Detection of Lake Sturgeon (*Acipenser fulvescens*) in the Winnipeg River using Environmental DNA (eDNA)

By

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Abstract

Lake Sturgeon (*Acipenser fulvescens*), a species of ecological and cultural significance in North America, face population declines due to habitat loss, overharvesting, and environmental changes. Effective monitoring tools are essential for conserving and managing their populations. This thesis explores the utility of environmental DNA (eDNA) as a non-invasive tool for detecting the presence and absence of Lake Sturgeon in the Winnipeg River system and its potential to identify spawning events.

The spatial and temporal distribution of Lake Sturgeon was examined using mitochondrial (Cyt-*b*) eDNA, revealing seasonal patterns that aligned with previously documented behaviours, such as spawning, foraging, and overwintering. Fifteen sites were sampled once a month from May through to October in both 2021 and 2022. A total of 55/78 samples (70.5%) amplified Lake Sturgeon DNA in 2021 and 45/65 samples (69.2%) in 2022. The relative location of the collection site to the hydroelectric generating stations was found to be a significant influence on the presence of eDNA.

The application of nuclear and mitochondrial eDNA for detecting Lake Sturgeon spawning events using a newly developed ITS-1 assay was found to be successful in a field setting. The nuclear assay only amplified Lake Sturgeon DNA in 14/57 samples (24.6%), all during the suspected spawning period when compared to ideal spawning conditions, successfully detecting the presence of reproductive adults during peak spawning periods. The eDNA results corresponded closely with conventional field observations, including egg deposition and adult sampling. The assay is sturgeon-specific but not species-specific, suggesting its broader applicability for monitoring other North American sturgeon species, though further validation across diverse environmental contexts is required.

The findings of this research underscore the versatility and promise of eDNA as a non-invasive tool for Lake Sturgeon conservation. By identifying critical habitats and reproductive events, eDNA provides an efficient and scalable approach to monitor sturgeon populations and inform targeted conservation strategies. This work lays a foundation for further research into refining eDNA techniques and integrating them into long-term monitoring programs to address ongoing challenges in freshwater species conservation.

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For my dad.

Thank you for pushing me to be the best version of myself, and always supporting me; no matter how far my dreams took me.

I miss you endlessly.

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Contribution of Authors

The ideas and methodology for the study were concieved by Morgan Anderson, Gary Anderson and Margaret Docker. All assay development and data collections were conducted by Morgan Anderson. Morgan Anderson led the data analysis and writing of the thesis, with James Roth contributing experitise to the data analysis. Gary Anderson, Margaret Docker, Ken Jeffries and James Roth provided revisions.

CHAPTER 1: General Introduction

1.1 eDNA AS AN ENVIRONMENTAL MONITORING TOOL

A species' genetic material is consistently being sloughed or shed into its natural environment, leaving traces of cellular material behind. While the species may have left an area, the shed DNA can still be detected using environmental DNA (eDNA) methodologies. eDNA concentrations are primarily used as a monitoring tool to detect the presence of a species within a given environment. The concept of extracting genetic material from the environment was first used in microbiology where DNA was extracted from marine sediments to determine and identify the microbes that could be found within (Ogram et al., 1987). Since then, the technique has expanded from the field of microbiology to all fields of biology and environmental studies using adapted methods and approaches.

Current uses of eDNA span a large variety of species and environments. These studies include topics such as the detection of aquatic or terrestrial invasive species (Gingera et al., 2016; Williams et al., 2018), detection of fecal contamination in water systems (Martellini et al., 2005; Ragot et al., 2023), terrestrial vertebrate detection using air samples (Lynggaard et al., 2022) and in paleogenetic studies using genetic materials found within the substrate or shore sediment deposits following a major environmental event (Anderson-Carpenter et al., 2011; Engel et al., 2020). While the target species or purpose behind these studies vary, the general principle of being able to detect a genus and/or species through molecular means remains the same. As a sampling method, extraction of eDNA from aquatic environments is reported to have a high success rate for detecting at-risk or rare aquatic species, or quantifying biodiversity (Jerde et al., 2011; Beng & Corlett, 2020).

1.2 eDNA AS A NON-INVASIVE SAMPLING MONITORING TECHNIQUE

Before eDNA was used as an environmental assessment tool, species distribution and biodiversity monitoring efforts traditionally fell to observational based studies, such as physical capture and sometimes mark recapture of the targeted aquatic species (Beng & Corlett, 2020). Capture-based monitoring in aquatic environments, such as the use of gillnets for fishes, can be difficult for the management of low-density species as increased time and effort may be needed to locate them within the system (Jerde et al., 2011; Bergman et al., 2016; Sepulveda et al., 2020; Wang et al., 2021). Furthermore, monitoring the spatial distribution of an aquatic species with traditional methods becomes increasingly more difficult as a species' range increases and as environmental conditions, such as ice cover in the winter, increased water flow during spring melt, or periods of high precipitation can impede capture techniques.

While these methods have advantages over eDNA practices, such as being able to determine fish health, sex, and growth, the physical capture and handling of a target species can result in negative health consequences that are not present with eDNA sampling methods (Rees et al., 2014). In addition to the increased possibility of detecting at-risk species using eDNA, the effort required to understand spatial distribution has the potential of being more time efficient and cost effective when compared with traditional methods of sampling within a closed system (Andres et al., 2023). However, larger scale open systems may result in numerous abiotic and biotic variables influencing the signal strength of the targeted eDNA (see Sect. 1.4); thus, significant research is required to understand how these variables influence the eDNA signal to ensure appropriate interpretation of the data.

1.3 MITOCHONDRIAL VS. NUCLEAR DNA

eDNA relies on the presence of either mitochondrial (mtDNA) or nuclear (nuDNA) DNA for detection of the targeted species. Mitochondrial DNA is arranged as a small, circular chromosome that is found within the mitochondria in a cell's cytoplasm. In most organisms, mtDNA is maternally inherited. An egg contains a variable amount of mtDNA copies; from 19 million in Zebrafish (*Dania rerio*) to 3 billion copies in Chinook Salmon (*Oncorhynchus tshawytscha*) (Wolff et al., 2011; Otten et al., 2016). The increased number of mtDNA copies makes it a popular choice when selecting target genes for eDNA studies, making detection from even a small number of individuals more likely (Barnes & Turner, 2016; Kim et al., 2022). mtDNA is also highly compact, containing no introns and little non-coding DNA, and its gene content and order are highly conserved among species (Seddigh & Darabi, 2018; Kim et al., 2022). Gene sequences are available for most vertebrate species—making it much easier to work with than the complex, highly variable, and relatively poorly characterized nuclear genome (Sanglard et al., 2022). Mitochondrial genes are used to develop assays to provide information on a variety of taxonomic groups or can be used to identify specific species within the studied environment. Many current species-specific markers rely on the mitochondrial genes cytochrome oxidase subunit I (COI) or cytochrome b (Cyt *b*) due to the large database of available sequences (Sanglard et al., 2022).

While the increase of genetic mitochondrial material available in a system is beneficial when determining presence/absence, it can lead to misinterpretations when looking at abundance or biomass of a species (Takahara et al., 2012; Minamoto et al., 2017b). Additionally, studies have found that mtDNA markers can have difficulties discriminating between highly

related genomes, creating intraspecies identification errors (Schwartz et al., 2007). For instance, in environments where two or more genetically similar species reside, it is possible that the DNA being detected is not from the targeted species but rather from a genetically similar counterpart (Thomsen et al., 2012; Hinlo et al., 2017; Wilcox et al., 2018; Spear et al., 2020). Research suggests the use of microsatellites or nuclear markers to combat this (Schwartz et al., 2007; Bylemans et al., 2017; Minamoto et al., 2017b; Dysthe et al., 2018).

Nuclear DNA is found within the nucleus and codes for most of the genome. In contrast to mtDNA, nuDNA is inherited from both parents (Wirgin & Waldman, 2005). While most nuclear genes are limited to a few copies per cell dependent on ploidy, the first internal transcribed spacer (ITS-1) has multiple copies in the nuclear genome (Long & Dawid, 1980). ITS-1 is located between the 18S and 28S subunits of the rRNA gene. ITS is evolutionary conserved as repeats evolve together within a species but remains diverse interspecifically. The high variability can assist in the discrimination among genetically similar species (Bylemans et al., 2017; Minamoto et al., 2017b; Jo et al., 2021). In a 2018 eDNA study on Bull Trout (*Salvelinus confluentus*), detection rates for the target species were tested using both Cyt *b* and ITS-1 assays (Dysthe et al., 2018). The assays were additionally tested against genetically similar species, such as Brook Trout (*Salvelinus fontinalis*), Arctic Char (*Salvelinus alpinus*) and Lake Trout (*Salvelinus namaycush*). The nuclear assay was found to be more specific than the mitochondrial assay as it did not cross amplify with the closely related species, while also having a higher rate of detection; supporting the findings of Minamoto et al. (Minamoto et al., 2017b; Dysthe et al., 2018).

However, studies have detected more rapid degradation of nuDNA compared to mtDNA (Jo et al., 2019a, 2019b; Wu et al., 2022). When the degradation of mtDNA and nuDNA was

compared using Japanese Jack Mackerel (*Trachurus japonicus*), both markers detected higher levels of DNA degradation with high temperatures and large biomass (Jo et al., 2019a, 2019b). Additionally, the ratio of nuDNA and mtDNA within a sample was depended on fish biomass, with nuDNA having a higher rate of detection and concentration than mtDNA (Jo et al., 2019a). The quick turnover of nuDNA in the water may allow for more accurate abundance estimates and decrease the chance of detecting old DNA in the system leading to false positives or inaccurate assumptions (Jo et al., 2022; Wu et al., 2022).

1.4 FACTORS INFLUENCING eDNA DETECTION IN AQUATIC SYSTEMS

1.4.1 Abiotic

Abiotic factors can influence the quantity and quality of eDNA available within the environment. These factors may have a positive effect; increasing the likelihood of detection rate, or a negative effect leading to the degradation of the DNA before extraction and measurement can be made. For example, higher water temperatures cause DNA degradation in the water column (Barnes et al., 2014; Curtis et al., 2021). In a recent study, samples stored at room temperatures resulted in a 0% positive detection rate of the target species, compared to 100% detection rate from the samples stored in a cooler environment over the 24-hour sampling period (Curtis et al., 2021). The same can be presumed to occur in a field setting as the surface temperature of a water body can increase rapidly on hot days, causing detection of eDNA from surface water samples differ from samples taken at a greater depth where there may be less temperature variation. UV radiation may also impact the presence and quality of DNA in the water, leading to degradation (Ravanat et al., 2001; Barnes et al., 2014).

An additional abiotic factor that can greatly impact the detection of DNA in a system is the effect of flow rate (Shogren et al., 2017). For example, turnover of water in ponds and lakes is typically less than streams and rivers and therefore may result in less movement of the DNA throughout the system (Jane et al., 2015; Shogren et al., 2017). Indeed, high stream flows were found to dilute eDNA concentrations and further decreased the detectability of the target species (Curtis et al., 2020). During a study conducted on a headwater stream, the targeted DNA could be detected up to of 239.5 m away from the original source regardless of flow (Jane et al., 2015). High flow did, however, impact the detectability of DNA collected close to the original source as the concentration of DNA was found to be at a significantly lower level compared to eDNA concentration in samples taken from the same location during a period of low flow (Jane et al., 2015). Extreme environmental events like flooding further complicate detectability rates. In a longitudinal study focused on the Asian Clam (*Corbicula fluminea*), during flooding events, eDNA concentrations were found to be diluted or absent at sites with previously high rates of detections (Curtis et al., 2020). In systems like the Winnipeg River, where seasonality plays a role in the rate of flow, the detection rate of eDNA may vary from the expected results (Curtis et al., 2020). These events could lead to false negatives in areas where a species may be commonly found. Furthermore, in environments such as large lakes where the rate of water turnover is relatively stable, sampling for eDNA may require increased water volumes to account for the total volume of the water body and obtain enough DNA for analysis and species detection (Rees et al., 2014; Strickland & Roberts, 2019).

1.4.2 Biotic

Biotic factors can also impact the ability to detect DNA from an environmental sample (Stewart, 2019). Seasonal events, such as spawning, overwintering and changes in body size, can influence the detectability of eDNA in a system. During a spawning event, the combination of shed somatic and germ cells increases the genetic material that can be found in the water; thus, increasing the possibility of detecting both nuDNA and mtDNA. The method in which the species spawns will also influence the detectability of the event; for instance, Lake Sturgeon (*Acipenser fulvescens*) who are broadcast spawners, release copious amounts of gametes into the water. A female Lake Sturgeon can release around 150,000 eggs while a male can release an estimated 1 billion sperm into the water (Bruch & Binkowski, 2002). These events will significantly increase the amount of genetic material that can be collected shortly after, creating a transient increase in concentration of both nuDNA and mtDNA. Therefore, increased detection of only mitochondrial related eDNA may create a false positive of spawning activity and should ideally be paired with increases in nuclear related eDNA and/or knowledge of the spawning periodicity of the population of interest (Maruyama et al., 2014). The presence of an increased concentration of mtDNA alone could be caused by a migration of the species into the area, a decaying animal or even the result of a fishing vessel bringing external DNA into the sampling area, none that particularly mean a spawning event has happened or will happen. In contrast, during times of low metabolic rates and sessile activity such as overwintering, an individual is less likely to be continuously contributing their genetic material to the water, which can lead to false negatives despite the animal being there (Maruyama et al., 2014). Therefore, it is important to consider

previous knowledge on the biology and movement patterns of the target species before interpreting eDNA results.

Movement patterns of a fish can be influenced by migratory requirements, feeding preferences and spawning behaviour. Many species will travel throughout an environment to meet their needs; however, the distances can differ greatly from one to the next. Knowledge pertaining to these movements is critical when creating an eDNA based study. If the locations selected for sampling mirror the expected patterns of the species, the chances of detection are higher. In contrast, if a species is expected to be in an area but remains undetectable through use of eDNA, the focus turns to potential environmental variables affecting the persistence of DNA in the water. Spatial distribution can be greatly affected by previously discussed environmental factors, such as flow rates. Spatial scales of detection can be determined by the initial quantity and quality of DNA being shed into the environment, and the environmental factors present that increase the degradation of the DNA molecules at varying speeds. During times of high flow, eDNA signals can be transported downstream from the original source and into areas where the species may not be expected. In a study conducted at the Experimental Lakes Area (IISD-ELA), Ontario, the effect that different types of flow had on eDNA signals was examined as the genetic material traveled through a chain of lakes and was detected in the water using metabarcoding techniques (Littlefair et al., 2023). Lakes were chosen based on the most complete historical datasets available for the fish and zooplankton populations dating back to the 1960s and more recent annual gillnetting and trap netting. The results of the study showed that the flow rate did not influence the predicted detections; rather, the number of inflows attached to the lake and the location of the sample compared to the outflow did (Littlefair et al., 2023).

While abundance is typically quantified as the number of individuals, the relationship between the quantity of species and amount of eDNA detected is not linear. The body size of a species additionally plays a role in the amount of DNA shed into the environment. More often, when comparing the release rate of eDNA to the body mass of the fish (g), larger-bodied species release less genetic material into the water than a smaller counterpart would (Maruyama et al., 2014; Mizumoto et al., 2018). In a study conducted by Yates et al. (2021), it was found that allometric scaling requires future research to better understand the relationships between the size of a species and the amount of eDNA they can shed into the environment before conclusions can be made about the relationships between DNA concentrations and species abundance when using eDNA.

1.5 DETERMINING SPATIAL AND TEMPORAL DISTRIBUTION USING eDNA

Determining the distribution of a species is important for understanding the regional and temporal abundance within its environment. In fisheries, traditional studies determine these distributions through echo location, electrofishing, telemetry, seine netting, gillnetting, and other methods (Jung & Houde, 2003; Ditya et al., 2018). In a comparative study, Handley et al. (2019) examined the spatial and temporal distribution of a variety of fish species found within Lake Windermere, England, using both eDNA and gillnetting. The study was repeated in the summer and winter to further test the reliability of eDNA with season. When the eDNA data was compared to species detected and relative abundance of species found in a gillnet, it was found to be consistent between the two methods (Handley et al., 2019). Gillnetting surveys and eDNA results from the offshore samples only detected Arctic Char during the summer sampling, and not during fall or winter attempts. Furthermore, eDNA extracted from water samples collected in

the winter, at greater depths in the middle of the lake, showed deepwater species, such as Arctic Char, were more evenly distributed in the water column compared to the summer samples where they were primarily detected at the bottom (Handley et al., 2019). These findings can be supported by temporal events such as seasonal stratification and seasonal mixing. Both should be considered when interpreting changes in detection of eDNA (Handley et al., 2019; Littlefair et al., 2021).

An additional study that focused on thermal preference and eDNA detection was conducted at the Experimental Lakes Area. The primary questions of the study were: does shed DNA remain in the species' respective thermal zone during stratification and does the DNA mix throughout the thermal zones during the bi-annual lake turnover (Littlefair et al., 2021)? The study found that during stratification, eDNA for each of the studied species was divided by the fishes' thermal preference; for example, Lake Trout eDNA was primarily found in the benthic samples, while Fathead Minnow (*Pimephales promelas*) eDNA could be detected closer to the surface (Littlefair et al., 2021). During the lake turnover, eDNA for all the species could be detected in all samples, regardless of depth. This work was further supported using Lake Trout telemetry data as the movement patterns of the trout matched the detected eDNA results during stratification, and contradicted them during the turnover (Littlefair et al., 2021).

Spatial and temporal distribution using eDNA has also been studied using a marine jellyfish species, the Japanese Sea Nettle (*Chrysaora pacifica*) (Minamoto et al., 2017a). This study used visual detection of the species at the study site in addition to surface and sea floor water sampling for spatial eDNA detections (Minamoto et al., 2017a). eDNA from the surface samples corresponded with the physical count of the detected species at the site, along with an

increased concentration of eDNA in the sea floor samples indicating an influx of jellyfish that were not visible to the researchers from the surface (Minamoto et al., 2017a). The determined eDNA concentration from each of the sampling periods was also found to significantly correlate with the observed number of jellyfish at the site (Minamoto et al., 2017a). This study provides evidence of the reliability of eDNA when paired with additional sampling methods and highlights its potential to expand to environments that were not previously accessible to the researcher.

1.6 DETERMINING SPAWNING EVENTS USING eDNA

Spawning events can be difficult to detect in unmonitored systems or areas with a low relative abundance of the target species. This information can be increasingly important when used to initiate preventive spawning measures for invasive species. For the invasive Grass Carp (*Ctenopharyngodon idella*) located in the Truman Reservoir of Missouri, eDNA results were compared to traditional methods of spawning detection, such as ichthyoplankton tows (Hayer et al., 2020); a positive correlation was found between Grass Carp eDNA detection and egg influx both temporally and spatially during the suspected spawning periods (Hayer et al., 2020). As eggs were released into the water, the concentrations of detectable eDNA increased as a result. The increased concentrations of eDNA are suggested to be a combination of both genetic material from the eggs and shed skin, scales and blood from the aggressive mating patterns seen in the Grass Carp (Hayer et al., 2020).

A similar study was conducted in 2016 on a variety of lamprey species to locate and reduce the spread of the invasive Sea Lamprey (*Petromyzon marinus*) in the Great Lakes (Gingera et al., 2016). This study used eDNA to detect Sea Lamprey spawning events in streams with high density spawning rates over a 3-month sampling period to determine how long a spawning signal

can remain within the water column. Detection of spawning events was based on a transient increase in species-specific eDNA concentration from water samples. Concentration peaked during known spawning times and decreased thereafter over a span of 2 weeks, providing evidence that a spawn could have occurred in the area (Gingera et al., 2016). While a spike in DNA concentration can be evidence that a spawn occurred, an increase in mtDNA alone does not always correlate with these events. For example, since lampreys are semelparous, the decomposition of their carcasses after spawning will increase the amount of mtDNA in the system post-spawn, regardless of presence or absence of gametes.

The development of nuclear based assays has created the opportunity to differentiate between the aggregation of a species and a possible spawning event. During a spawning event, broadcast spawners, such as Macquarie Perch (*Macquaria australasica*), Sea Scallops (*Placopecten magellanicus*), Largemouth Bass (*Micropterus salmoides*), Bluegill Sunfish (*Lepomis macrochirus*) and Lake Sturgeon, release copious amounts of eggs and sperm into the water column (Bylemans et al., 2017; Bayer et al., 2019; Wu et al., 2023). In the case of Lake Sturgeon, fertilized eggs will typically sink and adhere to rocky substrate (Bruch & Binkowski, 2002). The increase in genetic material creates a transient increase in the nuclear and mitochondrial DNA concentration that can be detected using targeted eDNA assays (Tsuji & Shibata, 2021). However, because sperm—each with a nucleus but little cytoplasm—contribute more nuclear material than mitochondrial during a spawning event. Thus, the ratio of nuDNA to mtDNA may be more indicative of the quantity of sperm related eDNA in a sample (Bylemans et al., 2017; Wu et al., 2023).

As described above, the ratio of nuclear to mitochondrial DNA can provide further support that the increase in genetic material is related to a spawn, rather than just an increased accumulation of a species in the sampled environment or the aftermath of a semelparous species' spawning event. The concurrent increase of mtDNA and nuDNA is followed by a quick degradation of nuDNA due to its unstable nature, as described in Sect. 1.3, while mtDNA can persist in the environment for longer (Jo et al., 2019a, 2019b). In the case of semelparous species, continuous addition of mtDNA and nuDNA into a system can occur for as long as the carcasses decay. Having knowledge on the target species' life history traits is important when interpreting the presence or absence of nuDNA and mtDNA within the system.

1.7 eDNA CONCENTRATIONS

Species-specific eDNA markers often rely on mtDNA over nuDNA when measuring the concentration of genetic material found within a sample. mtDNA assays often target the genes cytochrome b (Cyt *b*) and cytochrome c oxidase 1 (CO1) due to the large supporting database of DNA sequences that include these genes. Cyt *b* is a mitochondrial protein that plays an important role in the mitochondrial electron transport chain (ETC) (Seddigh & Darabi, 2018). It is an extensively sequenced gene in vertebrates and is useful in the context of species detection through eDNA given the differences in sequence between species (Tobe et al., 2010). CO1 is a subunit found in the final enzyme of the ETC (Rodrigues et al., 2017). It has a relatively fast mutation rate that allows closely related species to be distinguished, but still at a slower rate than other mtDNA counterparts (Rodrigues et al., 2017). The Lake Sturgeon mtDNA qPCR assay developed by Yusishen et al. (2020) utilized the mitochondrial genes Cyt *b* and CO1 creating a species-specific primer for Cyt *b*, while the CO1 assay is expected to cross amplify with some

other sturgeon species. As Lake Sturgeon are the only sturgeon species found within Manitoba, it is unlikely to get false positives through detection of off-target species, but additional testing should be conducted if used in areas where Lake Sturgeon may overlap with other sturgeon species, such as the Shortnose Sturgeon (*Acipenser brevirostrum*) (Yusishen et al., 2020).

Current aquatic eDNA studies primarily use assays that target mtDNA due to the increased density of mitochondrial material found within a sample compared to nuclear DNA (Marshall et al., 2021). However, nuDNA, when targeting repetitive ribosomal genes such as ITS-1, 18S or 28S, may also increase detection rates in some species (Bylemans et al., 2017; Minamoto et al., 2017b; Dysthe et al., 2018; Jo et al., 2019a; Marshall et al., 2021). ITS-1 (internal transcribed spacer 1) is a commonly used ribosomal RNA marker for nuclear assays (Jo et al., 2021). It is a nonfunctional RNA that is located between the structural ribosomal RNA 18S and 5.8S transcripts. In ITS-1, repeats evolve together promoting intragenomic homogeneity (Pleyte et al., 1992). This maintains high similarities within a species while also allowing for increased divergence among species due to the low evolutionary pressures acting on the noncoding spacer sequences (Pleyte et al., 1992). ITS-1 assays are commonly used in fungi and bacterial identification but have more recently been applied to ecological studies in aquatic environments; applications include spawning and species identification (Schoch et al., 2012; Bylemans et al., 2017; Minamoto et al., 2017b). A nuDNA assay has not been previously developed or validated for Lake Sturgeon.

1.8 LAKE STURGEON IN THE WINNIPEG RIVER

Lake Sturgeon populations across Canada vary in conservation concern (COSEWIC, 2017). COSEWIC (Committee on the Status of Endangered Wildlife in Canada) has four designatable

units (DU) that are defined by COSEWIC as “Species, subspecies, variety or geographically/genetically distinct population that may be assessed by COSEWIC, where such units are both discrete and evolutionarily significant”. The Saskatchewan-Nelson River population, which includes the Winnipeg River subpopulation of Lake Sturgeon (as well as the Nelson, Red, Assiniboine, English, and Rainy rivers), is classified as Designatable Unit 2 (DU2), which has been assessed as Endangered (COSEWIC, 2017).

The Winnipeg River stretches 260 km from Lake of the Woods, Ontario, emptying into Lake Winnipeg, in Traverse Bay, Manitoba. It is fragmented by six individual hydroelectric generating stations that affect the natural flow of the river (Struthers et al., 2017), and Lake Sturgeon populations are found at different levels of abundance across the isolated reservoirs (Barth & Anderson, 2015). Environmental factors such as food availability, water depth and intraspecific competition all play a role in the distribution of the species throughout the river (Barth et al., 2009, 2011, 2013; McDougall et al., 2013a; Barth & Anderson, 2015; Struthers et al., 2017). Common practice for tagging and monitoring this species is through gillnetting; previous studies have used telemetry as a tracking method to better understand adult and juvenile movements throughout the various reservoirs (Barth et al., 2011; Struthers et al., 2017).

Lake Sturgeon migrate upstream for spawning when their habitat remains uninterrupted by dams or impassable natural barriers (Auer, 1996a; McDougall et al., 2013b). During a spawning event, eggs are dispersed across rough surfaces, such as jagged bedrock, gravel, or boulders (Bruch & Binkowski, 2002; Gillespie et al., 2020). Male sturgeon release milt (i.e., seminal fluid containing sperm) into the water column surrounding the eggs, fertilizing on contact (Bruch & Binkowski, 2002). Spawning events can range over a few days depending on the

number of females in the area. In the Winnipeg River, Lake Sturgeon spawning events often occur near the base of hydroelectric dams as they act as physical barriers for further migration but also provide favourable spawning environments (McDougall et al., 2013a). One known spawning ground in the Winnipeg River is downstream of the Pointe du Bois generating station, making it an ideal study site for the comparison of eDNA concentrations associated with known spawning events within a population (Bruch & Binkowski, 2002; McDougall et al., 2013a; Struthers et al., 2017; Gillespie et al., 2020).

Spawning events in Lake Sturgeon are heavily influenced by environmental variables such as water temperature and discharge (Bruch & Binkowski, 2002; McDougall et al., 2014; Struthers et al., 2017). In the Winnipeg River, a positive correlation between water temperature, discharge and spawning behaviour is typically seen in the early spring as the fish begin to move toward the base of the dam (Struthers et al., 2017). The temperature range for Lake Sturgeon spawning in the Winnipeg River is between 10º and 12ºC (Zubair et al., 2012) and, in 2017, Lake Sturgeon were reported to remain within the spawning habitat below the Seven Sisters generating station for approximately 10 weeks (Struthers et al., 2017). However, it has also been reported that Lake Sturgeon spawning grounds may be restrictive habitats, with food sources being poor within fast-flowing water, resulting in a downstream migration shortly after a spawning event (Auer, 1996b; Struthers et al., 2017).

1.9 RESEARCH OBJECTIVES

In my thesis, I examined the effectiveness and reliability of Lake Sturgeon eDNA in understanding spatial and temporal distribution using the population in the Winnipeg River as a point of reference. In Chapter 2, I tested the hypothesis that Lake Sturgeon eDNA concentrations

will vary across space and time. To test this hypothesis, I sampled 15 sites across the Winnipeg River from May to October in 2021 and 2022. The results were compared against known distribution of Lake Sturgeon populations within the river based on previous gillnetting and telemetry data collected by other researchers. I predicted that distribution would be positively correlated between the study years as movement of Lake Sturgeon should not differ between years. Additionally, I hypothesized that environmental conditions, such as the change in flow rates in the river and the temperature of the water, would affect the detection rate of Lake Sturgeon between years. To test this hypothesis, hydrology data provided by Manitoba Hydro from the generating stations along the Winnipeg River and collected environmental data was compared to the positive and negative eDNA detections. A negative detection was determined when the DNA concentration in a sample was 0 in all the replicates. I predicted that an increase in water temperatures and flow rates would result in a decrease in the concentration of Lake Sturgeon DNA found in a sample.

In Chapter 3, I tested the hypothesis that gamete-related eDNA would be detectable during peak spawning times in the Winnipeg River. To test this hypothesis, I collected daily water samples from three locations above and below the Pointe du Bois generating station, a known spawning ground for Lake Sturgeon (Bruch & Binkowski, 2002; McDougall et al., 2014). A Lake Sturgeon specific nuDNA assay was developed targeting the ITS-1 region. The nuDNA assay was tested *in silico* (i.e., comparing Lake Sturgeon ITS-1 sequence to that of non-target species), *in vitro* (using tissue-derived DNA), and in the laboratory (using different concentrations of Lake Sturgeon milt) before analyzing the *in situ* samples. DNA concentrations in the samples were compared using both a mtDNA (Cyt *b*) (Yusishen et al., 2020) and the newly developed nuDNA

(ITS-1) assay using qPCR. I predicted that the amplification of Lake Sturgeon eDNA would increase and decrease as the Lake Sturgeon migrated to and from the spawning grounds, respectively. Additionally, I predicted that the concentrations and ratios of nuDNA and mtDNA would be higher during the suspected spawning events than during times when the fish were not spawning.

Chapter 2: Detecting Spatial and Temporal Distribution of Lake Sturgeon (*Acipenser fulvescens*) Using Environmental DNA.

2.1 INTRODUCTION

The spatial and temporal movement of fish is a critical area of study within freshwater biology, providing insights into behavioural patterns, habitat preferences, and population dynamics. Fish movement is influenced by a myriad of factors, including ecological interactions, seasonal changes, and anthropogenic impacts (Jung & Houde, 2003; Lucas & Baras, 2008; Block et al., 2011; Cooke et al., 2016). Understanding these patterns is essential for effective management and conservation of fish species, as it informs the design of protected areas, guides sustainable fishing practices, and aids in predicting the impacts of climate change on aquatic ecosystems (Bird et al., 2014; Cooke et al., 2016). Advanced methodologies, ranging from traditional tagging to sophisticated telemetry, have been employed to unravel the complexities of fish movement across different spatial and temporal scales (Cooke et al., 2013; Bird et al., 2014).

In recent years, environmental DNA (eDNA) has emerged as a powerful tool for tracking the spatial and temporal distribution of fish (Thomsen & Willerslev, 2015). By analyzing DNA fragments shed by organisms into their environment, eDNA methodologies allow researchers to detect the presence and abundance of fish species in various habitats without the need for physical capture (Handley et al., 2019; Beng & Corlett, 2020). This non-invasive technique has revolutionized aquatic monitoring, offering high sensitivity and specificity. eDNA studies have successfully mapped migration routes, identified spawning grounds, and monitored population trends, providing crucial data for conservation efforts (Maruyama et al., 2018; Thalinger et al., 2019; Muha et al., 2021). The application of eDNA for the spatial distribution of fish not only

enhances our understanding of aquatic biodiversity, but it also supports the development of more effective strategies for preserving aquatic ecosystems in the face of environmental change.

Lake Sturgeon (*Acipenser fulvescens*) are a large-bodied freshwater fish found in Canada and the United States of America. The temporal movement of sturgeon species is a complex interplay of life history traits, environmental cues, and ecological requirements (Auer, 1999). In spring, as temperatures begin to rise, Lake Sturgeon migrate upstream towards their spawning grounds. This migration is often triggered by specific temperature thresholds that signal the onset of the spawning season (Bruch & Binkowski, 2002; Boase et al., 2011). In the Winnipeg River, these temperatures range between 10º and 12ºC (Zubair et al., 2012). Lake Sturgeon seek out areas with moderate to high flow rates for spawning, which limit sedimentation and siltation and promote oxygenation which is conducive for successful egg development and hatching (Baril et al., 2018). Spawning behaviours are often concentrated in specific river reaches or tributaries, where sturgeon aggregate in large numbers for reproductive purposes (Boase et al., 2011; Kessel et al., 2017). The timing and duration of the spring migration can vary between populations and are influenced by factors such as water temperature, flow rates, and photoperiod. The timing of the spring migration and spawning events is also influenced by flow dynamics. Sturgeon may time their migration to coincide with periods of increased flow, such as spring runoff events or seasonal rainfalls, which can enhance flow rates and trigger reproductive behaviours (Lallaman et al., 2008; McDonald & Haxton, 2023). Conversely, alterations in flow regimes due to anthropogenic activities, such as dam operations or water withdrawals, may disrupt the natural cues and timing of spawning migrations, potentially impacting reproductive success (Auer, 1996a; McDougall et al., 2013b).

Following spawning, adult Lake Sturgeon transition into a period of summer foraging and growth. As temperatures continue to rise, sturgeon may move to deeper, cooler waters to seek out optimal foraging opportunities (Barth et al., 2013). During the summer months, sturgeon may exhibit localized movements within river systems, exploiting dynamic habitats such as river confluences, deep pools, and submerged structures (Knights et al., 2002; Struthers et al., 2017).

As temperatures begin to cool in the fall, Lake Sturgeon undergo another migratory phase, often moving towards overwintering areas characterized by stable thermal regimes and suitable habitat conditions. Overwintering habitats typically include deep pools, backwaters, and areas with minimal ice cover, where sturgeon can conserve energy and avoid potentially lethal low temperatures (Barth et al., 2013). The timing and extent of fall migrations can vary depending on local environmental conditions, including water temperature, flow dynamics, and food availability (McDougall et al., 2013a).

The movement patterns of Lake Sturgeon in the Winnipeg River are intricately linked to the presence of hydroelectric generating stations. These structures can influence their behaviour and habitat use in several ways. For example, dams create physical barriers to upstream migration, impeding Lake Sturgeon movement and restricting access to historical spawning and foraging habitats (McDougall et al., 2013b). Additionally, alterations in flow regimes and water temperatures downstream of dams can disrupt the natural cues and environmental conditions that trigger sturgeon migrations, potentially impacting the timing and success of reproductive events (Auer, 1996a, 1996b).

By collecting water samples from key habitats and analyzing the presence of sturgeon eDNA, we can infer the relative spatial and temporal distribution of sturgeon populations

throughout the year, offering valuable insights into their habitat use. In this study, I aim to examine the effectiveness and reliability of Lake Sturgeon eDNA, using the Cyt *b* assay from Yusishen et al. (2020) in understanding spatial and temporal distribution. I sampled water from the Winnipeg River in areas where the population of Lake Sturgeon is reasonably well described during the open water season of 2021 and 2022. I hypothesized that Lake Sturgeon eDNA concentrations would vary across space and time and that environmental conditions, such as the change in flow rates in the river and the temperature of the water, would affect the detection rate of Lake Sturgeon eDNA between the two years. I predicted that increases in eDNA would be detectable at known spawning times and locations and that high flow would result in reduced detections.

2.2 METHODS

2.2.1 Preparation

Eliminating the potential of false positives through contaminated samples is critical when conducting eDNA studies. For each sample site prior to going into the field, two 1 L bottles were sanitized using hot soapy water, wiped down with ELIMINase (Thermo Fisher Scientific, Waltham, MA, USA) followed by an ethanol rinse. Bottles were then placed in a 15-minute UV cycle in the AirClean 600 PCR Workstation (AirClean Systems, Creedmoor, NC, USA), along with nitrile gloves, large plastic freezer bags, and a sealed commercially purchased plastic 500 mL bottle of drinking water to act as a negative control. Upon completion of the sterile cycle, the two sampling bottles, nitrile gloves and 500 mL bottle of water were sealed in the labelled freezer bag. Steps were repeated dependent on the number of samples being collected. All other sampling gear such as

coolers, thermometers, and water quality testing devices were sanitized and kept in separate containers during water sample collection.

2.2.2 Sample Collection

Fifteen sites were selected along and around the Winnipeg River in areas of known Lake Sturgeon population abundance (Figure 2.1). These sites were selected based on previously collected data and monitoring efforts, previous tracking and gillnetting studies to determine movement patterns and abundance of Lake Sturgeon in the different sections of the river (Barth & Anderson, 2015; Manitoba Hydro, 2019; McDougall et al., 2013a, 2013b, 2014). Collection sites were limited to accessibility for public access points such as boat launches, public beaches, and areas off the highway with proximity to the Winnipeg River. Sites were visited mid-monthly from May to October in 2021 and 2022 apart from May 2022 during high water when many sites were inaccessible. Refer to Appendix A (Table A1) for site names and exact locations of each collection site.

Figure 2.1 Sampling sites for examining spatial distribution of Lake Sturgeon eDNA along the Winnipeg River (n =15). All sites are located at public beaches, public boat launches, or off the highway. Site codes are numbered from upstream (1) to downstream (15) with their corresponding site name (Table A1). Hydroelectric generating stations can be found at Pointe du Bois, Slave Falls and Seven Sisters Falls (as shown).

To detect possible contamination in 1 L sampling bottles or other equipment, at each sampling site, a sealed negative control bottle of water was emptied into a 1 L sample bottle then poured back into its original bottle for subsequent filtration and eDNA extraction. The two 1 L sample bottles were then submerged 0.5 m under the water at varying distances from shore. This distance was dependent on whether the site had access to a dock, allowing for water to be sampled farther away from the shoreline (0 m to 10 m from shore). If a dock was not available at the site, water was collected from the shore using a 3 m reaching pole. To avoid the collection of sediment and contamination of the samples, wading into the water was minimized. All samples

collected avoided the use of a motorized watercraft. The filled sample bottles and the negative control bottle were placed back into their corresponding plastic bags, sealed, and put into an ice-filled cooler.

Samples were collected in triplicate for all sites once a month on May 18th, June 15th, July 15th, August 16th, September 19th and October 18th (*n* = 78) in 2021; and on June 28th, July 29th, August 31st, September 26th and October 24th (*n* = 65) in 2022. Additional abiotic information collected at each site included: air temperature, water temperature, wind speed and direction, weather conditions (cloud cover, precipitation), conductivity (µS/cm), dissolved oxygen (DO), a photograph of the collection site, latitude and longitude, the relative proximity (i.e., at a public boat launch, next to a bridge, etc.), turbidity and the start/end time for the specific collection. A Total Dissolved Solids (TDS) and Electrical Conductivity (EC) meter (Health Metric, USA) (water temperature, conductivity, and Dissolved Oxygen (DO)), phone weather apps (air temperature, wind speed and direction, conditions), and the HydroColor phone application (turbidity) were used to determine these parameters. Water flow data was obtained from Manitoba Hydro data gathered at each of the three dams within the study area – from upstream to downstream, Pointe du Bois, Slave Falls and Seven Sisters Falls (Figure 2.1).

2.2.3 eDNA Filtering and Preservation

All samples were kept on ice in the dark prior to filtration in the laboratory within 24 hours of collection. Prior to opening and filtering the water samples, the lab bench was sterilized with ELIMINase. Samples were vacuum filtered through a Whatman 47 mm glass microfiber filter paper with a pore size of 1.5 µm (GE Whatman, Fairfield, CT). Filters were placed onto a 47 mm sealed fitted glass support base with sterile forceps. A 300 mL borosilicate glass funnel was

clamped onto the base with an aluminum clamp (Figure 2.2). 500 mL of the negative control sample was filtered first followed by the first of three 500 mL replicate samples taken from the 1 L sampling bottles. Sterile forceps were used to fold each filter paper in half three times (extraction side inwards) and then placed into a sterile 15 mL Falcon tube pre-filled with 8 mL of 90% ethanol for preservation of the sample. Forceps were dipped in ethanol and flamed prior to placing a new filter for the next sample filtration. All filters were placed within their own tubes and were labelled with the site code, filter date, and replicate number or negative control.

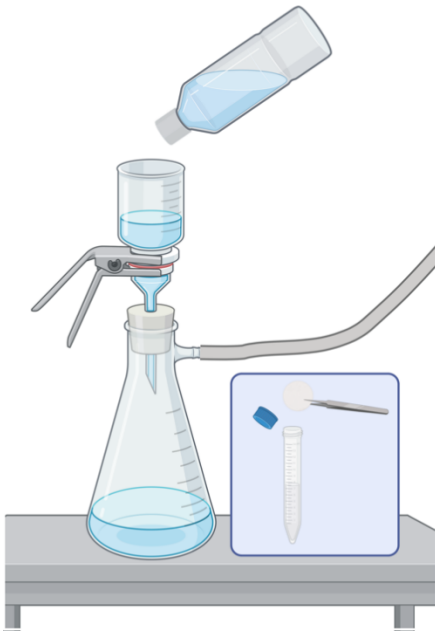

Figure 2.2 Example of the vacuum filtration setup used to filter the collected eDNA water samples. The 500 mL negative control is filtered first followed by the three biological replicates. Each corresponding filter paper is folded inwards and placed in a Falcon tube filled with ethanol (figure created with BioRender).

Before filtering samples from a different site, the lab bench was re-sterilized, and glassware, tubing and forceps were washed in hot soapy water, sprayed with ELIMINase, and rinsed with ethanol prior to being placed in an AirClean 600 PCR Workstation for a 15-minute UV

cycle. Falcon tubes containing the samples were stored in the freezer at -20º C until eDNA extraction.

2.2.4 eDNA Extractions

All extraction steps were conducted in an AirClean 600 PCR Workstation using the DNeasy Blood and Tissue Kit and QIAshredder (Qiagen Inc., Valencia, CA, USA). Filters were removed from their ethanol-filled Falcon tubes, unfolded, and placed on sterile petri dishes to air dry for 15 to 30 minutes prior to placing them in a 1.5 mL tube pre-filled with 450 µL of a tissue lysis buffer (Buffer ATL; Qiagen) and 40 µL of a proteolytic enzyme (proteinase K; ThermoFisher). An additional tube with no filter paper added was prepared as a lab negative control during this step. Samples were then placed in a thermomixer (ThermoFisher Scientific: 13687712) at 56º C for 18-24 h at a shaking speed of 350 rpm.

Following the incubation period, samples had 6 µL of RNase A (ThermoFisher) added to them and were then vortexed and centrifuged at 13,000 rpm for 10 seconds. Tubes were then incubated at 56º C for 5 minutes and centrifuged at 13,000 rpm for 10 seconds once complete. During this time, 100 µL per sample of elution buffer (Buffer AE; Qiagen) was prepared in a 1.5 mL tube and heated on a heating block at 70 ºC. Samples were then transferred to a QIAshredder spin column (Qiagen) (filter paper included); any additional liquid that was not absorbed by the filter paper was pipetted into the tube. Tubes were then centrifuged at 11,000 rpm for 2 minutes. Columns were removed and 500 µL of extracted flow-through liquid was transferred into a new 1.5 mL tube. 400 µL of a lysis buffer (Buffer AL; ThermoFisher) and 400 µL of 100% ethanol were then added to all tubes. Tubes were vortexed and centrifuged at 13,000 rpm for 10 seconds. Each sample was then assigned a DNeasy mini spin column (Qiagen). 650 µL

of mixture from the 1.5 mL corresponding tubes, including any formed precipitates, were then pipetted into the spin column and centrifuged at 8,000 rpm for 1 minute. Flow-through was discarded and the tube was placed back into the 2 mL collection tube. Remaining liquid from the 1.5 mL tube was added and centrifuged a second time at 8,000 rpm for 1 minute. Mini spin columns were then transferred to a new 2 mL collection tube. 500 µL of a wash buffer (Buffer AW1; Qiagen) was then added to the mini spin column and centrifuged at 8,000 rpm for 1 minute. Collection tubes were discarded, and the mini spin columns were moved to a new 2 mL collection tube. 500 µL of a second wash buffer (Buffer AW2; Qiagen) was added and then centrifuged at 14,000 rpm for 3 minutes. Collection tubes were discarded, and mini spin columns were then placed in a 1.5 mL tube. 100 µL of the previously heated Buffer AE (Qiagen) was pipetted directly onto the filter membrane. Samples were left to sit at room temperature for 5 minutes prior to being centrifuged at 8,000 rpm for 1 minute. Resulting flow-through from this step is the extracted DNA. Three aliquots were made from the 100 µL extracted DNA samples to avoid multiple freeze thaw cycles (one 1.5 mL tube with 50 µL DNA and two 0.6 mL tubes with 25 µL DNA). Extracted samples were stored at -20º C prior to performing quantitative polymerase chain reaction (qPCR) analysis.

2.2.5 Quantitative Polymerase Chain Reaction (qPCR)

qPCR was conducted using a StepOnePlus Real-Time PCR System (ThermoFisher Scientific). qPCR outputs the threshold cycle (C_t) value for each sample. The C_t value is the number of cycles required for a fluorescent signal to cross a predetermined threshold of detection. The lower the C_t value, the fewer cycles needed to detect DNA in the sample indicating a higher DNA concentration; higher C_t values indicating more cycles needed to cross

the threshold correspond to lower DNA concentrations. A value of 0 indicates a negative detection of Lake Sturgeon DNA. Zeros are expected to be found in lab, field, and qPCR negative controls. If a positive was found in the lab or field negative controls, subsequent detections found in the replicates were discarded due to the possibility of contamination (i.e., a false positive detection). qPCR negative controls were re-run with the samples; if a detection was found a second time, the data was omitted.

Sites with a qRT-PCR positive detection in the biological replicates were averaged across the replicates and compared against the standard curve to determine the concentration of DNA in each sample. A standard curve was created by conducting a 5-fold serial dilution using tissue-derived Lake Sturgeon DNA starting at a concentration of 7.5 ng/µL for 10 standards (Figure 2.3). C_t values were converted into concentrations using the line equation from the assay-specific standard curve ($y = mx + b$) where C_t is x, concentration is y, m is the slope and b is the intercept. Concentrations were used for further data analysis.

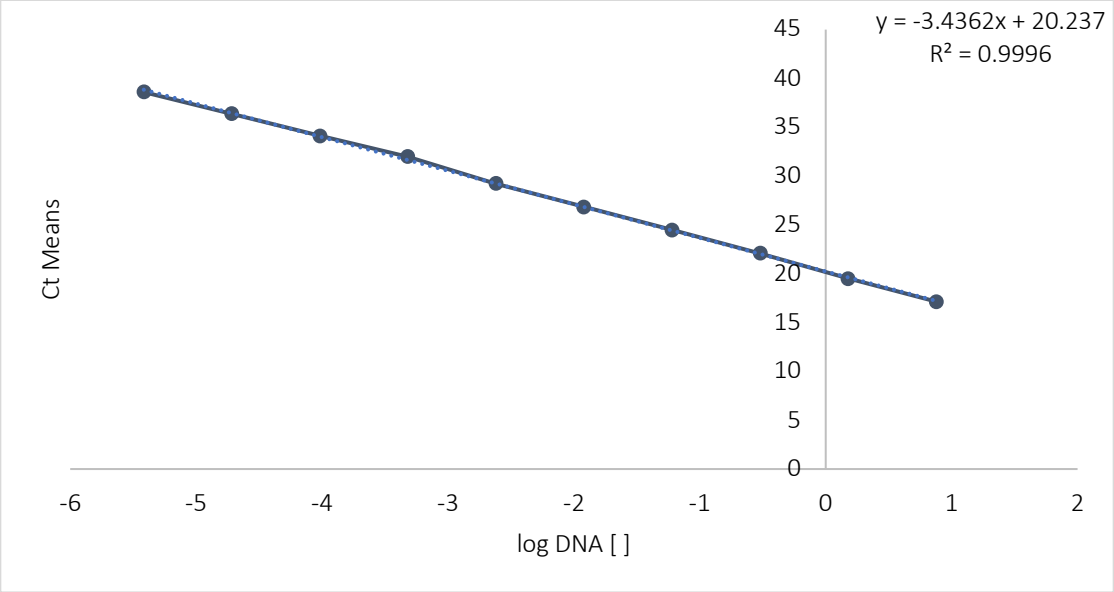

Figure 2.3 Standard curve for Cyt *b* Lake Sturgeon assay developed by Yusishen et al. (2020). Standards were created using 5-fold serial dilutions for 10 standards. Equation for the curve (y = -3.4362x + 20.237) is calculated by using the points on the graph.

DNA samples were thawed in the dark. All instruments and surfaces in the PCR station were wiped down with ELIMINase following a 15-minute UV cycle. The Cyt *b* Lake Sturgeon assay was used to detect the presence of species-specific DNA in the sample. Primer and probe sequences were used as previously described in Table 2.1 (Yusishen et al., 2020).

Table 2.1 Sequences for the forward and reverse primers and corresponding probe used to detect Lake Sturgeon DNA targeting the mitochondrial gene cytochrome *b*. Assay was developed by Yusishen et al. (2020). The designed probe used a 5' primer modification VIC as its reporter dye and a 3' primer modification MGB-NFQ which allows for detection of lower background signal, improving the precision of quantification when using the assay.

Gene	Identity	Sequence (5'-3')	Amplicon length (bp)
Cyt *b*	F primer	ATGAATCGGAGGCCAACCAG	105
	R primer	TAAGCCTGTCAGAGGGAGGG	
	Probe	VIC-CCTCCACAGTTTATTTTGCCCTATTCCT-MGBNFQ	

A TaqMan MGB probe (VIC; ThermoFisher Scientific) was used as the reporter dye at an annealing temperature of 58ºC and a reaction volume of 15 µL. All assays included a qPCR negative control, lab negative control, field negative control and extracted DNA samples. qPCR negative control samples consisted of 10 µL of prepared qPCR master mix and 5 µL of molecular grade water. qPCR master mix consisted of 1.75 µL of molecular grade water, 0.3 µL of forward primer (10 mM), 0.3 µL of reverse primer (10 mM), 7.5 µL of TaqMan Environmental Master Mix 2.0 (ThermoFisher Scientific), and 0.15 µL of the probe (10 mM) for a total volume of 10 µL. 10 µL of qPCR master mix and 5 µL of extracted DNA was added to each well on a 96 well sampling plate and each sample was assayed in triplicate. qPCR was run for a total of 40 cycles.

2.2.6 Data Analysis

Data analysis was performed using R version 4.1.1 (R Core Team, 2021). Collected eDNA concentrations were averaged across the 3 collected replicates taken during each sampling event. Environmental data collected from all sites were additionally averaged per month using the 'dplyr' package (Wickham et al., 2023). Data from Natalie Lake (NAL) and Keskinen Bay (KB) were excluded from the models due to lack of access in 2022, as they were only collected in 2021. Notably, all samples from NAL and KB in 2021 did not amplify Lake Sturgeon eDNA. Hourly hydrology data from Manitoba Hydro were matched with sampling times to estimate flow rates during sampling.

We first examined temporal variation in water temperature, flow rate, and eDNA concentration using two-way ANOVAs that included the interaction between month and year, with Site as a random effect. We then used model selection to compare a series of General

Linear Models to determine the environmental variables that best explained variation in DNA concentration. The independent variables used in this analysis included month, year, distance from the spawning grounds, distance from the dam, water temperature, and flow rate, with site collected from as a random effect. Distance from the spawning grounds and distance from the dam were highly correlated and so were not included in the same model to avoid multicollinearity. To meet model assumptions, DNA concentration was log-transformed using the 'car' package (Fox & Weisberg, 2019).

The models were fit using the lmer function from the lme4 package (Bates et al., 2015), and results were obtained using maximum likelihood estimation (REML). To explore the effects of the predictors on logDNA, all possible models were created by systematically including combinations of main effects and 2-way interactions of the independent variables. The models were compared and ranked based on Akaike Information Criterion (AIC). The best model was selected based on the lowest AIC value, which balances model fit and complexity.

Once the best model was selected, an ANOVA was performed using the Anova function from the ‘car’ package (Fox et al., 2012). If significant interactions or main effects were found, post-hoc pairwise comparisons were conducted. Estimated marginal means were calculated using the ‘emmeans’ package (Lenth, 2020), and pairwise comparisons were performed to assess the nature of these effects.

The random effect of Site was included in the model to account for variability in logDNA concentrations across different sampling locations. The ‘ranef’ function from the lme4 package was used to assess the random effect of Site and ensure that variability between sites was appropriately modeled. Relationships between variables were visualized using

'ggplot2' and 'gridExtra' to illustrate differences between 2021 and 2022 across sampled months (Wickham, 2016; Auguie, 2017).

2.3 RESULTS

Water temperature at time of sampling across all sites in 2021 was lowest in October (10.8ºC ± 1.18 SD) and highest in August (25.8ºC ± 1.15) (Figure 2.4a and Table A3). Similarly, water temperature at time of sampling in 2022 was lowest in October (13ºC ± 1.21) and highest in August (27.5ºC ± 1.4) (Figure 2.4a). A two-way ANOVA with Site as a random effect indicated that the main effects of Month ($\chi^2 = 1965.911$, $p < 0.001$), Year ($\chi^2 = 32.471$, $p < 0.001$), and their interaction ($\chi^2 = 47.272$, $p < 0.001$) significantly affected water temperature (Table A4).

Average water flow rate taken at time of sampling across the 3 dams in 2021 was lowest in August (156.5 m^3/s ± 24.95) and highest in May (659 m^3/s ± 91.84) (Figure 2.4b and Table A3). Water flow rate taken at the nearest upstream dam in 2022 was lowest in October (885.6 m^3/s ± 28.05) and highest in May (3590.6 m^3/s ± 106.31) (Figure 2.4b). The ANOVA indicated that the main effects of Month ($\chi^2 = 8070$, $p < 0.001$), Year ($\chi^2 = 38251$, $p < 0.001$), and their interaction ($\chi^2 = 5091$, $p < 0.001$) significantly affected flow rate (Table A5).

A total of 55/78 samples (70.5%) amplified Lake Sturgeon DNA in 2021 and 45/65 samples (69.2%) in 2022 (Table A2). In 2021, the month of August had the lowest average concentration of Lake Sturgeon eDNA across all sites (0.0004 ng/µL ± 0.0007), while in 2022, September had the lowest concentration (0.00047 ng/µL ± 0.001) (Figure 2.4c). The highest average concentration of Lake Sturgeon DNA in 2021 was in May (0.0337 ng/µL ± 0.06), and in 2022 was in July (0.0064 ng/µL ± 0.002) (Figure 2.4c). Overall, ANOVA indicated that there was no main effect of year on DNA concentration ($\chi^2 = 2.0239$, $p = 0.15$) (Figure 2.4c), but the effects of

month (χ^2 = 19.4066, p < 0.001) and the interaction between year and month (χ^2 = 16.9526, p < 0.001) were significant (Table A6). Both years resulted in an equal number of positive detections (May excluded).

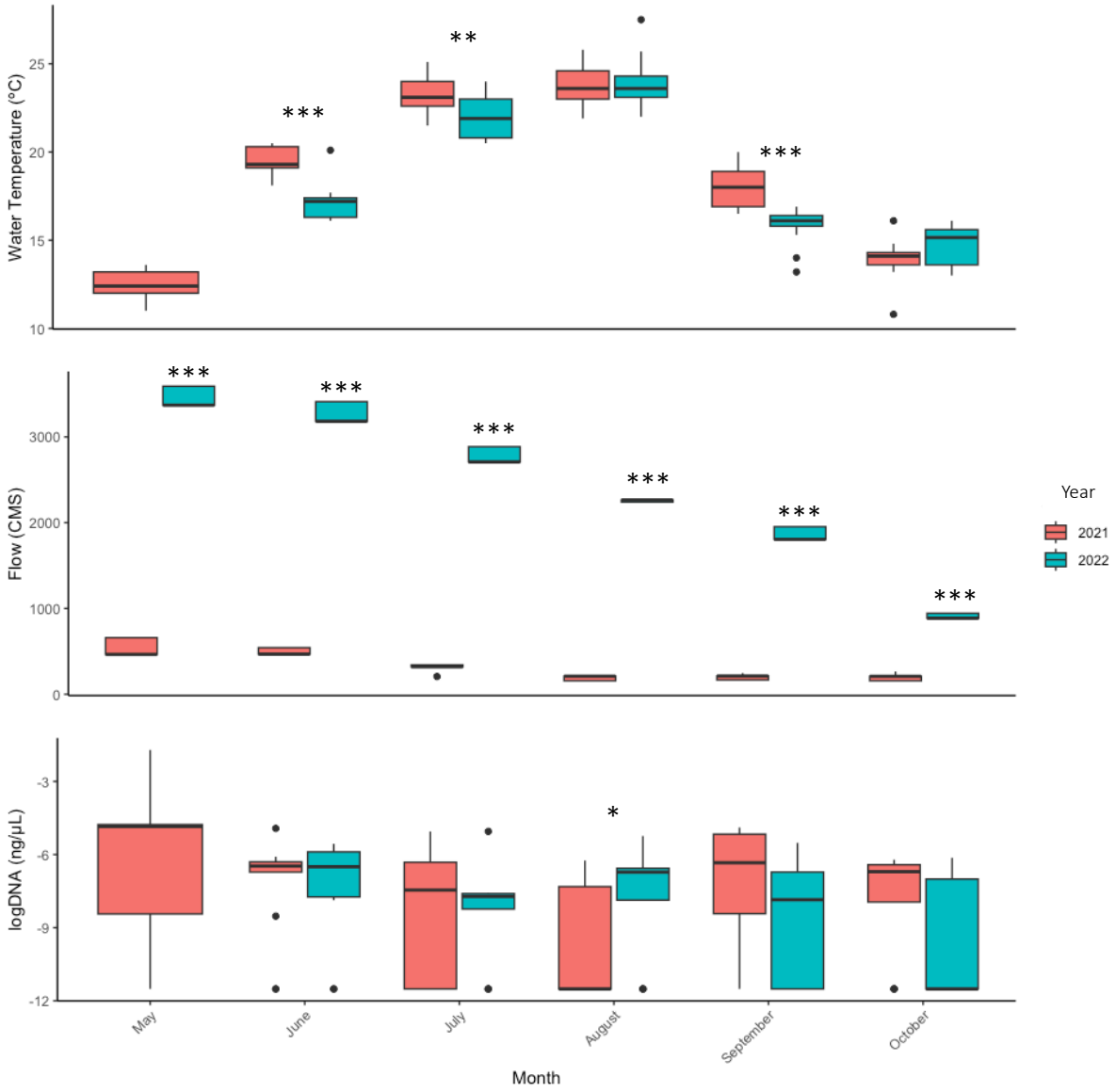

Figure 2.4 Box plots illustrating monthly average A) water temperature (ºC), B) flow rates (m^3/s) and C) eDNA concentration (log transformed) collected across all sites each month, excluding May 2022. Outliers are plotted outside of the whisker range. Significant differences between years of a given month (Tukey's HSD) are represented as (* = p < 0.05, ** = p < 0.01, *** = p < 0.001).

The model with the best support for explaining the concentration of eDNA at a site included month, year, distance from the dam and site (Table 2.1, Figure 2.5). The model explained 41% of the variation in the collected eDNA concentrations (conditional $r^2 = 0.41$) and the fixed effects explained 28% (marginal $r^2 = 0.28$). Water temperature ($p = 0.61$) and flow ($p = 0.630$) were not included in the best model.

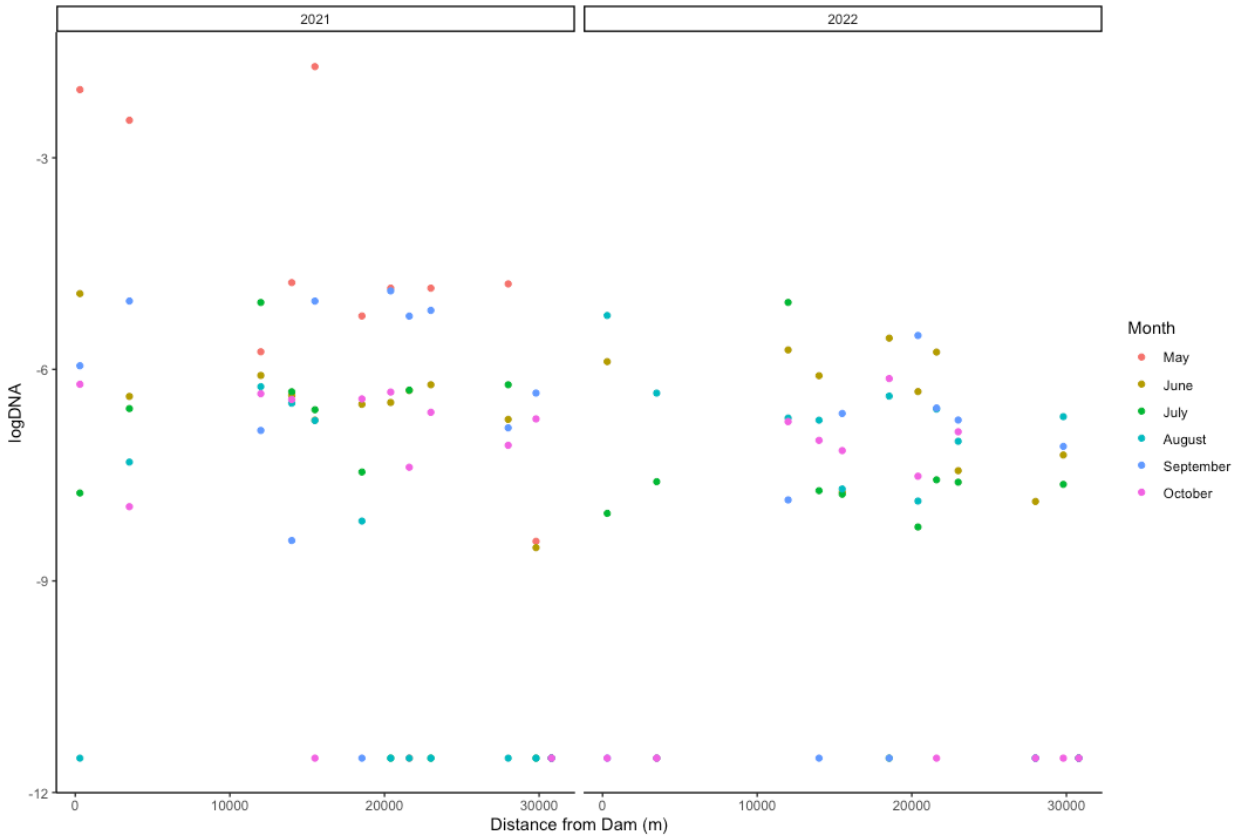

Figure 2.5 Average concentration of eDNA collected from each site from May to October in 2021 and 2022, relative to distance from the closest upstream dam.

Table 2.1 Comparison of the top five general linear mixed models, sorted by corrected Akaike Information Criterion (AIC_c), with the null model (only year, month, and site). Linear regression models were created by comparing the calculated logDNA to additive and interaction effects to the following variables: Water Temperature (ºC), Dam (distance from the closest upstream dam, m), Flow (rate of water flowing from the dam at the time of collection, CMS), Site, Year, and Month.

Ranking	Model	AIC_C	ΔAIC_C
1	logDNA ~ Year * Month + Dam + (1|Site)	601.0	0
2	logDNA ~ Year * Month + Flow + Dam + (1 | Site)	613.0	12.03
3	logDNA ~ Year * Month + Flow + (1 | Site)	619.9	18.89
4	logDNA ~ Year * Month + Flow + Water_Temp + (1 | Site)	620.2	19.17
5	logDNA ~ Year * Month * Dam * Water_Temp + (1 | Site)	622.5	21.45
11	logDNA ~ Year * Month + (1 | Site)	640.8	39.75

2.4 DISCUSSION

In this study, a total of 55/78 samples (70.5%) amplified Lake Sturgeon environmental DNA in 2021 and 45/65 samples (69.2%) in 2022. I hypothesized that Lake Sturgeon eDNA concentrations would vary across space and time and predicted that environmental conditions, such as flow rate and water temperature would be primary determinants of eDNA concentration from samples taken in the Winnipeg River. However, the data did not support this prediction as the relationships between eDNA concentration and the measured environmental conditions were not significant. Conversely, the locality of the sampling site to fast-flowing areas with known rocky substrate were a strong predictor of eDNA concentration. Further, there was a strong temporal trend of declining eDNA concentration as the year progressed from early spring through to late fall.

2.4.1 Life History of Lake Sturgeon in the Winnipeg River

The behaviour of adult and juvenile Lake Sturgeon has been extensively studied in the Winnipeg River (Barth et al., 2009, 2011, 2013; Barth & Anderson, 2015; McDougall et al., 2013a, 2013b, 2017a, 2017b; Struthers et al., 2017; Gillespie et al., 2020). Adult Lake Sturgeon exhibit distinct seasonal movements largely driven by spawning, foraging and overwintering behaviours. The in-depth understanding of these seasonally driven behaviours provided strong support for inferences I was able to make from the eDNA data collected in the present study.

2.4.1.1 Spring

Upstream migratory behaviour during the spring to spawn has been documented in several systems throughout the natural range of Lake Sturgeon (Lallaman et al., 2008; Boase et al., 2011; Gerig et al., 2011; Izzo et al., 2022). This behaviour is also evident in the Winnipeg River (McDougall et al., 2013a) and persists even when adults are transported past dams blocking their migratory route (McDougall et al., 2013b).

Adult Lake Sturgeon congregate in fast-flowing areas with rocky or gravel substrates (Struthers et al., 2017; Gillespie et al., 2020), often demonstrating strong site fidelity to spawning areas (Boase et al., 2011). These areas provide good conditions for subsequent embryogenesis as fertilized eggs adhere to the rocky substrate. In the Winnipeg River, sites with these characteristics are often found at the base of the dams and floodways, corresponding with collection sites Brookfield [BR] and Pointe du Bois [SF] in the present study (Struthers et al., 2017; Gillespie et al., 2020).

Environmental temperature is a strong predictor of spawning behaviour in Lake Sturgeon (Zubair et al., 2012; McDougall et al., 2013; Gillespie et al., 2020), and upstream movement by

adult Lake Sturgeon in the Winnipeg River normally occurs in early spring when water temperatures reach 10-12°C. In the present study, I detected high concentrations of eDNA from samples collected near the base of the dams (e.g. Pointe du Bois [SF] and Brookfield [BR]) and sites downstream of high-flow areas (e.g. Lac du Bonnet [LB]) during late May/early June in 2021. Note, I was unable to collect water at these sites in 2022 due to dangerously high water levels at that time of year. These high eDNA concentrations align well with known spawning areas for Lake Sturgeon at the base of Pointe du Bois generating station (McDougall et al., 2013b; Struthers et al., 2017; Gillespie et al., 2020) and Seven Sisters generating station (Struthers et al., 2017) and confirms that Lake Sturgeon utilize areas below these structures during early spring. However, increased mitochondrial eDNA concentration at these sites does not necessarily confirm spawning activity of Lake Sturgeon. Previous studies have examined changes in both mitochondrial and nuclear DNA in water samples, making the assumption that an increase in nuclear DNA is the result of a release of enormous amounts of gametes into the water column during a broadcast spawning event (Bylemans et al., 2017; Tsuji & Shibata, 2021). Initial high levels of both mitochondrial and nuclear eDNA are followed by a rapid decline in nuclear eDNA but a maintained heightened level of mitochondrial DNA (Bylemans et al., 2017; Tsuji & Shibata, 2021). Chapter 3 of this thesis describes the development of a nuclear eDNA assay for Lake Sturgeon and field testing in the Winnipeg River.

Post-spawn adult sturgeon can reside near the spawning grounds for a variable amount of time. Telemetry data from a study conducted in the St. Clair River, Michigan, USA, found that Lake Sturgeon reside at the spawning grounds for an average of 43 days before leaving to forage elsewhere (Boase et al., 2011). Conversely, adult Lake Sturgeon spawning at the base of the

Seven Sisters generating station on the Winnipeg River moved downstream of spawning areas within 10 days of spawning (Struthers et al., 2017). The authors presumed this was due to limited foraging opportunities in spawning areas because of increased flow rates and population density (Struthers et al., 2017). Indeed, adult fish were located near collection sites used in the present study (Lac du Bonnet [LB] and Winnipeg River A and B [WRA and WRB]) during the month of June, post-spawn.

The additional detections of Lake Sturgeon throughout the river in May 2021 (Lake Nutimik [NU], Barrier Bay [BB], Dorthy Lake [DLA and DLB], Otter Falls [OF], Eleanor Lake [ELA], and Winnipeg River A [WRA]) suggest a consistent distribution of Lake Sturgeon throughout the reservoirs as water temperatures begin to rise. However, an alternate explanation could be that Lake Sturgeon eDNA from upstream was carried downstream, resulting in higher concentrations of Lake Sturgeon eDNA at sites where they physically were not present. Indeed, the significance of the relationship between distance from a spawning ground and/or dam and eDNA concentration provides support for this explanation, particularly during the months of May and June. Furthermore, for both collection years, June provided the most positive detections across all sites (11/15 = 73.3%), further supporting eDNA mixing throughout the study area alongside downstream movement of adults post spawning as previously described (Struthers et al., 2017).

As experienced in 2022, springtime temperatures can result in large amounts of flooding as the winter snow melts and increased rainfall, resulting in an increased rate of flow throughout the river. While statistical analysis showed that increased flow rates from flooding did not significantly impact Lake Sturgeon eDNA detectability overall, detection rates across the collected replicates decreased at sites closest to dams and spillways. For example, samples at Brookfield

(BR) directly below Seven Sisters dam showed a high concentration of detectable Lake Sturgeon eDNA in June of 2021, and no eDNA detection in June of 2022 (Figure 2.4 and Table A1). A similar pattern was seen below the Pointe du Bois dam (SF) with decreased number of positive replicates seen in the 2022 June samples. Interestingly, the variability in detection rate between these sites was not evident in May, likely due to the influx of genetic material from the spawning events that may have been washed out by June in the high flow year of 2022.

To interpret negative eDNA detections, it is critical to understand the interplay between eDNA presence and environmental hydrology, as hydrological processes can significantly influence eDNA distribution within aquatic systems (URycki et al., 2024). Research highlights that studying hydrology alongside eDNA can reveal how water flow, turbulence, and channel morphology can impact eDNA transport and retention, which may contribute to variation in eDNA detection rates across sites (URycki et al., 2024). Understanding these flow patterns in a study system can provide insights into unexpected negative detections; for instance, a sampling location near an altered flow regime could redirect upstream eDNA away from the collection site, reducing detectability near the shore (URycki et al., 2024).

2.4.1.2 Summer

The behaviour of Lake Sturgeon in the Winnipeg River during summer is also influenced by variations in river flow and temperature, which can affect habitat availability and prey distribution. Adult Lake Sturgeon tend to disperse to deeper, slower-moving sections of the river to forage and evade the warming surface temperatures (McDougall et al., 2013a; Struthers et al., 2017). Summer habitats often include deeper pools and river stretches where water temperatures are cooler, oxygen levels are stable, and current flow is moderate, providing a

conducive environment for feeding and growth (Haxton & Findlay, 2008; Barth et al., 2011; Struthers et al., 2017). Telemetry data placed adult Lake Sturgeon further away from the dams in the middle and lower reaches of the reservoirs during the summer months of July and August, with 50% of the tagged fish favouring areas further downstream from site WRA and WRB (Struthers et al., 2017). Gill-net data from Nutimik [NU] and Numao Lakes indicates a high-density population of Lake Sturgeon in these reaches of the Winnipeg River during the summer months (McDougall et al., 2017a), and eDNA results from the present study supports these findings with a positive detection for Lake Sturgeon during each collection period in 2021 and 2022 across all collection months. An overall decline in detection was observed across all sites in July and August of both 2021 and 2022 collection years with some sites (e.g. Pointe du Bois [SF]) having zero detection levels in August and September. False negative eDNA detections can be the result of a variety of factors with temperature and UV degradation being key factors in the summer months degrading available eDNA in the water column at a faster rate compared to winter months (Barnes et al., 2014; Curtis et al., 2021). Furthermore, inhibitors of DNA detection such as heavy metals, humic acids or chlorine may impact the ability to detect eDNA in water samples as previously shown for detection of Brook Trout (*Salvelinus fontinalis*) eDNA (Jane et al., 2015). The impact of such factors on detection of Lake Sturgeon eDNA is unknown, but as it is genetic material, it is reasonable to assume similar effects would occur.

2.4.1.4 Fall

As water temperatures decline in the fall, Lake Sturgeon in the Winnipeg River tend to migrate from summer foraging habitat to overwintering habitat in the late fall. They will continue to forage on benthic organisms such as insect larvae, mollusks, and small fish, which are

abundant in deeper river sections (Rusak & Mosindy, 1997) as temperatures decline throughout the fall. However, as temperatures reach near zero levels, metabolic rate decreases and individuals transition towards winter dormancy, as they seek areas with stable temperatures, moderate flow and sufficient oxygen levels to over-winter (Izzo et al., 2020).

In the Winnipeg River, Lake Sturgeon are thought to return to the same overwintering sites annually, selecting habitat to survive more than 4 months of near zero temperature and reduced foraging opportunity (Barth et al., 2013). This behaviour has important conservation implications, as alterations in flow or habitat conditions in these areas could significantly impact the sturgeon population's ability to overwinter successfully (McDougall et al., 2013a). The presumed aggregation and reduced activity of adults and juveniles in the fall may explain the reduced eDNA detection levels across all sites in the present study. Such a phenomenon has previously been shown in sampled lake water; as temperatures drop, the metabolic rates of aquatic organisms also decrease, leading to reduced activity and consequently, lower rates of eDNA shedding (Eichmiller et al., 2016). Conversely, cooler water also causes a reduction in microbial activity responsible for breaking down eDNA, resulting in a slower rate of eDNA degradation that is balanced by the slower rate of eDNA release (Tsuji et al., 2017). However, it is likely that as temperatures decrease, there are reduced levels of Lake Sturgeon eDNA in our system in the fall compared to spring and summer due to decreased movement and activity (Thalinger et al., 2021).

An alternate explanation for reduced eDNA concentrations in the fall months could be due to the physical properties of water in cooler seasons. Colder water has higher density and greater stratification potential, which may limit the dispersion of eDNA particles throughout the

water column. This can make eDNA more localized or difficult to sample effectively in deeper or stratified water bodies in the fall (Yates et al., 2021). A previous study by Laramie et al. (2015) examined seasonality in eDNA detection for Bull Trout (*Salvelinus confluentus*) in the Pacific Northwest. The researchers found that eDNA detection was highest in spring, coinciding with spawning migrations, and decreased sharply in cooler seasons. This decline aligned with a reduction in fish movement and biological activity as water temperatures fell much like the present study.

2.4.2 Juvenile Lake Sturgeon in the Winnipeg River

While adult Lake Sturgeon distribution throughout the Winnipeg River varies seasonally, juvenile sturgeon have a much more limited range, typically defined by the presence of fast-flowing sections of the river (Barth et al., 2011; McDougall et al., 2013a). Depending on temperature, larval Lake Sturgeon hatch approximately 2 weeks following fertilization and will remain within the rocky substrate at the spawning grounds to absorb their yolk sac for a further 7–10 days as the rocky substrate provides protection from predation (Barth et al., 2009). Once the yolk sac is absorbed, juveniles emerge and drift downstream, settling on appropriate foraging habitat, typically gravel/sand, and will remain within this nursery habitat during the first summer of life (Barth et al., 2009, 2011). This results in large aggregations of juveniles in upstream reaches of the reservoir (Barth et al., 2009, 2011, 2015), resulting in a greater likelihood of a positive detection of Lake Sturgeon eDNA closer to dams and/or fast-flowing rapids. These habitat types can be observed at sites where I obtained the highest eDNA detections across both years of study (Pointe du Bois [SF], Otter Falls [OF] and Brookfield [BR]).

Interestingly, density dependent growth patterns have been observed throughout the Slave Falls reservoir on the Winnipeg River (Barth et al., 2015), where high density aggregations of juveniles in the upstream reaches of the reservoir exhibit lower growth rates than the lower density aggregations in the downstream reaches of the reservoir (Barth et al., 2015). The differences in resource accessibility may lead to an increased rate of mortality in Lake Sturgeon in the upper reaches of the river, further increasing the amount of DNA being sloughed into the environment at a given site and supporting the observed positive relationship between eDNA detection and proximity to dams and/or rapids in the present study.

2.5 CONCLUSION

eDNA analysis cannot differentiate between life stages of a species, limiting the ability to make precise inferences about the movement and distribution of specific demographic groups. Thus, interpreting eDNA data effectively requires a thorough understanding of the species' life history. For instance, while this study reliably detected Lake Sturgeon at sites known to be inhabited by juveniles, it remains challenging to determine whether adults are truly absent from these areas, or similarly, to confirm the predicted absence of juveniles in downstream regions. To fully comprehend the ecological roles of each life stage within an environment, conventional methods like gill netting and angling are still necessary. These traditional approaches provide demographic-specific data that eDNA alone cannot provide. However, eDNA can complement these methods, offering valuable insights when physical sampling is unfeasible and serve as a long-term monitoring tool to assess species presence and distribution across a given system.

CHAPTER 3: Detecting Lake Sturgeon (*Asipenser fulvescens*) Spawning Events Using Environmental DNA (eDNA).

3.1 INTRODUCTION

Lake Sturgeon (*Acipenser fulvescens*) is a freshwater fish endemic to North America. It is the only species of North American sturgeon to complete its entire life cycle in fresh water (Baril et al., 2017). During the spawning season, Lake Sturgeon can migrate up to 100's of kilometres to appropriate spawning habitats (Auer, 1999). While migratory behaviours have been seen in many populations, variables such as habitat preferences for foraging, spawning and over-wintering can make it difficult to generalize movement patterns in the species (Auer, 1999; Bruch & Binkowski, 2002; Boase et al., 2014). Although preferred habitat types may vary between these populations, the destruction and loss of spawning habitats remains a constant. In areas where rocky substrates were removed for industrialization, such as in the Detroit River, sturgeon populations plummeted because of loss of spawning habitat (Roseman et al., 2011). Protection of these spawning grounds is important for the longevity and future success of the species.

Physical barriers have also hindered the Lake Sturgeon's ability to migrate, resulting in restricted movement patterns when compared to Lake Sturgeon in open systems (Auer, 1999). In environments where human induced fragmentation has occurred, such as during the addition of dams, spawning habitats can be destroyed or become inaccessible to existing populations. If the remaining fish are not adapted to their new environments, or cannot use the necessary substrate required to spawn, population numbers can decline as a result. The importance of

spawning knowledge becomes critical if information on their preferred habitat can be protected or avoided when building new infrastructures.

The Winnipeg River in Manitoba, Canada, is part of the Saskatchewan River, Nelson River, Red-Assiniboine Rivers-Lake Winnipeg, Winnipeg River-English River, and Lake of the Woods-Rainy Riverpopulation of Lake Sturgeon. This population was assessed as Endangered in 2017 by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC, 2017). The Winnipeg River was altered by the addition of hydroelectric dams dating back to 1906 (Auer, 1996b). There are currently a total of six hydroelectric dams on the Winnipeg River that have created isolated reservoirs resulting in varying population sizes in each. While habitat destruction and fragmentation can be detrimental to a species' success, the isolated populations allow for research to be conducted in a more controlled manner when compared to an open system. The Lake Sturgeon in the Winnipeg River have been extensively studied with a focus on: habitat use, seasonal variation in diet, upstream movement for spawning, downstream movement behaviours and general health and population size assessments (Barth et al., 2009, 2011, 2013; McDougall et al., 2013a, 2013b, 2014; Manitoba Hydro, 2019).

As discussed in Chapter 2, a Lake Sturgeon's spatial and temporal distribution can be detected using mitochondrial DNA (mtDNA). While DNA is always present in an environment through the shedding of epidermal cells, the addition of germ cells provides genetic confirmation that a spawning event occurred when visual detection is not possible (Tsuji & Shibata, 2021). Confirmation of spawning events ideally require the paired detection of nuclear DNA (nuDNA) and mtDNA in the system (Bylemans et al., 2017), as an increase in mtDNA alone may not be sufficient to properly identify a spawning event; rather, a change in the ratio of mtDNA and

nuDNA in the water column is more appropriate because one would predict a transient increase nuDNA concentrations associated with the addition of gametes into the water. Spermatozoa in particular release copious amounts of nuDNA into the water as their nuclear components are stored differently than in somatic cells, resulting in more nuDNA than mtDNA (Bylemans et al., 2017). Lake Sturgeon are broadcast spawners, releasing billions of sperm and upwards of 150,000 eggs into the water during a single spawning event (Bruch & Binkowski, 2002). As seen previously with Macquarie Perch (*Macquaria australasica*), broadcast spawners can produce a spike in eDNA during a spawn, creating an invisible genetic indication of the events happening beneath the surface (Bylemans et al., 2017).

By pairing previous knowledge of Lake Sturgeon movement patterns and general life history characteristics with genetic monitoring efforts, spawning information can be gained pertaining to unstudied populations or habitats to better identify the locations and timing of spawning events. This knowledge would allow for protective measures of the spawning grounds to be put in place to prevent habitat alteration or destruction. Alternatively, the steps taken to create and validate a nuclear assay could be mirrored in other species of conservation or management concern to detect spawning events in unmonitored environments.

In this study, I developed a nuDNA assay for Lake Sturgeon and tested its effectiveness and reliability in a known Lake Sturgeon spawning location in the Winnipeg River at the base of the Pointe du Bois dam (Manitoba, Canada) using eDNA. I hypothesized that Lake Sturgeon mtDNA would be present in all samples collected, while nuDNA would only be detectable during the temperature predicted spawning window.

3.2 METHODS

To assess the presence of spawning individuals, I developed a nuDNA based eDNA assay that would be used concurrently with the mtDNA based eDNA assay (Yusishen et al., 2020) previously described in Chapter 2. As described below, the Lake Sturgeon nuclear assay was developed using the internal transcribed spacer (ITS-1) sequence, a non-coding region between the small-subunit (18S) ribosomal RNA (rRNA) and large-subunit (28S) rRNA genes, for this species from GenBank (FJ688022). Primers and assay were optimized using *in silico* validation (Primer-BLAST) and Sanger sequencing, *in vitro* validation (testing against a variety of species using tissue-derived DNA), and *in situ* validation (testing samples during an artificial and field spawning event (Klymus et al., 2020).

3.2.1 Assay Development

The selected GenBank sequence (FJ688022) was a partial sequence of the 28S – 18S region in Lake Sturgeon. Since there were no published annotated boundaries for the Lake Sturgeon ITS-1 region during the time the assay was being developed, the length of ITS-1 had to be inferred. ITS-1 is found between 18S and 5.8S (Figure 3.1). Since the available GenBank sequence only annotated the 28S and 18S regions, the developed primers favoured the sequences flanking 18S to avoid targeting 5.8S, which is more conserved than ITS-1 and less likely to yield species- or genus-specific primers (Figure 3.2).

Figure 3.1 General structure of a nuclear ribosomal RNA gene ITS-1 located within the internal transcribed spacer.

GTTTCACTGTACAAGCCCGTGTGTACTTAGACATGCATGGCTTAATCTTTGAGACAAGC
ATATGCTACTGGCAGGATCAACCAGGTAGCTGCGCCTTCCGCATCCCCCGGAGTCGG
GTGGAGGATGGTGGAACGAGCGTGATCCGACCCCCTGTTCCACCCCCCAAAGCGCCT
GCGAGAAGCGGATGGGGAAGCGCGGGAGGAAGGAACACGACGCGGAGCCCACCCG
CGAACTGGAGGGGGCTCGAGCGTGGCGGGTGTTCGAACGGAACCATCAAAAGCTAT

Figure 3.2 Lake Sturgeon ITS-1 assay sequence for version 7 (ITS1.7) of the new nuDNA assay. The underlined section is the known 18S region (inferred from 18S sequences in other fishes) and the non-underlined section is the suspected ITS-1 gene sequence. The forward primer can be seen in blue, reverse in green and the corresponding probe in pink.

When deciding on the optimal forward and reverse primer pairings, penalty scores, percentage of guanine (G) and cytosine (C), length of the primers, their melting temperatures and location on the genome played an important role. Lower penalty scores were favoured as they decreased the chances of the primers being unstable due to self and cross dimers or hairpins. Ideal %GC was between 30 and 80% to help promote binding. The target number of base pairs between the primers (i.e., amplicon length, including the primers) was between 50 and 150 base pairs, while the probes needed to be roughly 30 base pairs as recommended when making a TaqMan gene expression assay (ThermoScientific). Additionally, ideal melting

temperatures were between 58 and 60ºC. A total of seven forward and reverse primer pairs were designed in Primer Express (Applied Biosystems, v3.0.1) for further testing (Table 3.1).

Table 3.1 A series of forward and reverse sequences that were created using the GenBank sequence (FJ688022) for the 28S – 18S ribosomal RNA genes in Lake Sturgeon. Since the internal transcribed spacer (ITS-1) region in Lake Sturgeon is located between the 28S and 18S genes, some of the below sequences are partially flanking the ITS-1 region, sequences that are not flanking are entirely in the ITS-1 region. ITS1.7 was ultimately used for the experiment.

Identity	Name	Sequence (5' – 3')	Amplicon length (bp)	Tm (ºC)	Flanking?
F primer	ITS1.1F	TTTCCCCGGAGCTGAAATG	80	60	No
R primer	ITS1.1R	TGTTCGAACGGAACCATCAA		59	
Probe	ITS1.1P	TTCCTCTGGCATGCCT		70	
F primer	ITS1.2F	ATGGCTTAATCTTTGAGACAAGCA	102	58	Yes (18S)
R primer	ITS1.2R	CGCTCGTTCCACCATCCT		58	
Probe	ITS1.2P	TACTGGCAGGATCAAC		69	
F primer	ITS1.3F	TGTTCGAACGGAACCATCAA	80	59	No
R primer	ITS1.3R	TTTCCCCGGAGCTGAAATG		60	
Probe	ITS1.3P	ACCGCAGGCATGC		69	
F primer	ITS1.4F	GAACGGAACCATCAAAAGCTATG	150	59	No
R primer	ITS1.4R	GGGTGCCCTTCCATCTAGTG		58	
Probe	ITS1.4P	ACCGCAGGCATGC		69	
F primer	ITS1.5F	GAGCGTGGCGGGTGTTC	202	60	No
R primer	ITS1.5R	GGATGCCGACCTTTCCAA		58	
Probe	ITS1.5P	AACGGAACCATCAAAA		68	
F primer	ITS1.6F	TTCGAACGGAACCATCAAAAG	176	59	No
R primer	ITS1.6R	TTCCAAGCGAGGTCGAGAAG		59	
Probe	ITS1.6P	ACCGCAGGCATGC		69	
F primer	ITS1.7F	CAAGCCCGTGTGTACTTAGACATG	206	60	Yes (18S)
R primer	ITS1.7R	CGCGTCGTGTTCCTTCCT		58	
Probe	ITS1.7P	TGGCTTAATCTTTGAGACAAG		70	

3.3.2 *In Silico* Validation

3.3.2.1 Primer and Probe Design

The GenBank sequence for Lake Sturgeon 28S – 18S was compared against eight fish species found both within and outside of Manitoba with published ITS-1, 18S or 5.8S regions, including two other sturgeon species, Atlantic Sturgeon (*Acipenser oxyrinchus*) and Sterlet Sturgeon (*Acipenser ruthenus*) (Table 3.2). The sequence was first analysed using Primer-BLAST to compare against other ITS-1 sequences in the database to check for similarities in the genetic makeup of the markers. However, this was not done with other sturgeon species in Canada as complete ITS-1 sequences were not available. A full annotated genome was only available for the Sterlet Sturgeon. Instead, the Atlantic Sturgeon's 18S sequence was compared to the Lake Sturgeon's available data to infer the position of ITS-1 following the 18S gene. Using Geneious Prime, an alignment was made comparing the Lake Sturgeon's predicted ITS-1 gene to the other species (Table 3.2) to ensure the developed primers would be unlikely to amplify non-target DNA. The developed TaqMan primers and probes were further tested *in silico* using Primer Express (Applied Biosystems) (Klymus et al., 2020).

Table 3.2 Species used during *in silico* validation of the Lake Sturgeon ITS-1 assay. Sequences were used for a nucleotide alignment against the suspected ITS-1 region, including the flanking 18S and 5.8S regions, when possible, in Lake Sturgeon to determine if the assay would likely be genus-specific.

Species	GenBank ID	Region Available
Atlantic Salmon (*Salmo salar*)	HQ260441.1	18S- 5.8S
Atlantic Sturgeon (*Acipenser oxyrinchus*)	AF188373.1	18S
Burbot (*Lota lota*)	MT458302.1	ITS-1- 5.8S
Channel Catfish (*Ictalurus punctatus*)	GQ465242.1	18S- 28S
Common Carp (*Cyprinus carpio*)	JN628435.1	18S- 28S
Sterlet Sturgeon (*Acipenser ruthenus*)	CM021253.1	18S- 28S
Walleye (*Sander vitreus*)	MT458482.1	ITS-1
Yellow Perch (*Perca flavescens*)	MT458484.1	ITS-1- 5.8S

3.2.3 *In Vitro* Validation

3.2.3.1 PCR and Sanger Sequencing

Seven pairs of forward and reverse primers targeting the ITS-1 gene (Table 3.1) were then tested by conventional polymerase chain reaction (PCR) and Sanger sequencing, that is, the primers were used to amplify the fragment without the probe, and the size of the amplicon was determined using gel electrophoresis, after which (if it was the predicted size), it was sequenced to confirm identity. The PCR master mix solution consisted of 2 µL of extracted Lake Sturgeon DNA (diluted to 5 ng/µL), 2 µL of both the forward and reverse primers for each primer pair (10 µM/µL), 10 µL of EconoTaq DNA Polymerase (Biosearch Technologies) and 4 µL of molecular grade water. Cycling conditions were as follows: 94ºC for 2 minutes, followed by 34 cycles of a 3-

step cycle of 30 seconds at 94ºC, 30 seconds at 54ºC and 30 seconds at 72ºC. The resulting PCR products were run on a gel made up of 30 mL of Tris-acetate-EDTA buffer (TAE) (ThermoFisher) and 0.3 g of agarose. The agarose was dissolved by placing the solution in a microwave oven on high for 1 minute, stirring at the halfway point. 25 µL of EconoTaq DNA Polymerase (Biosearch Technologies), was then mixed into the agarose solution prior to it being poured into a casting tray and allowed to set at room temperature. 5 µL of the PCR product was added to each well and was run for 20 minutes at 120 V. Once complete, an image was taken of the gel to determine which of the primer combinations produced a product of appropriate size (Figure 3.3). Forward and reverse primers for primer sets 5 and 7 (Table 3.1) resulted in amplifying the most promising product of the correct size and quality and therefore were chosen for further sequencing.

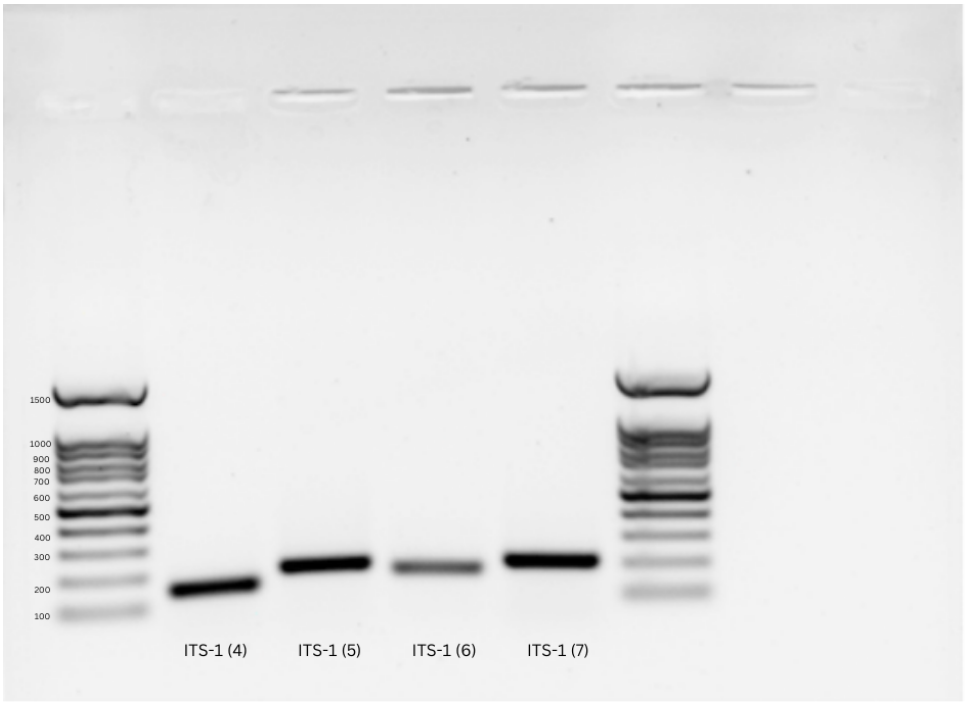

Figure 3.3 PCR gel with version 4, 5, 6 and 7 of the ITS-1 assays compared to a molecular size standard (measured in base pairs). Versions 5 and 7 were 202 and 206 base pairs, respectively.

A second round of PCR was run using the previously amplified PCR product for sets 5 and 7 of the primer pairs. 25 µL of EconoTaq DNA Polymerase (Biosearch Technologies), 4 µL of the previous PCR product, 5 µL of both the forward and reverse corresponding primers (10 µM/µL) for set 5 and 7, and 11 µL of molecular grade water was used. The corresponding gel required larger wells to account for the increased amount of product needed for sequencing. To accomplish this, three wells were taped together on either side, creating a larger reservoir. The gel was made and run in the same manner as previously mentioned except for 20 µL of reaction mixture was added to the newly created wells.

Approximately 0.5 g of the gel was cut out, targeting the section containing the amplified product, using a UV light to visualize the product following the E.Z.N.A (Omega Bio-tek) protocol. The cut gel and aliquots of the corresponding primers (diluted to 5 pmol) were then sent to the Centre for Applied Genomics at the Hospital for Sick Children (Toronto ON) for Sanger sequencing to confirm the designed primers (ITS-1.5 and ITS-1.7) were amplifying the correct target gene (Table 3.3).

Table 3.3 Sequence results from Sanger sequencing conducted on versions 5 and 7 of the Lake Sturgeon ITS-1 assay (ITS 1.5 and ITS 1.7 in Table 3.1). Sections in red represent the known 18S region. S indicates that the nucleotide could be either guanine (G) or cytosine (C), W indicates adenine (A) or thymine (T), K indicates guanine (G) or thymine (T), and R indicates adenine (A) or guanine (G).

Identity	Sequence (5' – 3')
ITS1.5F	GAAGAATGCATTTCAGCTCCGGGGAAAAGACGCGCACAACGGGGG ACTAGACCCGCCGGTCCTCCCCGGGCTCAAGCCAGACCACTAGATGG AAGGGCACCCGGACTTCTCGACCTCGCTTGGAAAGGTCSGCATCCAA
ITS1.5R	TCTAGTGGTCTGGCTTGAGCCCGGGGAGGACCGGCGGGTCTAGTCC CCCGTTGTGCGCGTCTTTTCCCCGGAGCTGAAATGCATTCTTCTGGCG TGCCTGCGGTCCCCGTACCGCATAGCTTTTGACGGTTCCGTTCGAACA CCCGCCACGCTCC
ITS1.7F	GCATATGCTACTGGCAGGATCAACCAGGTAGCCGCGCCTTCCGCATCC CCCGGAGTCGGGTGGAGGATGGTGGAACGAGCGTGATCCGACCCCC TGTTCCACCCCCCCAAAGCGCCKGCRARAAGSGRATGGGGAAGCGC GGGAGGAAGGAACACGACGCGG
ITS1.7R	ATCACGCTCGTTCCRCCWTCCTCCACCCRACTCCGGGGGATGCGGAA GGCSCGGCTACCTGGTTGATCCTGCCASTAGCATATGCTTGTCTCRAAR ATTAAGCCATGCATGTCTAARTACACACGGGCTT

3.2.3.2 Tissue Derived DNA Extraction

Extraction of genetic material was completed on fin clips taken from Lake Sturgeon (University of Manitoba), Atlantic Sturgeon (Acadian Sturgeon and Caviar), Shortnose Sturgeon (*Acipenser brevirostrum*) (Acadian Sturgeon and Caviar), and White Sturgeon (*Acipenser transmontanus*) (Vancouver Island University). Fin clips were stored in 90% ethanol prior to the extraction. All extraction steps were conducted in an AirClean 600 PCR Workstation used only for DNA extractions (AirCleansystems, Creedmoor, NC, USA) and in a separate room from all eDNA

work, using the DNeasy Blood and Tissue Kit and QIAshredder (Qiagen Inc., Valencia, CA, USA). Approximately 1 cm^2 of tissue was rinsed with molecular grade water prior to placing into a 1.5 mL tube pre-filled with 180 µL of a tissue lysis buffer (Buffer ATL; Qiagen) and 20 µL of a proteolytic enzyme (proteinase K; ThermoFisher). The workstation was thoroughly cleaned and exposed to UV light for 15 minutes prior to tissue extraction for each species. Completed tubes were placed in a thermomixer at 56ºC for 18-24 hours at a mixing speed of 350 rpm. Prior to starting day 2 protocols, 60 µL of elution buffer (Buffer AE; Qiagen) was heated at 70ºC. Samples were centrifuged at 13,000 rpm prior to adding 3 µL of RNase A (ThermoFisher). Samples were vortexed for 10 seconds and centrifuged at 13,000 rpm briefly. 200 µL of a lysis buffer (Buffer AL; ThermoFisher) and 200 µL of 100% ethanol were added to the tube. Tubes were vortexed and centrifuged at 13,000 rpm for 10 seconds. The sample was assigned a DNeasy mini spin column (Qiagen). 650 µL of mixture from the original tube was transferred into the new column, including any formed precipitates and centrifuged for 1 minute at 8,000 rpm. Flow-through was discarded and the remaining liquid was added to be centrifuged for an additional minute at 8,000 rpm. The mini spin column was then placed into a new 2 mL collection tube. 500µL of wash buffer (AW1; Qiagen) was added to the mini spin columns and centrifuged at 8,000 rpm for 1 minute. Collection tubes were discarded, and the mini spin columns were moved to a new 2 mL collection tube. 500 µL of a second wash buffer (Buffer AW2; Qiagen) was added and the solution was centrifuged at 14,000 rpm for 3 minutes. Collection tubes were discarded, and mini spin columns were each placed in a 1.5 mL tube. 60 µL of 70ºC Buffer AE (Qiagen) was pipetted directly onto the filter membrane. Samples were left to sit at room temperature for 5 minutes prior to being spun at 8,000 rpm for 1 minute. Resulting flow-through from this step is the

extracted DNA, and the mini spin columns were discarded. Extracted DNA was then quantified for each individual species using a Qubit dsDNA Broad Range Assay Kit (ThermoFisher) as per the manufactures protocol.

3.2.3.3 Sperm Derived DNA Extraction

DNA from Lake Sturgeon sperm, collected from spawning males in the Nelson River near Thompson, Manitoba, in 2022, was also extracted. 100 µL of sperm was used for the extraction. Methods were the same as the above protocol for tissue-derived DNA apart from the volume of ethanol added on day two; the sperm extractions required 250 µL of ethanol instead of 200 µL.

3.2.3.4 DNA Quantification

DNA quantification of the extracted tissues was completed using reagents and protocols from the ThermoFisher Scientific Qubit dsDNA Broad Range Assay Kit (ThermoFisher). For a single sample, 1 µL broad range reagent and 199 µL of broad range buffer were added to a 1.5 mL tube, creating the working solution, and the sample tube was inverted gently to mix the solution. To make the standards, two thin-walled microcentrifuge 0.5 mL tubes were needed. 10 µL of stock standard 1 and 190 µL of working standard were added and were gently inverted to mix. Steps were repeated in a new tube using 10 µL of stock standard 2. Samples were additionally prepared in 0.5 mL microcentrifuge tubes. 2 µL of DNA and 198 µL of working solution was added and inverted gently to mix. Samples and standards were incubated for 3 minutes at room temperature.

A Qubit 2.0 Fluorometer (ThermoFisher Scientific) was used to quantify the DNA. dsDNA Broad Range was selected as the assay type. Tubes were wiped down with a Kimwipe and were checked for any bubbles. Standard 1 was placed in the sample chamber and read, followed by

standard 2 to calibrate the machine. The DNA sample was then added into the chamber and read for roughly 3 seconds. Concentration value for the extracted DNA was recorded before additional samples were run.

3.2.3.5 Primer Efficiency

Primer efficiency was validated using a 4-fold dilution (starting at a concentration of 20 ng/µL and ending with 7.62939E-05 ng/µL) of DNA extracted from Lake Sturgeon fin clips and sperm, creating a standard curve for each assay option (ITS-1.5 and ITS-1.7) (Figure B1). qPCR was conducted using a StepOnePlus Real-Time PCR System (ThermoFisher Scientific) and TaqMan Environmental Master Mix 2.0 (ThermoFisher Scientific) for a total volume of 15 µL per sample (5 µL DNA + 10 µL master mix). qPCR conditions were the same as those mentioned in Sect. 2.3.5.

qPCR was also conducted using DNA extracted from fin tissue from the non-target species (Table 3.3) to determine if the newly developed assay was species-specific. The target gene was amplified from DNA extracted from White Sturgeon, Atlantic Sturgeon and Shortnose Sturgeon fin tissue, but it was not amplified in six non-sturgeon species native to Manitoba (Table B1). While this shows the assay was not species-specific for Lake Sturgeon, it did confirm the assay was specific to sturgeons (or at least genus *Acipenser*). Given that Lake Sturgeon is the only sturgeon species found in Manitoba, the risk of false detection resulting from cross-amplification with other species was considered negligible.

3.2.4 *In Situ* Validation

3.2.4.1 Laboratory detection of nuclear eDNA

To test the validity of the newly created nuclear eDNA assay and persistence of a nuDNA signal in the water, a lab experiment using field collected sperm was conducted. Prior to starting,

all equipment was sterilized using a 10-minute bleach bath (1:10), followed by 10-minute repeated rinse. Tanks were additionally wiped down using 90% ethanol. The 9-L flow-through tanks were equipped with air stones and a continual flow of water at 15ºC (Figure B2). To ensure all bleach and ethanol was removed from the tanks, water was run through the system for 3 days. On the third day, 500 mL of water was collected from each tank to test for potential Lake Sturgeon eDNA.

Following confirmation of a negative result (i.e., lack of eDNA detection), water flow was turned off and the respective volume of milt was added to each tank: Low (111.11 µL), Mid (333.33 µL) and High (1000 µL). 500 mL water samples were taken 20 minutes, 40 minutes, and 1, 3, 6, 9, 12, 24 and 48 hours post addition of sperm to each tank. Water was filtered, and DNA was extracted using the DNeasy Blood and Tissue Kit and QIAshredder (Qiagen Inc., Valencia, CA, USA) and quantified using qPCR as previously described in Chapter 2.

3.2.4.2 Field Spawning

The downstream side of the Pointe du Bois generating station and dam on the Winnipeg River is a well-known Lake Sturgeon spawning site with spawning adults typically present in late May to early June every year (Gillespie et al., 2020). In 2022, water samples were taken once daily starting on May 4th and ending on June 17th; however, the collected samples were affected by flooding that year and did not yield expected results. The study was therefore repeated in 2023 with collections spanning from May 8th to June 2nd. Samples taken between May 23rd and June 2nd in 2023 were collected twice daily; once in the morning between 9 and 10 am and once in the evening between 5 and 6 pm. Evening samples were only taken at the sites downstream of the dam. Samples were not collected during thunderstorms, resulting in a few missed collection

periods. Two collection sites were selected in 2022: one 300 m downstream of the spawning site and one 350 m upstream of the dam where Lake Sturgeon eDNA had not been previously detected in 2021 (Table A2). A third site immediately downstream of the generating station where Lake Sturgeon are known to spawn was added in 2023 (Figure 3.4). Sampling efforts followed the same procedures described in Chapter 2 (Sect. 2.2.2) with 1.5 L of water being collected in triplicate at each site. Environmental data was also collected every time a water sample was collected following the procedures described in Chapter 2 (Sect. 2.2.2).

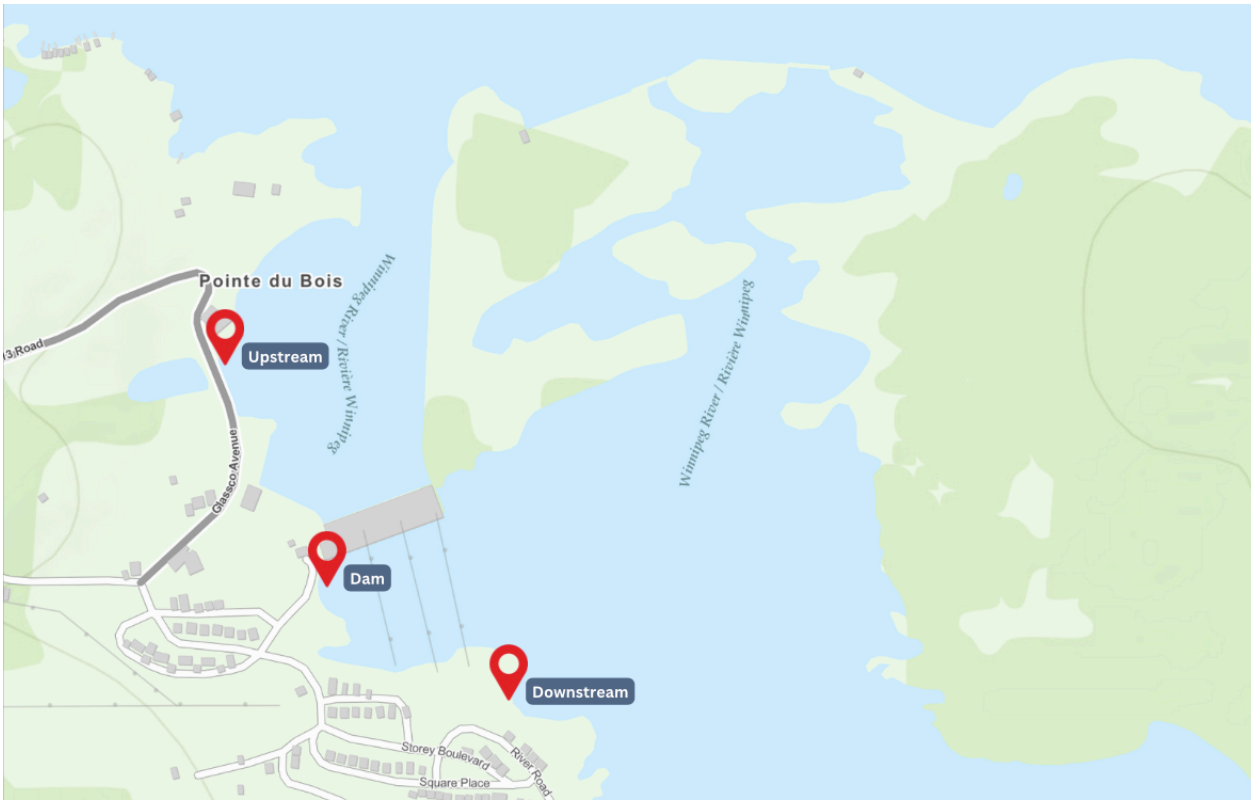

Figure 3.4 Map of the Pointe du Bois generating station located on the Winnipeg River in Manitoba, Canada. The 3 red markers indicate the locations where water was collected during the suspected spawning period: upstream from the dam (-350 m), at the base of the dam (0 m) and downstream from the dam (+300 m).

Samples were filtered on-site using a peristaltic pump system (Figure B3). All field sampling equipment (forceps, filter papers, gloves, filter holder, plastic casing and tubing) was prepared in advance as previously described (Sect. 2.3.1) to remove any Lake Sturgeon genetic material that would contaminate sampling equipment. Re-used items, such as the hand drill, were sanitized with ELIMINase and ethanol between each site to also reduce contamination. All filters were placed into sterile 15 mL Falcon tubes pre-filled with 8 mL of 90% ethanol and stored on ice until they could be transferred to a-20ºC freezer. DNA was extracted from each sample following the previously described protocols (Sect. 2.3.4). Nuclear eDNA concentration was measured using the assay described above targeting ITS-1 (Table 3.4); and mitochondrial eDNA was measured using the assay described previously targeting cytochrome *b* (Table 2.1; Yusishen et al., 2020).

Table 3.4 Final Lake Sturgeon ITS-1 primer (F = Forward and R = Reverse) and hydrolysis probe sequence using the ITS1.7 primer pair option.

Gene	Identity	Sequence (5' – 3')	Amplicon length (bp)	Tm (ºC)
ITS-1	F primer	CAAGCCCGTGTGTACTTAGACATG	206	58
	R primer	CGCGTCGTGTTCCTTCCT		
	Probe	VIC- TGGCTTAATCTTTGAGACAAG-MGBNFQ		

3.2.5 Data Analysis

Data analysis was performed using R version 4.1.1 (R Core Team, 2021). Collected eDNA concentrations were averaged across the 3 collected replicates taken during each sampling event in both the lab and field studies. Environmental data collected from all field sites were

additionally averaged per day using the 'dplyr' package (Wickham et al., 2023). Hourly hydrology data from Manitoba Hydro were matched with sampling times to estimate flow rates during sampling.

We first examined temporal variation in water temperature, flow rate, and eDNA concentration and tested whether time of day differed for each in 2023 using paired t-tests. A paired t-test was then conducted to compare the mean log-transformed DNA concentrations between the two assay types, MT and NU. We then used model selection to compare a series of General Linear Models to determine the environmental variables that best explained variation in DNA concentration. The independent variables used in this analysis included assay type, water temperature, flow rate, and date. To meet model assumptions, DNA concentration was log-transformed using the 'car' package (Fox & Weisberg, 2019).

The models were fit using the lm function from the base R package. To explore the effects of the predictors on logDNA, all possible models were created by systematically including combinations of main effects and 2-way interactions of the independent variables. The models were compared and ranked based on Akaike Information Criterion (AIC). The best model was selected based on the lowest AIC value, which balances model fit and complexity.
Once the best model was selected, an ANOVA was performed using the Anova function from the ‘car’ package (Fox et al., 2012). If significant interactions or main effects were found, post-hoc pairwise comparisons were conducted. Estimated marginal means were calculated using the ‘emmeans’ package (Lenth, 2020), and pairwise comparisons were performed to assess the nature of these effects. Relationships between variables were visualized using 'ggplot2' and

'gridExtra' to illustrate differences between 2021 and 2022 across sampled months (Wickham, 2016; Auguie, 2017).

3.3 RESULTS

3.3.1 Laboratory Detection of Nuclear eDNA

The three tanks (low, medium and high concentrations of Lake Sturgeon sperm) were sampled a total of nine times over 48 hours (Figure 3.5). mtDNA was detected in 100% (27/27) of the samples, while nuDNA was detected in 81% of the samples (22/27). Of the 19% of samples that did not detect Lake Sturgeon nuDNA, three were from the medium concentration tank (40 minutes, 9 hours and 48 hours) and two were from the high concentration tank (1 hour and 9 hours). Concentrations of Lake Sturgeon sperm assessed using the mtDNA-assay (Cyt *b*) and the nuDNA-assay (ITS-1) differed significantly (t (26) = 14.31, p = < 0.001).

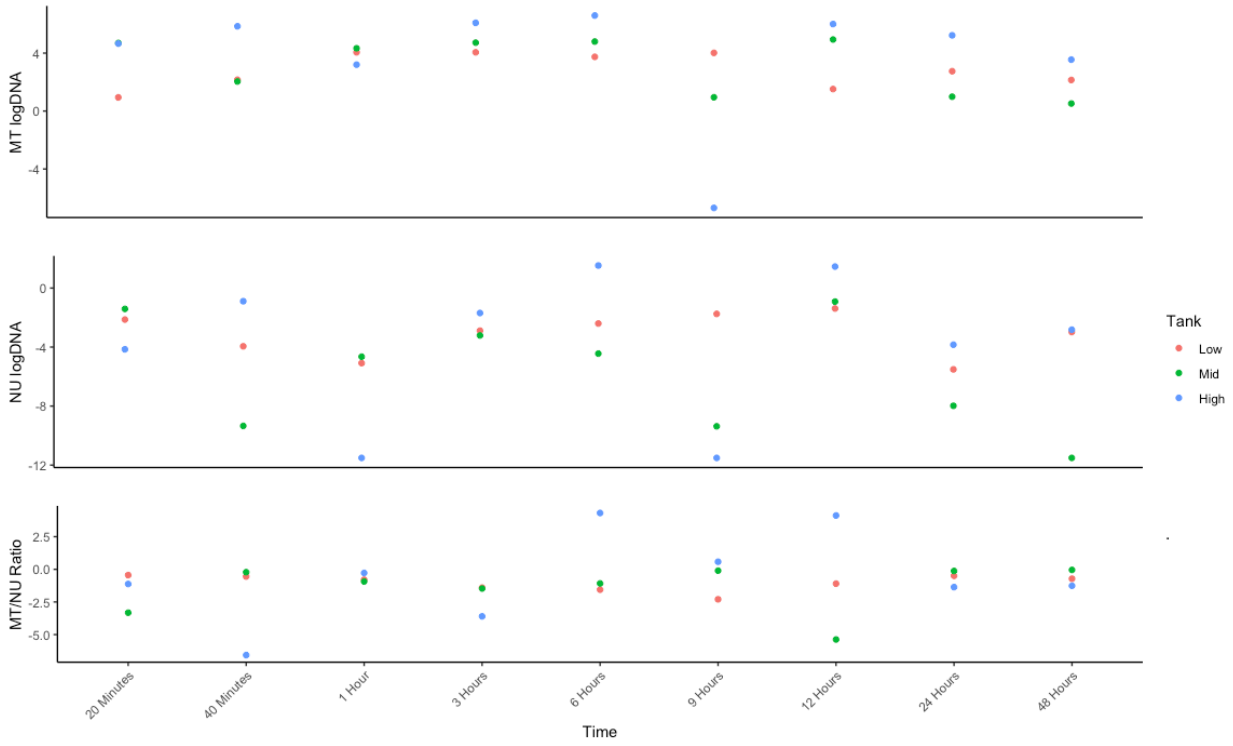

Figure 3.5 DNA from Lake Sturgeon sperm were detected using a mtDNA assay (Cyt b; MT) and a nuDNA assay (ITS-1; NU) in tanks of different concentrations: low, medium and high (111.1 µL (red), 333.3 µL (green), and 1000 µL (blue)). The top graph represents the average logged concentration of DNA across the three biological replicates sampled from each tank that was quantified using the Cyt b assay over the nine sampling events. The middle graph represents the average logged concentration of DNA from each tank were quantified using the ITS-1 assay over the nine sampling events. The bottom graph represents the ratio between the MT and NU data points.

3.3.2 Field Detection of Spawning Lake Sturgeon

Water temperature taken at time of sampling across all sites (mean ± SD) was lowest on May 8, 2023 (6.0 ± 2.5 ºC) and highest on June 2, 2023 (18.0 ± 2.5 ºC) (Figure 3.6). There were no significant differences in water temperature from samples taken in the morning versus the late afternoon in 2023 (t (23) =-1.45, p = 0.16).

Average water flow rate taken at time of sampling at the dam was lowest on May 28, 2023 (37.2 ± 0.2 CMS) and highest on May 8, 2023 (199.2 ± 0 CMS). There were no significant

differences in flow from samples taken in the morning versus the late afternoon in 2023 (t (23) = 0.57, p = 0.57).

A total of 48/57 samples from 2023 amplified Lake Sturgeon mtDNA (Table B2). Of the 9 samples with no detection of mtDNA, 6 were collected upstream of the dam and 3 were collected downstream of the dam. Overall, there was no significant difference in mtDNA concentration in samples collected in the morning and evening (t (13) =-0.922, p = 0.373).

A total of 14/57 samples (24.6%) amplified Lake Sturgeon nuDNA (Table B2). There was no detection of nuDNA in any of the above dam samples. Apart from samples taken on May 24th, all positive detections of nuDNA occurred between May 26th and June 2nd (Figure 3.6). Positive nuDNA concentration was the lowest on May 31, 2023 (0.006 ± 0.028 ng/µL), and the highest on May 28, 2023 (0.102 ± 0.047 ng/µL) (Figure 3.7) across the 3 sites. Overall, there was no significant difference in nuDNA concentration between samples collected in the morning and evening (t (20) = 0.116, p = 0.909). Concentrations were higher for nuDNA than mtDNA (t (56) = 6.29, $p < 0.001$).

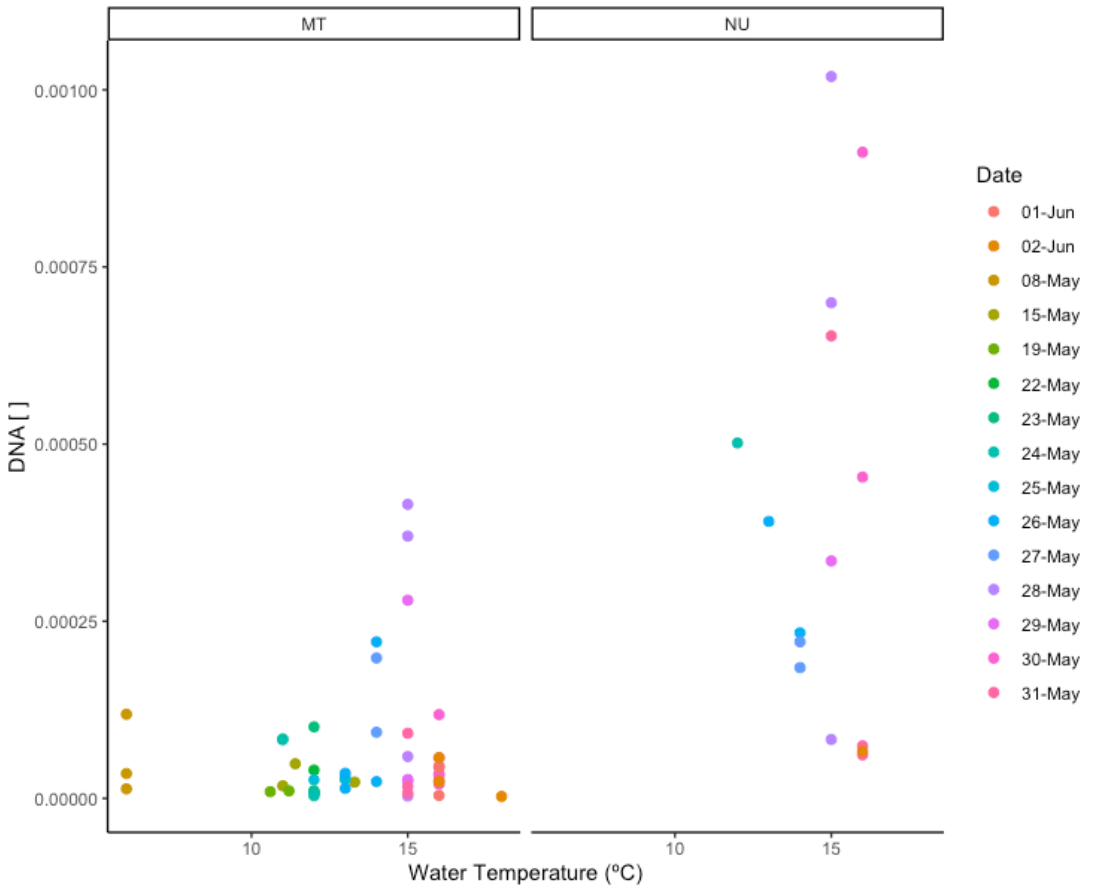

Figure 3.6 The concentrations of Lake Sturgeon eDNA at different water temperatures below the Pointe du Bois generating station, located in the Winnipeg River, MB. The figure is split between the two assay types: mitochondrial (Cyt *b*) and nuclear (ITS-1). Typically, Lake Sturgeon in this system will begin spawning when water temperatures are between 10ºC and 12ºC. The colours indicate the collection dates starting with yellow, going in rainbow order, and ending with red. Any sample that resulted in no detection of DNA has been removed from the plot.

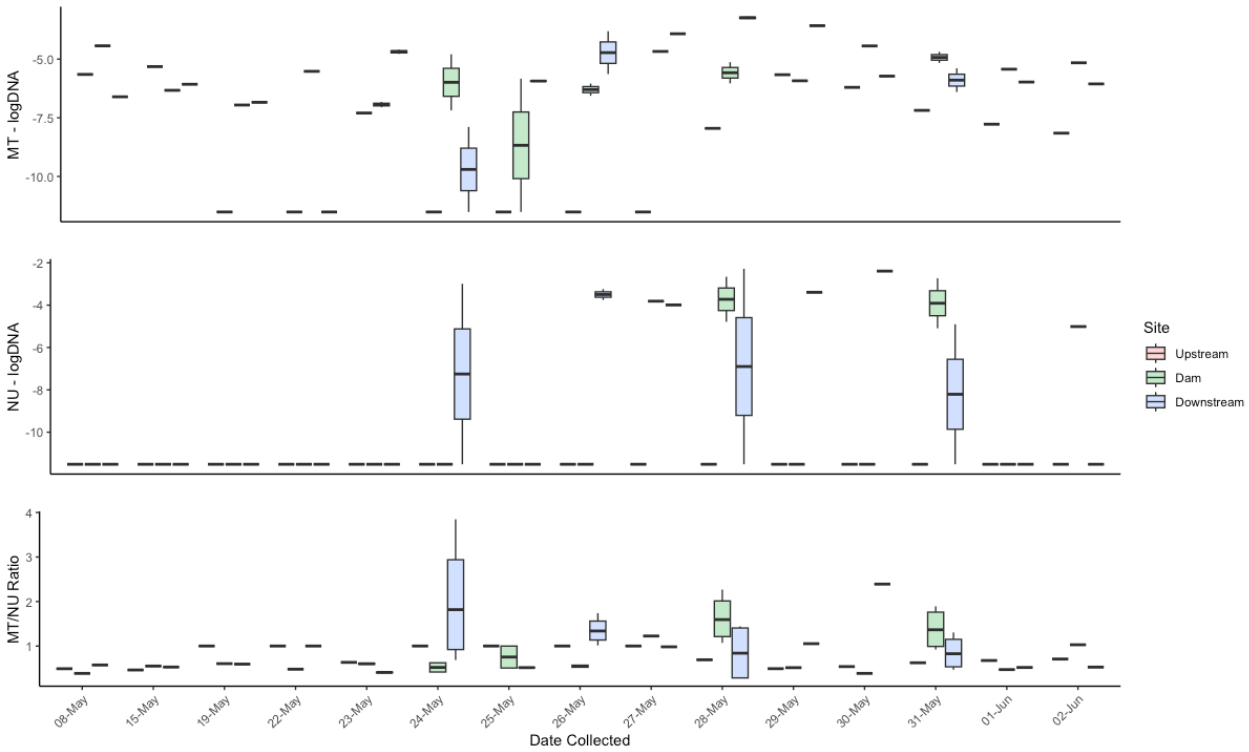

Figure 3.7 Detected DNA concentrations (log-transformed) for the detection of mitochondrial DNA (MT), nuclear DNA (NU), and the ratio of both (as seen above from top to bottom) for all the collection dates across the three collection sites comparing the morning and afternoon concentrations for samples collected from the dam (green) and downstream (blue). Upstream (red) samples were only collected in the morning.

3.3.3 Model Selection

The model with the best support for explaining the concentration of eDNA included water temperature, assay type and site (Table 3.5). The model was statistically significant ($F_{4, 109}$ = 13.73, $p < 0.001$), explaining approximately 33.5% of the variance in the dependent variable (R^2 = 0.335). eDNA concentration was positively affected by water temperature ($F_{1,109}$ = 6.85, p = 0.010), higher at the dam site ($F_{1,109}$ = 12.5, $p < 0.001$), and higher for nuDNA than mtDNA during suspected spawning periods ($F_{1,109}$ = 32.1, $p < 0.001$), and the interaction between water temperature and site was marginally significant ($F_{1,109}$ = 3.43, p = 0.067).

Table 3.5 Comparison of the top six general linear models to explain logDNA concentration, sorted by corrected Akaike Information Criterion (AIC_c). The general linear models were created using the following variables: Water Temperature (ºC), Assay type (mitochondrial or nuclear), Flow (rate of water flowing from the dam at the time of collection, CMS), Site (at or below the dam), and Date collected. *n* = 84 samples.

Ranking	Model	AIC_c	ΔAIC_c
1	logDNA ~ Water_Temp * Site + Assay	560.5	0.0
2	logDNA ~ Water_Temp * Assay	572.0	11.4
3	logDNA ~ Assay	575.6	15.1
4	logDNA ~ Time + Date + Assay + Water_Temp + Hydro + Site	578.0	17.4
5	logDNA ~ Date + Assay * Water_Temp	583.9	23.3
6	logDNA ~ Water_Temp * Site	578.9	27.2

3.4 DISCUSSION

In this thesis chapter, I have demonstrated the ability to detect presumptive Lake Sturgeon spawning events using a newly developed nuclear eDNA assay. I hypothesized that Lake Sturgeon mtDNA would be present in all samples collected, while nuDNA would only be detectable during the predicted spawning window. While testing the nuDNA assay in the lab, I observed a significant difference between samples tested using the mtDNA assay versus the nuDNA assay when sperm was present in the experimental tanks. I additionally observed an amplification of nuDNA in river samples during the suspected spawning period from May 24th to June 2nd. Finally, it was determined that environmental factors like water temperature and flow rate did not independently affect DNA amplification. However, when paired with the assay type, sampling location and water temperature became significant factors in influencing detection.

3.4.1 Assay Development

The newly developed nuDNA ITS-1 assay successfully amplified Lake Sturgeon DNA in both laboratory and field settings, indicating its potential for effectively detecting an increase in environmental nuDNA that could be related to spawning events. However, this assay also amplified DNA from other sturgeon species, such as White, Shortnose, and Atlantic Sturgeon, revealing its lack of species-specificity. Lake Sturgeon is the only sturgeon species in the Saskatchewan-Nelson River systems draining into Hudson's Bay (COSEWIC, 2017), but within the St. Lawrence drainage, Lake Sturgeon has the potential to overlap with Atlantic and Shortnose sturgeons (COSEWIC, 2011; 2015). Therefore, although cross-reactivity with other species is a limitation in these areas if species-specificity is required, it could present an opportunity to monitor spawning in other sturgeon species. That said, validation and testing is required to determine the assay's applicability in systems beyond the Winnipeg River in identifying presence of sturgeon to the species level and to avoid misidentification and/or false positives for any one species.

3.4.2 Lab Study

In the lab study consisting of three concentrations of added Lake Sturgeon sperm and conducted under controlled environmental conditions (air and water temperatures), mtDNA was detected consistently using the Cyt-*b* marker in 100% of samples, while nuDNA detection using the ITS-1 marker was only found 81% of the time. This discrepancy raises questions about the inconsistent detection of nuDNA, particularly since the experimental tanks were kept under uniform conditions.

False negatives in eDNA studies are not uncommon, and several factors documented in the literature could help explain this inconsistency. One possibility is the differential degradation rates between mitochondrial and nuclear DNA. Mitochondrial DNA is often more abundant and resistant to degradation than nuclear DNA due to its smaller size, circular structure, and higher copy number per cell (Jo et al., 2019; Turner et al., 2014). Jo et al. (2020) found that nuDNA degraded faster than mtDNA in all treatments when comparing water temperature and biomass, which was predicted to be caused by the structural differences in the two types of DNA (Kornberg, 1974; Lindahl 1993). However, the unpredictable nature of detection using the nuDNA assay in water samples from the same experimental tanks in the present study suggests that complete degradation of nuDNA was unlikely in this case.

Another factor to consider is the potential for eDNA to become unevenly distributed in the water column. Turner et al. (2014) observed that eDNA concentrations can fluctuate due to factors such as water currents, organismal behaviour, and sampling methodology. In the lab study, even small-scale water movement or sampling inconsistencies could have caused variable detection of nuDNA, resulting in false negatives. Additionally, the cellular origin of eDNA might play a role; nuclear DNA is often associated with intact cells, which may settle out of the water column or degrade more quickly than cell-free mtDNA fragments, further contributing to patchy distribution and detection (Thomsen & Willerslev, 2015). Indeed, the structural integrity of the nuDNA used for this study may have been compromised as the sperm was previously frozen. Future studies should consider the freeze thaw cycles of the sperm samples prior to starting the experiment.

These observations underscore the complexity of interpreting eDNA data and highlight the need for further studies to optimize sampling methods and understand eDNA dynamics in aquatic environments. A better understanding of these factors will enhance the reliability of eDNA assays for monitoring sturgeon populations and other aquatic species.

3.4.3 Field Detection of Spawning Lake Sturgeon

3.4.3.1 Assay Type

mtDNA was detected in 48 out of the 57 field samples (84%) collected between May 24th and June 2nd, 2023, whereas nuDNA was detected in 14 of the 57 samples (25%). In both cases, peak detection occurred in samples collected on May 28th, 2023. Our sampling area, just downstream of Pointe du Bois generating station, is a known spawning area for Lake Sturgeon (Gillespie et al., 2020), and previous studies have shown an increase in presence of spawning adults within the sampling timeframe we used in this study (McDougall et al., 2014). The shorter detection window for nuDNA, contrasted with the extended detection of mtDNA, can be explained by a more rapid degradation of nuDNA in comparison with mtDNA. However, the increased detection of both mtDNA and nuDNA can be explained by an increase in cellular debris released from Lake Sturgeon in the area, with the increased detectability of nuDNA suggestive of spawning (Bylemans et al., 2017). To confirm the link between nuDNA and spawning in any species of fish, prior knowledge of the species' life history is critical for appropriate interpretation of the data.

In a previous study, spawning events were determined via transient increases in the mtDNA concentration of the targeted species (Tsuji & Shibata, 2020). Using two species of medaka (*Oryzias latipes* and *Oryzias sakaizumi*), the researchers were able to validate when the

fish spawned in a laboratory setting as they visually confirmed spawning behaviour at the same time as sampling the water. However, increases in mtDNA alone may not be sufficient to identify spawning events as it can be explained by an increase in biomass of the target species but not necessarily an increase in release of gametes into the water column (Tsuji & Shibata, 2020).

Inference of spawning in wild populations of fish is much stronger when mtDNA detection is paired with nuDNA detection. For example, studies on Macquarie Perch, Common Carp (*Cyprinus carpio*), Largemouth Bass (*Micropterus salmoides*), and Bluegill Sunfish (*Lepomis macrochirus*) have all employed the paired assay method to detect spawning events in the field (Bylemans et al., 2017; Wu et al., 2022). Similar to our findings, Wu (2022) observed that mtDNA levels for Common Carp, Largemouth Bass, and Bluegill Sunfish increased as these species entered the area to prepare for spawning. However, a transient increase in both mtDNA and nuDNA was only detected in Common Carp, not in Largemouth Bass or Bluegill Sunfish during a known spawning event (Wu et al., 2022). It was predicted that an increase in nuDNA from the other species was not seen due to differences in spawning behaviour (Wu et al., 2022). Macquarie Perch samples showed a similar pattern to our findings for Lake Sturgeon mtDNA and nuDNA in the Winnipeg River. Increases in both mtDNA and nuDNA concentrations were detected from field samples during the predicted 2-day spawning event, both of which were significantly higher than detected concentrations from 3 weeks prior (Bylemans et al., 2017).

Since nuDNA degrades shortly after spawning while mtDNA persists, detecting both types of DNA is crucial for confirming spawning events that might otherwise go undetected. Consequently, consideration of nuDNA and mtDNA decay rates is useful when determining the most suitable time for water sampling and possible detection of a spawning event is particularly

relevant when considering species that may spawn within a narrow temperature range (Bylemans et al., 2017; Tsuji and Shibata, 2021).

3.4.3.2 Water Temperature

Since there was no visual confirmation of a spawning event during the sampling period in the present study, relying on prior knowledge of Lake Sturgeon spawning behaviour is essential. Previous research indicates that Lake Sturgeon in this system initiate spawning when water temperatures reach between 10ºC and 12ºC (Zubair et al., 2012). In this study, eDNA concentrations of Lake Sturgeon increased alongside rising water temperatures, with mtDNA detection occurring across a broader temperature range. However, nuDNA was only detected between 12ºC and 16ºC (Figure 3.9). As water temperature measurements were taken at the surface, it is likely that the benthic temperatures were few degrees cooler, aligning with preferred temperatures for spawning in Lake Sturgeon. Given that nuDNA was detected at the dam and downstream between May 24th and June 2nd, it can be inferred that the Pointe du Bois spawning event occurred within this window, following a trend from previous studies (Gillespie et al., 2020). AIC modeling further supports the role of water temperature as a key factor influencing the detection of the spawning event via eDNA.

The distribution of a species may additionally be affected by water temperatures in the lead up to a spawning event. Species that migrate for spawning events are typically driven by environmental cues. The Ayu (*Plecoglossus altivelis*) alters its spatiotemporal distribution based on the temperature of the water (Nagayama et al., 2023). While the temperature requirements to initiate spawning for this species remain relatively the same, the area in which they spawn in the Nagara River, Japan, varies from year to year. This poses a challenge when selecting areas for

sampling efforts to take place as temperature can vary in flood years, and increased flow rates (Nagayama et al., 2023).

Lake Sturgeon are known to be poor swimmers (McDougall et al., 2014) and, as such, are unable to ascend reaches of the river with significant elevation change and high flow. However, it is well known that rapids and areas of high flow downstream of hydroelectric dams often provide good spawning habitat for Lake Sturgeon (Barth et al., 2011). During spawning events, as broadcast spawners, a female Lake Sturgeon can release upwards of 700,000 eggs in high flow areas at the base of rapids or dams (Barth et al., 2011; Gillespie et al., 2020). Males will release sperm concurrently, and the fertilized eggs disperse and adhere onto rocky substrate to develop, prior to larvae emerging and drifting downstream to suitable habitat for foraging (Barth et al., 2009). These spawning events are temperature dependent and lead to an enormous amount of species-specific DNA released into the environment. Thus, coupling knowledge of ideal spawning habitat with eDNA collection enhances the likelihood of positive detection.

3.4.3.3 Rate of Flow

Flow rates within a system can distort the accuracy of sampling sites, making it difficult to pinpoint the exact location of a spawning event. However, in our study, flow rate did not have a statistically significant impact on DNA presence during the sampling period. Although models including flow rate ranked low based on AIC, the large amount of DNA released during the spawning event likely overshadowed any effects of flow. Both mtDNA and nuDNA were detectable at the dam and the downstream collection site during the presumed spawning period. Research conducted by Gillespie and colleagues (2020) found that the bulk of eggs released by Lake Sturgeon takes place at the base of the Pointe du Bois dam. While some eggs do manage to

travel downstream, much of the spawning event is localized (Gillespie et al., 2020). eDNA can travel even further than Lake Sturgeon eggs, increasing the distance in which a spawning event may be detectable (Jane et al., 2015). Because of this, the distance from the dam and/or spawning grounds plays an important role in the accurate detection of a species downstream of these areas (as previously discussed in Chapter 2).

Several factors can mitigate the influence of flow, most notably the amount of DNA present in the system, which can counterbalance any effect of dilution that might normally occur outside of a spawning event (Curtis et al., 2020). Our research supports that during such events, the substantial reproductive output likely overwhelms any dilution effects from river flow, maintaining detectable nuDNA concentrations even in areas with faster-moving water. Studies have shown that the greater the initial amount of eDNA released, the less impact flow has on detection rates (Pilliod et al., 2014; Wilcox et al., 2016). Moreover, the persistence and transport of eDNA in flowing waters have been well-documented. Deiner and Altermatt (2014) found that mtDNA from invertebrate species in rivers remained detectable over 9 km downstream from the original source. Similarly, a study by Jane et al. (2015) corroborates these findings, showing that eDNA can travel significant distances downstream, maintaining detectability even under complex flow regimes. Jane et al. (2015) found that when caged Brook Trout (S*alvelinus fontinalis*) were introduced into a fishless headwater stream, their DNA could be detected at their furthest sampling site (239.5 m downstream) and predicted that the DNA would be present even further downstream with added sampling sites.

Given the high DNA output and eDNA persistence in flowing waters, flow appeared not to be a limiting factor for detecting Lake Sturgeon eDNA during reproductive periods. Flow,

however, may lead to false positive detections at sites downstream. For example, an unexpected increase in eDNA concentration at a downstream site may not necessarily indicate local spawning activity but could instead reflect a spawning event that occurred upstream (Takahara et al., 2012). This underscores the importance of understanding hydrodynamic factors and developing spatially explicit sampling strategies when using eDNA to monitor spawning events.

To address these challenges, future studies should incorporate more frequent sampling at multiple sites, both upstream and downstream, to better understand the transport dynamics of eDNA and identify the true sources of genetic material. Additionally, integrating hydrological modeling with eDNA sampling could help predict how DNA is dispersed in flowing waters, improving the accuracy of identifying the specific locations of spawning events (Wu et al., 2022). Such approaches are crucial for advancing the utility of eDNA as a tool for monitoring sturgeon and other species in complex aquatic environments.

3.4.3.4 Collection Timing

In this study, the time of day did not significantly affect Lake Sturgeon eDNA detection. As broadcast spawners, Lake Sturgeon release large quantities of genetic material during spawning, resulting in consistent eDNA presence throughout the day (Bylemans et al., 2017). This contrasts with species that exhibit diel activity patterns, where eDNA concentrations fluctuate based on behaviour (Jensen et al., 2022; Tsuji et al., 2024). For instance, Jensen et al. (2022) found that the detection of eDNA for each of the targeted species varied dependent on the time of day the sample was taken, likely due to diurnal patterns of organismal activity. These types of behavioural patterns could lead to false negative detections if the species resides in the area but does not visit the specific habitat type during the time of eDNA collection. Tsuji et al. (2024) observed diel

variation in eDNA concentrations of Japanese Jack Mackerel (*Trachurus japonicus*) with higher concentrations detected during the night, likely due to the nocturnal spawning tendencies of this species.

These studies indicate that, for species with more pronounced diel activity patterns or those that release DNA more gradually, the time of day can significantly influence eDNA detection. However, for broadcast spawners, the large-scale release and dispersion of genetic material during spawning events may override any diurnal variation in eDNA concentrations. By recognizing the differences in eDNA dynamics related to the behaviour of the target species, researchers can tailor their sampling strategies more effectively. While some species require time-specific sampling to account for diel variations, Lake Sturgeon eDNA monitoring can likely proceed with less concern about the time of day, focusing instead on the location of spawning events.

4. CONCLUSION

This thesis investigated the spatial and temporal distribution of Lake Sturgeon in the Winnipeg River system by comparing the presence of eDNA to known movement patterns and evaluating the capabilities of nuDNA as a tool to detect spawning events. The findings presented in Chapters 2 and 3 provide complementary insights into the biology, behaviour, and monitoring of this species, contributing valuable knowledge to ongoing conservation efforts.

In Chapter 2, the spatial and temporal distribution of Lake Sturgeon was examined through field surveys and data analysis. The eDNA results mirrored key patterns in habitat use across different life stages, with distinct seasonal movements aligned with spawning, foraging, and overwintering behaviours (Barth et al., 2009, 2011, 2013; Barth & Anderson, 2015;

McDougall et al., 2013a, 2013b, 2017a, 2017b; Struthers et al., 2017; Gillespie et al., 2020). Previously studied critical habitats, including spawning sites, were confirmed using eDNA, underscoring the importance of preserving these areas to support population recovery (Lallaman et al., 2008; Boase et al., 2011; Gerig et al., 2011; Struthers et al., 2017; Gillespie et al., 2020; Izzo et al., 2022). The data also revealed potential environmental or anthropogenic influences affecting sturgeon distribution, which may inform targeted management strategies when using eDNA as a monitoring tool in the Winnipeg River.

Building upon these spatial findings, Chapter 3 explored the application of eDNA for detecting Lake Sturgeon spawning events. The study demonstrated that eDNA is a sensitive and non-invasive method for monitoring reproductive activity, capable of detecting the presence of spawning sturgeon with high specificity and temporal resolution (Bylemans et al., 2017; Tsuji & Shibata, 2020). This approach successfully captured the timing of spawning events, aligning with observations from conventional field methods such as direct sampling of spawning adults and egg deposition surveys (Struthers et al., 2017; Gillespie et al., 2020). Importantly, the nuDNA assay developed in this study was not species-specific, meaning it could potentially detect other sturgeon species in North America. This characteristic offers exciting opportunities for broader applications, including monitoring of endangered sturgeon species such as the Atlantic Sturgeon or White Sturgeon. However, additional testing is required to validate the assay's effectiveness across different species and environmental contexts (Klymus et al., 2020).

The findings from this thesis highlight the promise of eDNA as a complementary tool in Lake Sturgeon conservation. Its scalability and non-invasive nature make it particularly useful for monitoring populations in remote or otherwise inaccessible areas. When combined with

traditional field methods, eDNA can provide a more comprehensive picture of sturgeon presence and reproductive behaviour, helping to address knowledge gaps and inform management decisions.

Future studies should focus on refining the use of eDNA to improve detection accuracy and expand its applications. Specifically, research into the environmental factors that influence eDNA degradation and transport in freshwater systems is critical (Eichmiller et al., 2016; Tsuji et al., 2017; Jo et al.,2019a, 2019b; Wu et al., 2022). Parameters such as water flow, temperature, and microbial activity can affect how long eDNA persists in the environment and how far it travels from its source (Jane et al., 2015; Shogren et al., 2017). Understanding these dynamics will allow researchers to interpret eDNA results more precisely, particularly in river systems like the Winnipeg River. Additionally, while the assay used in this study was able to detect Lake Sturgeon presence during spawning, further validation across other sturgeon populations and environments is necessary. This could involve testing the assay in regions with sympatric sturgeon species to confirm its ability to distinguish among closely related taxa. Integrating eDNA with other molecular tools, such as metabarcoding, could also allow for simultaneous monitoring of multiple species, providing valuable insights into community-level interactions in sturgeon habitats.

The findings in this study represent a snapshot of Lake Sturgeon distribution and spawning behaviour. Long-term monitoring programs are essential to assess interannual variability and detect trends over time. For example, climate change is expected to have significant impacts on freshwater ecosystems, potentially altering the timing and success of sturgeon spawning events. eDNA could play a pivotal role in tracking these changes and

identifying emerging threats to sturgeon populations. Broad-scale applications of eDNA could also enable monitoring of sturgeon populations across their range in North America, particularly in regions where traditional survey methods are logistically challenging. By applying a consistent eDNA protocol across sites, researchers and managers could compare data from different river systems, identifying range-wide patterns in sturgeon behaviour and habitat use.

From a conservation management perspective, eDNA offers a powerful tool to evaluate the effectiveness of protected areas and restoration projects. For example, eDNA could be used to monitor the presence of reproductive adults in spawning sanctuaries or protected areas over time, providing a non-invasive means of assessing these habitats' effectiveness. Similarly, restoration projects aimed at improving habitat connectivity, such as fish passage structures near hydroelectric dams, could be evaluated using eDNA to monitor sturgeon movement and spawning success. Hydroelectric development remains a significant challenge for sturgeon conservation, altering flow regimes and limiting access to critical habitats. eDNA could support the development of mitigation measures, such as flow management strategies or fish passage technologies, by providing data on sturgeon activity upstream and downstream of barriers.

By combining ecological insights with innovative molecular techniques, this thesis demonstrates the value of eDNA as a tool for monitoring and conserving Lake Sturgeon. The ability of eDNA to detect sturgeon presence, track reproductive activity, and identify critical habitats reinforces its potential as a complementary approach to traditional survey methods. These findings provide a strong foundation for future research and management initiatives aimed at ensuring the sustainability of Lake Sturgeon populations in the Winnipeg River system and beyond. Continued advancements in eDNA methodologies, along with their integration into long-

term monitoring frameworks, will further enhance our ability to adaptively manage sturgeon populations in the face of environmental change. This work exemplifies the importance of combining traditional and molecular tools to address conservation challenges, offering a pathway toward more effective strategies for freshwater species conservation.

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APPENDIX A

Table A1. List of sample sites with abbreviations and locations located along the Winnipeg River. Site names are listed from upstream to downstream.

Site Name	Latitude	Longitude
Keskinen Bay (KB)	50.31038	-95.56529
Pointe du Bois (PB)	50.30066	-95.50830
Eight Foot Falls (SF)	50.24451	-95.54060
Lake Nutimik (NU)	50.14910	-95.66592
Barrier Bay (BB)	50.17284	-95.70002
Dorothy Lake A (DLA)	50.16179	-95.74739
Dorothy Lake B (DLB)	50.15198	-9575606
Otter Falls (OF)	50.14589	-95.80619
Eleanor Lake A (ELA)	50.11704	-95.83506
Eleanor Lake B (ELB)	50.10963	-95.86791
Natalie Lake (NAL)	50.11344	-95.98111
Brookfield (BR)	50.14095	-96.05352
Lac du Bonnet (LB)	50.24451	-96.05860
Winnipeg River A (WRA)	50.28401	-95.99842
Winnipeg River B (WRB)	50.35263	-96.02179

Table A2. Three replicates were taken at each sampling site and were tested for the presence or absence of Lake Sturgeon DNA using qPCR in 2021 (left side) and 2022 (right side). The final row of the table indicates the total amount of positive detections per month across all the sites (2021/2022).

Site Code	Positive Detection (Rep) 2021/2022					
	May	June	July	August	September	October
1-KB	N (0) / NA	N (0) / NA	N (0) / NA	N (0) / NA	N (0) / NA	N (0) / NA
2-PB	N (0) / NA	N (0) / Y (1)	N (0) / Y (1)	N (0) / N (0)	N (0) / Y (1)	N (0) / N (0)
3-SF	Y (2) / NA	Y (3) / Y (1)	Y (1) / Y (1)	N (0) / Y (1)	Y (2) / N (0)	Y (3) / N (0)
4-NU	Y (3) / NA	Y (3) / Y (3)	Y (2) / Y (3)	Y (1) / Y (1)	Y (3) / Y (3)	Y (3) / Y (2)
5-BB	Y (3) / NA	Y (2) / Y (1)	Y (2) / Y (1)	Y (2) / Y (3)	Y (1) / N (0)	Y (2) / Y (2)
6-DLA	Y (3) / NA	Y (2) / Y (3)	Y (2) / NA	Y (1) / Y (2)	N (0) / N (0)	Y (2) / Y (1)
7-DLB	Y (3) / NA	Y (1) / Y (2)	N (0) / Y (2)	N (0) / Y (3)	Y (2) / Y (2)	Y (3) / Y (1)
8-OF	Y (3) / NA	Y (2) / Y (1)	N (0) / Y (3)	N (0) / Y (3)	Y (2) / Y (3)	Y (3) / Y (2)
9-ELA	Y (3) / NA	Y (1) / Y (2)	Y (3) / NA	N (0) /NA	Y (1) / NA	Y (3) / N (0)
10-ELB	N (0) / NA	N (0) / N (0)	N (0) / N (0)	N (0) / N (0)	N (0) / Y (2)	N (0) / N (0)
11-NAL	N (0) / NA	N (0) / NA	N (0) / NA	N (0) / NA	N (0) / NA	N (0) / NA
12-BR	Y (3) / NA	Y (2) / N (0)	Y (2) / Y (1)	Y (1) / Y (2)	Y (1) / N (0)	Y (1) / N (0)
13-LB	Y (3) / NA	Y (2) / Y (2)	Y (2) / Y (2)	Y (2) / Y (3)	Y (1) / Y (3)	N (0) / Y (1)
14-WRA	N (0) / NA	Y (3) / Y (2)	Y (1) / Y (3)	N (0) / Y (1)	Y (2) / Y (3)	Y (1) / N (0)
15-WRB	Y (1) / NA	Y (1) / Y (3)	N (0) / Y (2)	Y (1) / Y (3)	Y (2) / Y (2)	Y (2) / N (0)
Total Positive:	10 / NA	11 / 11	8 / 10	6 / 10	10 / 8	10 / 6

Table A3. Monthly minimum, maximum, mean and median values for water temperature (ºC), Flow (CMS) and DNA concentration (ng/µL) for each collected month in 2021 and 2022. Flow rates collected from the dams were supplied by Manitoba Hydro. Rates of flow are measured in cubic meters per second. The concentration of DNA as determined by a mitochondrial (Cyt *b*) assay was converted from the critical threshold value (C_t) to concentration using the predetermined equation based on its standard curve.

MAY					
Year	Variable	Min	Max	Mean	Median
2021	H_2O Temp (ºC)	11	13.6	12.49	12.4
2022	H_2O Temp (ºC)	-	-	-	-
2021	Flow (CMS)	464.1	659	527	464.1
2022	Flow (CMS)	-	-	-	-
2021	DNA [ ] (ng/µL)	0	0.181	0.0337	0.0078
2022	DNA [ ] (ng/µL)	-	-	-	-
JUNE					
Year	Variable	Min	Max	Mean	Median
2021	H_2O Temp (ºC)	18.1	20.5	19.5	19.3
2022	H_2O Temp (ºC)	16.1	20.1	17.1	17.2
2021	Flow (CMS)	467.2	542.9	493	497.2
2022	Flow (CMS)	3182.4	3410.5	3252.6	3182.4
2021	DNA [ ] (ng/µL)	0	0.0072	0.0017	0.0015
2022	DNA [ ] (ng/µL)	0	0.0038	0.0016	0.0015
JULY					
Year	Variable	Min	Max	Mean	Median
2021	H_2O Temp (ºC)	21.5	25.1	23.2	23.1
2022	H_2O Temp (ºC)	20.5	24	21.9	21.9
2021	Flow (CMS)	207.1	341.7	309	319.5
2022	Flow (CMS)	2707.7	2885.3	2762.3	2707.7
2021	DNA [ ] (ng/µL)	0	0.0063	0.0012	0.0005
2022	DNA [ ] (ng/µL)	0	0.0064	0.0008	0.0004

AUGUST					
Year	Variable	Min	Max	Mean	Median
2021	H_2O Temp (ºC)	21.9	25.8	23.7	23.6
2022	H_2O Temp (ºC)	22	27.5	24	23.6
2021	Flow (CMS)	156.6	210.2	192.2	210.2
2022	Flow (CMS)	2240.7	2264.2	2256.9	2264.2
2021	DNA [ ] (ng/µL)	0	0.0019	0.0004	0
2022	DNA [ ] (ng/µL)	0	0.0053	0.0012	0.0012
SEPTEMBER					
Year	Variable	Min	Max	Mean	Median
2021	H_2O Temp (ºC)	16.5	20	17.9	18
2022	H_2O Temp (ºC)	13.2	16.9	15.8	16.1
2021	Flow (CMS)	166.7	246.7	203.7	212.5
2022	Flow (CMS)	1805.3	1952.5	1805.3	1850.59
2021	DNA [ ] (ng/µL)	0	0.0075	0.0029	0.0017
2022	DNA [ ] (ng/µL)	0	0.0039	0.0007	0.0004
OCTOBER					
Year	Variable	Min	Max	Mean	Median
2021	H_2O Temp (ºC)	10.8	16.1	13.9	14.1
2022	H_2O Temp (ºC)	13	16.1	14.6	15.1
2021	Flow (CMS)	156.5	265.6	201.6	209
2022	Flow (CMS)	885.6	944	903.6	885.6
2021	DNA [ ] (ng/µL)	0	0.0019	0.001	0.0012
2022	DNA [ ] (ng/µL)	0	0.0021	0.0005	0

Table A4. Tukey HSD Results – Water temperature and month.

Comparison	Mean Difference	Lower CI	Upper CI	*p*-Value
June-May	5.796154	4.4821681	7.1101396	<0.001
July-May	10.080769	8.7667835	11.3947550	<0.001
August-May	11.350000	10.0360142	12.6639858	<0.001
September-May	4.365385	3.0513989	5.6793704	<0.001
October-May	1.792308	0.4783219	3.1062934	<0.05
July-June	4.284615	3.2117505	5.3574803	<0.001
August-June	5.553846	4.4809813	6.6267110	<0.001
September-June	-1.430769	-2.5036341	-0.3579044	<0.05
October-June	-4.003846	-5.0767110	-2.9309813	<0.001
August-July	1.269231	0.1963659	2.3420956	<0.05
September-July	-5.715385	-6.7882495	-4.6425197	<0.001
October-July	-8.288462	-9.3613264	-7.2155967	<0.001
September-August	-6.984615	-8.0574803	-5.9117505	<0.001
October-August	-9.557692	-10.6305572	-8.4848274	<0.001
October-September	-2.573077	-3.6459418	-1.5002120	<0.001

Table A5. Tukey HSD Results – Flow and month.

Comparison	Mean Difference	Lower CI	Upper CI	*p*-Value
June-May	1345.79173	361.64575	2329.93771	<0.001
July-May	1008.67000	24.52402	1992.81598	0.0410963
August-May	697.54892	-286.59706	1681.69490	0.3206575
September-May	500.11492	-484.03106	1484.26090	0.6845875
October-May	25.54881	-958.59717	1009.69479	0.9999997
July-June	-337.12173	-1140.67356	466.43010	0.8300445
August-June	-648.24281	-1451.79464	155.30902	0.1886057
September-June	-845.67681	-1649.22864	-42.12498	0.0328940
October-June	-1320.24292	-2123.79475	-516.69110	<0.001
August-July	-311.12108	-1114.67290	492.43075	0.8727190
September-July	-508.55508	-1312.10690	294.99675	0.4505221
October-July	-983.12119	-1786.67302	-179.56936	<0.001
September-August	-197.43400	-1000.98583	606.11783	0.9804440
October-August	-672.00012	-1475.55194	131.55171	0.1575766
October-September	-474.56612	-1278.11794	328.98571	0.5295281

Table A6. Tukey HSD Results – DNA [ ] and month.

Comparison	Mean Difference	Lower CI	Upper CI	*p*-Value
June-May	-3.204565e-02	-0.04945568	-0.01463563	<0.001
July-May	-3.269925e-02	-0.05010927	-0.01528922	<0.001
August-May	-3.288708e-02	-0.05029710	-0.01547705	<0.001
September-May	-3.185520e-02	-0.04926522	-0.01444517	<0.001
October-May	-3.294381e-02	-0.05035383	-0.01553378	<0.001
July-June	-6.535908e-04	-0.01486882	0.01356163	0.9999941
August-June	-8.414215e-04	-0.01505665	0.01337380	0.9999793
September-June	1.904581e-04	-0.01402477	0.01440568	1.0000000
October-June	-8.981512e-04	-0.01511338	0.01331707	0.9999713
August-July	-1.878308e-04	-0.01440306	0.01402739	1.0000000
September-July	8.440488e-04	-0.01337118	0.01505927	0.9999789
October-July	-2.445604e-04	-0.01445979	0.01397067	1.0000000
September-August	1.031880e-03	-0.01318335	0.01524711	0.9999430
October-August	-5.672962e-05	-0.01427196	0.01415850	1.0000000
October-September	-1.088609e-03	-0.01530383	0.01312662	0.9999257

APPENDIX B

Table B1. Non-target species used for *in vitro* validation and DNA concentrations extracted from fin tissue. Extracted DNA was used to test the specificity and efficiency of the ITS-1 assay. Table indicates if the DNA was amplified during qPCR.

Species	Amplified with ITS-1
Atlantic Sturgeon (*Acipenser oxyriynchus*)	Yes
Cisco (*Coregonus artedi*)	No
Common Carp (*Cyprinus carpio*)	No
Fathead Minnow (*Pimephales promelas*)	No
Muskellunge (*Esox masquinongy*)	No
Shortnose Sturgeon (*Acipenser brevirostrum*)	Yes
Silver Lamprey (*Ichthyomyzon unicuspis*)	No
Walleye (*Sander vitreus*)	No
White Sturgeon (*Acipenser transmontanus*)	Yes

Table B2. Three replicates were taken at each sampling site and were tested for the presence or absence of Lake Sturgeon DNA using qPCR for mitochondrial (left side) and nuclear DNA (right side). The final row of the table indicates the total amount of positive detections per assay across all the sampled dates (MT / NU).

Date Collected	Positive Detection (Rep) MT/NU		
	Upstream 3	Dam 1	Downstream 2
May 8	Y (3) / N (0)	Y (3) / N (0)	Y (1) / N (0)
May 15	Y (1) / N (0)	Y (3) / N (0)	Y (2) / N (0)
May 19	N (0) / N (0)	Y (1) / N (0)	Y (2) / N (0)
May 22	N (0) / N (0)	Y (1) / N (0)	N (0) / N (0)
May 23 – AM	Y (1) / N (0)	Y (2) / N (0)	Y (3) / N (0)
May 23 – PM	NA	Y (1) / N (0)	Y (3) / N (0)
May 24 – AM	N (0) / N (0)	Y (3) / N (0)	N (0) / Y (1)
May 24 – PM	NA	Y (2) / N (0)	Y (1) / N (0)
May 25 – AM	N (0) / N (0)	N (0) / N (0)	Y (1) / N (0)
May 25 – PM	NA	Y (2) / N (0)	Y (3) / N (0)
May 26 – AM	N (0) / N (0)	Y (3) / N (0)	Y (3) / Y (1)
May 26 – PM	NA	Y (2) / N (0)	Y (3) / Y (2)
May 27	N (0) / N (0)	Y (3) / Y (3)	Y (1) / Y (1)
May 28 – AM	Y (1) / N (0)	Y (3) / Y (3)	Y (3) / Y (1)
May 28 – PM	NA	Y (3) / Y (2)	Y (3) / N (0)
May 29	Y (2) / N (0)	Y (2) / N (0)	Y (3) / Y (3)
May 30	Y (2) / N (0)	Y (3) / N (0)	Y (3) / Y (1)
May 31 – AM	Y (3) / N (0)	Y (3) / Y (1)	Y (2) / N (0)
May 31 – PM	NA	Y (3) / Y (1)	Y (3) / Y (2)
June 1	Y (1) / N (0)	Y (3) / N (0)	Y (3) / N (0)
June 2	Y (1) / N (0)	Y (3) / Y (2)	Y (3) / N (0)
Total Positive:	9 / 0	20 / 6	19 / 8

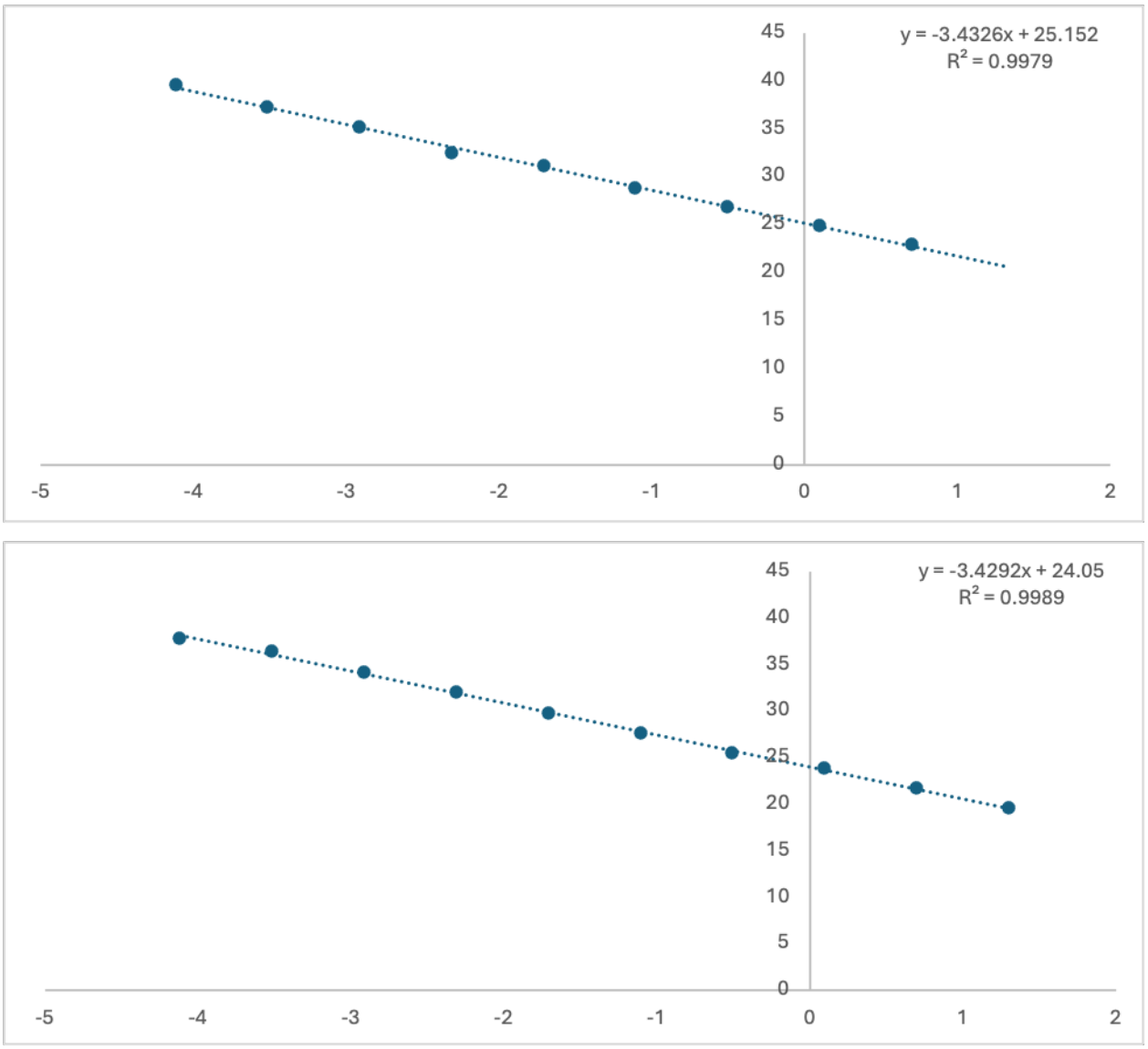

Figure B1. Lake Sturgeon standard curves using milt (top) and tissue-extracted DNA (bottom) using the ITS-1 assay (version 7). Curves were made using a 4-fold dilution series.

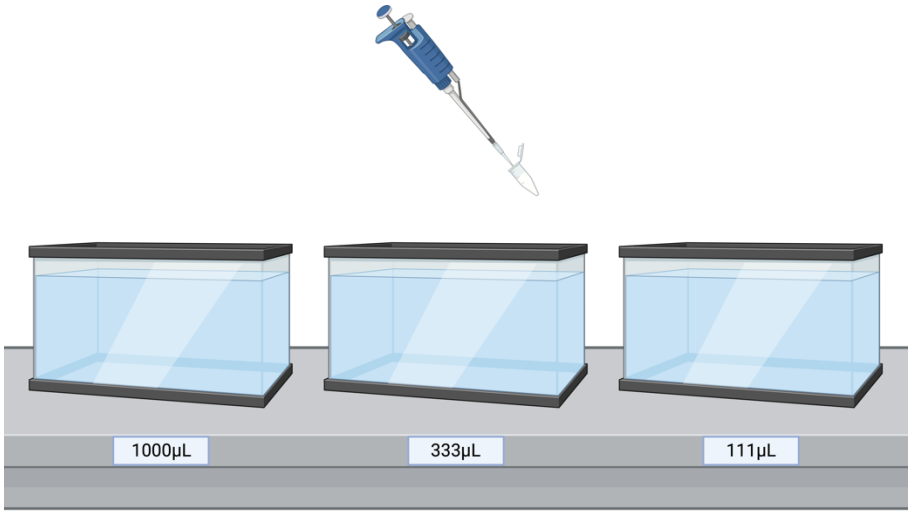

Figure B2. To test the rate of degradation of Lake Sturgeon nuclear eDNA in milt, identical 9-L tanks were set up in an environmental chamber. Three experimental replicates were created for each concentration: low (111 µL milt), mid (333 µL), and high (1000 µL). 500 mL of water was collected 20 minutes, 40 minutes, and 1, 3, 6, 9, 12, 24 and 48 hours post addition from each tank.

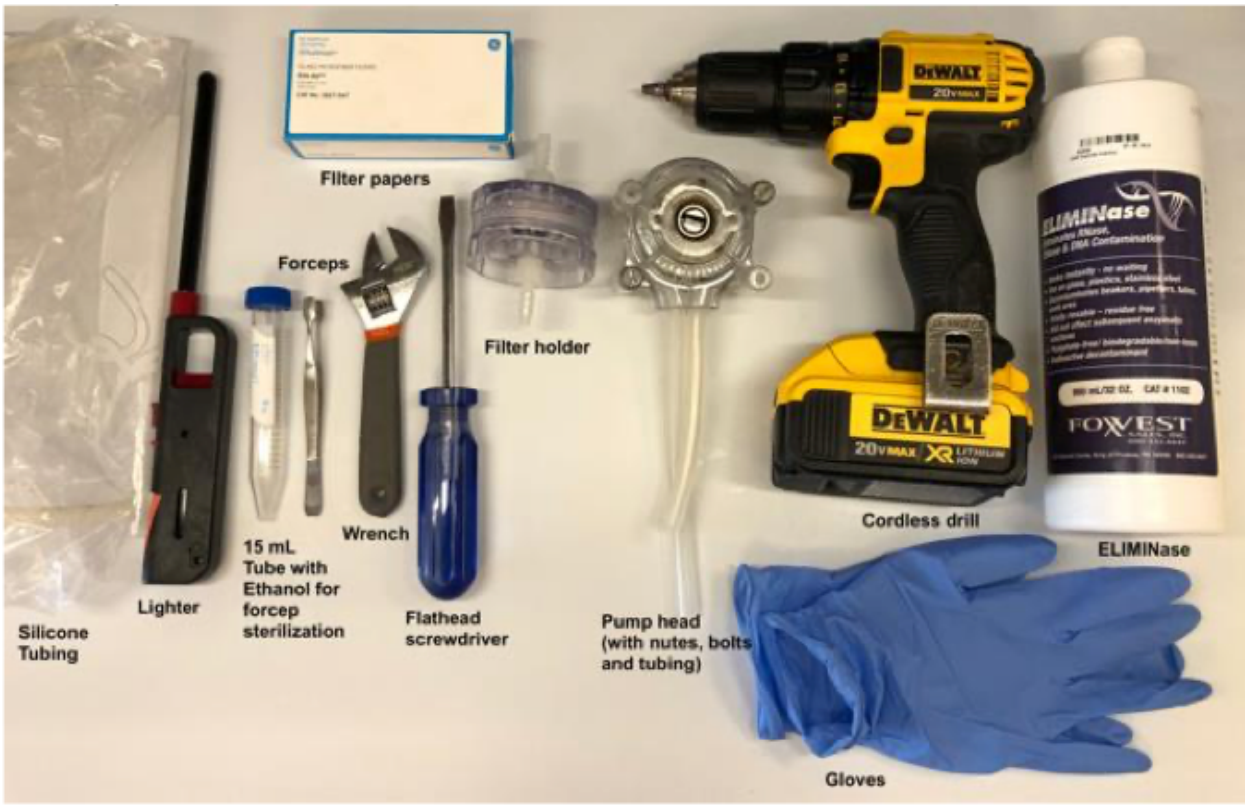

Figure B3. Materials for assembling and using the DIY peristaltic pump (GEN-FISH; https://gen-fish.ca/).