Monitoring Protocol for the Nearshore Ecosystem in the South Basin of Lake Winnipeg

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2020

Canadian Technical Report of
Fisheries and Aquatic Sciences 3386

Fisheries and Oceans Canada | Pêches et Océans Canada

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Canadian Technical Report of Fisheries and Aquatic Sciences

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MONITORING PROTOCOL FOR THE NEARSHORE ECOSYSTEM IN THE SOUTH BASIN OF LAKE WINNIPEG

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Cat. No. Fs97-6/3386E-PDF ISBN 978-0-660-35424-8 ISSN 1488-5379

Correct citation for this publication:

Aminot, M., Caskenette, A.L., and Enders, E.C. 2020. Monitoring protocol for the nearshore ecosystem in the south basin of Lake Winnipeg. Can. Tech. Rep. Fish. Aquat. Sci. 3386: viii + 36 p.

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Since their introduction, Aquatic Invasive Species (AIS) have posed major threats to Canadian waterbodies, industries, and tourism. As AIS continue to invade and change Canada's native ecosystems, provincial, territorial, and federal governments are acting through the implementation of new measures and laws in hopes of controlling the spread. However, the lack of public awareness and scientific knowledge of AIS is hindering these efforts, and therefore the management of their spread remains a key issue that needs to be addressed. Since 2013, Zebra Mussels (*Dreissena polymorpha*) have invaded Lake Winnipeg and are thought to affect the lake's ecosystem. The purpose of this technical report is to present a standardized monitoring protocol of the nearshore ecosystem of the south basin of Lake Winnipeg in order to determine the effects of Zebra Mussels on the nearshore food web. This report provides detailed sampling methods for the collection of water, fishes, zooplankton, benthic invertebrates, mussels, and sediment, as well as monitoring guidelines for environmental variables such as water depth, wave frequency, light penetration, and water temperature. Data emerging from this field work will benefit the monitoring and the control of AIS as well as provide insight on the population status of native species in Lake Winnipeg.

RÉSUMÉ

Aminot, M., Caskenette, A.L., and Enders, E.C. 2020. Monitoring protocol for the nearshore ecosystem in the south basin of Lake Winnipeg. Can. Tech. Rep. Fish. Aquat. Sci. 3386: viii + 36 p.

Depuis leur introduction, les espèces aquatiques envahissantes (AIS) représentent une menace majeure pour les eaux, les industries, et le tourisme au Canada. Alors que les AIS continuent d'envahir et de modifier les écosystèmes indigènes du Canada, les gouvernements provinciaux, territoriaux et fédéraux du Canada agissent par la mise en œuvre de nouvelles mesures et lois dans l'espoir de contrôler la propagation. Cependant, la lacune de sensibilisation du public et de connaissances scientifiques sur les AIS entrave ces efforts et, par conséquent, la gestion de leur propagation reste un problème clé qui doit être résolu. Depuis 2013, la moule zébrée (*Dreissena polymorpha*) a envahi le lac Winnipeg et elle est crue affecter l'écosystème du lac. Le but de ce rapport technique est de présenter un protocole de surveillance normalisé pour l'écosystème côtier du bassin sud du lac Winnipeg afin de déterminer les effets des moules zébrées sur le réseau trophique littoral. Ce rapport fournit des méthodes d'échantillonnage détaillées pour la collecte de l'eau, des poissons, du zooplancton, des invertébrés benthiques, des moules et du sédiment, ainsi que des directives de surveillance des variables environnementales telles que la profondeur de l'eau, la fréquence des vagues, la pénétration de la lumière et la température de l'eau. Les données issues de ce travail sur le terrain profiteront à la surveillance et au contrôle des AIS et fourniront un aperçu de l'état des populations d'espèces indigènes du lac Winnipeg.

ACKNOWLEDGMENTS

We wish to acknowledge the contributions of the members of the DFO Riverine Ecology lab who collected and processed the field samples, the DFO Chemistry lab for the analysis of water and sediment samples, and Environment and Climate Change Canada (ECCC) for the analysis of Zebra Mussels. A warm thanks to Darcy McNicholl and James Marcaccio for the detailed review and comments on the report manuscript.

1.0 INTRODUCTION

Canada is home to approximately two million lakes (Boyce 2006). These water sources house a multitude of aquatic species such as plants, fish, invertebrates, and planktons. In addition, Canada's waterbodies are crucial to the country's economy as they contribute to its annual multi-million dollar fishing industry, its tourism, as well as other essential industries such as hydro-electric power, and the distribution of potable water (DFO 2004; Wasssenaar and Rao 2012; ECCC 2020). However, Canadian waterbodies are facing environmental consequences that cannot be overlooked, namely the introduction of Aquatic Invasive Species (AIS). AIS are plants, animals, and micro-organisms that, when introduced, can pose major threats to novel aquatic ecosystems (DFO 2018). These invaders often share common characteristics with native species, resulting in frequent competitions concerning reproduction, predation, and the ability to adapt to new environments (DFO 2018). For centuries, Canada's waters have been affected by the presence of hundreds of AIS (DFO 2004; Wassenaar and Rao 2012). As the volume and speed of the global trade increases, so does the rate of invasions in Canadian waters (Briski *et al.* 2012; Sylvester and MacIsaac 2013; Seebens *et al.* 2019). These new invasions are primarily seen as a result of AIS presence on ocean-going shipping vessels, specifically on the ship's hull, and in ballast water and sediments (Wonham *et al.* 2005; Sylvester and MacIsaac 2013; Chan *et al.* 2015). However, other introduction pathways such as fish stocking, recreational boating, releasing of live bait, opening of canals and waterways, and the escape of organisms from aquariums also contribute to this issue (Lindgren 2006; ECCC 2011). Alien species originating from countries or regions with similar climates to Canada are especially detrimental to Canadian waters as they have a better chance of invasion, posing threats to Canada's native aquatic ecosystem (DFO 2004).

In 1993, the Government of Canada recognized the threats of invasive species, and has since implemented control measures to stop the spread (DFO 2004), such as mandatory mid-ocean ballast-water exchanges for all vessels coming from foreign and USA ports (Wonham *et al.* 2005; Rup *et al.* 2010; Sylvester and MacIsaac 2013). Despite these measures, AIS continue to invade Canadian waters. The rate of 15 new alien species has been recorded to occur in Canada's coastal and inland waters every decade (DFO 2004). Although Bailey *et al.* (2011) have shown a more recent decrease of AIS invasions seen in the Great Lakes since the implementation of ballast-water regulations, the numerous other pathways for AIS introduction and dispersal remain an issue as these invaders continue to threaten Canada's ecosystem. In fact, AIS are affecting the fishing industry by endangering and eliminating indigenous fish, and are responsible for damage to human infrastructure (DFO 2004). This loss of revenue and the implementation of control measures against AIS are costing billions of dollars annually to the government (DFO 2004). Today, there are numerous provincial, territorial, and federal programs that focus on the threat of AIS. In Manitoba, the province is focused on public outreach and education in order to prevent the introduction of AIS into provincial waters. This includes the distribution of written material, presentations, surveys, and boat launch signs (Lindgren 2006; ECCC 2011). In addition, they have implemented monitoring programs and have developed policies to improve provincial preparedness for AIS introduction (ECCC 2011).

Despite the recent efforts to control the introduction and spread of AIS, the current lack of effective tools and procedures available to target alien species is responsible for a shortage of knowledge of environmental, social, and economic consequences attributed to the presence of AIS (DFO 2004; Wassenaar and Rao 2012). In an effort to address this issue, and in compliance with the province's efforts to improve AIS awareness, this report aims to provide a detailed scientific description of the Lake Winnipeg Nearshore

Monitoring Program. This report offers several detailed sampling methods, namely water, sediment, fish, zooplankton, benthic invertebrate, and mussel samples, in order to effectively assess the consequences attributed to the presence of invasive species, primarily that of Zebra Mussels (ZM, *Dreissena polymorpha* (Pallas, 1771)), in three Lake Winnipeg nearshore locations.

The implementation of the sampling methods presented in this technical report can contribute to both scientific and public knowledge on the current state of Lake Winnipeg's nearshore communities. Data emerging from this field work will benefit the monitoring and the control of AIS as well as provide insight on the population status of native species.

2.0 LAKE WINNIPEG

With boundaries extending 436 km from north to south, covering a total area of 23,750 km^2, Lake Winnipeg (LWPG) is the 10^{th} largest freshwater body in the world (Wassenaar and Rao 2012; ECCC 2020), and the 6^{th} largest in Canada (Nag 2019; ECCC 2020). The lake consists of three subdivisions, that is, the north basin, south basin, and the Narrows (Figure 1). The north basin covers the largest portion of LWPG, namely 74% of its area coverage (ECCC 2011). With the water current flowing from south to north, the north basin holds approximately 81% of the lake's volume, and is the deeper of the two basins with an average depth of 13.3 m compared to the south's more shallow average depth of 9 m (ECCC 2011). The two basins differ in water clarity, nutrient concentrations, and in abundance and composition of phytoplankton communities (ECCC 2011). Given these differences, the two basins are considered as distinct water bodies (ECCC 2020). The Narrows is the channel separating the two basins, and represents 15% of LWPG's area with depths extending to nearly 60 m north of Black Island (Pip 2006; ECCC 2011).

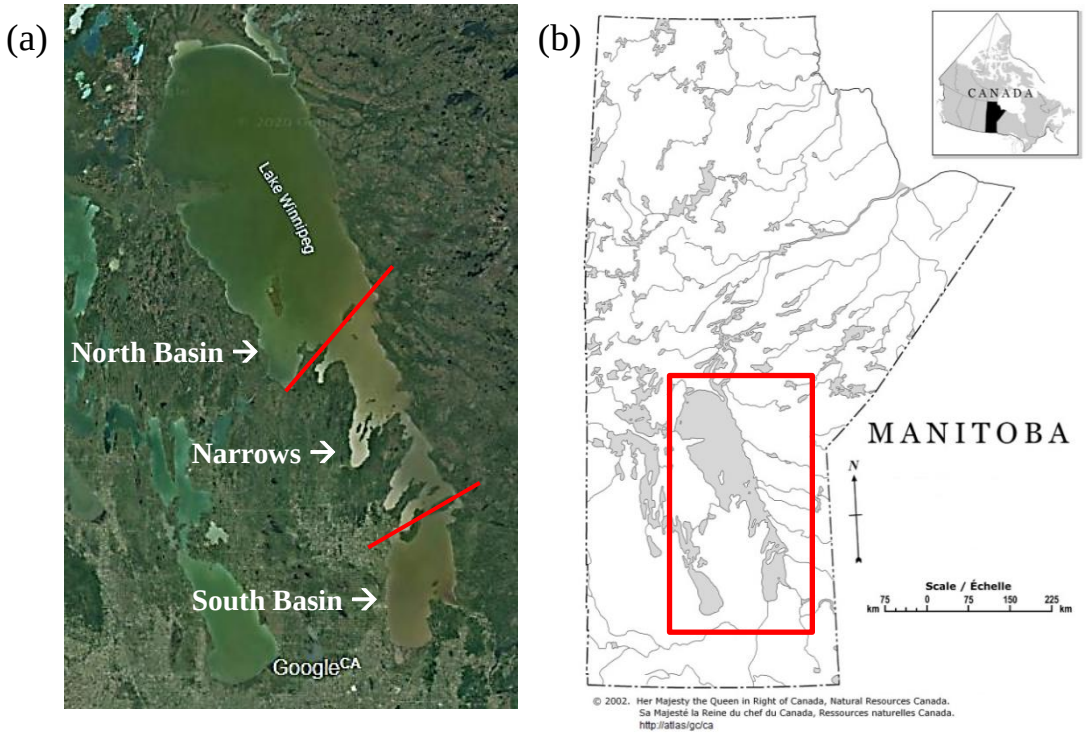

Figure 1. (a) Google Earth (version 9.3.111.1) satellite image of Lake Winnipeg (b) in Manitoba, Canada. Lake Winnipeg consists of three subdivisions, namely the north and south basins linked by the Narrows. The north basin is the largest subdivision of the lake, followed by the Narrows, and the south basin.

Several lakes and rivers contribute to LWPG's watershed. In fact, its tributaries stretch along several provinces (i.e., Alberta, Saskatchewan, Manitoba, and Ontario) and states (i.e., Montana, North Dakota, South Dakota, and Minnesota) (ECCC 2020). Amongst these lakes and rivers contributing to LWPG's water levels, its main water sources originate from the Saskatchewan, Red, Winnipeg, and Dauphin rivers (Pip 2006; Wassenaar and Rao 2012; Nag 2019). With an approximate watershed-to-lake ratio of 40:1, LWPG faces some unique legal (i.e., inter-jurisdictional and international) and environmental (e.g., climate variability) challenges in assuring its overall state (ECCC 2011).

Over the years, LWPG's boreal forests, rivers, limestone cliffs, bat caves, and sandy beaches have contributed to its recreational and leisure use (Nag 2019). However, the state of LWPG's ecosystem has recently declined, which has impacted residents and lake users (ECCC 2011). Over the past few decades, accelerated nutrient enrichment from the LWPG watershed has increased the severity and frequency of algal blooms in LWPG, which have been negatively impacting the water quality of the lake (Wassenaar and Rao 2012; Nag 2019; ECCC 2020). In addition, the lake is facing threats caused by the presence of AIS (Nag 2019).

3.0 INVASIVE SPECIES

Aquatic Invasive Species are notable for significantly altering an ecosystem's natural dynamics. Being plants, animals, or micro-organisms originating from other regions, novel ecosystems occupied by AIS

often lack the invader's natural predators and diseases, giving the invaders an advantage in competing for food and other natural resources (ECCC 2011; DFO 2019). As AIS reproduce and disperse over time, the threats that accompany them also increase, leading to long-term consequences for the dynamic of aquatic ecosystems (Strayer *et al.* 1999; DFO 2018).

Along with the common means of entry for AIS in ecosystems (e.g., boating, live bait release), the geographic positioning of LWPG makes it vulnerable to other pathways for AIS. It has a large watershed with many tributaries such as diversions, canals, and rivers that act as entry pathways for AIS into the lake (ECCC 2020). In addition, AIS also find their way into LWPG through other watersheds due to the small land barriers separating them from its waters (ECCC 2011). Moreover, once invaders have entered LWPG's waters, inter-basin transfers pose significant threats of AIS dispersal throughout the lake (ECCC 2011). The presence of an invasive species found in LWPG, namely the Zebra Mussel, is discussed below.

3.1 ZEBRA MUSSELS

3.1.1 Description

Zebra Mussels (ZM) are small triangular (or D shaped) bivalve mollusks with tufts of hair-like filaments, called byssal threads, that extend from the bottom of their shell (Woodward and Quinn 2011). While most of them have distinct rays stretching along their shell, others may vary in appearance with a lack of stripes and a plain, cream, or even black shell colour (Woodward and Quinn 2011; Figure 2). Though adult ZM shells can range from 6–45 mm in length, not exceeding a length of 5 cm (Woodward and Quinn 2011), ZMs are normally 1–3 cm in size (MWFB 2020). These variations are due to the concentrations of algae present in the water and to the temperature of the water, as they affect shell growth (Jantz and Neumann 1998; DFO 2014). Jantz and Neumann (1998) showed that the relationship between shell growth rates and water temperature follows an optimal curve. Furthermore, the amplitude of the curve depends on the availability of algae (measured as Chlorophyll *a* concentrations; Jantz and Neumann 1998). Shell lengths increased to a maximum rate at Chlorophyll *a* concentrations of 20–40 $\mu g \cdot l^{-1}$ and water temperatures of 15–20 °C (Jantz and Neumann 1998). Adult ZMs have little growth potential when water temperatures lie below 10 °C or above 32 °C (Schloesser and Nalepa 2013). In addition, the lifespan of ZMs is temperature dependent. Though ZMs can survive up to 6–9 years, they have an average lifespan of 3–4 years, with warmer lake temperatures making for a shorter lifespan (DFO 2014). ZMs cannot survive when exposed to water temperatures reaching > 24 °C for extended periods of time, and water temperatures > 30 °C can be lethal to ZMs (Woodward and Quinn 2011; Schloesser and Nalepa 2013).

ZM can survive for up to a month out of water, depending on the temperature and humidity levels (MWFB 2020). A number of studies (Ricciardi *et al.* 1995; Ussery and McMahon 1995; Schloesser and Nalepa 2013) observed the effects of relative humidity (RH) and temperature on the survivorship of ZMs in air (i.e., increased out-of-water ZM survival with low temperatures and high RH). Individual ZM specimens can survive up to 10 d out of water in optimal conditions (10 °C, 95% RH) and may survive longer in small clusters or monolayers (Ricciardi *et al.* 1995). Schloesser and Nalepa (2013) reported an estimate of 27.9 d are required for 100% mortality of an unidentified number of ZMs at 5 °C and RH >95%. Larval ZMs, called veligers, however, cannot survive out of water (MWFB 2020).

Figure 2. Image showing three different shell appearances of freshwater Zebra Mussels (*Dreissena polymorpha*). Zebra Mussels normally demonstrate a zebra-like pattern along their shell (left). Some exhibit no stripes, with a solid cream-coloured (right), or dark pigmented (center) shell. (Source: Lake George Association 2020)

3.1.2 Origin and Introduction History

ZMs are native to Eastern Europe and Western Asia, originating from the Black, Caspian, and Azov seas (Woodward and Quinn 2011; ECCC 2011; MWFB 2020). It is thought that ZMs were introduced into North America through the release of freshwater ballasts by commercial ships, resulting in the first discovery of ZMs in Lake St. Clair in 1988 (ECCC 2011; Woodward and Quinn 2011). Despite their discovery being made in the late 80s, it is probable that they had been established and remained undetected in Lake St. Clair 2–3 years prior (Woodward and Quinn 2011). In September of 2009, ZMs were found in the LWPG watershed, in Big Pelican Lake in Minnesota, and a veliger was found on a dam in the Red River the following year (ECCC 2011). In 2013, the first ZM was discovered in LWPG (Jansen, *et al.* 2017; Hann *et al.* 2017) and they have since been found throughout the lake. Juvenile and adult ZMs attach themselves to hard surfaces such as boats and trailers through the means of their byssal threads, resulting in their dispersal throughout waterways (Woodward and Quinn 2011; DFO 2014). In their larval stage, however, ZM veligers cannot yet bind to hard surfaces, and therefore disperse through passive drifting (Woodward and Quinn 2011; ECCC 2011; Karatayev *et al.* 2015).

3.1.3 Habitat

ZMs are found in freshwater waterbodies such as lakes, rivers, ponds, and canals (Woodward and Quinn 2011; DFO 2014). ZMs can be found attached to several different hard substrates, a few examples include rocks, metal, other mollusk shells, crustacean exoskeletons, plants, wood, boats, and manmade products such as PVC pipes (Woodward and Quinn 2011; DFO 2014). With the optimal water temperatures for growth and reproduction ranging between 16–26 °C, they usually settle in relatively shallow water ranging from 2–9 m in depth (Woodward and Quinn 2011; Schloesser and Nalepa 2013). They are rarely found in depths exceeding 50 m due to the lack of large sediment and cold water temperatures (~4 °C; DFO 2014). In fact, water temperatures outside of the optimal range result in a considerable decline in rates of growth and reproduction (Schloesser and Nalepa 2013). Water temperatures <10 °C result in little growth potential, and temperatures exceeding 30 °C, or freezing temperatures are lethal to ZMs (Woodward and Quinn 2011; Schloesser and Nalepa 2013). In addition to water temperature, other factors such as oxygen-availability, calcium-ion concentrations, and pH levels contribute to ZM growth (Woodward and Quinn 2011). ZMs thrive in waters with calcium-ion concentrations of 45–55 $mg \cdot l^{-1}$ and in pH levels of 7.4–8.0 (Woodward and Quinn 2011). They struggle, however, in oxygen-poor waters (Woodward and Quinn 2011). In favorable conditions, ZMs gather, leading to densities as big as 1,000,000 individuals per square meter (DFO 2014). These large clusters of ZMs are called druses (Woodward and Quinn 2011).

3.1.4 Diet

ZMs, in their larval, juvenile, and adult form, are filter feeders (Strayer *et al.* 1999; DFO 2004; Woodward and Quinn 2011). These mollusks consume algae, zooplankton, bacteria, detritus, as well as silt found in the water column (Strayer et al. 1999; Woodward and Quinn 2011). Though ZMs can filter particles of 0.4–450 µm in diameter (Jantz and Neumann 1998; Schloesser and Nalepa 2013), they show optimal filtration rates for particles ranging from 5–35 µm in diameter (Sprung and Rose 1988; Jantz and Neumann 1998; Schloesser and Nalepa 2013). Filtered particles > 50 µm in diameter are often rejected by the production of either feces or pseudofeces (Sprung and Rose 1988; Naddafi *et al.* 2007; Schloesser and Nalepa 2013). Phosphorus (P), nitrogen (N), and carbon (C) are often found in the seston ingested by ZMs (Naddafi *et al.* 2008). These nutrients are either to digest or egest by ZMs, which may alter the nutrient regime of the water column (Naddafi *et al.* 2008; Woodward and Quinn 2011; Schloesser and Nalepa 2013).

3.1.5 Life History

ZMs have three life stages, the larval, juvenile, and adult periods (Woodward and Quinn 2011). The adult stage begins at the age of 1–2 years, once the mollusk has attained sexual maturity (Woodward and Quinn 2011; DFO 2014). Once sexually mature, the female ZM can release up to one million eggs for each spawning occurrence (DFO 2014). As soon as the eggs enter the water column, they can be fertilized by the sperm released by the males (Woodward and Quinn 2011). The fertilized eggs then develop into veligers within a few days (3–5 d) and are free-swimming until they form a clam-like shell and settle on a hard substrate (Woodward and Quinn 2011; DFO 2014). This free-swimming period can last up to a month (DFO 2014). Once attached onto a substrate, the ZM veliger undergoes metamorphosis and develops a small (1–3 mm) triangular shell (Woodward and Quinn 2011). Once the shell is complete, the veliger is then considered a juvenile ZM (Woodward and Quinn 2011). Depending on the water temperature at the time of fertilization, it can take between 8–180 d for the gametes to become juvenile ZMs (Woodward and Quinn 2011). Colder water temperatures make for a slower development time (Woodward and Quinn 2011). For this reason, most spawning occurs between June and September, where the water temperatures lie between the threshold of 12 °C, and the maximum temperature of 20 °C for optimal reproduction and growth (Woodward and Quinn 2011).

3.1.6 Environmental Impacts

The presence of ZMs in a foreign waterbody has numerous impacts on the aquatic environment and its inhabitants. Toxic algal blooms, reduced phytoplankton communities, augmentations in benthic populations, and even variations in the foraging and abundance of native fish populations have been observed in waterbodies invaded by ZMs (Strayer *et al.* 1999; DFO 2004; Woodward and Quinn 2011; Jansen *et al.* 2017). In the lower Great Lakes, ZM filter-feeding activities have resulted in augmentations of water clarity, which, after several years, have led to a significant growth and spread of aquatic vegetation (DFO 2004). The result of several years of increases in light penetration and aquatic vegetation in these lakes has amplified the frequency and severity of toxic algal blooms (DFO 2004). The feeding activities of ZMs also affect phytoplankton populations, leading to changes in zooplankton and fish communities. With large numbers of ZMs in an aquatic environment, phytoplankton populations dramatically decrease in number as they are targeted by ZMs (Woodward and Quinn 2011). With decreased numbers of phytoplankton to feed on, zooplankton populations, upon which many planktivorous fish depend, also diminish (Strayer *et al.* 1999; Woodward and Quinn 2011). However, large ZM druses produce a lot of waste, which increases benthos and bacterial communities who feed on the feces (Strayer *et al.* 1999;

Woodward and Quinn 2011; Burlakova *et al.* 2014; Jansen *et al.* 2017). The presence of additional bacteria produces more food for the ZMs, whereas the additional benthos communities increase the number of benthic-feeding fishes (Woodward and Quinn 2011; Burlakova *et al.* 2014). These fish, namely Lake Sturgeon (*Acipenser fulvescens*), Yellow Perch (*Perca flavescens*), and Freshwater Drum (*Aplodinotus grunniens*), also prey on ZMs (Woodward and Quinn 2011). Consequently, variations in native fish populations may occur as benthic-feeding fish replace those that prey on zooplankton (Woodward and Quinn 2011). Finally, ZMs also affect native mollusks, such as freshwater clams. By latching onto the clam's shell or obstructing its opening, ZMs affect the clam's ability to move through sediment, and prevent the release of gametes, the defensive closing of the shell, and food intake (Schloesser and Nalepa 1994; Woodward and Quinn 2011).

4.0 SAMPLING PROTOCOL

The following sampling protocol uses elements of two existing protocols: (1) the Lake Winnipeg MV Namao fisheries trawl protocol conducted by DFO in collaboration with the Lake Winnipeg Research Consortium and (2) the Lake Winnipeg shoreline and littoral monitoring program protocol conducted by ECCC in the frame of Lake Winnipeg Basin Program.

4.1 Field Sampling

4.1.1 Sampling Site Specifications

Three sites located on the east shore in the south basin of LWPG were chosen for sampling based on the relative abundance of hard substrates (very low, low, and medium). These sites are the following: Patricia Beach, Sunset Beach, and Lester Beach (Figure 3, Table 1). These three sites are sampled five times, starting the 26th week of the year, then every three weeks following. The sites are sampled over a period of one to two days with the first site being randomly selected. Each sampling site is the area encompassed by 180 m along the shoreline out to 20 m.

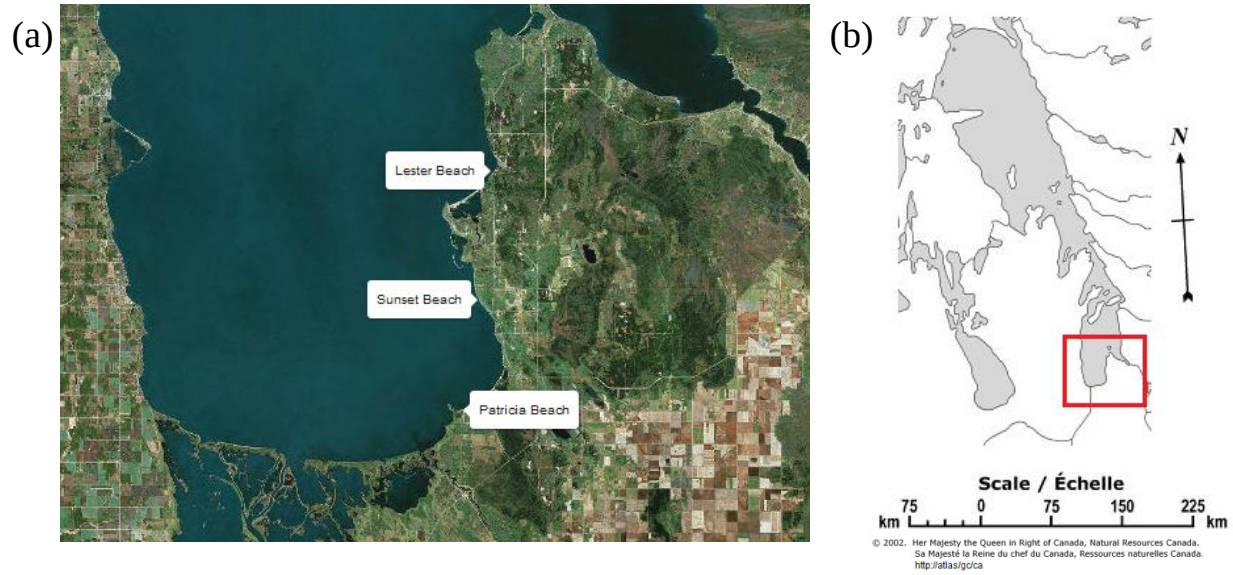

Figure 3. (a) Map of sampling locations in the south basin of (b) Lake Winnipeg. Samples collected for the Nearshore Surveying Program are taken at three beaches, chosen for their relative abundance of hard substrates, on the east shore of Lake Winnipeg; Lester Beach (low abundance), Sunset Beach (medium abundance), and Patricia Beach (very low abundance).

Table 1. Table of nearshore sampling locations. Coordinates, site description, and road accessibility is presented for all three nearshore sampling sites (i.e., Lester, Sunset, and Patricia beaches).

Location	Coordinates	Description	Access
Lester Beach	50.581073, -96.584610	Long sandy beach with some cobble and boulders.	Via trail from Lester Blvd
Sunset Beach	50.496262, -96.598824	Long beach with several groynes, gravel substrate.	Via Rd 102N (off PR 500)
Patricia Beach	50.423570, -96.616360	Large open sandy beach, east of Netley marsh and southwest of another smaller wetland.	Via Provincial Rd 319

4.1.2 Field Equipment and Preparation

Consult Appendix 1 for a complete list of safety, field, processing, and repair gear needed for the nearshore field work.

Before heading to the field site to conduct field work, prepare the following:

- Collect field gear and check equipment for damage
- Refill any items depleted in previous sampling rounds
- Fill four bottles with reverse osmosis water
- Make labels and label sample vials

- Download previous data and reset temperature/light loggers
- Generate random integers for the following, and enter into the field book:
    - The order for three sites to be sampled in
    - One number between 1–9 for the transect that will contain the wave action logger, temperature/light logger, slope measured, and have water sample taken
    - Four numbers from 1–9 (excluding the logger transect number) for the benthic invertebrates and Zebra Mussel transects
        - Randomly generate either 1 or 2 four times; these indicate if the benthic invertebrate transects are completed in the first 10 m from shore or the second 10 m segment (from 10–20 m from shore)
    - Two numbers from 1–9 (excluding the logger transect number) for sediment core chemistry analysis
    - Four numbers from 1–9 (excluding the logger transect number) for the zooplankton transects

- Fill out a field book entry for each site. To save time during the busy sampling day, this can be done in advance. Labels can also be prepared in advance. Write down in the field book the following:

  Date (Day, Month, and Year):
  Site:
  Collector Initials:
  Weather (cloud cover, wind):
  Time the temperature/light logger was set:
  Depth at 5, 10, 15, and 20 m from shore:

  Transect #: (1–9)
  Waypoint (on shore):
  Fish haul #
  Catch (number of bags):
  Zooplankton tow: Y/N
  Kick Net: Y/N
  Sediment Sample: Y/N
  Notes:

4.1.3 Field Procedures

Each site is broken into nine 20 m wide transects. One transect is randomly chosen for wave, temperature, and light measurements. The remaining eight transects are seined with a subset of these transects sampled for the zooplankton and benthic invertebrates.

Upon arrival at the field site, make note of the weather, water, and air temperatures in the field book. If the site's conditions differ from the norm (e.g., new large wood debris), make a note of this in the field book. Starting at the GPS's listed location coordinates (Table 1), measure out and mark the leading edge of the nine transects on the shore. Turn on the GPS before measuring transects to allow it to find the satellites, this may take longer on cloudy days. Latitude and longitude are to be recorded in Decimal Degrees (format = DD.DDDDD°). The map datum must be set to WGS84.

To record the changes in water levels throughout the year(s), the distance of the shoreline from a chosen landmark is to be measured at each site. The position of the following landmarks is represented in Figure 4:

- Lester Beach – Aspen tree
- Sunset Beach – Stair post
- Patricia Beach – Beach sign

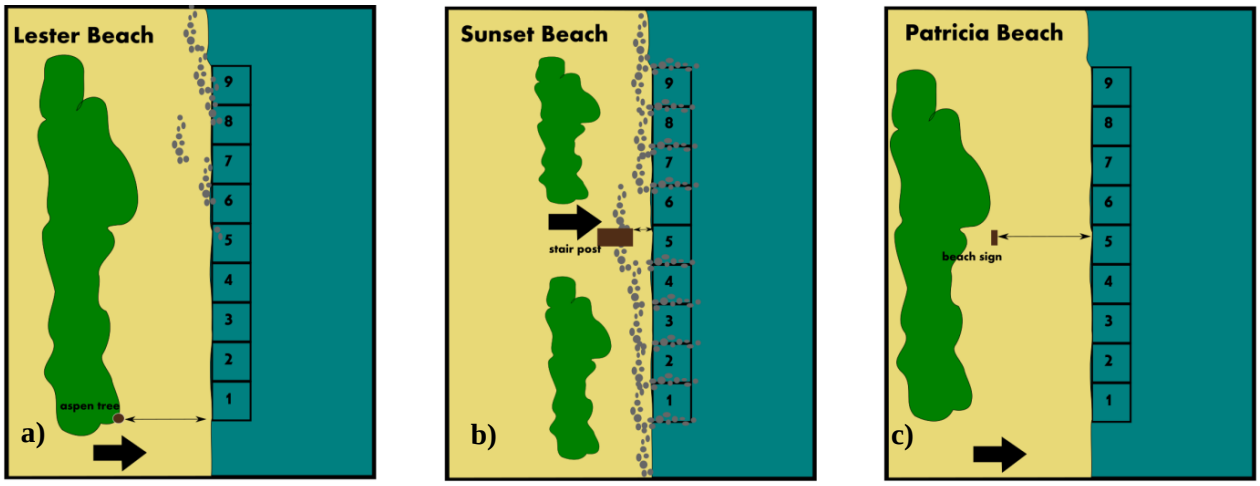

Figure 4. Each site possesses a landmark used to measure the distance to the shoreline. (a) The landmark at Lester Beach is an aspen tree aligned with the outer edge of the first transect. (b) The landmark at Sunset Beach is a stair post aligned with the outer edge of the fifth transect. (c) The landmark at Patricia Beach is a beach sign aligned with the inner edge of the fifth transect. The large black arrow indicates the point of access to the site.

At each site, record in the field book the "distance from landmark".

Sampling of Abiotic Environmental Variables

In the designated transect, record in the field book the water depth at 5, 10, 15, and 20 m from the shoreline. In the same transect, set up the temperature/light logger (HOBO Pendant® MX Temperature/Light Data Logger, MX2202, Onset, Bourne, MA, USA) as per Appendix 2 and the wave logger as per the pre-existing protocol: An inexpensive instrument for measuring wave exposure and water velocity (Figurski *et al.* 2011). Deploy the loggers with an anchor where the water depth reaches 30 cm. In the field book, record the time of day (24 h clock) the loggers were deployed, and make sure they are functioning properly. Do not remove the loggers until all the other samples have been taken at the site. The loggers should be submerged a minimum of 1 h. Record in the field book the time the loggers were removed from the water. Wind direction and strength was measured at each site using a WEATHERmeter (WeatherFlow, Scotts Valley, CA, USA). A WEATHERmeter is a miniature weather station connected to a smartphone application via Bluetooth that collects real-time wind, temperature, humidity, and pressure readings.

Water Sampling

Water is sampled for factors such as Chlorophyll *a* and phosphorus, which may be affected by Zebra Mussel establishment. At each site, 1 l of water is collected by wading into the water, and collecting the sample with a 1 l bottle on an extendable sampling pole to prevent contamination of the sample. Chlorophyll *a* is used as a proxy for phytoplankton abundance.

Before collecting the water, organize the supplies needed (full list of supplies in Appendix 1):

- Extendable sampling pole
- Cooler
- 1 l Nalgene® bottles (opaque, wide-mouth)
- Ice packs
- Permanent markers
- Bottle brush

Prior to collecting water for water chemistry, label a 1 l Nalgene® bottle as indicated in the table below (Table 2). Attach the 1 l sample bottle to the extendable sampling pole. Tighten the ring sufficiently enough to ensure that the bottle is secure. Extend the pole to its maximal extent and fill the bottle about 90% full by utilizing the swivel on the pole; this helps in the proper mixing of the water prior to filtering for analysis. Water samples are to be taken in the same transect as the water logger. So as not to disturb the water column, take the water sample perpendicular to the transect in undisturbed water at the distance furthest from shore. The water samples are to be kept cool (e.g., in a cooler with ice packs) until their return to the laboratory.

Table 2. Nalgene® bottle label specifications for all three nearshore sampling sites. All water chemistry sample bottles must be labelled accordingly prior to collection of sample.

Location	Label
Lester Beach	LWPG-LB-Epi dd/mm/yy Transect #
Sunset Beach	LWPG-SB-Epi dd/mm/yy Transect #
Patricia Beach	LWPG-PB-Epi dd/mm/yy Transect #

Fish Sampling

Before each trawl, assure that there are no tears in the seine net and organize the supplies needed to sort the catch (full list of supplies in Appendix 1).

- Beach seine net
- Whirlpak® bags
- Mini cooler
- Ice packs
- Permanent markers
- Pencil
- Hanging weigh scale
- Measuring board
- Mesh bag
- Miscellaneous aquarium fish nets
- Fish ID book
- Labels
- Field book
- Waterproof camera
- Live fish photographing chamber

In addition, using a permanent marker, label eight Whirlpak® bags with the date, site, haul #, round #, and the appropriate transect #.

A total of eight 20 m long hauls are conducted at each site. Each haul is treated as a separate replicate.

Beach Seine Specifications

Beach seining is the act of sampling an area of water with a net operated from shore. This net is composed of a bag and long wings (5 mm mesh, 9 m long x 2 m high with a 2 x 2 m bag). Floats are attached to the head ropes, which keeps it on the surface, whereas the foot rope is in permanent contact with the substrate. Consequently, the beach seine prevents fish from escaping from the area enclosed by the net.

Putting the seine in the water requires at least two persons. One person is to be positioned on each end of the net. While keeping the net vertical, the two net operators should remain as far apart as possible and move as quickly as possible. The bottom of the net should remain on the substrate at all times. Make note in the field book if contact with the substrate was lost at any point due to obstructions (e.g., boulders, logs).

Once the shore is reached, empty the fish from the net into a bucket of ice water. A quick examination of the fish should be performed before placing the fish in the Whirlpak® bag. If fish listed under the *Species at Risk Act* (SARA) are caught (Appendix 3), they should be photographed, measured (total length L_t, fork length L_f), and weighed before being released. Any caught fish with a fork length >200 mm are also to be released. Prior to doing so, record the species, body mass, and length of all fish who exceed this length in the field book.

Fill each Whirlpak® with fish just under ¾ full, such that there is a maximum of two to three layers of fish in the bag when lying flat. This assures that the specimens in the bag freeze quickly and conserve well for later identification. Place the bags in a cooler with ice-packs while sampling the other transects and transfer all the fish samples into a freezer as soon as possible.

If any Spiny Water Fleas (*Bythotrephes longimanus*) are found in the seines, they are to be placed in Whirlpak® bags labeled using permanent marker with the date, site, transect #, initials of the collector, and "Spiny Water Fleas in seines". Place the bags in a cooler with ice-packs and transfer into a freezer as soon as possible.

LWPG has several Aquatic Invasive Species. Consult Appendix 4 for details on proper identification of such species. If any of these species are encountered, make a note in the field book.

Zooplankton Sampling

Before deploying the zooplankton net, check that it is clear of debris and there are no visible tears. Organize the following supplies as needed (full list of supplies in Appendix 1).

- Four sample bottles
- Four sample containers
- Spray bottle
- 95% ethanol
- Permanent markers
- Pencil
- Labels
- Field book
- Scoop
- Tweezers
- Zooplankton net (500 µm mesh, 30.5 cm Ø opening, 100 cm long) with collection bucket

In addition, using a permanent marker, label four sample bottles with the site, date, "Zoop", round #, and appropriate transect #. Add a paper label in each of the sample bottles with the same information written in pencil.

Zooplankton sampling requires two persons and the samples are to be taken from the four randomly designated transects. The first person must stand onshore while the second, equipped with the zooplankton net and attached collection bucket, walks out 20 m into the water while holding the end of the net above the surface of the water. The first person will then walk out to 10 m. Both people will take three steps (in the same direction) perpendicular to the transect in order to sample undisturbed water, then the first person will pull the cord attached to the net towards them at a quick enough pace to keep the zooplankton net at,

but not above, the surface of the water column. If the water is too shallow to submerge the full zooplankton net, record in the field book the percentage of the net that was in the water.

Rinse the net from the outside with a bucket of water to make sure all of the zooplankton on the net fall into the attached sampling jar. Once on the shore, remove the jar from the net and move its contents into a sample bottle by using a spray bottle with clean water, a scoop, and/or tweezers. If the sample is too large to fit in the sample bottle, use a larger sample container instead. Cover the sample with 95% ethanol. Make sure that all zooplankton are removed from the net and the sampling jar before re-deploying the zooplankton net.

If any Spiny Water Fleas are found in the zooplankton trawl, make a note of it on the sample container. Do not separate them from the sample.

Benthic Invertebrate Sampling

Before sampling, organize the supplies needed (full list of supplies in Appendix 1).

- Four sample bottles
- Four sample containers
- Spray bottle
- Reverse osmosis (RO) water
- 95% ethanol
- Permanent markers
- Pencil
- Labels
- Field book
- CABIN Protocol kick net (400 µm mesh size with collection bucket)
- Scoop

In addition, using a permanent marker, label the sample bottle with the site, date, and the appropriate transect #. Add a paper label in each of the sample bottles with the same information written in pencil.

Benthic invertebrate sampling requires at least two persons and the samples are to be taken from the four randomly designated transects. The numbers (1 or 2) previously written down in the field book for each of the four transects sampled for benthic invertebrates indicate whether the sample is taken 10 or 20 m from shore. In the case where the sample is taken at 20 m, the person holding the kick net must walk out 20 m from the shore into the water, and the second person must then walk 10 m towards the first. In the other case (i.e., 10 m), the person holding the kick net must walk out 10 m from the shore into the water, while the second person stays on the shore. In both cases, once the person holding the kick net has walked out the appropriate distance, they must then take three steps to the side and then shuffle backwards, disturbing the sediment and catching it in the kick net until they reach the 10 m endpoint marked by the second person.

Use a scoop to transfer the contents from the net into a labelled sample bottle ensuring it is not more than 50% full. If needed, use a second sample bottle or a larger sample container and ensure they are no more than 50% full. Cover the contents with 95% ethanol and seal shut. Any additional sample bottles or containers must be labelled with the site, date, and the appropriate transect #, and must contain a paper label with the same information written in pencil. The paper label must go in the additional sample bottle or container.

If any mussels listed under SARA (Appendix 3) are caught in the benthic invertebrate samples, their lengths must be recorded in the field book, and they must be returned to the spot they were found.

Mussel Sampling

Before sampling, organize the supplies needed (full list of supplies in Appendix 1).

- Shovel
- Metal sheet with holes (4.75 mm hole diameter)
- Wire template (10 cm^2)
- Whirlpak® bags
- Aluminum foil
- Zip top bags
- Permanent marker
- Scraper

Zebra Mussel sampling requires at least two persons and the samples are to be taken from the four designated benthic transects. Boulders (if applicable), cobble, and pebble must be searched for mussels at each designated transect, and must be selected at random. Three samples of each substrate must be taken at each of the selected transects. Each of the samples from the three types of substrates are numbered sequentially from 1–3 during the sampling. The water depth and distance from the shoreline must be measured for each of the mussel sampling sites and recorded in the field note book.

Any rocks that are too large to be lifted out of the water are considered boulders. Once a boulder is selected at random, place a wire template (10 cm^2) on the substrate; this represents the sampling area that will be searched for mussels. Carefully remove any mussels found within the selected search area on the boulder and place them in a Whirlpak® bag. With a permanent marker, label the bag with the following; boulder #, site, transect #, depth, distance from shore, and the date. Place the bag in a cooler with ice-packs. Repeat twice more with new boulders chosen at random.

Cobbles are considered to be a minimum of 6.35 cm up to anything larger that can be lifted out of water. Once a rock is selected at random, determine whether or not there are >100 mussels attached. If there are <100 mussels on the rock, remove all mussels and place them into a Whirlpak® bag. With a permanent marker, label the bag with the following; cobble #, site, transect #, depth, distance from shore, and the date. Otherwise, use the wire template (10 cm^2) to isolate a representative area of the rock surface with mussels and only remove the mussels inside the template area and place them into a labelled Whirlpak® bag. Place the bag in a cooler with ice-packs. Repeat twice more with new cobbles chosen at random. Once all three cobbles have been searched for mussels, they must be measured. Place a sheet of aluminum foil over the searched surface of the cobble and cut off any extra foil. Carefully fold the foil and place it in a small labelled Whirlpak® bag. These foils will then be weighed in the lab, after which a regression of foil weight versus surface area is used to estimate the rock surface area (A_r):

$$A_r = \left(\frac{A_k}{W_k}\right) \cdot W_{rf}$$

Where A_k is the known area of the foil, W_k is the known weight of the foil, and W_{rf} is the weight of the 'rock' foil (Steinman *et al.* 2017).

Pebble size substrate is anything less than 6.35 cm. In a randomly selected area of the designated transects, shovel, in one motion, from the bottom substrate an area of approximately 40 cm long and place on a metal sheet with holes (Figure 5). Carefully remove any mussels that are attached to pebble, taking care not to crush or damage them, and place them into a Whirlpak® bag. With a permanent marker, label the bag with the following; pebble #, site, transect #, depth, distance from shore, and the date. Place the bag in a cooler

with ice-packs. Repeat twice more with new pebble areas chosen at random. Record the width of the shovel to determine the area sampled.

Figure 5. Metal sheet with 4.75 mm holes used to sift through pebble for mussel sampling. Approximately 40 cm of bottom substrate is shoveled onto the metal sheet. The pebbles remaining on the sheet are inspected for Zebra Mussels (*Dreissena polymorpha*). Any Zebra Mussel found is placed in a bag and transferred on ice.

Sediment Chemistry Sampling

Before sampling, organize the supplies needed (full list of supplies in Appendix 1).

- Core tube
- Turkey baster
- Syringe
- Rubber stoppers
- Whirlpak® bag
- Permanent markers

At the two predetermined transects, take a sediment sample at 30 cm water depth by vertically inserting a rinsed core tube ~5 cm into the sediment. Seal the tube by putting a rubber stopper at both ends. Keep the tube in an upright position at all times. If tilted, return the tube to an upright position and let the sediment settle before removing the sample. Remove the rubber stopper at the top of the tube and use a turkey baster to carefully remove the water in the core tube, making sure not to remove any sediment. Remove the bottom stopper and let out some of the sediment, leaving only the top ~1 cm of sediment in the tube. Scoop the top 1 cm of sediment in the core tube into a labelled Whirlpak® (date, site, round #, and appropriate transect #). Rinse any sediment remaining on the core tube into the Whirlpak® using the rinse bottle. Close the Whirlpak® and place it in the cooler.

4.1.4 Sample Storage

At the end of the day, all samples are to be brought back to the lab. All frozen fish and mussels must be placed in a freezer, and the water and sediment samples must be placed in a fridge. Verify that all supplies are replenished for the next field outing.

4.1.5 Field Equipment Cleanup

On the last day of the survey:

- Clean the beach seine, make sure there are no dead fish in the netting and check it over to see if it needs any repair.
- Spread the seine net out to dry. Due to the occurrence of Spiny Water Flea in LWPG, the net and all equipment used in LWPG will have to be dried in hot sun for ~48 h to ensure eggs are killed.

4.2 Laboratory Analysis

4.2.1 Laboratory Equipment

Consult Appendix 5 for a complete list of supplies needed in the laboratory for analysis of samples collected from the nearshore field work.

4.2.2 Fish Analysis

Fish samples must be thawed prior to being measured and weighed. Thaw fish samples by submerging Whirlpak® bag(s) in cold water. Do not use warm or hot water to thaw the sample, as this may damage the sample. Once samples are thawed don't delay their processing.

Once thawed, empty the Whirlpak® bag's contents onto an enamel tray. Fishes must first be sorted by species. Stewart and Watkinson (2004) *The Freshwater Fishes of Manitoba* serves as a useful identification guide for freshwater fishes found in LWPG. The guide describes general features and measurements of fishes, as well as identification descriptions, biological notes, and species distributions for freshwater fishes found in Manitoba. Once sorted by species, fish are then to be sorted into size groups (i.e., young-of-the-year, small, medium, and large) based on the fish size chart (Table 3). Size classes are used to sub-sample when there are more than 50 fish of the same species and of the same size class from a haul. The first 50 individuals of the same species and of the same size class are individually measured by both fork and total length, and are weighed. The rest are counted and bulk weighed. While measuring and weighing fish from one tray, the remaining trays of fish can be covered with a cool, damp paper towel and be put in the fridge until ready for processing.

Table 3. Size chart for fish frequently sampled in the laboratory. This size chart specifies the size classes, based on the fork length (mm), of different fish species commonly found in the Nearshore seine trawls. Fish species can be classified as; young-of-the-year (YOY), small, medium, or large.

Size class length (mm) / Species	YOY	Small	Medium	Large
Emerald Shiner (*Notropis atherinoides*)	<45	45–55	55–70	>70
Cisco (*Coregonus artedi*)	N/A	<100	100–150	>150
Rainbow Smelt *(Osmerus mordax*)	<50	50–70	70–100	>100
Goldeye (*Hiodon alosoides*)	<50	50–110	110–170	>170
Troutperch *(Percopsis omiscomaycus*)	N/A	35–60	60–80	>80
Ninespine Stickleback *(Pungitius pungitius*)	<35	35–40	40–45	>45
White Bass (*Morone chrysops*)	<40	40–100	100–200	>200
Yellow Perch (*Perca flavescens*)	<50	50–100	100–200	>200
Walleye (*Sander vitreus*)	<100	100–200	N/A	N/A
Sauger (*Sander canadensis*)	<100	100–200	N/A	N/A

Range of length within and between size groups will vary depending on the species and the time of year the samples were collected (spring, summer, or fall).

Computer Data Entry

In the lab, data is entered directly into a Microsoft Excel spreadsheet. The columns should be labelled as follows:

- Date Processed
- Date Collected
- Site
- Haul
- Transect
- Round
- Sample code
- Preservation code
- Species code
- Size code
- Total number
- Fork length (mm)
- Total length (mm)
- Body mass (g)
- Comments

Prior to measuring the fish, enter into the spreadsheet all known data about the sample in the appropriate cells. Data from each row represents a single fish, with the exception of the bulk mass.

Processing the Samples

Once individual fish are arranged by species and by size class on enamel trays, their length and body mass can be measured and entered in the spreadsheet. Work sequentially from one end of the tray to the other. In order to ensure that length and body mass correspond, fish must be measured and weighed in the same order.

Individual Fork Length Measurements:

Fork length (L_f) is measured from the tip of the snout to the fork of the tail. Use 0–150 mm digital calipers to measure fork length to the nearest millimeter. If fish length is >150 mm, use a large measuring board and measure to the nearest mm. Enter the length into the spreadsheet

Individual Total Length Measurements:

Total length (L_t) is measured from the tip of the snout to the end of the caudal fins. Use 0–150 mm digital calipers to measure total length to the nearest millimeter. If fish length is >150 mm, use a large measuring board and measure to the nearest mm. Enter the length into the spreadsheet.

Individual Body Mass Measurements:

Turn on the scale and place the corresponding Whirlpak® bag on the weigh pan. Tare the scale to zero the weigh pan. Using tweezers, place in the bag the first fish for which the length was measured. Measure the body mass of the fish to the nearest tenth of a gram (+/-0.1 g) and enter it into the spreadsheet. Tare the scale before proceeding to the next fish.

Bulk Mass Measurements:

Once 50 individuals of the same species and size groups are individually measured and weighed, the remaining fish of that species and size class are counted and bulk weighed. Bulk mass is measured after all individual fish body mass measurements. Tare the scale to zero the weigh pan on which is placed the Whirlpak® bag of fish. Place all of the counted bulk mass fish into the Whirlpak® bag and measure the bulk mass to the nearest tenth of a gram (+/-0.1 g). Enter the bulk mass into the spreadsheet.

Once a sample has been processed, mark “Done” on the Whirlpak® bag(s) and return the sample to the freezer. Record “Done” beside the round number under the site in the lab notebook for all processed samples.

Walleye and Sauger:

Prior to identifying, measuring, and weighing the Walleye (*Sander vitreus*) and Sauger (*Sander canadensis*), enter into the spreadsheet all known data about the sample in the appropriate cells. Data from each row represents a single fish.

To identify Walleye and Sauger, the pyloric caeca found in the stomach cavity of the fish are counted. Using scissors, carefully cut along the underbelly of the fish to access the stomach cavity. Walleye have 2–3 pyloric caeca as long as the stomach and Sauger have 4–5 shorter pyloric caeca.

Once identified, the individual Walleye/Sauger are measured and weighed as described for the frozen fish above.

End of the day

At the end of the day, wash all enamel trays, tweezers, and dissecting tools in hot, soapy water. Wipe down calipers, fish measuring board, countertops, and scale.

4.2.3 Zooplankton and Benthic Invertebrate Analysis

To analyze the zooplankton and benthic invertebrate samples collected, first set up a plate filled with pre-weighed foil tins. The identified invertebrates and detritus from the collected sample will then go into their appropriate tins. Identify the sample information from the selected sample by writing down on the plate template sheet (Figure 6) the following:

- Sample type (zooplankton or benthic invertebrate)
- Site
- Round
- Transect
- Date collected
- Date processed
- Initials of person processing the sample

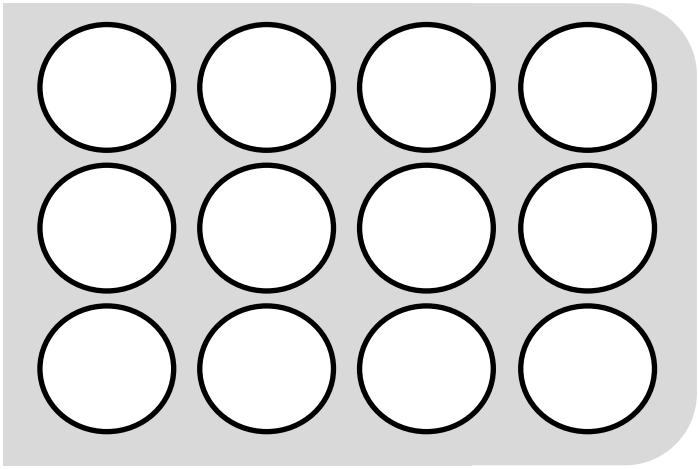

Figure 6. Information sheet used to record the zooplankton and benthic sample information. Each circle represents a well in the plate where a pre-weighed foil tin rests. Microorganisms from the zooplankton and benthic invertebrate samples are organized by species and placed into their appropriate tin.

Empty the sample container's contents into a petri dish and observe under magnification. Make sure that the sample is always submerged in 95% ethanol while sorting. All zooplankton and benthic invertebrates are to be sorted to order, with the exception of a few select groups. Annelid worms found in the samples are to be sorted as Oligochaeta, and the species presented in Table 4 should be identified to family. If any organisms found in Table 4 cannot be identified to one of the two family taxa, they are to be sorted to order as for the rest of the organisms.

Table 4. Table of benthic invertebrates that can be identified to the family taxa. Organisms under the order of Diptera or Hemiptera are to be classified at the next level of taxonomy (i.e., family). If such identification cannot be made, they must be classified by order as are the rest of the organisms.

Order	Family
Diptera	Chironomidae Culicidae
Hemiptera	Notonectidae Corixidae

Count and place the sorted organisms in the appropriate tin. The following information should be written in each used circle of the plate:

- Foil tin starting weight
- Species name (Family/Order)
- Total species count

Only count to 1,000 for each species. Excess organisms of that species can be bulk weighed in an unused foil tin labeled as "*Species*, excess".

All of the detritus must be collected in a weighting tin, but not counted. All sand and rocks are to be removed.

Once the sample is completely sorted, ensure all species counts are written on the information sheet. Rest the sheet on the closed plate, and place the bundle in the oven to dry. The oven temperature should be set to 60 °C. All samples must dry in the oven no less than 24 h before they can be weighed. Once each tin from a sample has been individually weighed and the final mass written on the information sheet, the tins can be thrown out. Enter the Data into an Excel spreadsheet.

4.2.4 Sample Analysis by External Sources

All mussel samples are provided to ECCC for further analysis. Water and sediment chemistry samples are analyzed by DFO's Chemistry lab. Water samples are analyzed for the following:

- Suspended nitrogen (SUSPN) $\mu g \cdot l^{-1}$
- Suspended phosphorus (SUSPP) $\mu g \cdot l^{-1}$
- Suspended carbon (SUSPC) $\mu g \cdot l^{-1}$
- Chlorophyll-*a* (CHLA and HPLC) $\mu g \cdot l^{-1}$
- Conductivity at 25 °C ($\mu S \cdot cm^{-1}$)

Sediment samples are analyzed for nitrogen, phosphorus, and carbon, as well as a measure of detritus (i.e., "loss of ignition").

5.0 DISCUSSION AND RECOMMENDATIONS

The nearshore monitoring protocol for LWPG was developed, tested, and executed for two field seasons. Baseline data from those two field seasons will be made available on the Government of Canada's Open Data Portal (https://open.canada.ca/en/open-data) and be available for comparison of future monitoring efforts and adaptive management efforts.

An effective and successful monitoring program requires proper consideration of metrics, number, and frequency of sampling efforts (Hewitt *et al.* 2003). Such considerations also include a specific purpose and objectives for the experiment, an understanding of the ecosystem in question, as well as a detailed model of the study, which not only allows the observation of ecological changes amongst the site but also is open to new information or questions that may appear during the study (Braun *et al.* 2019). A number of recommendations that apply to the nearshore monitoring program presented in this report are discussed below.

Proper understanding of the studied ecosystem is crucial for any study. Although often time consuming, having baseline data for the ecosystem in question can benefit the monitoring program (Braun *et al.* 2019), especially if it includes the effects of invasive species as does this nearshore monitoring program. Additional knowledge from baseline data is especially useful for data analysis. Knowledge of indigenous species and their biology can contribute to proper analysis of the effects of the introduction of new species. For example, a sudden increase in abundance of aquatic life could be incorrectly assessed as a positive response (Braun *et al.* 2019). However, the sudden increase in abundance of aquatic life could be a result of nutrient enrichment, or may be only observed in stress tolerant species (Braun *et al.* 2019). Therefore, baseline data is a useful starting point for comparing the severity of changes observed over time in the studied ecosystem.

Baseline data should contain as much information about the community within the targeted ecosystem. This allows for a more accurate picture of the mechanisms by which the ecosystem is being affected. Though the nearshore monitoring program focuses on the impacts of Zebra Mussels on the fish populations of LWPG, samples and data are collected and studied on multiple trophic levels. Studying only the variations in fish communities after the introduction of ZMs may lead to erroneous conclusions since the interaction between the two may not be direct, but rather linked to the modifications of lower trophic levels on which the fishes rely (Woodward and Quinn 2011). Therefore, the study of multiple trophic levels at the studied nearshore sites provides robust baseline data for the LWPG nearshore ecosystem; serving as a point of comparison for each additional year of monitoring and may be useful for future adaptive management efforts.

The number of sites, site visits in a year, and samples must also be taken into account when planning a monitoring program. In general, increasing the number of sites and sampling within sites, the frequency of sampling, and the number of years the study will take place, increases the statistical power of the study, yielding more telling data (Braun *et al.* 2019). Samples collected in the frame of the nearshore monitoring protocol were planned accordingly.

5.1 NUMBER OF SITES

Since the nearshore sampling could not take place at sufficient locations to be representative of LWPG as a whole or for its entire south basin, the sampling sites were chosen as to represent a range of ideal substrates

for ZM colonization. ZMs are known to colonize hard substrates, however, they have also been shown to directly colonize soft sediments such as sandy substrates (Berkman *et al.* 1998; Schloesser and Nalepa 2013). They do so by latching onto individual grains of sand and binding them together into aggregates (Berkman *et al.* 1998). Successive colonization of the soft substrate may then lead to carpets of ZMs (Schloesser and Nalepa 2013). For this reason, the three sites chosen for the nearshore sampling were based on their relative abundance of hard substrate (i.e., very low, low, and medium). This allows for the surveying of changes in ZM abundance in different types of habitats. Generally, an increase in the number of sites sampled is reflected in a greater statistical power of the analysis (Braun *et al.* 2019). Therefore, if there are fewer time or budget constraints, we suggest increasing the number of sites for the study.

5.2 SAMPLING WITHIN SITES

Each sampling site is broken down into nine transects of equal dimensions in which eight are sampled for fish, and four are sampled for zooplankton and benthic invertebrates. There are 4–5 persons who participated in the sampling of nearshore sites per sampling day. Since either these participants or their tasks may vary during the sampling, there are certain limitations that are introduced while sampling, especially with regard to the sampling of fish, zooplankton, and benthic invertebrates. In order to minimize the risk of variabilities amongst samples, there are sampling guidelines which the field staff should follow; (1) during the benthic invertebrate sampling, while walking backwards in the 10 m sampling area, a sampling rhythm of two steps per second should be maintained; (2) while collecting the zooplankton samples, the rope attached to the zooplankton net should be pulled at a constant speed to ensure that the net remains completely submerged in the water and without contact with the sediment; (3) the tension in the beach seine net should also be maintained and both ends pulled with the same speed to keep the net parallel to the shore at all times. These guidelines help reduce any variabilities associated to the sampling methods both during the field season and between sampling years.

There are also limitations apart from variabilities in sampling methods, namely the number of beachgoers at the site on the day of sampling and the presence of large obstacles in the water such as boulders, large rocks, or large woody debris. Sampling should always take place in undisturbed water, however, this may be challenging when there are a large number of beachgoers in the water. It is also possible that there are transects with large obstacles in the water that may affect the efficacy of seining. In such a case, field staff should try to maneuver over these obstacles all while continuing to walk towards the shore at a constant speed and keeping the seine net fully extended and parallel to the shore. Whenever there is an obstacle during the sampling, it is essential to take note in the field book. The collection of samples from multiple transects increases the replication at each site. These replicates are important since they allow for the capture of any within-site variabilities associated with any of the above limitations, or with the clustering nature of fish, zooplankton, and benthic invertebrates. In addition, these replicates make for a greater statistical power of the analysis.

5.3 FREQUENCY OF SAMPLING

The fish community changes depending on the time of year sampled. Some fish species spawn earlier than others, making for variations in life history stages between species caught in the seine net. Depending on the species, various size classes of fishes can be found within the same haul. In fact, within the scope of the sampling period, certain species of fish will have had the chance to reach several stages of development, which can either be inside or outside the size targeted by the seine net (i.e., too small, ideal, too large). Consequently, the field sampling is repeated five times each field season. The frequency of sampling has

allowed for the creation of a dataset with two years' worth of information on the fish species caught in the seines during the nearshore sampling. Lab processing of fishes has provided information on population density, average growth rates, and species of fish found at the sampled nearshore sites. In addition, stomach content analyses provide information on fish diet. Changes in the diet, size, and abundance of fishes could be a response to variations in lower trophic levels, a known consequence attributed to the presence of ZMs in other invaded waterbodies (Woodward and Quinn 2011). The analysis of zooplankton and benthic invertebrate samples also provides insight on possible changes within lower trophic levels. Therefore, it is important to continue sampling to determine changes associated with a prolonged presence of ZMs in LWPG.

5.4 NUMBER OF YEARS

Though ZMs have been in LWPG since 2013, and have a high potential of impacting its ecosystem (DFO 2014), current data from the nearshore surveys have shown low abundances in the sampled locations compared to their potential (Enders *et al.* 2019). Thus, the data emerging from the two years of nearshore monitoring can act as a baseline for the remainder of the study. In order to properly identify any trends due to ZMs in LWPG, and to increase the statistical power of the analysis, additional years of data are needed.

Variability in time and environmental conditions should be considered when sampling, since similarities between sampling dates and conditions make for a more accurate annual comparison of the ecosystem's dynamics (Braun et al 2019).With the exception of days where the weather was extreme, sampling took place on the designated sampling date. While this resulted in a range of environmental conditions, efforts were taken to measure possible environmental drivers to account for them in the analyses. Wind, for example, can cause changes in fish behavior by changing the strength and frequency of the waves. Variation in wave action was quantified with the wave logger. The nearshore monitoring was carried out at three different sites, with more than one site sampled per day. To reduce the influence of diurnal changes in environmental conditions, the site sampling order was chosen at random. To reduce the influence of time of day even further, future sampling may be spread out over three days during a specified time frame.

6.0 CONCLUSION

Canadian aquatic ecosystems are facing environmental consequences attributed to the introduction of AIS into its waterbodies. Since their introduction into LWPG, ZMs are thought to affect the native fishes of the lake by impacting the lake's lower trophic levels. This report presents several sampling methods for a subset of the LWPG nearshore habitat to study the impacts of ZMs. With continuous monitoring, the data emerging from the nearshore sample analyses will allow us to: (1) determine the local colonization of ZMs; (2) determine the relationship between ZMs, nutrient availability, and the community composition and biomass of the lower trophic levels; (3) determine the relationship between relative abundances of ZMs and relative abundances and composition of the nearshore small fish community, and; (4) identify changes in the relative contribution of the pelagic and benthic prey diet, trophic position, ontogenetic shifts, and species specific interactions. Additional monitoring will be necessary before being able to draw conclusions regarding the impacts of ZMs in the sampled nearshore sites in LWPG. However, data generated using this standardized protocol provides a baseline for future monitoring and adaptive management efforts.

7.0 REFERENCES

Bailey, S.A., Deneau, M.G., Jean, L., Wiley, C.J., Leung, B., and MacIsaac, H.J. 2011. Evaluating efficacy of an environmental policy to prevent biological invasions. Environmental Science and Technology. 45(7): 2554-2561. https://doi.org/10.1021/es102655j

Berkman, P.A., Haltuch, M.A., Tichich, E., Garton, D.W., Kennedy, G.W., Gannon, J.E., Mackey, S.D., Fuller, J.A., and Liebenthal, D.L. 1998. Zebra Mussels invade Lake Erie muds. Nature. 393: 27-28. https://doi-org.uml.idm.oclc.org/10.1038/29902

Boyce, F.M. 2006. Citing online sources: The Canadian Encyclopedia – Lake [online]. Available from https://www.thecanadianencyclopedia.ca/en/article/lake [accessed 5 April 2020].

Braun, D.C., Smokorowski, K.E, Bradford, M.J., and Glover, L. 2019. A review of functional monitoring methods to assess mitigation, restoration, and offsetting activities in Canada. DFO Canadian Science Advisory Secretary Research Document. 2019/057. vii + 75 p.

Briski, E., Bailey, S.A., Casas-Monroy, O., DiBacco, C., Kaczmarska, I., Levings, C., MacGillivary, M.L., McKindsey, C.W., Nasmith, L.E., Parenteau, M., Piercey, G.E., Rochon, R., Roy, S., Simard, N., Villac, M.C., Weise, A.M., and MacIsaac, H.J. 2012. Relationship between propagule pressure and colonization pressure in invasion ecology: a test with ships' ballast. Proceedings of the Royal Society B. 279(1740): 2990–2997. http://doi.org/10.1098/rspb.2011.2671

Burlakova, L.E., Karatayev, A.Y., Pennuto, C., Mayer, C. 2014. Changes in Lake Erie benthos over the last 50 years: Historical perspectives, current status, and main drivers. Journal of Great Lakes Research. 40(3): 560-573. https://doi.org/10.1016/j.jglr.2014.02.008.

Chan, F.T., MacIsaac, H.J., and Bailey, S.A. 2015. Relative importance of vessel hull fouling and ballast water as transport vectors of nonindigenous species to the Canadian Arctic. Canadian Journal of Fisheries and Aquatic Sciences. 72(8): 1230-1242. https://doi.org/10.1139/cjfas-2014-0473

[COSEWIC] Committee on the Status of Endangered Wildlife in Canada. 2016. Assessment and status on the Mapleleaf, *Quadrula quadrula*, Great Lakes – Upper St. Lawrence population, Saskatchewan – Nelson Rivers population, in Canada. COSEWIC, committee on the Status of Endangered Wildlife in Canada, Ottawa, ON, Canada.

[DFO] Department of Fisheries and Oceans Canada. 2004. Citing online sources: A Canadian Action Plan to address the threat of Aquatic Invasive Species [online]. Available from https://waves-vagues.dfo-mpo.gc.ca/Library/365581.pdf [accessed 5 April 2020]

[DFO] Department of Fisheries and Oceans Canada. 2014. Citing online sources: Lake Winnipeg Zebra Mussel treatment [online]. Available from

http://publications.gc.ca/collections/collection_2014/mpo-dfo/Fs70-7-2014-31-eng.pdf [accessed 10 April 2020]

DFO] Department of Fisheries and Oceans Canada. 2016. Recovery strategy and action plan for the Mapleleaf (*Quadrula quadrula*) in Canada (Great Lakes-Western St. Lawrence population) [Proposed]. Species at Risk Act Recovery Strategy Series. Fisheries and Oceans Canada, Ottawa. vi + 57 pp.

[DFO] Department of Fisheries and Oceans Canada. 2018. Citing online sources: Aquatic Invasive Species [online]. Available from www.dfo-mpo.gc.ca/oceans/publications/soto-rceo/2012/page06-eng.html [accessed 5 April 2020]

[DFO] Department of Fisheries and Oceans Canada. 2019. Citing online sources: Aquatic Invasive Species Program [online]. Available from www.inter.dfo-mpo.gc.ca/ATIP/PIA/AIS [accessed 5 April 2020]

[ECCC] Environment and Climate Change Canada, and Manitoba Agriculture and Research Development. 2020. State of Lake Winnipeg, 2nd Edition. Available from https://www.gov.mb.ca/sd/pubs/water/lakes-beaches-rivers/state_lake_wpg_report_tech.pdf [accessed 21 April 2020]

[ECCC] Environment and Climate Change Canada, and Manitoba Water Stewardship. 2011. State of Lake Winnipeg: 1999 to 2007. Available from https://deslibris.ca/ID/229271 [accessed 5 April 2020]

Enders, E.C., Charles, C., Caskenette, A.L., Rudolfsen, T.A., and Watkinson, D.A. 2019. Distribution patterns of the early invasions of Zebra Mussels, *Dreissena polymorpha* (Pallas, 1771), in the south basin of Lake Winnipeg. BioInvasions Records. **8**(2): 329-342. doi:10.3391/bir.2019.8.2.15

Figurski, J.D., Malone, D., Lacy, J.R., and Denny, M. 2011. An inexpensive instrument for measuring wave exposure and water velocity. Limnology and Oceanography: Methods. 9(5): 204-214. https://doi-org.uml.idm.oclc.org/10.4319/lom.2011.9.204

Hann, B., Wishart, M., and Watson, S. 2017. Long-term trends in benthic invertebrate populations (1929-2013) in Lake Winnipeg. Journal of Great Lakes Research. **43**(5): 938-952. https://doi.org/10.1016/j.jglr.2017.06.005

Hewitt, L.M., Dubé, M.G., Culp, J.M., MacLatchy, D.L., and Munkittrick, K.R. 2003 A proposed framework for investigation of cause for environmental effects monitoring. Human and Ecological Risk Assessment. **9**(1): 195-211. doi:10.1080/713609859.

Jansen, W., Gill, G., and Hann, B. 2017. Rapid geographic expansion of spiny water flea (*Bythotrephes longimanus*) in Manitoba, Canada 2009-2015. Aquatic Invasions. **12**(3): 287-297. https://doi.org/10.3391/ai.2017.12.3.03

Jantz, B., and Neumann, D. 1998. Growth and reproductive cycle of the zebra mussel in the River Rhine as studied in a river bypass. Oecologia. **114**(2): 213-225.

Karatayev, A.Y., Burlakova, L.E., Mastitsky, S.E., and Padilla, D.K. 2015. Predicting the spread of aquatic invaders: Insight from 200 years of invasions by Zebra Mussels. Ecological Applications. **25**(2): 430-440. https://doi-org.uml.idm.oclc.org/10.1890/13-1339.1

Lake George Association. 2020. Citing online source: How to identify the Zebra Mussel [online]. Available from https://www.lakegeorgeassociation.org/educate/science/lake-george-invasive-species/zebra-mussel/ [accessed 13 April 2020]

Lindgren, C.J. 2006. Angler Awareness of Aquatic Invasive Species in Manitoba. Journal of Aquatic Plant Management**. 44**: 103-108. Available from: http://www.apms.org/wp/wp-content/uploads/2012/10/v44p103.pdf [accessed 17 April 2020]

[MN DNR] Minnesota Department of Natural Resources. 2020. Citing online sources: Spiny Waterflea (*Bythotrephes longimanus*). Available from https://www.dnr.state.mn.us/invasives/aquaticanimals/spinywaterflea/index.html [accessed 28 April 2020]

[MWFB] Manitoba Wildlife and Fisheries Branch. 2020. Citing online sources: Fish and Wildlife [online]. Available from https://www.gov.mb.ca/sd/environment_and_biodiversity/invasive_species/fish_and_wildlife/index.html [accessed 10 April 2020]

Naddafi, R., Pettersson, K., and Eklöv, P. 2007. The effect of seasonal variation in selective feeding by zebra mussels (*Dreissena polymorpha*) on phytoplankton community composition. Freshwater Biology. **52**(5): 823-842. https://doi-org.uml.idm.oclc.org/10.1111/j.1365-2427.2007.01732.x

Naddafi, R., Pettersson, K., and Eklöv, P. 2008. Effects of the Zebra Mussel, an exotic freshwater species, on seston stoichiometry. Limnology and Oceanography. **53**(5): 1973-1987. https://doi-org.uml.idm.oclc.org/10.4319/lo.2008.53.5.1973

Nag, O.S. 2019. Citing online sources: The largest lakes in Canada [online]. Available from https://www.worldatlas.com/articles/which-are-the-largest-lakes-in-canada.html [accessed 5 Arpil 2020]

Ontario Invading Species Awareness Program. 2012. Citing online source: Spiny and Fishhook Water Fleas [online]. Available from www.invadingspecies.com [accessed 17 April 2020].

Pip, E. 2006. Littoral mollusc communities and water quality in southern Lake Winnipeg, Manitoba, Canada. Biodiversity and Conservation. **15**: 3637-3652. doi:10.1007/s10531-005-1877-y

Ricciardi, A., Serrouya, R., and Whoriskey, F.G. 1995. Aerial exposure tolerance of zebra and quagga mussels (Bivalvia: Dreissenidae): implications for overland dispersal. Canadian Journal of Fisheries and Aquatic Sciences. **52**(3): 470-477. https://doi.org/10.1139/f95-048

Rup, M.P., Bailey, S.A., Wiley, C.J., Minton, M.S., Miller, A.W., Ruiz, G.M., and MacIsaac, H.J. 2010. Domestic ballast operations on the Great Lakes: potential importance of Lakers as a vector for introduction and spread of nonindigenous species. Canadian Journal of Fisheries and Aquatic Sciences. **67**(2):256-268. https://doi.org/10.1139/F09-180

Seebens, H., Briski, E., Ghabooli, S., Shiganova, T., MacIsaac, H.J., and Blasius, B. 2019. Non-native species spread in a complex network: The interaction of global transport and local population dynamics determines invasion success. Proceedings of the Royal Society B. **286**(1901): 20190036. http://doi.org/10.1098/rspb.2019.0036

Schloesser, D.W., and Nalepa, T.F. 1994. Dramatic decline of unionid bivalves in offshore waters of western Lake Erie after infestation by zebra mussel, *Dreissena polymorpha*. Canadian Journal of Fisheries and Aquatic Sciences. **51**: 2234-2242

Schloesser, D.W., and Nalepa, T.F. 2013. Quagga and Zebra Mussels: Biology, impacts, and control (2nd edition). CRC Press LLC, Boca Raton, FL, USA.

Sprung, M., and Rose, U. 1988. Influence of food size and quantity on the feeding of the mussel *Dreissena polymorpha*. Oecologia. **77**(4): 526-532

Steinman, A.D., Lamberti, G.A., Leavitt, P.R., and Uzarski, D.G. 2017. Biomass and pigments of benthic algae. *In* Methods in stream ecology: Volume 1: Ecosystem structure. *Edited by* R.F. Hauer and G.A. Lamberti. Academia Press. 233 pp.

Stewart, K.W., and Watkinson, D.A. 2004. The freshwater fishes of Manitoba. University of Manitoba Press, Winnipeg, MB.

Strayer, D. L., Caraco, N.F., Cole, J.J., Findlay, S., and Pace, M.L. 1999. Transformation of Freshwater Ecosystems by Bivalves: A case study of Zebra Mussels in the Hudson River. BioScience. **49**(1): 19-27. doi:10.1525/bisi.1999.49.1.19

Sylvester, F., and MacIsaac, H.J. 2013. Is vessel hull fouling an invasion threat to the Great Lakes? Diversity and Distributions. **19**(12): 1578-1579. https://doi.org/10.1111/j.1472-4642.2009.00622.x

Ussery, T.A., and McMahon, R.F. 1995. Comparative study of the desiccation resistance of Zebra Mussels (*Dreissena polymorpha*) and quagga mussels (*Dreissena bugensis*). Technical Report EL-95-6, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS, USA.

Wassenaar L.I., and Rao, Y.R. 2012. Lake Winnipeg: The forgotten great lake. Journal of Great Lakes Research. **38**(3): 1-5. https://doi.org/10.1016/j.jglr.2012.04.004

Wonham, M., Bailey, S.A., MacIsaac, H.J., and Lewis, M.A. 2005. Modelling the invasion risk of diapausing organisms transported in ballast sediments. Canadian Journal of Fisheries and Aquatic Sciences **62**: 2386-2398. doi:10.1139/F05-146

Woodward, S.L., and Quinn, J.A. 2011. Encyclopedia of invasive species: From Africanized Honey Bees to Zebra Mussels (Vol. 1). ABC-CLIO LLC, Santa Barbara, CA.

Appendices

Appendix 1. Field Checklist

Safety/Field gear

- Life jackets
- Waders
- Raincoat
- Rain pants
- Gloves
- First Aid Kit
- Emergency contact phone numbers

Processing gear

- Boxes with lids
- Fish identification book
- Scientific Collection Permit
- Sampling protocol (in binder)
- Field book
- GPS
- Open reel tape measure (0–100 m)
- Coolers for transporting samples
- Ice packs
- Pencils/sharpeners
- Permanent markers
- Labels (on waterproof paper)
- Sample bottles for zooplankton
- Sample bottles for benthic invertebrates
- Additional sample containers
- Whirlpak® bags (4, 18 and 42 ounce)
- Zip top bags
- Reverse osmosis water
- 95 % ethanol
- Spray bottles for RO water and ethanol
- Temperature/light logger set-up
- Sediment and water sampling sheets
- Extendable sampling pole
- 1 l Nalgene® bottles (opaque, wide-mouth)
- Bottle brush
- Hanging scale (0–10 kg)
- Mesh Bag for hanging scale
- Measuring board (0–60 cm)
- Miscellaneous aquarium fish nets
- Waterproof Camera with case and float strap
- Live fish photographing chamber
- Zooplankton net (500 µm mesh, 12" opening, 39" long) with collection bucket
- Scoop
- Tweezers
- CABIN protocol kick net (400 µm mesh size with collection bucket)
- Shovel
- Metal sheet with holes (4.75 mm hole diameter)
- Wire template (10 cm^2)
- Aluminum foil
- Scraper
- Core tube
- Turkey baster
- Syringe
- Rubber stoppers
- Side cutters
- Lighter
- Knife

Net/Repairs

- Beach seine (5 mm mesh, 9 m long x 2 m high with a 2 x 2 m bag)
- Cable ties for net repairs

Appendix 2. Temperature/Light Logger Set-up

Hobo pendant MX 2202

Download the HOBOmobile application to phone/ tablet – enable Bluetooth and open app.

In the app **settings,** change units to SI.

1) Press the circular button in the center of the logger to wake it up. LEDs will flash. Tap the HOBO icon in the app (bottom left) and then tap the logger in the list to connect it.
2) **Configure > Logging Interval** (Set to 10s) > **Done.** Logging duration should now be set to ~5.5 d.
3) Hit **Start Logging > On Date/Time >** input desired date and time to start logging **> Done**.

Note: Put the Date and Time of start as the same for all Loggers so that they start recording data at the same time.

4) **Stop Logging >** Hit **"When Memory Fills" "On Button Push"** and under Stop Logging (Time Options) **"After…"** select duration of logging **> Done**.

Note: Similar to 'start logging' (3), make sure these are all set to stop recording after the same period of time.

5) Select **Start** from the upper right hand corner of the configuration page. This will take a few seconds to configure. Select **OK** from the pop-up after configuration to continue.
6) Hit the >
7) **HOBOs** button in the top left corner, and select the next logger to configure it using steps 1–4.
8) This brings you back to the connected page. **Start Logging** should say "awaiting delayed start." Once the Logger starts recording this button changes to **Stop Logging.**

Note: You can view data recording in real time with the **Full Status Details,** which also allows you to view the current configuration settings.

9) Hit **Stop Logging** to stop earlier or wait until duration of logging is over.
10) Once stopped the **Stop Logging** button changes to **Start Logging (reconfigure to start)**
11) Hit **Readout > OK** to save file.

Note: To start logging again, start back at step 2)

12) To see files hit **Data Files > "Close Connection" > Yes**
13) To download hit **Select > tap files to select > Share > CSV > Export symbol** (upper right) > select mail or other app to send/save to.

Appendix 3. Aquatic Species at Risk in Manitoba

The following information and images were retrieved from the book *The Freshwater Fishes of Manitoba* (Stewart and Watkinson 2004).

Silver Chub (*Macrhybopsis storeriana)*

- **Status:** Species of Special Concern (COSEWIC, SARA)
- **Synopsis:** Manitoba may be home to one of the last healthy and abundant populations of Silver Chub in North America. It is a member of the minnow family. It is usually found in slow moving water, over soft substrates. Silver Chub are abundant in the Red and Assiniboine rivers, and may also be found in the south basin of Lake Winnipeg. Silver Chub may live to the age of 3. They spawn in summer. Populations are adversely affected by low oxygen levels in the water and fluctuations in water temperature.
- **Description:** Silver Chub have large scales, silver sides, and olive green back, silver-white under parts, a faint lateral band, an inferior mouth and a pale streak along the lower edge of the caudal fin. The average adult total length is 150–210 mm.

Carmine Shiner (*Notropis percobromus*)

- **Status:** Endangered (COSEWIC, SARA)
- **Synopsis:** Within Canada, Carmine Shiner is only found in Manitoba. It has one of the most limited distributions of fish species at risk in Manitoba. It is found in the Whitemouth River watershed and the Winnipeg River. It generally requires clear, fast-flowing streams with clean gravel bottoms. Carmine Shiner is especially sensitive to habitat disruption, including alterations of water flow and siltation. They spawn in spring.
- **Description:** Carmine Shiner is distinguished by having the origin of the dorsal fin located behind a line drawn upward from the insertion of the pelvic fins. Carmine Shiner also lacks the fleshy keel on the abdomen and lacks the strongly decurved lateral line. It also has a snout length equal to the eye diameter. In addition, Carmine Shiner has black pigment outlining the scale pockets. Carmine

Shiner is a silvery minnow, with pinkish or rosy pigment on the opercula and cheek. Breeding males develop a bright red hue around the head, gills and pectoral fins which remains for the rest of its life. They can grow to 55–60 mm total length, with a maximum age of three years. It has a large mouth and transparent fins.

Bigmouth Buffalo (*Ictiobus cyprinellus)*

- **Status:** Species of special concern (COSEWIC, SARA)
- **Synopsis:** Bigmouth Buffalo is currently the largest native sucker in Canada. A mid-water to bottom feeding sucker, the Bigmouth Buffalo lives in warm, large, slow-moving rivers, lakes, and marshes. They can be found in the Red River and its tributaries, and the southern part of Lake Manitoba. Competition for habitat from Common Carp (*Cyprinus carpio*) can decrease the abundance of Bigmouth Buffalo and siltation can cover eggs. They spawn in spring.
- **Description:** Bigmouth Buffalo is a robust, deep-bodied, large-scaled sucker with a long dorsal fin. It lacks spines at the front of the dorsal and anal fins and lacks two barbells on each side of the upper jaw. It has a terminal, oblique mouth that lacks fleshy expansions of the lips and has anterior dorsal rays three or fewer times longer than the posterior rays. The Bigmouth Buffalo is similar to the carp in shape and can range from 358–690 mm total length. They have a bluish-green back shading to coppery-blue sides with a light bluish-grey belly.

Lake Sturgeon (*Acipenser fulvescens)*

- **Status:** Endangered (COSEWIC)
- **Synopsis:** Lake Sturgeon are considered living fossils, having changed little from their ancestors. They are the largest freshwater fish in Manitoba. Lake Sturgeon are bottom-dwelling fish found in large rivers and lakes including the Red and Assiniboine rivers, as well as Lake Winnipeg. They spawn in spring and may live over 100 years. Human activities represent the most important threat to sturgeon. Overfishing and construction of dams have resulted in the decline of Lake Sturgeon. These threats result in habitat degradation and fragmentation.

- **Description:** Lake Sturgeon are the only freshwater fish in Manitoba with a shark-like caudal fin, rows of large plates down the back and sides, the mouth is located on the underside of a long snout, and there is a row of four barbels across the snout in front of the mouth. Lake Sturgeon are dark to light brown on the back and sides, with a lighter belly. Lake Sturgeon can grow to 1200–1400 mm total length.

Shortjaw Cisco (*Coregonus zenithicus)*

- **Status:** Threatened (COSEWIC, SARA)

- **Synopsis:** Shortjaw Cisco is only found in North America. They are usually found near the bottom of large deep lakes, between 50–150 m. The Shortjaw Cisco has been recorded in Lake Winnipeg and Lake Athapapuskow. Shortjaw Cisco have declined due to commercial over-fishing and threats from non-native species of fish. Nothing is known about Shortjaw Cisco spawning in Manitoba, but most reports suggest they spawn in fall.

- **Description:** Shortjaw Cisco has a terminal mouth and the maxillary reach to below the eye. It has fewer and shorter gill rakers, and the lower jaw is usually included rather than terminal or projecting. In Manitoba, Shortjaw Cisco is smaller than the Cisco. Shortjaw Cisco have a green or blue back, silvery sides with a pink or purple sheen, and white bellies. Shortjaw Cisco has a second back fin near its tail called an adipose fin. They can grow to a length of 100–300 mm.

Chestnut Lamprey (*Ichthyomyzon castaneus)*

- **Status:** Species of Special Concern (COSEWIC, SARA)

- **Synopsis:** Chestnut Lamprey is found in most streams and lakes in southern Manitoba, including Lake Winnipeg. Siltation can degrade spawning habitat, and eutrophication can kill larvae. They spawn in late spring to early summer.

- **Description:** Chestnut Lamprey is distinguished from Northern Brook Lamprey (*Ichthyomyzon fossor*) by having the oral disc as wide as or wider than the body. They also have one or more bicuspid teeth in the inner lateral tooth row. They are olive coloured and have a single dorsal fin with a notch. Adults can range in size from 265–282 mm.

Mapleleaf Mussel *(Quadrula quadrula)*

- **Status:** Endangered (COSEWIC, SARA)

- **Synopsis:** Mapleleaf Mussel populations in Manitoba are located along the Red River and the lower reaches of its tributaries, and Lake Winnipeg. This mussel is in decline and appears to be limited to the Red, Assiniboine, Roseau and Bloodvein rivers. In Canada, the Mapleleaf is usually found in medium-to-large rivers with slow-to-moderate currents and firmly packed sand, coarse gravel or clay/mud bottoms (substrates). Like all species of freshwater mussels, the Mapleleaf filters its food from the water. Bacteria and algae are its primary food sources. Mapleleaf populations in Canada are threatened by invasive species, habitat loss and degradation, and siltation (more sediment in the water), which can bury, smother and starve filter-feeding mussels. In Manitoba, deteriorating water quality due to non-point source pollution and possible invasion of Zebra Mussels (*Dreissena polymorpha*) are major concerns.

- **Description:** Mapleleaf Mussel is a medium-sized freshwater mussel. This mussel is found in both Manitoba and Ontario, and is named for its somewhat square shape, which resembles a maple leaf. It has the following features:

    - Thick, square, yellowish-green (juvenile) or mostly brown (adult) shell;
    - Two rows of raised nodules form a V-shape along the outside of the shell;
    - Inside of the shell (nacre) is pearly white;
    - Raised part at the top of the shell (beak) is small and slightly raised above the hinge line;
    - Heavy hinge teeth;
    - Well-defined growth lines in juvenile mussels, crowded and difficult-to-discern growth lines in adults; and
    - Adults can grow to 12 cm in length.

(DFO 2016; COSEWIC 2016; photo credits to Douglas Watkinson)

Appendix 4. Aquatic Invasive Species

Zebra Mussel (*Dreissena polymorpha*)

Zebra Mussels are small, clam-like, aquatic animals that are a significant environmental and economic concern to Manitoba. Native to Eastern Europe and Western Asia, Zebra Mussel have caused millions of dollars in damage to the Laurentian Great Lakes area and have cost the North American economy billions of dollars to control.

- Usually 1–3 cm long.
- Triangular, or "D"- shaped shell.
- Most have light and dark brown bands on shells.
- Adult shells have very strong tufts of hair-like filaments, called byssal threads.
- Usually grow in clusters containing numerous individuals.

Zebra Mussels are the only freshwater mussel that firmly attaches itself to solid objects, including rocks, watercraft hulls, etc. Native mussels will bury into soft substrates on lake and river bottoms.

Unlike adults, larval Zebra Mussel, called veligers, are free-swimming and microscopic; they are difficult to see with the naked eye.

(MWFB 2020)

Spiny Water Flea (*Bythotrephes longimanus*)

- Spiny Water Flea is a small invertebrate that consumes large quantities of food. Populations expand quickly as they are not eaten by smaller fish, due to their long, barbed tail. It is a nuisance to anglers by entangling fishing line and downrigger cables.
- Adults range from 6–16 mm long.

- Spiny Water Fleas have a single long tail with small spines along its length.
- They collect in gelatinous globs on fishing lines and downrigger cables.

(Ontario Invading Species Awareness Program 2012)

Appendix 5. Laboratory Checklist

- Electric calipers
- Extra caliper batteries
- Paper towels
- Permanent markers
- Forceps (various sizes)
- Dissecting kit (scissors, scalpels)
- Fish size chart
- Fish identification book
- Lab notebook
- Lab protocol
- Pens/pencils
- Nitrile gloves
- Whirlpak® bags (various sizes)
- Large measuring board (0–60 cm)
- Cleaning supplies (cloths, brushes, dish detergent)
- Scale
- Lab coats
- Enamel tray
- Computer