGTTAAACGAGAGGCCCTAGTTGATAGTGCAACGGCGTAAAGGGTGGTTAAGGATAGTGAAATAATAAAGTCGAATGGCC
 CCTTGGCTGTCATACGCTTCTAGGTGTCCGAAGCCCAATATACGAAAGTAGCTTTAAGAAAGCCACCTGACCCACGAAAAGTGA
 GAAA

>AF038492
 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Phenacobius|Phenacobius mirabilis

CACCGCGGTTAAACGAGAGGCCCTAGTTGATTAAATAGCGGCGTAAAGGGTGGTTAAGAATAACATAATGATAAAGTCAAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCACTAACACGAAAGTAACCTTAAGCAAGTCTATCTGACCCACGAAACCTG
AGAAA

>DQ536431

Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Phenacobius|Phenacobius
mirabilis

CACCGCGGTTAAACGAGAGGCCCTAGTTGATTAAATAGCGGCGTAAAGGGTGGTTAAGAATAACATAATGATAAAGTCAAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCACTAACACGAAAGTAACCTTAAGCAAGTCTATCTGACCCACGAAACCTG
AGAAA

>NC_015364 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Phoxinus|Phoxinus
eos

CACCGCGGTTAAACGAGAGGCCCTAGTTAACAATACAACGGCGTAAAGGGTGGTTAAGGGGTGCAAACAATAAGCCGAATGGCCC
TTTGGCTGTCATACGCTTCTAGGTGTCCGAAGCCCAACATACGAAAGTAGCTTTAACAAAGCCCACCTGACCCACGAAAGCTGAG
AAA

>AF038490 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Phoxinus|Phoxinus
erythrogaster

CACCGCGGTTAAACGAGAGGCCCTAGTTAACAATACAACGGCGTAAAGGGTGGTTAAGGAGTGCAAAACAATAAGCCGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGTGTCCGAAGCCCAACATACGAAAGTAGCTTTAGCAAAATCCACCTGACCCACGAAAGCTGA
GAAA

>AF038493 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Phoxinus|Phoxinus
neogaeus

CACCGCGGTTAAACGAGAGGCCCTAGTTAATGATACAACGGCATAAAGGGTGGTTAAGGAAAGCAATATAATAAAGTCAAATGGCC
CTTTGGCTGTCATACGCTTCTAGGTGTCCGAAGCCCAACATACGAAAGTAGCTTTAGAAAAGCCCACCTGATCCCACGAAAGCTGA
GAAA

>AP012101

Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Pimephales|Pimephales
notatus

CACCGCGGTTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGATAGTGATATAATAAAGTCGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCACTATACGAAAGTAACCTTAAGAGAACCAACCTGACCCACGAAACTGA
GAAA

>AY216555

Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Pimephales|Pimephales
notatus

CGCCGCGGTTAAACGAGAGGCCCTAGTTGATAAATAGCGGCGTAAAGGGTGGTTAAGGATAATAAATAATAAAGTCGCATGGCCCT
TTGGCTGTCATACGCTTCTGGGAGCCCGAAGTCCAATATACGAAAGTAACCTTAAGAAAAGCCCACCTGACCCACGAAAGCTGAGA
CA

>MG570457

Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Pimephales|Pimephales
notatus

CACCGCGGTTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGATAGTGATATAATAAAGTCGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCACTATACGAAAGTAACCTTAAGAGAACCCACCTGACCCACGAAACTGA
GAAA

>MG570458
Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Pimephales|Pimephales
notatus
CACCGCGGTTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGATAGTGATATAATAAAGTCGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCAGTATACGAAAGTAACCTTTAAGAGAACCCACCTGACCCACGAAAAGTGA
GAAA

>MG570420
Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Pimephales|Pimephales
notatus
CACCGCGGTTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGATAGTGATATAATAAAGTCGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCAGTATACGAAAGTAACCTTTAAGAGAACCCACCTGACCCACGAAAAGTGA
GAAA

>MG570450
Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Pimephales|Pimephales
notatus
CACCGCGGTTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGATAGTGATATAATAAAGTCGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCAGTATACGAAAGTAACCTTTAAGAGAACCCACCTGACCCACGAAAAGTGA
GAAA

>MG570452
Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Pimephales|Pimephales
promelas
CACCGCGGTTAAACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGACAGCAAATCAATAAAGTCGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCAATATACGAAAGTAACCTTTAAGAATGCCACCTGACCCACGAAAGCTGA
GAAA

>MG570454
Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Pimephales|Pimephales
promelas
CACCGCGGTTAAACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGACAGCAAATCAATAAAGTCGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCAATATACGAAAGTAACCTTTAAGAATGCCACCTGACCCACGAAAGCTGA
GAAA

>KT289925
Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Pimephales|Pimephales
promelas
CACCGCGGTTAAACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGACAGCAAATCAATAAAGTCGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCAATATACGAAAGTAACCTTTAAGAATGCCACCTGACCCACGAAAGCTGA
GAAA

>KT278765
Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Pimephales|Pimephales
promelas
CACCGCGGTTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGACAGCGAATCAATAAAGTCGAATGGCC
CTTTGGCTGTTATACGCTTCTAGGAGTCCGAAGTCCAATATACGAAAGTAACCTTTAAGAAGCGCCACCTGACCCACGAAAGCTGA
GAAA

>AP011279

Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Pimephales|Pimephales
promelas
CACCGCGTTAAACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGACAGCAAATCAATAAAGTCGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGTCCAATATACGAAAGTAACCTTAAGAATGCCACCTGACCCACGAAAGCTGA
GAAA

>MG570416

Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Rhinichthys|Rhinichthys
obtusus
CACCGCGGTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGATAGTAAATTAATAAAGTCGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCAATATACGAAAGTAGCTTTAAGAAAGCCACCTGACCCACGAAAGCTGA
GAAA

>MG570417

Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Rhinichthys|Rhinichthys
obtusus
CACCGCGGTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGATAGTAAATTAATAAAGTCGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCAATATACGAAAGTAGCTTTAAGAAAGCCACCTGACCCACGAAAGCTGA
GAAA

>MG570448

Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Rhinichthys|Rhinichthys
cataractae
CACCGCGGTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGACAGCGAAATAATAAAGTCGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCGATATACGAAAGTAGCTTTAGAAAAGCCACCTGACCCACGAAAGCTGA
GAAA

>AP012105

Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Rhinichthys|Rhinichthys
cataractae
CACCGCGGTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGACAGCGAAATAATAAAGTCGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCGATATACGAAAGTAGCTTTAGAAAAGCCACCTGACCCACGAAAGCTGA
GAAA

>MG570446

Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Rhinichthys|Rhinichthys
cataractae
CACCGCGGTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGACAGCGAAATAATAAAGTCGAATGGCC
CTTTGGCTGTCATACGCTTCTAGGAGTCCGAAGCCCGATATACGAAAGTAGCTTTAGAAAAGCCACCTGACCCACGAAAGCTGA
GAAA

>MG570418 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Semotilus|Semotilus
atromaculatus
CACCGCGGTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGGTGAACAAAATAAAGCCGAATGGCCCT
TTGGCTGTCATACGCTTCTAGGTGCCTGAAGCCCAACATACGAAAGTCGCTTTAAAGTAGCCTACCTGACCCACGAAACTGAGA
AA

>MG570419 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Semotilus|Semotilus
atromaculatus
CACCGCGGTTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGGTGAACAAAATAAGCCGAATGGCCCT
TTGGCTGTCATACGCTTCTAGGTGCCTGAAGCCCAACATACGAAAGTCGCTTTAAAGTAGCCTACCTGACCCACGAAAACCTGAGA
AA

>AP012107 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Semotilus|Semotilus
atromaculatus
CACCGCGGTTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGGTGAACAAAATAAGCCGAATGGCCCT
TTGGCTGTCATACGCTTCTAGGTGCCTGAAGCCCAACATACGAAAGTCGCTTTAAAGTAGCCTACCTGATCCCACGAAAACCTGAGA
AA

>AF023199 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Semotilus|Semotilus
atromaculatus
CACCGCGGTTAAACGAGAGGCCCTAGTTGATAGTACAACGGCGTAAAGGGTGGTTAAGGGTGAACAAAATAAGCCGAATGGCCCT
TTGGCTGTCATACGCTTCTAGGTGCCTGAAGCCCAACATACGAAAGTCGCTTTAAAGTAGCCTACCTGATCCCACGAAAACCTGAGA
AA

>MG570441
Metazoa|Chordata|Actinopteri|Cypriniformes|Catostomidae|Catostomus|Catostomus
catostomus
CACCGCGGTTATACGAGAGGCCCTAGTTGATAAACACGGCGTAAAGGGTGGTTAAGGGAGTATATAAATAAGCCGAAGGACCCTC
TGGCCGTTATACGCTTCTAGGCACCCGAAGCCCAACACGAAAGTAGCTTTAATTTAGCCACCTGACCCACGAAAACCTGAGAAA

>MG570442
Metazoa|Chordata|Actinopteri|Cypriniformes|Catostomidae|Catostomus|Catostomus
catostomus
CACCGCGGTTATACGAGAGGCCCTAGTTGATAAACACGGCGTAAAGGGTGGTTAAGGGAGTATATAAATAAGCCGAAGGACCCTC
TGGCCGTTATACGCTTCTAGGCACCCGAAGCCCAACACGAAAGTAGCTTTAATTTAGCCACCTGACCCACGAAAACCTGAGAAA

>AF333595
Metazoa|Chordata|Actinopteri|Cypriniformes|Catostomidae|Catostomus|Catostomus
catostomus
CACCGCGGTTATACGAGAGGCCCTAGTTGATAAACACGGCGTAAAGGGTGGTTAAGGGAGTATATGAATAAGCCGAAGGACCCTC
TGGCCGTTATACGCTTCTAGGCACCCGAAGCCCAACACGAAAGTAGCTTTAATTTAGCCACCTGACCCACGAAAACCTGAGAAA

>AF333596
Metazoa|Chordata|Actinopteri|Cypriniformes|Catostomidae|Catostomus|Catostomus
commersonii
CACCGCGGTTATACGAGAGGCCCTAGTTGATAAGCACGGCGTAAAGGGTGGTTAAGGGAGTACATAAATAAGCCGAAGGGCCCTC
TGGCCGTTATACACTTCTAGGCGCCCGAAGCCCAACACGAAAGTAACTTTAATTTAGCCACCTGACCCACGAAAACCTAAGAAA

>KP013114
Metazoa|Chordata|Actinopteri|Cypriniformes|Catostomidae|Catostomus|Catostomus
commersonii
CACCGCGGTTATACGAGAGGCCCTAGTTGATAAGCACGGCGTAAAGGGTGGTTAAGGGAGTACATAAATAAGCCGAAGGGCCCTC
TGGCCGTTATACGCTTCTAGGCGCCCGAAGCCCAACACGAAAGTAGCTTTAATTTAGCCACCTGACCCACGAAAACCTGAGAAA

>AB127394
Metazoa|Chordata|Actinopteri|Cypriniformes|Catostomidae|Catostomus|Catostomus
commersonii
CACCGCGTTATACGAGAGGCCCTAGTTGATAAGCACGGCGTAAAGGGTGGTTAAGGGAGTACATAAATAAGCCGAAGGGCCCTC
TGGCCGTTATACGCTTCTAGGCGCTCGAAGCCCAACACGAAAGTAGCTTTAATTTAGCCACCTGACCCACGAAAACCTGAGAAA
>MG570423

Metazoa|Chordata|Actinopteri|Cypriniformes|Catostomidae|Catostomus|Catostomus
commersonii
CACCGCGTTATACGAGAGGCCCTAGTTGATAAGCACGGCGTAAAGGGTGGTTAAGGGAGTACATAAATAAGCCGAAGGGCCCTC
TGGCCGTTATACGCTTCTAGGCGCCGAAGCCCAACACGAAAGTAGCTTTAATTTAGCCACCTGACCCACGAAAACCTGAGAAA
>MG570424

Metazoa|Chordata|Actinopteri|Cypriniformes|Catostomidae|Catostomus|Catostomus
commersonii
CACCGCGTTATACGAGAGGCCCTAGTTGATAAGCACGGCGTAAAGGGTGGTTAAGGGAGTACATAAATAAGCCGAAGGGCCCTC
TGGCCGTTATACGCTTCTAGGCGCCGAAGCCCAACACGAAAGTAGCTTTAATTTAGCCACCTGACCCACGAAAACCTGAGAAA
>AF333606

Metazoa|Chordata|Actinopteri|Cypriniformes|Catostomidae|Hypentelium|Hypentelium
nigricans
CACCGCGTTATACGAGAGGCCCTAGTTGATAAGCACGGCGTAAAGGGTGGTTAAGGGAGCACAAAAATAAGCCGAAGGGCCCTC
TGGCTGTTATACGCTTCCGGGTGCCCAGGCCCAACACGAAAGTAGCTTTAATTAAGCCACCTGACCCACGAAAACCTGAGAAA
>AB242169

Metazoa|Chordata|Actinopteri|Cypriniformes|Catostomidae|Hypentelium|Hypentelium
nigricans
CACCGCGTTATACGAGAGGCCCTAGTTGATAAGCACGGCGTAAAGGGTGGTTAAGGGAGCACAAAAATAAGCCGAAGGGCCCTC
CGGCTGTTATACGCTTCCGGGTGCCCAGGCCCAACACGAAAGTAGCTTTAATTAAGCCACCTGACCCACGAAAACCTGAGAAA
>AY009143

Metazoa|Chordata|Actinopteri|Cypriniformes|Catostomidae|Ictiobus|Ictiobus
cyprinellus
CACCGCGTTATACGAGAGGCCCTAGTTGATAGACACGGCGTAAAGGGTGGTTAAGGAAAGCAAAAAATAAGCCGAAGGGCCCTTG
GCCGTATACGCTTCTGAGTGTCCGAAGGCCCAACACGAAAGTAGCTTTAATTTAGCCACCTGACCCACGAAAACCTGAGAAA
>KP306894

Metazoa|Chordata|Actinopteri|Cypriniformes|Catostomidae|Ictiobus|Ictiobus
cyprinellus
CACCGCGTTATACGAGAGGCCCCAGTTGATAAACACGGCGTAAAGGGTGGTTAAGGGAGCATAAGAATAAGCCGAAGGGCCCTT
TGGCCGTTATACGCTTCTAGGTGTCCGAAGGCCCAACACGAAAGTAGCTTTAGTTAAGCCACCTGACTCCACGAAAACCTGAGAAA
>AB242166

Metazoa|Chordata|Actinopteri|Cypriniformes|Catostomidae|Minytrema|Minytrema
melanops
CACCGCGTTATACGAGAGGCCCTAGTTGATAAATACGGCGTAAAGGGTGGTTAAGGGAGTATAAAAAATAAGCCGAAGGGTTCTC
TAGCCGTTATACGCTTCCAGACACCCGAAGTCCAAGCACGAAGGTAGCTTTAATTCAGCCACCTGACCCACGAAAACCTGAGAAA
>MG570430

Metazoa|Chordata|Actinopteri|Cypriniformes|Catostomidae|Minytrema|Minytrema
melanops

CACCGCGGTTATACGAGAGGCCCTAGTTGATAAATACGGCGTAAAGGGTGGTTAAGGGAGTATAAAAAATAAGCCGAAGGGTCCTC
TAGCCGTTATACGCTTCCAGGCACCCGAAGCCCAGACACGAAAGTAGCTTTAATTCAGCCACCTGACCCACGAAAACTGAGAAA
>MG570436

Metazoa|Chordata|Actinopteri|Cypriniformes|Catostomidae|Minytrema|Minytrema
melanops

CACCGCGGTTATACGAGAGGCCCTAGTTGATAAATACGGCGTAAAGGGTGGTTAAGGGAGTATAAAAAATAAGCCGAAGGGTCCTC
TAGCCGTTATACGCTTCCAGGCACCCGAAGCCCAGACACGAAAGTAGCTTTAATTCAGCCACCTGACCCACGAAAACTGAGAAA
>AF333599

Metazoa|Chordata|Actinopteri|Cypriniformes|Catostomidae|Minytrema|Minytrema
melanops

CACCGCGGTTATACGAGAGGCCCTAGTTGATAAATACGGCGTAAAGGGTGGTTAAGGGAGTATAAAAAATAAGCCAAAGGGTTCTC
TAGCCGTTATACGCTTCCAGACACCCGAAGTCCAATCACGAAAGTAGCTTTAATTCAGCCACCTGACCCCTCGAAAACTGAGAAA
>DQ536432

Metazoa|Chordata|Actinopteri|Cypriniformes|Catostomidae|Minytrema|Minytrema
melanops

CACCGCGGTTATACGAGAGGCCCTAGTTGATAAATACGGCGTAAAGGGTGGTTAAGGGAGTATAAAAAATAAGCCGAAGGGTTCTC
TAGCCGTTATACGCTTCCAGACACCCGAAGTCCAAGCACGAAGGTAGCTTTAATTCAGCCACCTGACCCACGAAAACTGAGAAA
>AF333600

Metazoa|Chordata|Actinopteri|Cypriniformes|Catostomidae|Moxostoma|Moxostoma
anisurum

CACCGCGGTTATACGAGAGGCCCTAGTTGATAAGCACGGCGTAAAGGGTGGTTAGAGGAGTGAAAAATAAGCTGAAGGACCCTC
TGGCCGTTATACGCTTCCAGGTACCCGAAACCAACACGAAAGTAGCTTTAATTCACCCTTGACCCACGAAAACTGAGAAA
>AF333601

Metazoa|Chordata|Actinopteri|Cypriniformes|Catostomidae|Moxostoma|Moxostoma
carinatum

CACCGCGGTTATACGAGAGGCCCTAGTTGATAAGTACGGCGTAAAGGGTGGTTAGAGGAGTACAAAAATAAGCTGAAGGACCCTT
TGGCCGTTATACGCTTCCAGGTACCCGAAATCCAAATACGAAAGTAGCTTTAATTTAGATCACTTGACCCACGAAAACTGAGGAA
>JN015532

Metazoa|Chordata|Actinopteri|Siluriformes|Ictaluridae|Ameiurus|Ameiurus
melas

CACCGCGGTTATACGAAAGACCCTAGTTGCTAGCCACGGCGTAAAGGGTGGTTAAGGACAACAACAAATAAGCTAAAGATCCCCT
AAGCCGTCATACGCATTCCGGGGGCACGAAGCCCTAACACGAAAGTAGCTTTAAAAAATATACCTGACCCACGAAAGCTAAGAAA
>KT804702

Metazoa|Chordata|Actinopteri|Siluriformes|Ictaluridae|Ameiurus|Ameiurus
melas

CACCGCGGTTATACGAAAGACCCTAGTTGCTAGCCACGGCGTAAAGGGTGGTTAAGGACAACAACAAATAAGCTAAAGATCCCCT
AAGCCGTCATACGCATTCCGGGGGCACGAAGCCCTAACACGAAAGTAGCTTTAAAAAATATACCTGACCCACGAAAGCTAAGAAA
>MF621735

Metazoa|Chordata|Actinopteri|Siluriformes|Ictaluridae|Ameiurus|Ameiurus
natalis

CACCGCGGTTATACGAAAGGCCCTAGTTGCTAGCCACGGCGTAAAGGGTGGTTAAGGACAATAATAATAAGCCAAAGATCCCCT
AGGCCGTCATACGCATTCCGGGGGCACGAAGCCCTAACACGAAAGTAGCTTTAAAAATTTACCTGACCCACGAAAGCTAAGAAA
>MG570406

Metazoa|Chordata|Actinopteri|Siluriformes|Ictaluridae|Ameiurus|Ameiurus
natalis

CACCGCGTTATACGAAAGGCCCTAGTTGCTAGCCACGGCGTAAAGGGTGGTTAAGGACAATAATAAATAAGCCAAAGATCCCCT
AGGCCGTCATACGCATTCCGGGGGCACGAAGCCCTAACACGAAAGTAGCTTTAAAAATTTACCTGACCCACGAAAGCTAAGAAA
>JX899750 Metazoa|Chordata|Actinopteri|Siluriformes|Ictaluridae|Ameiurus|Ameiurus
nebulosus

CACCGCGTTATACGAAAGGCCCTAGTTGCTAGCCACGGCGTAAAGGGTGGTTAAGGACAACAACAAATAAAGCTAAAGATCCCCT
AAGCCGTCATACGCATTCCGGGGGCACGAAGCCCTAACACGAAAGTAGCTTTAAAAATATACCTGACCCACGAAAGCTAAGAAA
>MF621731 Metazoa|Chordata|Actinopteri|Siluriformes|Ictaluridae|Ameiurus|Ameiurus
nebulosus

CACCGCGTTATACGAAAGACCCTAGTTGCTAGCCACGGCGTAAAGGGTGGTTAAGGACAACAACAAATAAAGCTAAAGATCCCCT
AAGCCGTCATACGCATTCCGGGGGCACGAAGCCCTAACACGAAAGTAGCTTTAAAAATATACCTGACCCACGAAAGCTAAGAAA
>MF621733 Metazoa|Chordata|Actinopteri|Siluriformes|Ictaluridae|Ameiurus|Ameiurus
nebulosus

CACCGCGTTATACGAAAGGCCCTAGTTGCTAGCCACGGCGTAAAGGGTGGTTAAGGACAACAACAAATAAAGCTAAAGATCCCCT
AAGCCGTCATACGCATTCCGGGGGCACGAAGCCCTAACACGAAAGTAGCTTTAAAAATATACCTGACCCACGAAAGCTAAGAAA
>MF621734 Metazoa|Chordata|Actinopteri|Siluriformes|Ictaluridae|Ameiurus|Ameiurus
nebulosus

CACCGCGTTATACGAAAGGCCCTAGTTGCTAGCCACGGCGTAAAGGGTGGTTAAGGACAACAACAAATAAAGCTAAAGATCCCCT
AAGCCGTCATACGCATTCCGGGGGCACGAAGCCCTAACACGAAAGTAGCTTTAAAAATATACCTGACCCACGAAAGCTAAGAAA
>MF621716 Metazoa|Chordata|Actinopteri|Siluriformes|Ictaluridae|Ictalurus|Ictalurus
punctatus

CACCGCGTTATACGAAAGACCCTAGTTGCTAGCCACGGCGTAAAGGGTGGTTAAGGACAACAATGAATAAAGCTAAAGACCCCCT
GGGCCGTCATACGCATTTCCGGGGGCACGAAACCCTAACACGAAAGTAGCTTTAAAAACACACCTGACCCACGAAAGCTAAGAAA
>MF621717 Metazoa|Chordata|Actinopteri|Siluriformes|Ictaluridae|Ictalurus|Ictalurus
punctatus

CACCGCGTTATACGAAAGACCCTAGTTGCTAGCCACGGCGTAAAGGGTGGTTAAGGACAACAATGAATAAAGCTAAAGACCCCCT
GGGCCGTCATACGCATTTCCGGGGGCACGAAACCCTAACACGAAAGTAGCTTTAAAAACACACCTGACCCACGAAAGCTAAGAAA
>MF621718 Metazoa|Chordata|Actinopteri|Siluriformes|Ictaluridae|Ictalurus|Ictalurus
punctatus

CACCGCGTTATACGAAAGACCCTAGTTGCTAGCCACGGCGTAAAGGGTGGTTAAGGACAACAATGAATAAAGCTAAAGACCCCCT
GGGCCGTCATACGCATTTCCGGGGGCACGAAACCCTAACACGAAAGTAGCTTTAAAAACACACCTGACCCACGAAAGCTAAGAAA
>MF621720 Metazoa|Chordata|Actinopteri|Siluriformes|Ictaluridae|Ictalurus|Ictalurus
punctatus

CACCGCGTTATACGAAAGACCCTAGTTGCTAGCCACGGCGTAAAGGGTGGTTAAGGACAACAATGAATAAAGCTAAAGACCCCCT
GGGCCGTCATACGCATTTCCGGGGGCACGAAACCCTAACACGAAAGTAGCTTTAAAAACACACCTGACCCACGAAAGCTAAGAAA
>MF621721 Metazoa|Chordata|Actinopteri|Siluriformes|Ictaluridae|Ictalurus|Ictalurus
punctatus

CACCGCGTTATACGAAAGACCCTAGTTGCTAGCCACGGCGTAAAGGGTGGTTAAGGACAACAATGAATAAAGCTAAAGACCCCCT
GGGCCGTCATACGCATTTCCGGGGGCACGAAACCCTAACACGAAAGTAGCTTTAAAAACACACCTGACCCACGAAAGCTAAGAAA
>MF621722 Metazoa|Chordata|Actinopteri|Siluriformes|Ictaluridae|Ictalurus|Ictalurus
punctatus

CACCGCGTTATACGAAAGACCCTAGTTGCTAGCCACGGCGTAAAGGGTGGTTAAGGACAACAATGAATAAAGCTAAAGACCCCCT
GGGCCGTCATACGCATTTCCGGGGGCACGAAACCCTAACACGAAAGTAGCTTTAAAAACACACCTGACCCACGAAAGCTAAGAAA

>AF482987 Metazoa|Chordata|Actinopteri|Siluriformes|Ictaluridae|Ictalurus|Ictalurus punctatus
 CACCGCGGTTATACGAAAGACCCTAGTTGCTAGCCACGGCGTAAAGGGTGGTTAAGGACAACAATGAATAAAGCTAAAGACCCCCT
 GGGCCGTCATACGCATTTTCGGGGGCACGAAACCCTAACACGAAAGTAGCTTTAAAAACACACCTGACCCACGAAAGCTAAGAAA
 >JN015531 Metazoa|Chordata|Actinopteri|Siluriformes|Ictaluridae|Ictalurus|Ictalurus punctatus
 CACCGCGGTTATACGAAAGACCCTAGTTGCTAGCCACGGCGTAAAGGGTGGTTAAGGACAACAATGAATAAAGCTAAAGACCCCCT
 GGGCCGTCATACGCATTTTCGGGGGCACGAAACCCTAACACGAAAGTAGCTTTAAAAACACACCTGACCCACGAAAGCTAAGAAA
 >JN015534 Metazoa|Chordata|Actinopteri|Siluriformes|Ictaluridae|Noturus|Noturus gyrinus
 CACCGCGGTTATACGAAAGACCCGAGTTGCTAGCCACGGCGTAAAGGGTGGTTAGGGATCAAAATAAATAAAGCCAAAGACCCCCT
 AAGCCGTCATACGCACTACGGAGGCACGAGGCCCTACACGAAAGTAGCTTTAAGCACTCTGACCCACGAAAGCTAAGAAA
 >KP013095 Metazoa|Chordata|Actinopteri|Esociformes|Umbridae|Umbra|Umbra limi
 CACCGCGGTTATACGAGAGGCCCTAGTTGATAGCCGTCGGCGTAAAGAGTGGTTAAGGAATTTAAATTTATAAAGTTGAATAACCC
 TTTAGCTGTTATACGCACCTTGGGCTATGAAATCTTCCGCGAAAGCAGCTTTATGCCCCCTGAACCCACGACGTTATGACA
 >JN015533 Metazoa|Chordata|Actinopteri|Siluriformes|Ictaluridae|Pylodictis|Pylodictis olivaris
 CACCGCGGTTATACGAAAGACCCCAGTTGCTAGCCACGGCGTAAAGGGTGGTTAAGGACAACAATAAATAAAGCTAAAGACCCCCT
 AAGCCGTCATACGCATTCCGGGGGCACGAGACCCTAACACGAAAGTAGCTTTAAAAACATACCTGACCCACGAAAGCTAAGAAA
 >MF621730 Metazoa|Chordata|Actinopteri|Siluriformes|Ictaluridae|Pylodictis|Pylodictis olivaris
 CACCGCGGTTATACGAAAGACCCCAGTTGCTAGCCACGGCGTAAAGGGTGGTTAAGGACAACAATAAATAAAGCTAAAGACCCCCT
 AAGCCGTCATACGCATTCCGGGGGCACGAGACCCTAACACGAAAGTAGCTTTAAAAACATACCTGACCCACGAAAGCTAAGAAA
 >MF621727 Metazoa|Chordata|Actinopteri|Siluriformes|Ictaluridae|Pylodictis|Pylodictis olivaris
 CACCGCGGTTATACGAAAGACCCCAGTTGCTAGCCACGGCGTAAAGGGTGGTTAAGGACAACAATAAATAAAGCTAAAGACCCCCT
 AAGCCGTCATACGCATTCCGGGGGCACGAGACCCTAACACGAAAGTAGCTTTAAAAACATACCTGACCCACGAAAGCTAAGAAA
 >MF621728 Metazoa|Chordata|Actinopteri|Siluriformes|Ictaluridae|Pylodictis|Pylodictis olivaris
 CACCGCGGTTATACGAAAGACCCCAGTTGCTAGCCACGGCGTAAAGGGTGGTTAAGGACAACAATAAATAAAGCTAAAGACCCCCT
 AAGCCGTCATACGCATTCCGGGGGCACGAGACCCTAACACGAAAGTAGCTTTAAAAACATACCTGACCCACGAAAGCTAAGAAA
 >MF621729 Metazoa|Chordata|Actinopteri|Siluriformes|Ictaluridae|Pylodictis|Pylodictis olivaris
 CACCGCGGTTATACGAAAGACCCCAGTTGCTAGCCACGGCGTAAAGGGTGGTTAAGGACAACAATAAATAAAGCTAAAGACCCCCT
 AAGCCGTCATACGCATTCCGGGGGCACGAGACCCTAACACGAAAGTAGCTTTAAAAACATACCTGACCCACGAAAGCTAAGAAA
 >AP004103 Metazoa|Chordata|Actinopteri|Esociformes|Esocidae|Esox|Esox lucius

CACCGCGGTTATACGAGAGGCCCTAGTTGATAATTGTCGGCGTAAAGAGTGGTTTAGAAAATAATTTAATAAAGCCGAACACCTCC
 TCAGTTGTTATACACATTTGAAGATATGAAGCCCTGCCGCGAAAGCAGCTTTAAGTATCCTGAACCCACGACAGCTATGGTA
 >KT124232 Metazoa|Chordata|Actinopteri|Esociformes|Esocidae|Esox|Esox lucius
 CACCGCGGTTATACGAGAGGCCCTAGTTGATAATTGTCGGCGTAAAGAGTGGTTTAGAAAATAATTTAATAAAGCCGAACACCTCC
 TCAGTTGTTATACACATTTGAAGATATGAAGCCCTACCGCGAAAGCAGCTTTAAGTATCCTGAACCCACGACAGCTATGGTA
 >KT124233 Metazoa|Chordata|Actinopteri|Esociformes|Esocidae|Esox|Esox lucius
 CACCGCGGTTATACGAGAGGCCCTAGTTGATAATTGTCGGCGTAAAGAGTGGTTTAGAAAATAATTTAATAAAGCCGAACACCTCC
 TCAGTTGTTATACACATTTGAAGATATGAAGCCCTACCGCGAAAGCAGCTTTAAGTATCCTGAACCCACGACAGCTATGGTA
 >KT124234 Metazoa|Chordata|Actinopteri|Esociformes|Esocidae|Esox|Esox lucius
 CACCGCGGTTATACGAGAGGCCCTAGTTGATAATTGTCGGCGTAAAGAGTGGTTTAGAAAATAATTTAATAAAGCCGAACACCTCC
 TCAGTTGTTATACACATTTGAAGATATGAAGCCCTACCGCGAAAGCAGCTTTAAGTATCCTGAACCCACGACAGCTATGGTA
 >KT124235 Metazoa|Chordata|Actinopteri|Esociformes|Esocidae|Esox|Esox lucius
 CACCGCGGTTATACGAGAGGCCCTAGTTGATAATTGTCGGCGTAAAGAGTGGTTTAGAAAATAATTTAATAAAGCCGAACACCTCC
 TCAGTTGTTATACGATTTGAAGATATGAAGCCCTACCGCGAAAGCAGCTTTAAGTATCCTGAACCCACGACAGCTATGGTA
 >HM106493 Metazoa|Chordata|Actinopteri|Osmeriformes|Osmeridae|Osmerus|Osmerus
 mordax
 CACCGCGGTTATACGAGTGGCCCAAGTTGAAAGTTACCGGCGTAAAGAGTGGTTAGGGAAATAATAAACTAAAGCCGAACACCTCC
 TAGGCTGTTATACGCTTCTGACGGCACGAAGCCCCACTACGAAAGTGCTTTAATACACCTGAACCCACGACAACTAAGACA
 >MF621765 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Coregonus|Coregonus
 artedi
 CACCGCGGTTATACGAGAGGCCCTAGTTGATAATCACCGGCGTAAAGAGTGGTTAGGAATTATATTTAATAAAGCCGAACACCCCC
 TTGGCTGTCATACGCACCTGGGGGCACGAAGCCCCACTGCGAAAGCAGCTTTAATCAACACCTGAACCCACGACAGCTATGACA
 >MF621766 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Coregonus|Coregonus
 artedi
 CACCGCGGTTATACGAGAGGCCCTAGTTGATAATCACCGGCGTAAAGAGTGGTTAGGAATTATATTTAATAAAGCCGAACACCCCC
 TTGGCTGTCATACGCACCTGGGGGCACGAAGCCCCACTGCGAAAGCAGCTTTAATCAACACCTGAACCCACGACAGCTATGACA
 >JQ390060 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Coregonus|Coregonus
 clupeaformis
 CACCGCGGTTATACGAGAGGCCCTAGTTGATAATTACCGGCGTAAAGAGTGGTTAGGAATTATATTTAATAAAGCCGAACACCCCC
 TTGGCTGTCATACGCACCTGGGGGCACGAAGCCCCACTGCGAAAGCAGCTTTAATCACCACCTGAACCCACGACAGCTATGATA
 >JQ661482 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Coregonus|Coregonus
 clupeaformis
 CACCGCGGTTATACGAGAGGCCCTAGTTGATAATCACCGGCGTAAAGAGTGGTTAGGAATTATATTTAATAAAGCCGAACACCCCC
 TTGGCTGTCATACGCACCTGGGGGCACGAAGCCCCACTGCGAAAGCAGCTTTAATCACCACCTGAACCCACGACAGCTATGATA
 >JQ661483 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Coregonus|Coregonus
 clupeaformis
 CACCGCGGTTATACGAGAGGCCCTAGTTGATAATCACCGGCGTAAAGAGTGGTTAGGAATTATATTTAATAAAGCCGAACACCCCC
 TTGGCTGTCATACGCACCTGGGGGCACGAAGCCCCACTGCGAAAGCAGCTTTAATCACCACCTGAACCCACGACAGCTATGATA
 >JQ661484 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Coregonus|Coregonus
 clupeaformis
 CACCGCGGTTATACGAGAGGCCCTAGTTGATAATCACCGGCGTAAAGAGTGGTTAGGAATTATATTTAATAAAGCCGAACACCCCC
 TTGGCTGTCATACGCACCTGGGGGCACGAAGCCCCACTGCGAAAGCAGCTTTAATCACCACCTGAACCCACGACAGCTATGATA

>JQ661485 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Coregonus|Coregonus clupeaformis
 CACCGCGGTTATACGAGAGGCCCTAGTTGATAATCACCGGCGTAAAGAGTGGTTAGGAATTATATTTAATAAAGCCGAACACCCCC
 TTGGCTGTCATACGCACCTGGGGGCACGAAGCCCCACTGCGAAAGCAGCTTTAATCACACCTGAACCCACGACAGCTATGATA

>JQ661486 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Coregonus|Coregonus clupeaformis
 CACCGCGGTTATACGAGAGGCCCTAGTTGATAATCACCGGCGTAAAGAGTGGTTAGGAATTATATTTAATAAAGCCGAACACCCCC
 TTGGCTGTCATACGCACCTGGGGGCACGAAGCCCCACTGCGAAAGCAGCTTTAATCACACCTGAACCCACGACAGCTATGATA

>JQ661487 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Coregonus|Coregonus clupeaformis
 CACCGCGGTTATACGAAAGGCCCTAGTTGATAATCACCGGCGTAAAGAGTGGTTAGGAATTATATTTAATAAAGCCGAACACCCCC
 TTGGCTGTCATACGCACCTGGGGGCACGAAGCCCCACTGCGAAAGCAGCTTTAATCACACCTGAACCCACGACAGCTATGATA

>MG924469 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Coregonus|Coregonus zenithicus
 CACCGCGGTTATACGAGAGGCCCTAGTTGATGATCACCGGCGTAAAGAGTGGTTAGGAATTATATTTAATAAAGCCGAACACCCCC
 TTGGCTGTCATACGCACCTGGGGGCACGAAGCCCCACTGCGAAAGCAGCTTTAATCACACCTGAACCCACGACAGCTATGACA

>EF455489 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Oncorhynchus|Oncorhynchus gorbuscha
 CACCGCGGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTATGGAAAATATTTAATAAAGCCGAACACCCCC
 CTCAGCCGTCATACGCATCTGGGAGCACGAAGACCTACTGCGAAAGCAGCTTTAATTATGCCTGACCCACGACAGCTAAGAAA

>EF126369 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Oncorhynchus|Oncorhynchus kisutch
 CACCGCGGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTATGGAAAGATATTTAATAAAGCCGAACACCCCC
 CTCAGCCGTCATACGCACCTGGGAGCACGAAGACCTACCGCGAAAGCAGCTTTAATTACGCCTGACCCACGACAGCTAAGAAA

>MF621749 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Oncorhynchus|Oncorhynchus kisutch
 CACCGCGGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTATGGAAAGATATTTAATAAAGCCGAACACCCCC
 CTCAGCCGTCATACGCACCTGGGAGCACGAAGACCTACCGCGAAAGCAGCTTTAATTACGCCTGACCCACGACAGCTAAGAAA

>MF621751 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Oncorhynchus|Oncorhynchus kisutch
 CACCGCGGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTATGGAAAGATATTTAATAAAGCCGAACACCCCC
 CTCAGCCGTCATACGCACCTGGGAGCACGAAGACCTACCGCGAAAGCAGCTTTAATTACGCCTGACCCACGACAGCTAAGAAA

>LC050735 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Oncorhynchus|Oncorhynchus mykiss
 CACCGCGGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTATGGAAAATATTTAATAAAGCCGAACACCCCC
 CTCAGCCGTCATACGCACCTGGGAGCACGAAGACCTACTGCGAAAGCAGCTTTAATTATGCCTGACCCACGACAGCTAAGAAA

>KP013084
Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Oncorhynchus|Oncorhynchus
mykiss
CACCGCGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTATGGAAAAATATTTAATAAGCCGAACACCCC
CTCAGCCGTCATACGCACCTGGGAGCACGAAGACCTACTGCGAAAGCAGCTTTAACTATGCCTGACCCACGACAGCTAAGAAA
>KP085590
Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Oncorhynchus|Oncorhynchus
mykiss
CACCGCGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTATGGAAAAATATTTAATAAGCCGAACACCCC
CTCAGCCGTCATACGCACCTGGGAGCACGAAGACCTACTGCAAAAGCAGCTTTAACTATGCCTGACCCACGACAGCTAAGAAA
>HQ167664
Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Oncorhynchus|Oncorhynchus
mykiss
CACCGCGTTATACGAGAGACCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTATGGAAAAATATTTAATAAGCCGAACACCCC
CTCAGCCGTCATACGCACCTGGGAGCACGAAGACCTACTGCGAAAGCAGCTTTAACTATGCCTGACCCACGACAGCTAAGAAA
>DQ288268
Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Oncorhynchus|Oncorhynchus
mykiss
CACCGCGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTATGGAAAAATATTTAATAAGCCGAACACCCC
CTCAGCCGTCATACGCACCTGGGAGCACGAAGACCTACTGCAAAAGCAGCTTTAACTATGCCTGACCCACGACAGCTAAGAAA
>DQ288269
Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Oncorhynchus|Oncorhynchus
mykiss
CACCGCGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTATGGAAAAATATTTAATAAGCCGAACACCCC
CTCAGCCGTCATACGCACCTGGGAGCACGAAGACCTACTGCGAAAGCAGCTTTAACTATGCCTGACCCACGACAGCTAAGAAA
>DQ288270
Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Oncorhynchus|Oncorhynchus
mykiss
CACCGCGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTATGGAAAAATATTTAATAAGCCGAACACCCC
CTCAGCCGTCATACGCACCTGGGAGCACGAAGACCTACTGCGAAAGCAGCTTTAACTATGCCTGACCCACGACAGCTAAGAAA
>DQ288271
Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Oncorhynchus|Oncorhynchus
mykiss
CACCGCGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTATGGAAAAATATTTAATAAGCCGAACACCCC
CTCAGCCGTCATACGCACCTGGGAGCACGAAGACCTACTGCGAAAGCAGCTTTAACTATGCCTGACCCACGACAGCTAAGAAA
>MF621750
Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Oncorhynchus|Oncorhynchus
mykiss
CACCGCGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTATGGAAAAATATTTAATAAGCCGAACACCCC
CTCAGCCGTCATACGCACCTGGGAGCACGAAGACCTACTGCGAAAGCAGCTTTAACTATGCCTGACCCACGACAGCTAAGAAA

>AF392054
Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Oncorhynchus|Oncorhynchus
tshawytscha
CACCGCGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTATGGAAAAATATTTAATAAGCCGAACACCCC
CTCAGCCGTCATACGCACCTGGGGGCACGAAGACCTACTGCGAAAGCAGCTTTAATTACACCTGACCCACGACAGCTAAGAAA
>HQ167665
Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Oncorhynchus|Oncorhynchus
tshawytscha
CACCGCGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTATGGAAAAATATTTAATAAGCCGAACACCCC
CTCAGCCGTCATACGCACCTGGGGGCACGAAGACCTACTGCGAAAGCAGCTTTAATTACACCTGACCCACGACAGCTAAGAAA
>AP013049 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Prosopium|Prosopium
cylindraceum
CACCGCGTTATACGAGAGGCCCTAGTTGATAGCCGTCGGGCGTAAAGAGTGGTTAAGGAATTTAAATTTATAAAGTTGAATAACCC
TTTAGCTGTTATACGCACCTTGGGCTATGAAATTCTTCGCGAAAGCAGCTTTATGCCCTGAACCCACGACAGTTATGACA
>MF621759 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Prosopium|Prosopium
cylindraceum
CACCGCGTTATACGAGAGGCCCAAGTTGATAACTACCGGCGTAAAGAGTGGTTAGGGATAATACTTAATAAAGCCGAACACCCCC
TAGGCTGTCATACGCACCTGGGGGCACGAAGCCCCACTGCGAAAGCAGCTTTAACCACCACCTGAACCCACGACAGCTATGATA
>MF621764 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Prosopium|Prosopium
cylindraceum
CACCGCGTTATACGAGAGGCCCAAGTTGATAACTACCGGCGTAAAGAGTGGTTAGGGATAATACTTAATAAAGCCGAACACCCCC
TAGGCTGTCATACGCACCTGGGGGCACGAAGCCCCACTGCGAAAGCAGCTTTAACCACCACCTGAACCCACGACAGCTATGATA
>MF621767 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Prosopium|Prosopium
cylindraceum
CACCGCGTTATACGAGAGGCCCAAGTTGATAACTACCGGCGTAAAGAGTGGTTAGGGATAATACTTAATAAAGCCGAACACCCCC
TAGGCTGTCATACGCACCTGGGGGCACGAAGCCCCACTGCGAAAGCAGCTTTAACCACCACCTGAACCCACGACAGCTATGATA
>MF621768 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Prosopium|Prosopium
cylindraceum
CACCGCGTTATACGAGAGGCCCAAGTTGATAACTACCGGCGTAAAGAGTGGTTAGGGATAATACTTAATAAAGCCGAACACCCCC
TAGGCTGTCATACGCACCTGGGGGCACGAAGCCCCACTGCGAAAGCAGCTTTAACCACCACCTGAACCCACGACAGCTATGATA
>JQ390062 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Prosopium|Prosopium
cylindraceum
CACCGCGTTATACGAGAGGCCCAAGTTGATAACTACCGGCGTAAAGAGTGGTTAGGGATAATACTTAATAAAGCCGAACACCCCC
TAGGCTGTCATACGCACCTGGGGGCACGAAGCCCCACTGCGAAAGCAGCTTTAACCACCACCTGAACCCACGACAGCTATGATA
>MG924478 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Prosopium|Prosopium
coulterii
CACCGCGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTAGGGTAATACTTAATAAAGCCGAACACCTCC
TAGGCTGTCATACGCACCTGGAGACACGAAGCCCCACTGCGAAAGCAGCTTTAATCACCACCTGAATCCACGACAGCTATGATA
>AF133701 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salmo|Salmo salar
CACCGCGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTACGGAAAAATATTTAATAAGCCGAACACCCC
CTCAGCCGTCATACGCACCTGGGGGCACGAAGATCTACTACGAAAGCAGCTTTAATTATACCTGAACCCACGACAGCTACGACA
>HQ167667 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salmo|Salmo salar

CACCGCGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTACGGAAAAATATTTAATAAAGCCGAACACCCC
 CTCAGCCGTCATACGCACCTGGGGGCACGAAGATCTACTACGAAAGCAGCTTTAATTATACCTGAACCCACGACAGCTACGACA
 >KF791036 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salmo|Salmo salar
 CACCGCGTTATACGAGAGGCCCAAATTGATAGCCAGCGGCGTAAAGAGTGGTTAGGGATAACCCCACTAAAGTCGAACGCCTTCA
 GAGCTGTTATACGCTCCCGAAGGCAAGAAGCCCTACTACGAAAGTAACCTTTAAACCACCTGACTCCACGAAAGCTGTGAAA
 >U12143 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salmo|Salmo salar
 CACCGCGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTACGGAAAAATATTTAATAAAGCCGAACACCCC
 CTCAGCCGTCATACGCACCTGGGGGCACGAAGATCTACTACGAAAGCAGCTTTAATTATACCTGAACCCACGACAGCTACGACA
 >LC012541 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salmo|Salmo salar
 CACCGCGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTACGGAAAAATATTTAATAAAGCCGAACACCCC
 CTCAGCCGTCATACGCACCTGGGGGCACGAAGATCTACTACGAAAGCAGCTTTAATTATACCTGAACCCACGACAGCTACGACA
 >EU643688 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salmo|Salmo salar
 CACCGCGTTATACGAGAGGCCCTAGTTGATAATTACCGGCGTAAAGAGTGGTTACGGAAAAATATTTAATAAAGCCGAACACCCC
 CTCAGCCGTCATACGCACCTGGGGGCACGAAGATCTACTACGAAAGCAGCTTTAATTATACCTGAACCCACGACAGCTACGACA
 >EU643689 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salmo|Salmo salar
 CACCGCGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTACGGAAAAATATTTAATAAAGCCGAACACCCC
 TCAGCCGTCATACGCACCTGGGGGCACGAAGATCTACTACGAAAGCAGCTTTAATTATACCTGAACCCACGACAGCTACGACA
 >EU643690 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salmo|Salmo salar
 CACCGCGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTACGGAAAAATATTTAATAAAGCCGAACACCCC
 CTCAGCCGTCATACGCACCTGGGGGCACGAAGATCTACTACGAAAGCAGCTTTAATTATACCTGAACCCACGACAGCTACGACA
 >JQ390055 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salmo|Salmo salar
 CACCGCGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTACGGAAAAATATTTAATAAAGCCGAACACCCC
 CTCAGCCGTCATACGCACCTGGGGGCACGAAGACCTACTACGAAAGCAGCTTTAATTGTACCTGAACCCACGACAGCTACGACA
 >JQ390056 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salmo|Salmo salar
 CACCGCGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTACGGAAAAATATTTAATAAAGCCGAACACCCC
 CTCAGCCGTCATACGCACCTGGGGGCACGAAGATCTACTACGAAAGCAGCTTTAATTATACCTGAACCCACGACAGCTACGACA
 >JQ390057 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salmo|Salmo trutta
 CACCGCGTTATACGAGAGACCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTACGGAAAAATATTCAATAAAGCCGAACACCCC
 CTCAGCCGTCATACGCACCTGGGGGCACGAAGATCTACTGCGAAAGCAGCTTTAATTATGCCTGAACCCACGACAGCTACGACA
 >HQ167666 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salmo|Salmo trutta
 CACCGCGTTATACGAGAGACCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTACGGAAAAATATTCAATAAAGCCGAACACCCC
 CTCAGCCGTCATACGCACCTGGGGGCACGAAGATCTACTGCGAAAGCAGCTTTAATTATGCCTGAACCCACGACAGCTACGACA
 >KT633607 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salmo|Salmo trutta
 CACCGCGTTATACGAGAGACCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTACGGAAAAATATTCAATAAAGCCGAACACCCC
 CTCAGCCGTCATACGCACCTGGGGGCACGAAGATCTACTGCGAAAGCAGCTTTAATTATGCCTGAACCCACGACAGCTACGACA
 >KT634053 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salmo|Salmo trutta
 CACCGCGTTATACGAGAGACCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTACGGAAAAATATTCAATAAAGCCGAACACCCC
 CTCAGCCGTCATACGCACCTGGGGGCACGAAGATCTACTGCGAAAGCAGCTTTAATTATGCCTGAACCCACGACAGCTACGACA
 >GU233801 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salmo|Salmo trutta
 CACCGCGTTATACGAGAGACCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTACGGAAAAATATTCAATAAAGCCGAACACCCC
 CTCAGCCGTCATACGCACCTGGGGGCACGAAGATCTACTGCGAAAGCAGCTTTAATTATGCCTGAACCCACGACAGCTACGACA
 >AM910409 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salmo|Salmo trutta

CACCGCGGTTATACGAGAGACCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTACGGAAAAATATTCAATAAAGCCGAACACCCC
 CTCAGCCGTCATACGCACCTGGGGGCACGAAGATCTACTGCGAAAGCAGCTTTAATTATGCCTGAACCCACGACAGCTACGACA
 >MF621760 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salmo|Salmo trutta
 CACCGCGGTTATACGAGAGACCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTACGGAAAAATATTCAATAAAGCCGAACACCCC
 CTCAGCCGTCATACGCACCTGGGGGCACGAAGATCTACTGCGAAAGCAGCTTTAATTATGCCTGAACCCACGACAGCTACGACA
 >MF621761 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salmo|Salmo trutta
 CACCGCGGTTATACGAGAGACCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTACGGAAAAATATTCAATAAAGCCGAACACCCC
 CTCAGCCGTCATACGCACCTGGGGGCACGAAGATCTACTGCGAAAGCAGCTTTAATTATGCCTGAACCCACGACAGCTACGACA
 >MF621762 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salmo|Salmo trutta
 CACCGCGGTTATACGAGAGACCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTACGGAAAAATATTCAATAAAGCCGAACACCCC
 CTCAGCCGTCATACGCACCTGGGGGCACGAAGATCTACTGCGAAAGCAGCTTTAATTATGCCTGAACCCACGACAGCTACGACA
 >MF621763 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salmo|Salmo trutta
 CACCGCGGTTATACGAGAGACCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTACGGAAAAATATTCAATAAAGCCGAACACCCC
 CTCAGCCGTCATACGCACCTGGGGGCACGAAGATCTACTGCGAAAGCAGCTTTAATTATGCCTGAACCCACGACAGCTACGACA
 >LC011387 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salmo|Salmo trutta
 CACCGCGGTTATACGAGAGACCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTACGGAAAAATATTCAATAAAGCCGAACACCCC
 CTCAGCCGTCATACGCACCTGGGGGCACGAAGATCTACTGCGAAAGCAGCTTTAATTATGCCTGAACCCACGACAGCTACGACA
 >AF154850
 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salvelinus|Salvelinus
 fontinalis
 CACCGCGGTTATACGAGAGGCCCTAGTTGATAAATACCGGCGTAAAGAGTGGTTACGAAAAATGTTTAATAAAGCCGAACACCCC
 CTCAGCCGTCATACGCACCTGGAGGCACGAAGACCTACTGCGAAAGCAGCTTTAATTATACCCGAATCCACGACAGCTACGACA
 >HQ167669
 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salvelinus|Salvelinus
 fontinalis
 CACCGCGGTTATACGAGAGGCCCTAGTTGATAAATACCGGCGTAAAGAGTGGTTACGAAAAATGTTTAATAAAGCCGAACACCCC
 CTCAGCCGTCATACGCACCTGGAGGCACGAAGACCTACTGCGAAAGCAGCTTTAATTATACCCGAATCCACGACAGCTACGACA
 >MF621737
 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salvelinus|Salvelinus
 fontinalis
 CACCGCGGTTATACGAGAGGCCCTAGTTGATAAATACCGGCGTAAAGAGTGGTTACGAAAAATGTTTAATAAAGCCGAACACCCC
 CTCAGCCGTCATACGCACCTGGAGGCACGAAGACCTACTGCGAAAGCAGCTTTAATTATACCCGAATCCACGACAGCTACGACA
 >MF621738
 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salvelinus|Salvelinus
 fontinalis
 CACCGCGGTTATACGAGAGGCCCTAGTTGATAAATACCGGCGTAAAGAGTGGTTACGAAAAATGTTTAATAAAGCCGAACACCCC
 CTCAGCCGTCATACGCACCTGGAGGCACGAAGACCTACTGCGAAAGCAGCTTTAATTATACCCGAATCCACGACAGCTACGACA
 >MF621739
 Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salvelinus|Salvelinus
 fontinalis
 CACCGCGGTTATACGAGAGGCCCTAGTTGATAAATACCGGCGTAAAGAGTGGTTACGAAAAATGTTTAATAAAGCCGAACACCCC
 CTCAGCCGTCATACGCACCTGGAGGCACGAAGACCTACTGCGAAAGCAGCTTTAATTATACCCGAATCCACGACAGCTACGACA

>MF621742
Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salvelinus|Salvelinus
namaycush
CACCGCGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTACGGAAAAATGTTTAATAAGCCGAACACCCC
CTCAGCCGTCATACGCACCTGGGGGCACGAAGACCTACTGCGAAAGCAGCTTTAATTGTACCCGAATCCACGACAGCTACGACA
>MF621744
Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salvelinus|Salvelinus
namaycush
CACCGCGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTACGGAAAAATGTTTAATAAGCCGAACACCCC
CTCAGCCGTCATACGCACCTGGGGGCACGAAGACCTACTGCGAAAGCAGCTTTAATTGTACCCGAATCCACGACAGCTACGACA
>MF621745
Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salvelinus|Salvelinus
namaycush
CACCGCGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTACGGAAAAATGTTTAATAAGCCGAACACCCC
CTCAGCCGTCATACGCACCTGGGGGCACGAAGACCTACTGCGAAAGCAGCTTTAATTGTACCCGAATCCACGACAGCTACGACA
>MF621746
Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salvelinus|Salvelinus
namaycush
CACCGCGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTACGGAAAAATGTTTAATAAGCCGAACACCCC
CTCAGCCGTCATACGCACCTGGGGGCACGAAGACCTACTGCGAAAGCAGCTTTAATTGTACCCGAATCCACGACAGCTACGACA
>MF621747
Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salvelinus|Salvelinus
namaycush
CACCGCGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTACGGAAAAATGTTTAATAAGCCGAACACCCC
CTCAGCCGTCATACGCACCTGGGGGCACGAAGACCTACTGCGAAAGCAGCTTTAATTGTACCCGAATCCACGACAGCTACGACA
>MF621748
Metazoa|Chordata|Actinopteri|Salmoniformes|Salmonidae|Salvelinus|Salvelinus
namaycush
CACCGCGTTATACGAGAGGCCCTAGTTGATAACTACCGGCGTAAAGAGTGGTTACGGAAAAATGTTTAATAAGCCGAACACCCC
CTCAGCCGTCATACGCACCTGGGGGCACGAAGACCTACTGCGAAAGCAGCTTTAATTGTACCCGAATCCACGACAGCTACGACA
>AF049731
Metazoa|Chordata|Actinopteri|Percopsiformes|Percopsidae|Percopsis|Percopsis
omiscomaycus
CACCGCGTTATACGAGAGGCTCAAGTTGATTCTACCGGCGTAAAGGTGGCTAGGGTAATTTATATAGGGCCGAACCCCCTCT
AGGCTGTTATACGCATCCGAAGAATGAAGCACCAACACGAAAGTAGCCCTACCTCCCCGAACCCACGAAAGCCATAAAA
>AP004403
Metazoa|Chordata|Actinopteri|Percopsiformes|Aphredoderidae|Aphredoderus|Aphredo
derus sayanus
CACCGCGTTATACGAGCGACTCAAGTTGACAAACACCGGCGTAAAGCGTGATTAAGGTTTACATTAAGTAGAGCCAAACCACTTC
TAAGCCGTTATACGAATCGAAGAAATGAAGCCCTATCAGGAAAGTAGCTCTACATACCCGAACCCACGAAATCCATATAA
>AP004412 Metazoa|Chordata|Actinopteri|Gadiformes|Lotidae|Lota|Lota lota

CACCGCGTTATACGAGAGGCCCAAGTTGATGAAAAACGGCGTAAAGCGTGGTTAAGAAAACAAGAAAAATAGGGCCGAACAGCCT
 CAAAGCAGTTATACGCATTTCGAGGCCACGAAGCTCAATCACGAAAGTGGCCCTACAAATCTCTGATTCCACGAAAGCCATAAAA
 >KM201364 Metazoa|Chordata|Actinopteri|Gadiformes|Lotidae|Lota|Lota lota
 CACCGCGTTATACGAGAGGCCCAAGTTGATGAAAAACGGCGTAAAGCGTGGTTAAGAAAACAAGAAAAATAGGGCCGAACAGCCT
 CAAAGCAGTTATACGCATTTCGAGGCCACGAAGCTCAATCACGAAAGTGGCCCTACAAATCTCTGATTCCACGAAAGCCATAAAA
 >KC844053 Metazoa|Chordata|Actinopteri|Gadiformes|Lotidae|Lota|Lota lota
 CACCGCGTTATACGAGAGGCCCAAGTTGATGAAAAACGGCGTAAAGCGTGGTTAAGAAAACAAGAAAAATAGGGCCGAACAGCCT
 CAAAGCAGTTATACGCATTTCGAGGCCACGAAGCTCAATCACGAAAGTGGCCCTACAAATCTCTGATTCCACGAAAGCCATAAAA
 >KM363244 Metazoa|Chordata|Actinopteri|Gadiformes|Lotidae|Lota|Lota lota
 CACCGCGTTATACGAGAGGCCCAAGTTGATGAAAAACGGCGTAAAGCGTGGTTAAGAAAACAAGAAAAATAGGGCCGAACAGCCT
 CAAAGCAGTTATACGCATTTCGAGGCCACGAAGCTCAATCACGAAAGTGGCCCTACAAATCTCTGATTCCACGAAAGCCATAAAA
 >FJ445395
 Metazoa|Chordata|Actinopteri|Cyprinodontiformes|Fundulidae|Fundulus|Fundulus
 diaphanus
 CACCGCGTTAAACGAGAGGCTCAAGTTGATAGCCTTCGGCGTAAAGAGTGGTTAAGGAATTAATAAACTAGAGTCGAATTTTCTC
 ATGGCTGTTATACGCACCCGAGAACATGAAGCCCAAACACGAAAGTGGCTTTAAATAACCTGACCCACGAAAGCTGTGAAA
 >NC_028293
 Metazoa|Chordata|Actinopteri|Cyprinodontiformes|Fundulidae|Fundulus|Fundulus
 notatus
 CACCGCGTTAAACGAGAGGCTCGAGTTGATAGCCTTCGGCGTAAAGAGTGGTTAAGGAGTTATTAGGCTAAAGTCGAATCTCTC
 ATGGCTGTTATACGCACCCGAGAGCATGAAGTCCAAACGCGAAAGTAACTTTAAATATCCTGACCCACGAAAGCTGTGAAA
 >AB445125 Metazoa|Chordata|Actinopteri|Perciformes|Gasterosteidae|Culaea|Culaea
 inconstans
 CACCGCGTTATACGAGAGACCCAAGTTGATAAACACGGCGTAAAGAGTGGTTAAGTTAAATGAAAACTAAAGCCGAACGCCCC
 CAAGGCTGTTATACGCATCCGGAGGTAAGAAGTTCAACCACGAAAGTGACTTTATGTAACCTGAACCCACGAAAGCTAAGATA
 >AP002944
 Metazoa|Chordata|Actinopteri|Perciformes|Gasterosteidae|Gasterosteus|Gasteroste
 us aculeatus
 CACCGCGTTATACGAGAGGCCCAAGTTGATGAATTCCGGCGTAAAGAGTGGTTAAGCTAAAATTAATAAACTAAAGCCGAACGCCCC
 CAAAGCTGTTATACGCATCCGGAGGTGAGAAGTTCAACCACGAAGGTGGCTTTATTAACTGAACCCACGAAAGCTACGGCA
 >AB445130
 Metazoa|Chordata|Actinopteri|Perciformes|Gasterosteidae|Pungitius|Pungitius
 pungitius
 CACCGCGTTATACGAGAGGCCCAAGTTGATGAACATCGGCGTAAAGAGTGGTTAAGCTAAAATTAATAAACTAAAGCCGAACGTCCC
 CAAAGCTGTTATACGCACCCGGGGTAAGAAGTTCAACCACGAAGGTGGCTTTATTAACTGAACCCACGAAAGCTACGGCA
 >KT989571
 Metazoa|Chordata|Actinopteri|Perciformes|Gasterosteidae|Pungitius|Pungitius
 pungitius
 CACCGCGTTATACGAGAGGCCCAAGTTGATGAACATCGGCGTAAAGAGTGGTTAAGCTAAAATTAATAAACTAAAGCCGAACGTCCC
 CAAAGCTGTTATACGCACCCGGGGTAAGAAGTTCAACCACGAAGGTGGCTTTATTAACTGAACCCACGAAAGCTACGGCA
 >KM057993 Metazoa|Chordata|Actinopteri|Perciformes|Cottidae|Cottus|Cottus bairdii

CACCGCGGTTATACGAGAGGCCCAAGTTGACAAACACCGGCGTAAAGCGTGGTTAAGTTAAAAATCGTACTAAAGCCAAACATCTT
CAAGACTGTTATACGTAACCGAAGACAGGAAGTTCAACCACGAAAGTCGCTTTATCTGATCTGAATCCACGAAAGCTAAGGAA
>KP013090 Metazoa|Chordata|Actinopteri|Perciformes|Cottidae|Cottus|Cottus bairdii
CACCGCGGTTATACGAGAGGCCCAAGTTGACAAACACCGGCGTAAAGCGTGGTTAAGTTAAAAATCGTACTAAAGCCAAACATCTT
CAAGACTGTTATACGTAACCGAAGACAGGAAGTTCAACCACGAAAGTCGCTTTATCTGATCTGAATCCACGAAAGCTAAGGAA
>AB188190 Metazoa|Chordata|Actinopteri|Perciformes|Cottidae|Cottus|Cottus cognatus
CACCGCGGTTATACGAGAGGCCCAAGTTGACAAACACCGGCGTAAAGCGTGGTTAAGTTAAAAATCGTACTAAAGCCAAACATCTT
CAAGACTGTTATACGTAACCGAAGACAGGAAGTTCAACCACGAAAGTCGCTTTATCTGATCTGAATCCACGAAAGCTAAGGAA
>MG924474 Metazoa|Chordata|Actinopteri|Perciformes|Cottidae|Cottus|Cottus ricei
CACCGCGGTTATACGAGAGGCCCAAGTTGACAAACACCGGCGTAAAGCGTGGTTAAGTTAAAAATCATACTAAAGCCAAACATCTT
TCAAGACTGTTATACGTAACCGAAGACAGGAAGTTCAACCACGAAAGTCGCTTTATCTGATCTGAATCCACGAAAGCTAAGGAA
>KU641485 Metazoa|Chordata|Actinopteri|<not present>|Moronidae|Morone|Morone
americana
CACCGCGGTTAAACGGGAGGCTCAAGTTGATAGACATCGGCGTAAAGCGTGGTTAGGGGCCAACTTTAACTAAAGCCGAACGC
TCTTAGAACAGTTATACGTAAGAGTAAGAAGATCAATTACGAAAGTAGCTTTATTCAAACCTGACTCCACGAAAGCTAAGGCA
>AY372812 Metazoa|Chordata|Actinopteri|<not present>|Moronidae|Morone|Morone
chrysops
CACCGCGGTTAAACGGGAGGCTCAAGTTGATAGACATCGGCGTAAAGCGTGGTTAAGGGCCAACTTTTCTACTAAAGCCGAACGCT
TTTAGAGCAGTTATACGCACTGAGAGTAAGAAGATCAATTACGAAAGTAGCTTTATTCAAACCTGACCCACGAAAGCTAAGGCA
>AF055589 Metazoa|Chordata|Actinopteri|<not present>|Moronidae|Morone|Morone
chrysops
CACCGCGGTTAAACGGGAGGCTCAAGTTGATAGACATCGGCGTAAAGCGTGGTTAAGGGCCAACTTTTCTACTAAAGCCGAACGCT
TTTAGAGCAGTTATACGCACTGAGAGTAAGAAGATCAATTACGAAAGTAGCTTTATTCAAACCTGACCCACGAAAGCTAAGGCA
>KY660677
Metazoa|Chordata|Actinopteri|Centrarchiformes|Centrarchidae|Ambloplites|Ambloplites
rupestris
CACCGCGGTTATACGAGAGGCCCAAGTTGATTAACCCCGGCGTAAAGAGTGGTTAAGATGAGCTATAAACTAAAGCCGAATGCCCT
CAAAGCTGTTATACGCACCCGAAGTAAGAAGCTCAATCACGAAAGTGGCTTTACACCCCTGAACCCACGAAAGCTATGATA
>KY815235
Metazoa|Chordata|Actinopteri|Centrarchiformes|Centrarchidae|Ambloplites|Ambloplites
rupestris
CACCGCGGTTATACGAGAGGCCCAAGTTGATTAACCCCGGCGTAAAGAGTGGTTAAGATGAGCTATAAACTAAAGCCGAATGCCCT
CAAAGCTGTTATACGCACCCGAAGTAAGAAGCTCAATCACGAAAGTGGCTTTACACCCCTGAACCCACGAAAGCTATGATA
>KC427094
Metazoa|Chordata|Actinopteri|Centrarchiformes|Centrarchidae|Lepomis|Lepomis
cyaneus
CACCGCGGTTATACGAGAGGCTCAAGTTGATAAACCCCGGCGTAAAGAGTGGTTAAGGAAAAATAGAACTAAAGCCGAATGCTTT
CAAGACTGTTATACGTTCCCGAGAGTAAGAAGACCAATTACGAAAGTGGCTTTACTTTACCTGACCCACGAAAGCTACGACA
>KP013087
Metazoa|Chordata|Actinopteri|Centrarchiformes|Centrarchidae|Lepomis|Lepomis
cyaneus

CACCGCGGTTATACGAGAGGCTCAAGTTGATAAACCCCGGCGTAAAGAGTGGTTAAGGAAAATAGAACTAAAGCCGAATGCTTT
CAAGACTGTTATACGTTCCGAGAGTAAGAAGACCAATTACGAAAGTGGCTTTACTTTACCTGACCCACGAAAGCTACGACA
>KP013097

Metazoa|Chordata|Actinopteri|Centrarchiformes|Centrarchidae|Lepomis|Lepomis
gibbosus

CACCGCGGTTATACGAGAGGCTCAAGTTGATAAACCCCGGCGTAAAGAGTGGTTAAGGAAGATTAATACTAAAGCCGAATGCTTT
CAAAGCTGTTATACGTTCCGAGAGTAAGAAGACCACTACGAAAGTGACTTTATAGCCCCTGACCCACGAAAGCTATGGCA
>MF621724

Metazoa|Chordata|Actinopteri|Centrarchiformes|Centrarchidae|Lepomis|Lepomis
gibbosus

CACCGCGGTTATACGAGAGGCTCAAGTTGATAAACCCCGGCGTAAAGAGTGGTTAAGGAAGATTAATACTAAAGCCGAATGCTTT
CAAAGCTGTTATACGTTCCGAGAGTAAGAAGATCACTACGAAAGTGACTTTATAGCCCCTGACCCACGAAAGCTATGGCA
>MF621725

Metazoa|Chordata|Actinopteri|Centrarchiformes|Centrarchidae|Lepomis|Lepomis
gibbosus

CACCGCGGTTATACGAGAGGCTCAAGTTGATAAACCCCGGCGTAAAGAGTGGTTAAGGAAGATTAATACTAAAGCCGAATGCTTT
CAAAGCTGTTATACGTTCCGAGAGTAAGAAGATCACTACGAAAGTGACTTTATAGCCCCTGACCCACGAAAGCTATGGCA
>MF621726

Metazoa|Chordata|Actinopteri|Centrarchiformes|Centrarchidae|Lepomis|Lepomis
gibbosus

CACCGCGGTTATACGAGAGGCTCAAGTTGATAAACCCCGGCGTAAAGAGTGGTTAAGGAAGATTAATACTAAAGCCGAATGCTTT
CAAAGCTGTTATACGTTCCGAGAGTAAGAAGATCACTACGAAAGTGACTTTATAGCCCCTGACCCACGAAAGCTATGGCA
>AY372810

Metazoa|Chordata|Actinopteri|Centrarchiformes|Centrarchidae|Lepomis|Lepomis
humilis

CACCGCGGTTATACGAGAGGCTCAAGTTGATAAACTTCGGCGTAAAGAGTGGTTAAGGGAGATAATACTAAAGCCGAATGCTTTC
AAAGCTGTTATACGTTCCGAAAGTAAGAAGATTCAATCACGAAAGTGGCTTTACTTCCCCTGACCCACGAAAGCTATGACA
>MF621712

Metazoa|Chordata|Actinopteri|Centrarchiformes|Centrarchidae|Lepomis|Lepomis
macrochirus

CACCGCGGTTATACGAGAGGCTCAAGTTGATGAACCCCGGCGTAAAGAGTGGTTAAGGGAGATCAATACTAAAGCCGAATGCTTTC
AAAGCTGTTATACGTTCCGAAAGTAAGAAGCTCAATCACGAAAGTGGCTTTACTTTACCTGACCCACGAAAGCTACGACA
>MF621713

Metazoa|Chordata|Actinopteri|Centrarchiformes|Centrarchidae|Lepomis|Lepomis
macrochirus

CACCGCGGTTATACGAGAGGCTCAAGTTGATGAACCCCGGCGTAAAGAGTGGTTAAGGGAGATCAATACTAAAGCCGAATGCTTTC
AAAGCTGTTATACGTTCCGAAAGTAAGAAGCTCAATCACGAAAGTGGCTTTACTTTACCTGACCCACGAAAGCTACGACA
>MF621714

Metazoa|Chordata|Actinopteri|Centrarchiformes|Centrarchidae|Lepomis|Lepomis
macrochirus

CACCGCGGTTATACGAGAGGCTCAAGTTGATGAACCCCGGCGTAAAGAGTGGTTAAGGGAGATCAATACTAAAGCCGAATGCTTTC
AAAGCTGTTATACGTTCCGAAAGTAAGAAGCTCAATCACGAAAGTGGCTTTACTTTACCTGACCCACGAAAGCTACGACA

>JN389795
Metazoa|Chordata|Actinopteri|Centrarchiformes|Centrarchidae|Lepomis|Lepomis
macrochirus
CACCGCGTTATACGAGAGGCTCAAGTTGATGAACCCCGGCGTAAAGAGTGGTTAAGAGAGATCAAACTAAAGCCGAATGCTTTC
AAAGCTGTTATACGCTTCCGAAAGTAAGAAGCCCAATCACGAAAGTGGCTTTACTTTACCTGACCCACGAAAGCTACGACA
>KP013118
Metazoa|Chordata|Actinopteri|Centrarchiformes|Centrarchidae|Lepomis|Lepomis
macrochirus
CACCGCGTTATACGAGAGGCTCAAGTTGATGAACCCCGGCGTAAAGAGTGGTTAAGAGAGATCAAACTAAAGCCGAATGCTTTC
AAAGCTGTTATACGCTTCCGAAAGTAAGAAGCCCAATCACGAAAGTGGCTTTACTTTACCTGACCCACGAAAGCTACGACA
>AP005993
Metazoa|Chordata|Actinopteri|Centrarchiformes|Centrarchidae|Lepomis|Lepomis
macrochirus
CACCGCGTTATACGAGAGGCTCAAGTTGATGAACCCCGGCGTAAAGAGTGGTTAAGAGAGATCAAACTAAAGCCGAATGCTTTC
AAAGCTGTTATACGCTTCCGAAAGTAAGAAGCCCAATCACGAAAGTGGCTTTACTTTACCTGACCCACGAAAGCTACGACA
>MF621710
Metazoa|Chordata|Actinopteri|Centrarchiformes|Centrarchidae|Micropterus|Micropt
erus dolomieu
CACCGCGTTATACGAGAGGCCCAAGTTGACAAACCCCGGCGTAAAGAGTGGTTAAGGAAAATTTAAACTAAAGCCGAATGCCCC
CAAAGCTGTTATACGCACCCGAGGGTAAGAAGCCCAATCACGAAAGTGGCTTTACACTACCTGAACCCACGAAAGCTATGAAA
>MF621711
Metazoa|Chordata|Actinopteri|Centrarchiformes|Centrarchidae|Micropterus|Micropt
erus dolomieu
CACCGCGTTATACGAGAGGCCCAAGTTGACAAACCCCGGCGTAAAGAGTGGTTAAGGAAAATTTAAACTAAAGCCGAATGCCCC
CAAAGCTGTTATACGCACCCGAGGGTAAGAAGCCCAATCACGAAAGTGGCTTTACACTACCTGAACCCACGAAAGCTATGAAA
>AB378749
Metazoa|Chordata|Actinopteri|Centrarchiformes|Centrarchidae|Micropterus|Micropt
erus dolomieu
CACCGCGTTATACGAGAGGCCCAAGTTGACAAACCCCGGCGTAAAGAGTGGTTAAGGAAAATTTAAACTAAAGCCGAATGCCCC
CAAAGCTGTTATACGCACCCGAGGGTAAGAAGCCCAATCACGAAAGTGGCTTTACACTACCTGAACCCACGAAAGCTATGAAA
>AB378750
Metazoa|Chordata|Actinopteri|Centrarchiformes|Centrarchidae|Micropterus|Micropt
erus dolomieu
CACCGCGTTATACGAGAGGCCCAAGTTGACAAACCCCGGCGTAAAGAGTGGTTAAGGAAAATTTAAACTAAAGCCGAATGCCCC
CAAAGCTGTTATACGCACCCGAGGGTAAGAAGCCCAATCACGAAAGTGGCTTTACACTACCTGAACCCACGAAAGCTATGAAA
>HQ391896
Metazoa|Chordata|Actinopteri|Centrarchiformes|Centrarchidae|Micropterus|Micropt
erus salmoides
CACCGCGTTATACGAGAGGCCCAAGTTGACAAACCCCGGCGTAAAGAGTGGTTAAGAAAATTTAAACTAAAGCCGAATGCCCT
CAAAGCTGTTATACGCACCCGAAGTAAGAAGCCCAATCACGAAAGTGGCTTTACATTTCCGAACCCACGAAAGCTATGAAA

>AP014536
Metazoa|Chordata|Actinopteri|Centrarchiformes|Centrarchidae|Micropterus|Micropterus salmoides
CACCGCGTTATACGAGAGACCCAAGTTGATAGACATCGGCGTAAAGAGTGGTTAAGACAAATTAAGACTAAAGCCGAAGCCCCT
CAGAGCTGTTATACGCACCCGAGAGCAAGAAGTTCAATCACGAAAGTGGCTTTACCCCCCCCCCTGAACCCACGAAAGCTATG
ATA

>DQ536425
Metazoa|Chordata|Actinopteri|Centrarchiformes|Centrarchidae|Micropterus|Micropterus salmoides
CACCGCGTTATACGAGAGGCCCAAGTTGACAAACCCGCGTAAAGAGTGGTTAAGAAAAATTTAAACTAAAGCCGAATGCCCT
CAAAGCTGTTATACGCACCCGAAGGTAAGAAGCCCAATCACGAAAGTGGCTTTACATTTCTGAACCCACGAAAGCTATGAAA

>KP013112
Metazoa|Chordata|Actinopteri|Centrarchiformes|Centrarchidae|Pomoxis|Pomoxis nigromaculatus
CACCGCGTTATACGAGAGGCTCAAGTTGATTAACCCCGCGTAAAGAGTGGTTAAGATGAACATAAACTAAAGCCGAATGCCCT
CAAAGCCGTTATACGCACTCGAAGGAAAGAAGCTCAATCACGAAAGTAGCTTTACATCACCTGAACCCACGAAAGCTATGAAA

>MF621715
Metazoa|Chordata|Actinopteri|Centrarchiformes|Centrarchidae|Pomoxis|Pomoxis nigromaculatus
CACCGCGTTATACGAGAGGCTCAAGTTGATTAACCCCGCGTAAAGAGTGGTTAAGATGAACATAAACTAAAGCCGAATGCCCT
CAAAGCCGTTATACGCACTCGAAGGAAAGAAGCTCAATCACGAAAGTAGCTTTACATCACCTGAACCCACGAAAGCTATGAAA

>MF621719
Metazoa|Chordata|Actinopteri|Centrarchiformes|Centrarchidae|Pomoxis|Pomoxis nigromaculatus
CACCGCGTTATACGAGAGGCTCAAGTTGATTAACCCCGCGTAAAGAGTGGTTAAGATGAACATAAACTAAAGCCGAATGCCCT
CAAAGCCGTTATACGCACTCGAAGGAAAGAAGCTCAATCACGAAAGTAGCTTTACATCACCTGAACCCACGAAAGCTATGAAA

>AY372766 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Ammocrypta|Ammocrypta pellucida
CACCGCGTTATACGAGAGGCCCGAGTTGATAGACGCCGCGTAAAGCGTGGTTAGGATTTCTATAAACTAAAGCCGAACACCCCT
CAGAGCTGTTATACGCACCCGAAGGCTTGAAGCTCAACCACGAAAGTGGCTTTACAACCCGAACCCACGAAAGCTAAGACA

>AY372771 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Etheostoma|Etheostoma blennioides
CACCGCGTTATACGAGAGGCCCAAGTTGATAGACACCGCGTAAAGCGTGGTTAAGATTTTCACAGAAATAAGCCGAACACCTT
CAGGGCTGTTATACGCACCCGAAGGCAAGAAGATCAACTACGAAGGTGGCTTTACAATCTGAACCCACGAAAGCTACGACA

>KY660678 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Etheostoma|Etheostoma caeruleum
CACCGCGTTATACGAGAGGCCCAAGCTGATAGACACCGCGTAAAGCGTGGTTAAGGTTCTTCCAAAATAAGCCGAACACCT
TCAGAACTGTTATACGCACCCGAAGGTATGAAGACCAACAACGAAAGTGGCTTTATAATTCTGAACCCACGAAAGCTACGACA

>AY372773 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Etheostoma|Etheostoma caeruleum
CACCGCGTTATACGAGAGGCCCAAGCTGATAGACACCGCGTAAAGCGTGGTTAAGGTTCTTCCAAAATAAGCCGAACACCT
TCAGAACTGTTATACGCACCCGAAGGCATGAAGACCAACAACGAAAGTGGCTTTATAATTCTGAACCCACGAAAGCTACGACA

>NC_042248 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Etheostoma|Etheostoma flabellare
CACC GCG GTTATACGAGAGGCCCAAGCTGATAGACACCGGCGTAAAGCGTGGTTAAGACTATAACAGACTAAAGCCAAACACCTTC
ACAGCTGTTATACGCACCCGAAGGCAAGAAGACCAACCACGAAAGTAGCTTTAAACTCTGAACCCACGAAAGCTATGACA
>AY372781 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Etheostoma|Etheostoma nigrum
CACC GCG GTTATACGAGAGGCCCAAGTTGATAGACACCGGCGTAAAGCGTGGTTAAGATTTTTTCAAACTAAAGCCGAACACCTT
CAAGGCTGTTATACGCACCCGAAGGCAAGAAGATCAACCACGAAAGTGCTTTACAGCCCTGAATCCACGAAAGCTACGACA
>KT289926 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Etheostoma|Etheostoma nigrum
CACC GCG GTTATACGAGAGGCCCAAGTTGATGGACACCGGCGTAAAGCGTGGTTAAGATTTTTTCAAACTAAAGCCGAACACCTT
CAGGGCTGTTATACGCACCCGAAGGCAAGAAGATCAACCACGAAAGTGCTTTACAGCCCTGAATCCACGAAAGCTACGACA
>AY372790 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Etheostoma|Etheostoma spectabile
CACC GCG GTTATACGAGAGGCCCAAGTTGATATATGCCGGCGTAAAGCGTGGTTAAGATTTTTATGCAACTAAAGCCAAACACCTT
CAAAGCTGTTATACGCACCCGAAGATAAGAGGATCAATCACGAAAGTGCTTTACTATCTGAACCCACGAAAGCTACGACA
>AP005994 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Etheostoma|Etheostoma zonale
CACC GCG GTTATACGAGAGGCCCAAGCTGATAGACACCGGCGTAAAGCGTGGTTAGGATTTTTATAAACTAAAGCCGAACACCTT
CAGAGCTGTTATACGCACCCGAAGGTAAGAAGACCAACCACGAAAGTGCTTTACAACGCTGAACCCACGAAAGCTATGATA
>KM978956 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Gymnocephalus|Gymnocephalus cernua
CACC GCG GTTATACGAGAGGCCCAAGCTGATAGATATCGGCGTAAAGCGTGGTTAAGATTTTAAATATAAACTAAAGCCGAACACCT
TCAAAGCTGTTATACGCACCCGAAGATAAGAAGTTCAACCACGAAAGTGCTTTATAACCCCTGAACCCACGAAAGCTACGACA
>MF621736 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Perca|Perca flavescens
CACC GCG GTTATACGAGAGGCCCAAGTTGATTGACATCGGCGTAAAGCGTGGTTAAGGCCAAAAATGAACTAAAGCCGAACACCT
TCAGAGCTGTTATACGCATCCGAAGGCAAGAAGTTCAACCACGAAGGTGGCTTTATAACCCCTGAACCCACGAAAGCTAAGATA
>JX629442 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Perca|Perca flavescens
CACC GCG GTTATACGAGAGGCCCAAGTTGATTGACATCGGCGTAAAGCGTGGTTAAGGCCAAAAATGAACTAAAGCCGAACACCT
TCAGAGCTGTTATACGCATCCGAAGGCAAGAAGTTCAACCACGAAGGTGGCTTTATAACCCCTGAACCCACGAAAGCTAAGATA
>JX629443 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Perca|Perca flavescens
CACC GCG GTTATACGAGAGGCCCAAGTTGATTGACATCGGCGTAAAGCGTGGTTAAGGCCAAAAATGAACTAAAGCCGAACACCT
TCAGAGCTGTTATACGCATCCGAAGGCAAGAAGTTCAACCACGAAGGTGGCTTTATAACCCCTGAACCCACGAAAGCTAAGATA
>JX629444 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Perca|Perca flavescens
CACC GCG GTTATACGAGAGGCCCAAGTTGATTGACATCGGCGTAAAGCGTGGTTAAGGCCAAAAATGAACTAAAGCCGAACACCT
TCAGAGCTGTTATACGCATCCGAAGGCAAGAAGTTCAACCACGAAGGTGGCTTTATAACCCCTGAACCCACGAAAGCTAAGATA
>JX629445 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Perca|Perca flavescens
CACC GCG GTTATACGAGAGGCCCAAGTTGATTGACATCGGCGTAAAGCGTGGTTAAGGCCAAAAATGAACTAAAGCCGAACACCT
TCAGAGCTGTTATACGCATCCGAAGGCAAGAAGTTCAACCACGAAGGTGGCTTTATAACCCCTGAACCCACGAAAGCTAAGATA
>JX629446 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Perca|Perca flavescens

CACCGCGTTATACGAGAGGCCCAAGTTGATTGACATCGGCGTAAAGCGTGGTTAAGGCCAAAAATGAACTAAAGCCGAACACCT
 TCAGAGCTGTTATACGCATCCGAAGGCAAGAAGTTCAACCACGAAGGTGGCTTTATAACCCCTGAACCCACGAAAGCTAAGATA
 >JX629447 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Perca|Perca flavescens
 CACCGCGTTATACGAGAGGCCCAAGTTGATTGACATCGGCGTAAAGCGTGGTTAAGGCCAAAAATGAACTAAAGCCGAACACCT
 TCAGAGCTGTTATACGCATCCGAAGGCAAGAAGTTCAACCACGAAGGTGGCTTTATAACCCCTGAACCCACGAAAGCTAAGATA
 >JX629448 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Perca|Perca flavescens
 CACCGCGTTATACGAGAGGCCCAAGTTGATTGACATCGGCGTAAAGCGTGGTTAAGGCCAAAAATGAACTAAAGCCGAACACCT
 TCAGAGCTGTTATACGCATCCGAAGGCAAGAAGTTCAACCACGAAGGTGGCTTTATAACCCCTGAACCCACGAAAGCTAAGATA
 >AY372797 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Percina|Percina
 caprodes
 CACCGCGTTATACGAGAGGCCCAAGTTGATAATTACCGGCGTAAAGCGTGGTTAAGATTTTTATAGAACTAAAGCCGAACCCCTT
 CAGAGCTGTTATACGCACCCGAAGGTAAGAAGTTCAACCACGAAAGTGCTTTACAGCCCCGAACCCACGAAAGCTAAGATA
 >AY372798 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Percina|Percina
 copelandi
 CACCGCGTTATACGAGAGGCCCAAGTTGATAATTACCGGCGTAAAGCGTGGTTAAGATTTTTATAGAACTAAAGCCGAACCCCTT
 CAGAGCTGTTATACGCACCCGAAGGTAAGAAGTTCAACCACGAAAGTGCTTTACAGCCCCGAACCCACGAAAGCTAAGATA
 >AY372801 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Percina|Percina
 maculata
 CACCGCGTTATACGAGAGGCCCAAGTTGATAATTACCGGCGTAAAGCGTGGTTAAGATTTTTATACATCTAAAGCCGGACCCCTT
 CAGAGCTGTTATACGCTCCCGAAGATAAGAAGTTCAACCACGAAAGTGCTTTACAGCCCCGAACCCACGAAAGCTAAGATA
 >KC663435 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Sander|Sander
 canadensis
 CACCGCGTTATACGAGAGGCCCAAGTTGATAGACATCGGCGTAAAGCGTGGTTAAGACAAAAACAAAATAAGCCGAACACCTT
 CAGAGCTGTTATACGCACCCGAAGGTAAGAAGTTCAACCACGAAAGTGCTTTATTACCCCTGAACCCACGAAAGCTACGATA
 >KT211477 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Sander|Sander
 canadensis
 CACCGCGTTATACGAGAGGCCCAAGTTGATAGCCATCGGCGTAAAGCGTGGTTAAGATAAAGACTAAAATAAGCCGAACACCC
 TCAGAGCTGTTATACGCACCCGAAGGTAAGAAGTTCAATCACGAAAGTGCTTTATAGACCCCGAACCCACGAAAGCTACGATA
 >KT211478 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Sander|Sander
 canadensis
 CACCGCGTTATACGAGAGGCCCAAGTTGATAGCCATCGGCGTAAAGCGTGGTTAAGATAAAGACTAAAATAAGCCGAACACCC
 TCAGAGCTGTTATACGCACCCGAAGGTAAGAAGTTCAATCACGAAAGTGCTTTATAGACCCCGAACCCACGAAAGCTACGATA
 >MG599474
 Metazoa|Chordata|Actinopteri|Perciformes|Sciaenidae|Aplodinotus|Aplodinotus
 grunniens
 CACCGCGTTATACGAGAGGCCCAAGTCGATAGTCAACGGCGTAAAGAGTGGTTAGAAAGAACACTATTACTAAAGCCGAACGCCC
 TCAAAGCTGTTATACGCATCCGAGAGTGAGAAGCCCATCCACGAAAGTGCTTTACAACCTTGAACCCACGAAAGCTATGGCA
 >KU755530 Metazoa|Chordata|Actinopteri|Gobiiformes|Gobiidae|Neogobius|Neogobius
 melanostomus
 CACCGCGTTATACGAGAGGCCCAAGTTGACAAAAATCGGCGAAAAGCGTGGTTAGTGATATCACCTCCACTAAAGCCAAACACCT
 TCAAGGCTGTAATACGCCCCGAAGACAGGAAGCCCAATCACGAAAGTAGCTTTAATTTTCATGAAGCCACGAAAGCTAGGAAA

[illegible]

[illegible]

[illegible]

>KJ564183 Metazoa|Chordata|Actinopteri|Anguilliformes|Anguillidae|Anguilla|Anguilla
rostrata
CACCGCGGTTATACGAGGGGCTCAAATTGATATTACACGGCGTAAAGCGTGATTAAAAATAAACAACTAAAGCCAAACACTTCC
CAAGTGTCATACGCTACCGGACAAAACGAAGCCCTATAACGAAAGTAGCTTTAACACCTTTGAACTCACGACAGTTGAGGAA

>KJ564184 Metazoa|Chordata|Actinopteri|Anguilliformes|Anguillidae|Anguilla|Anguilla
rostrata
CACCGCGGTTATACGAGGGGCTCAAATTGATATTACACGGCGTAAAGCGTGATTAAAAATAAACAACTAAAGCCAAACACTTCC
CAAGTGTCATACGCTACCGGACAAAACGAAGCCCTATAACGAAAGTAGCTTTAACATCTTTGAACTCACGACAGTTGAGGAA

>KJ564185 Metazoa|Chordata|Actinopteri|Anguilliformes|Anguillidae|Anguilla|Anguilla
rostrata
CACCGCGGTTATACGAGGGGCTCAAATTGATATTACACGGCGTAAAGCGTGATTAAAAATAAACAACTAAAGCCAAACACTTCC
CAAGTGTCATACGCTACCGGACAAAACGAAGCCCTATAACGAAAGTAGCTTTAACACCTTTGAACTCACGACAGTTGAGGAA

>KJ564186 Metazoa|Chordata|Actinopteri|Anguilliformes|Anguillidae|Anguilla|Anguilla
rostrata
CACCGCGGTTATACGAGGGGCTCAAATTGATATTACACGGCGTAAAGCGTGATTAAAAATAAACAACTAAAGCCAAACACTTCC
CAAGTGTCATACGCTACCGGACAAAACGAAGCCCTATAACGAAAGTAGCTTTAACATCTTTGAACTCACGACAGTTGAGGAA

>KJ564187 Metazoa|Chordata|Actinopteri|Anguilliformes|Anguillidae|Anguilla|Anguilla
rostrata
CACCGCGGTTATACGAGGGGCTCAAATTGATATTACACGGCGTAAAGCGTGATTAAAAATAAACAACTAAAGCCAAACACTTCC
CAAGTGTCATACGCTACCGGACAAAACGAAGCCCTATAACGAAAGTAGCTTTAACATCTTTGAACTCACGACAGTTGAGGAA

>KJ564188 Metazoa|Chordata|Actinopteri|Anguilliformes|Anguillidae|Anguilla|Anguilla
rostrata
CACCGCGGTTATACGAGGGGCTCAAATTGATATTACACGGCGTAAAGCGTGATTAAAAATAAACAACTAAAGCCAAACACTTCC
CAAGTGTCATACGCTACCGGACAAAACGAAGCCCTATAACGAAAGTAGCTTTAACACCTTTGAACTCACGACAGTTGAGGAA

>KJ564271 Metazoa|Chordata|Actinopteri|Anguilliformes|Anguillidae|Anguilla|Anguilla
rostrata
CACCGCGGTTATACGAGGGGCTCAAATTGATATTACACGGCGTAAAGCGTGATTAAAAACAAAAAACTAAAGCCAAACACTTCC
CAAGTGTCATACGCTACCGGAACATAAACGAAGCCCTATAACGAAAGTAGCTTTAACACCTTTGAACTCACGACAGTTGAGGAA

>AF266496 Metazoa|Chordata|Actinopteri|Anguilliformes|Anguillidae|Anguilla|Anguilla
rostrata
CACCGCGGTTATACGAGGGGCTCAAATTGATATTACACGGCGTAAAGCGTGATTAAAAATAAACAACTAAAGCCAAACACTTCC
CAAGTGTCATACGCTACCGGACAAAACGAAGCCCTATAACGAAAGTAGCTTTAACACCTTTGAACTCACGACAGTTGAGGAA

>AF266497 Metazoa|Chordata|Actinopteri|Anguilliformes|Anguillidae|Anguilla|Anguilla
rostrata
CACCGCGGTTATACGAGGGGCTCAAATTGATATTACACGGCGTAAAGCGTGATTAAAAATAAACAACTAAAGCCAAACACTTCC
CAAGTGTCATACGCTACCGGACAAAACGAAGCCCTATAACGAAAGTAGCTTTAACACCTTTGAACTCACGACAGTTGAGGAA

>AB021901 Metazoa|Chordata|Actinopteri|Anguilliformes|Anguillidae|Anguilla|Anguilla
rostrata
CACCGCGGTTATACGAGGGGCTCAAATTGATATTACACGGCGTAAAGCGTGATTAAAAATAAACAACTAAAGCCAAACACTTCC
CAAGTGTCATACGCTACCGGACAAAACGAAGCCCTATAACGAAAGTAGCTTTAACATCTTTGAACTCACGACAGTTGAGGAA

>KJ476998 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACTTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
A

>MF443764 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACTTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
A

>MF443765 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACTTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
A

>MF443766 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACTTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
A

>MF443767 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACTTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
A

>MF443768 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACTTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
A

>MF443769 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACTTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
A

>MF443770 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACTTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
A

>MF443771 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
A

>MF443758 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
A

>MF443759 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
A

>MF443760 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
A

>MF443761 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
A

>MF443762 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
A

>MF443763 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
A

>JN105355 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTTAATAAACCCACCTGACCCACGAAAGCTGAGGA
A

>KJ874428 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
 CTTGGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
 A

>KJ874429 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
 auratus
 CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
 CTTGGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
 A

>KJ874430 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
 auratus
 CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
 CTTGGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
 A

>KJ874431 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
 auratus
 CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
 CTTGGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
 A

>EF483931 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
 auratus
 CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
 CTTGGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
 A

>KF147851 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
 auratus
 CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
 CTTGGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
 A

>HQ875340 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
 auratus
 CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
 CTTGGCCGTCATACGCTTCTAGGTGTCCGAAGCCCTAATACGAAAGTAACCTTTAATAAGCCACCTGACCCACGAAAGCTGAGGA
 A

>KX505165 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
 auratus
 CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
 CTTGGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
 A

>KM657132 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
 auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTAATGAACCCACCTGACCCACGAAAGCTGAGAA
A

>KM657133 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
A

>KM657134 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTAATAAGCCACCTGACCCACGAAAGCTGAGGA
A

>KM659025 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
A

>AB379915 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
A

>AB379916 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
A

>AB379917 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
A

>AB379918 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
A

>AB379919 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
A

>AB379920 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
A

>AB379921 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
A

>AB111951 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTAATAAACCACCTGACCCACGAAAGCTGAGGA
A

>KT756205 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
A

>AB006953 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTAATAAACCACCTGACCCACGAAAGCTGAGAA
A

>KU146528 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
A

>GU086395 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTAATAAACCACCTGACCCACGAAAGCTGAGGA
A

>GU086396 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTTAATAAACCCACCTGACCCACGAAAGCTGAGGA
A

>GU086397 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTTAATAAACCCACCTGACCCACGAAAGCTGAGGA
A

>AP011236 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAATAAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTTAATGAACCCACCTGACCCACGAAAGCTGAGGA
A

>AP011239 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Carassius|Carassius
auratus

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGCGTCCGAAGCCCTAATACGAAAGTAACCTTTAATAAACCCGCCTGACCCACGAAAGCTGAGAA
A

>KJ511882 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
carpio

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGAGTCCGAAGCCCTAATACGAAAGTAACCTTTAATAAACCCACCTGACCCACGAAAGCTGAGAA
A

>KJ511883 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
carpio

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGAGTCCGAAGCCCTAATACGAAAGTAACCTTTAATAAACCCACCTGACCCACGAAAGCTGAGAA
A

>JN105352 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
carpio

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGAGTCCGAAGCCCTAATACGAAAGTAACCTTTAATAAACCCACCTGACCCACGAAAGCTGAGAA
A

>JN105353 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
carpio

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGAGTCCGAAGCCCTAATACGAAAGTAACCTTTAATAAACCCACCTGACCCACGAAAGCTGAGAA
A

>JN105354 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
carpio

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGAGTCCGAAGCCCTAATACGAAAGTAACTTTAATAAACCCACCTGACCCACGAAAGCTGAGAA
A

>JN105357 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
carpio
CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGAGTCCGAAGCCCTAATACGAAAGTAACTTTAATAAACCCACCTGACCCACGAAAGCTGAGAA
A

>MG570426 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
carpio
CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGAGTCCGAAGCCCTAATACGAAAGTAACTTTAATAAACCCACCTGACCCACGAAAGCTGAGAA
A

>MG570427 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
carpio
CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGAGTCCGAAGCCCTAATACGAAAGTAACTTTAATAAACCCACCTGACCCACGAAAGCTGAGAA
A

>MG570435 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
carpio
CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGAGTCCGAAGCCCTAATACGAAAGTAACTTTAATAAACCCACCTGACCCACGAAAGCTGAGAA
A

>X61010 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
carpio
CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGAGTCCGAAGCCCTAATACGAAAGTAACTTTAATAAACCCACCTGACCCACGAAAGCTGAGAA
A

>JX188253 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
carpio
CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGAGTCCGAAGCCCTAATACGAAAGTAACTTTAATAAACCCACCTGACCCACGAAAGCTGAGAA
A

>JX188254 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
carpio
CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGAGTCCGAAGCCCTAACACGAAAGTAACTTTAATAAACCCACCTGACCCACGAAAGCTGAGAA
A

>KP013086 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
carpio

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGAGTCCGAAGCCCTAATACGAAAGTAACCTTTAATAAACCCACCTGACCCACGAAAGCTGAGAA
A

>KX710076 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
carpio
CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGAGTCCGAAGCCCTAATACGAAAGTAACCTTTAATAAACCCACCTGACCCACGAAAGCTGAGAA
A

>KU301745 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
carpio
CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGAGTCCGAAGCCCTAATACGAAAGTAACCTTTAATAAACCCACCTGACCCACGAAAGCTGAGAA
A

>KU050703 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
carpio
CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGAGTCCGAAGCCCTAATACGAAAGTAACCTTTAATAAACCCACCTGACCCACGAAAGCTGAGAA
A

>KU146529 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
carpio
CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGAGTCCGAAGCCCTAATACGAAAGTAACCTTTAATAAACCCACCTGACCCACGAAAGCTGAGAA
A

>KU146530 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
carpio
CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGAGTCCGAAGCCCTAATACGAAAGTAACCTTTAATAAACCCACCTGACCCACGAAAGCTGAGAA
A

>KU159761 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
carpio
CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGAGTCCGAAGCCCTAATACGAAAGTAACCTTTAATAAACCCACCTGACCCACGAAAGCTGAGAA
A

>KP993136 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
carpio
CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGAGTCCGAAGCCCTAATACGAAAGTAACCTTTAATAAACCCACCTGACCCACGAAAGCTGAGAA
A

>KP993137 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
carpio

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
 CTTGGCCGTCATACGCTTCTAGGAGTCCGAAGCCCTAATACGAAAGTAACCTTTAATAAACCCACCTGACCCACGAAAGCTGAGAA
 A

>KP993138 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
 carpio
 CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
 CTTGGCCGTCATACGCTTCTAGGAGTCCGAAGCCCTAATACGAAAGTAACCTTTAATAAACCCACCTGACCCACGAAAGCTGAGAA
 A

>KP993139 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
 carpio
 CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
 CTTGGCCGTCATACGCTTCTAGGAGTCCGAAGCCCTAATACGAAAGTAACCTTTAATAAACCCACCTGACCCACGAAAGCTGAGAA
 A

>AP009047 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
 carpio
 CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTATAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
 CTTGGCCGTCATACGCTTCTAGGAGTCCGAAGCCCTAATACGAAAGTAACCTTTAATAAACCTACCTGACCCACGAAAGCTGAGAA
 A

>AP017328 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
 carpio
 CGCCGCGGTTATACGAGAGGCTCAAGTTGATAGTTGCCGGCGTAAAGAGTGGTTACGGAAGTTATTAAGTAAAGCCAAACACCTC
 CTAGGCTGTACATACGACCTGAAGGCACGAAGTCCCCTACGAAAGTAGCTTTACCCCCCCCCGAACCCACGACAGCTATGTCA
 >AP014757 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
 carpio
 CACCGCGGTTATACGAGGGGCCCAAGTTGATAAACGCCGGCGTAAATGTGGTTAATATAGTATTGCACTAAAGCCAAACATCTTC
 AAAGTGTTATACCATATGAAGACAGGAAGCACTTCCACGAAAGTGCTTTAAATAATGTTATCCACTAAAGCTAGGAGA
 >AP017364 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
 carpio
 CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
 CTTGGCCGTCATACGCTTCTAGGAGTCCGAAGCCCTAATACGAAAGTAACCTTTAATAAACCCACCTGACCCACGAAAGCTGAGAA
 A

>KF927167 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
 carpio
 CACCGCGGTTAAACGAGAGGCCCTAGTTGATAACACCACGGCGTAAAGGGTGGTTAAGGAGAGCAAGATAATTTTTAAAGCCAAAT
 GGCCCTTTGGCCGTCATACGCTTCTAGGTGTCCGAAGCCCAATCATACGAAAGCAGCTTTAACAAGCCACCTGACCCACGAA
 GCTGAGAAA

>KF873612 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
 carpio
 CACCGCGGTTATACGAGAGGCTCAAGTTGATAAACCTCGGCGTAAAGTGTGGTTAAGGAAATTTTTAACTAAAGCCGAACGCCTG
 TAGAAGCAGTAGCACGCTTATTAAGGTATGAAGCTACCCACGAAAGTGCTTTACAAAACCCGACTCCACGAAAGCTAAGAAA
 >KF856964 Metazoa|Chordata|Actinopteri|Cypriniformes|Cyprinidae|Cyprinus|Cyprinus
 carpio

CACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCGTAAAGGGTGGTTAAGGATAAACAAAAATAAAGTCAAATGGCCC
CTTGCCGTCATACGCTTCTAGGAGTCCGAAGCCCTAATACGAAAGTAACTTTAATAAACCCACCTGACCCACGAAAGCTGAGAA
A

>AF357585 Metazoa|Chordata|Actinopteri|Cypriniformes|Cobitidae|Misgurnus|Misgurnus
anguillicaudatus

CACCGCGGTTATACGAGAGGCCCCAGTTGATGAACACGGCGTAAAGGGTGGTTAAGGTTTAACTAAAATAAAGTCAAAAGACTTCT
TGGCCGTCATACGCCCTGAACATCTGAAGCTCATATACGAAAGTAACTTTAATATTAGCCACCTGACCCACGAAAGCTGAGAA
A

>KC509900 Metazoa|Chordata|Actinopteri|Cypriniformes|Cobitidae|Misgurnus|Misgurnus
anguillicaudatus

CACCGCGGTTATACGAGAGGCCCCAGTTGATGGACACGGCGTAAAGGGTGGTTAAGGTTTAGTAAAAATAAAGCCAAAAGACCTCT
TGGCTGTCATACGCCCTGAGTCTCCGAAGCCCACATACGAAGGTAGCTTTAATACTATATTTACCTGACCCACGAAAGCTGAGA
AA

>KC509901 Metazoa|Chordata|Actinopteri|Cypriniformes|Cobitidae|Misgurnus|Misgurnus
anguillicaudatus

CACCGCGGTTATACGAGAGGCCCCAGTTGATGGACACGGCGTAAAGGGTGGTTAAGGCTTAGTAAAAATAAAGCCAAAAGACCTCT
TGGCTGTCATACGCCCTGAGTCTCCGAAGCCCACATACGAAGGTAGCTTTAATACTATATTCACCTGACCCACGAAAGCTGAGA
AA

>EU670804 Metazoa|Chordata|Actinopteri|Cypriniformes|Cobitidae|Misgurnus|Misgurnus
anguillicaudatus

CACCGCGGTTATACGAGAGGCCCTAGTTGATGGACACGGCGTAAAGGGTGGTTAAGGTCCAATATAAATAAAGCCAAAAGACCTCT
TGGCTGTCATACGCCCTGAGTTTCCGAAGCCCACATACGAAAGTAGCTTTAATACCATTACCTGACCCACGAAAGCTGAGAAA

>KC734881 Metazoa|Chordata|Actinopteri|Cypriniformes|Cobitidae|Misgurnus|Misgurnus
anguillicaudatus

CACCGCGGTTATACGAGAGGCCCCAGTTGATGGACACGGCGTAAAGGGTGGTTAAGGTTTAGTAAAAATAAAGCCAAAAGACCTCT
TGGCTGTCATACGCCCTGAGTCTCCGAAGCCCACATACGAAGGTAGCTTTAATACTATATTCACCTGACCCACGAAAGCTGAGA
AA

>KC762740 Metazoa|Chordata|Actinopteri|Cypriniformes|Cobitidae|Misgurnus|Misgurnus
anguillicaudatus

CACCGCGGTTATACGAGAGGCCCCAGTTGATGGACACGGCGTAAAGGGTGGTTAAGGTTTAGTAAAAATAAAGCCAAAAGACCTCT
TGGCTGTCATACGCCCTGAGTCTCCGAAGCCCACATACGAAGGTAGCTTTAATACTATATTCACCTGACCCACGAAAGCTGAGA
AA

>KC823274 Metazoa|Chordata|Actinopteri|Cypriniformes|Cobitidae|Misgurnus|Misgurnus
anguillicaudatus

CACCGCGGTTATACGAGAGGCCCCAGTTGATGGGCACGGCGTAAAGGGTGGTTAAGGTTTAGTAAAAATAAAGCCAAAAGACCTCT
TGGCTGTCATACGCCCTGAGTCTCCGAAGCCCACATACGAAGGTAGCTTTAATACTGTATTACCTGACCCACGAAAGCTGAGA
AA

>KC881110 Metazoa|Chordata|Actinopteri|Cypriniformes|Cobitidae|Misgurnus|Misgurnus
anguillicaudatus

CACCGCGGTTATACGAGAGGCCCCAGTTGATGGGCACGGCGTAAAGGGTGGTTAAGGTTTAGTAAAAATAAAGCCAAAAGACCTCT
TGGCTGTCATACGCCCTGAGTCTCCGAAGCCCACATACGAAGGTAGCTTTAATACTGCATTACCTGACCCACGAAAGCTGAGA
AA

>KC884745 Metazoa|Chordata|Actinopteri|Cypriniformes|Cobitidae|Misgurnus|Misgurnus anguillicaudatus
 CACCGCGGTTATACGAGAGGCCCCAGTTGATGGACACGGCGTAAAGGGTGGTTAAGGTTTAGTAAAAATAAGCCAAAAGACCTCT
 TGGCTGTCATACGCCCTGAGTCTCCGAAGCCCACATACGAAGGTAGCTTTAATACTGTATTCACCTGACCCACGAAAGCTGAGA
 AA

>KM186181 Metazoa|Chordata|Actinopteri|Cypriniformes|Cobitidae|Misgurnus|Misgurnus anguillicaudatus
 CACCGCGGTTATACGAGAGGCCCCAGTTGATGGGCACGGCGTAAAGGGTGGTTAAGGTTTAGTAAAAATAAGCCAAAAGACCTCT
 TGGCTGTCATACGCCCTGAGTCTCCGAAGCCCACATACGAAGGTAGCTTTAATACTGTATTCACCTGACCCACGAAAGCTGAGA
 AA

>HM856629 Metazoa|Chordata|Actinopteri|Cypriniformes|Cobitidae|Misgurnus|Misgurnus anguillicaudatus
 CACCGCGGTTATACGAGAGGCCCCAGTTGATGGACACGGCGTAAAGGGTGGTTAAGGTTTAGAATAAATAAGCCAAAAGACCTCT
 TGGCTGTCATACGCCCTGAGTTTCCGAAGCCCATATACGAAAGTAGCTTTAATATTATACTACCTGACCCACGAAAGCTGAGAA
 A

>DQ026434 Metazoa|Chordata|Actinopteri|Cypriniformes|Cobitidae|Misgurnus|Misgurnus anguillicaudatus
 CACCGCGGTTATACGAGAGGCCCCAGTTGATGAACACGGCGTAAAGGGTGGTTAAGGTTTAACTAAAATAAGTCAAAAGACTTCT
 TGGCCGTCATACGCCCTGAACATCTGAAGCTCATATACGAAAGTAACTTTAATATTAGCCACCTGACCCACGAAAAGCTGAGAA
 A

>AP011291 Metazoa|Chordata|Actinopteri|Cypriniformes|Cobitidae|Misgurnus|Misgurnus anguillicaudatus
 CACCGCGGTTATACGAGAGGCCCTAGTTGATGGACACGGCGTAAAGGGTGGTTAAGGTCCAATACAAATAAGCCAAAAGACCTCT
 TGGCTGTCATACGCCCTGAGTTTCTGAAGCCCACATACGAAAGTAGCTTTAATATTATTCACCTGACCCACGAAAGCTGAGAAA

>KP013098 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Sander|Sander vitreus
 CACCGCGGTTATACGAGAGGCCCAAGTTGATAGCTACCGGCGTAAAGCGTGGTTAAGATAAAGACTAAGACTAAAGCCGAACGCC
 TCAGAGCTGTTATACGCACCCGATGGTGAGAAGTTCAATCACGAAAGTGCTTTATAAATCCTGAACCCACGAAAGCTAAGATA

>KT211456 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Sander|Sander vitreus
 CACCGCGGTTATACGAGAGGCCCAAGTTGATAGCTACCGGCGTAAAGCGTGGTTAAGATAAAGACTAAGACTAAAGCCGAACGCC
 TCAGAGCTGTTATACGCACCCGATGGTGAGAAGTTCAATCACGAAAGTGCTTTATAAATCCTGAACCCACGAAAGCTAAGATA

>KT211457 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Sander|Sander vitreus
 CACCGCGGTTATACGAGAGGCCCAAGTTGATAGCTACCGGCGTAAAGCGTGGTTAAGATAAAGACTAAGACTAAAGCCGAACGCC
 TCAGAGCTGTTATACGCACCCGATGGTGAGAAGTTCAATCACGAAAGTGCTTTATAAATCCTGAACCCACGAAAGCTAAGATA

>KT211455 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Sander|Sander vitreus
 CACCGCGGTTATACGAGAGGCCCAAGTTGATAGCTACCGGCGTAAAGCGTGGTTAAGATAAAGACTAAGACTAAAGCCGAACGCC
 TCAGAGCTGTTATACGCACCCGATGGTGAGAAGTTCAATCACGAAAGTGCTTTATAAATCCTGAACCCACGAAAGCTAAGATA

>KT211458 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Sander|Sander vitreus
 CACCGCGGTTATACGAGAGGCCCAAGTTGATAGCTACCGGCGTAAAGCGTGGTTAAGATAAAGACTAAGACTAAAGCCGAACGCC
 TCAGAGCTGTTATACGCACCCGATGGTGAGAAGTTCAATCACGAAAGTGCTTTATAAATCCTGAACCCACGAAAGCTAAGATA

>KT211459 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Sander|Sander vitreus
 CACCGCGGTTATACGAGAGGCCCAAGTTGATAGCTACCGGCGTAAAGCGTGGTTAAGATAAAGACTAAGACTAAAGCCGAACGCC
 TCAGAGCTGTTATACGCACCCGATGGTGAGAAGTTCAATCACGAAAGTGCTTTATAAATCCTGAACCCACGAAAGCTAAGATA

[illegible]

>KT211446 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Sander|Sander vitreus
 CACCGCGTTATACGAGAGGCCCAAGTTGATAGCTACCGGCGTAAAGCGTGGTTAAGATAAAGACTAAGACTAAAGCCGAACGCCC
 TCAGAGCTGTTATACGCACCCGATGGTGAGAAGTTCAATCACGAAAGTGGCTTTATAAATCCTGAACCCACGAAAGCTAAGATA

>KT211447 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Sander|Sander vitreus
 CACCGCGTTATACGAGAGGCCCAAGTTGATAGCTACCGGCGTAAAGCGTGGTTAAGATAAAGACTAAGACTAAAGCCGAACGCCC
 TCAGAGCTGTTATACGCACCCGATGGTGAGAAGTTCAATCACGAAAGTGGCTTTATAAATCCTGAACCCACGAAAGCTAAGATA

>KT211448 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Sander|Sander vitreus
 CACCGCGTTATACGAGAGGCCCAAGTTGATAGCTACCGGCGTAAAGCGTGGTTAAGATAAAGACTAAGACTAAAGCCGAACGCCC
 TCAAAGCTGTTATACGCACCCGATGGTGAGAAGTTCAATCACGAAAGTGGCTTTATAAATCCTGAACCCACGAAAGCTAAGATA

>KT211449 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Sander|Sander vitreus
 CACCGCGTTATACGAGAGGCCCAAGTTGATAGCTACCGGCGTAAAGCGTGGTTAAGATAAAGACTAAGACTAAAGCCGAACGCCC
 TCAGAGCTGTTATACGCACCCGATGGTGAGAAGTTCAATCACGAAAGTGGCTTTATAAATCCTGAACCCACGAAAGCTAAGATA

>KT211450 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Sander|Sander vitreus
 CACCGCGTTATACGAGAGGCCCAAGTTGATAGCTACCGGCGTAAAGCGTGGTTAAGATAAAGACTAAGACTAAAGCCGAACGCCC
 TCAGAGCTGTTATACGCACCCGATGGTGAGAAGTTCAATCACGAAAGTGGCTTTATAAATCCTGAACCCACGAAAGCTAAGATA

>KT211451 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Sander|Sander vitreus
 CACCGCGTTATACGAGAGGCCCAAGTTGATAGCTACCGGCGTAAAGCGTGGTTAAGATAAAGACTAAGACTAAAGCCGAACGCCC
 TCAGAGCTGTTATACGCACCCGATGGTGAGAAGTTCAATCACGAAAGTGGCTTTATAAATCCTGAACCCACGAAAGCTAAGATA

>KT211452 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Sander|Sander vitreus
 CACCGCGTTATACGAGAGGCCCAAGTTGATAGCTACCGGCGTAAAGCGTGGTTAAGATAAAGACTAAGACTAAAGCCGAACGCCC
 TCAGAGCTGTTATACGCACCCGATGGTGAGAAGTTCAATCACGAAAGTGGCTTTATAAATCCTGAACCCACGAAAGCTAAGATA

>KT211453 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Sander|Sander vitreus
 CACCGCGTTATACGAGAGGCCCAAGTTGATAGCTACCGGCGTAAAGCGTGGTTAAGATAAAGACTAAGACTAAAGCCGAACGCCC
 TCAGAGCTGTTATACGCACCCGATGGTGAGAAGTTCAATCACGAAAGTGGCTTTATAAATCCTGAACCCACGAAAGCTAAGATA

>KT211454 Metazoa|Chordata|Actinopteri|Perciformes|Percidae|Sander|Sander vitreus
 CACCGCGTTATACGAGAGGCCCAAGTTGATAGCTACCGGCGTAAAGCGTGGTTAAGATAAAGACTAAGACTAAAGCCGAACGCCC
 TCAGAGCTGTTATACGCACCCGATGGTGAGAAGTTCAATCACGAAAGTGGCTTTATAAATCCTGAACCCACGAAAGCTAAGATA