



FishPass Assessment Plan

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1. Introduction

This assessment plan is intended to describe and coordinate appropriate monitoring and assessment techniques to address FishPass objectives and achieve biological goals (summarized in the Project Overview document). The plan also establishes standardized monitoring techniques, data units, data storage protocols, and quality-assurance/quality control procedures. All assessment activities are reviewed by the Science Team and approved by the FishPass Advisory Board. As the Science Team further develops (2018-2021) and the Advisory Board implements (2021) a FishPass research plan, new monitoring and assessment needs will arise; therefore, this plan is considered a living document that will be periodically updated and maintained on the FishPass website.

FishPass is being designed and implemented as a real-scale adaptive management experiment. Data on fish community structure and dynamics, fish movement/passage, contaminant loading, sea lamprey populations, habitat and habitat use, water quality, and stream morphology will be collected pre-, during-, and post-construction at representative reaches of the Boardman River. This assessment design will allow managers to make annual adjustments to adapt FishPass operations in terms of the number of individuals and species passed to effectively enhance fishery production in the Boardman River. Monitoring techniques will encompass direct (e.g., telemetry), indirect (e.g., fish surveys), and abiotic measurements.

The purpose of this assessment plan is to address broad monitoring efforts associated with overall project objectives and goals; however, it does not explicitly address monitoring requirements for individual research projects within the facility. While individualized monitoring plans will need to be developed for each research project (both internal and externally led), and integrated via the FishPass Advisory Board into this assessment plan, project leaders are encouraged to use the monitoring resources detailed herein. All collection permits and fish handling protocols are to be addressed by the Principle Investigator (PI) for each project and subject to the conditions and requirements of the PI's institution.

The assessment plan is organized to provide a preliminary list of target species and current knowledge of their movement, a compendium of past assessment and monitoring efforts in the Boardman River, and a description of monitoring programs associated with project objectives and metrics.

2. Target species and known movements

Fifty-eight fish species are known to occur in the Boardman River for part of their life cycle. However, a finite list of target species has been identified for study because not all species are relevant to fish passage studies in the lowermost reach of the river, have sufficient abundance to detect effects, or are easily obtained and/or tagged. Target species of the assessment plan are listed in Table 2-1, and categorized by

presence relative to the Union Street Dam: (1) downstream only, (3) upstream only, and (3) bi-directionally.

Lake sturgeon are a target species, but abundance is currently very low in the Boardman River. Only a few individuals have been observed downstream of the Union Street Dam in the last decade. Lake sturgeon will be included in indirect measurement efforts (e.g., fish surveys, DIDSON), fin clip taken for genetic testing, scanned for any existing tags, tagged if not already tagged, and released. No decisions have been made regarding potential stocking or local rearing efforts of rare species like lake sturgeon or arctic grayling.

The movement phenology of adults of many target species in the Boardman River is generally understood (Fig. 2-1); however, site specific timing and movement cues are unknown. The Union Street Dam acts as a barrier to nearly all upstream fish movement. However, sea lamprey larvae have been observed upstream of the Union Street Dam and periodic lampricide treatments have occurred since 1963. The dam has a pool and weir type fishway, but velocity conditions and step heights preclude passage by most native fishes; only introduced Pacific salmonids and some brown trout have been observed passing. In 2018, the lowermost step of the fishway will be blocked with a screen to prevent passage of any fish, including salmon, during the removal of Sabin Dam.

Table 2-1. Target species for study based on current presence up- or down-stream of Union Street Dam.

Common name	Scientific name	Location	
American Brook Lamprey	<i>Lethenteron appendix</i>		Up
Sea Lamprey*	<i>Petromyzon marinus</i>	Down	
Lake Sturgeon	<i>Acipenser fulvescens</i>	Down	
Coho Salmon	<i>Oncorhynchus kisutch</i>	Down	
Chinook Salmon	<i>Oncorhynchus tshawytscha</i>	Down	
Rainbow Trout	<i>Oncorhynchus mykiss</i>	Down	
Brown Trout	<i>Salmo trutta</i>	Down	Up
Brook Trout	<i>Salvelinus fontinalis</i>		Up
Lake Trout	<i>Salvelinus namaycush</i>	Down	
Common Carp	<i>Cyprinus carpio</i>	Down	
White Sucker	<i>Catostomus commersonii</i>	Down	Up
Rock Bass	<i>Ambloplites rupestris</i>	Down	Up
Smallmouth Bass	<i>Micropterus dolomieu</i>	Down	Up
Yellow Perch	<i>Perca flavescens</i>	Down	Up
Walleye	<i>Sander vitreus</i>	Down	Up
Round Goby	<i>Neogobius melanostomus</i>	Down	Up

*Sea lamprey larval assessments occur upstream of Union Street Dam

Approximately 0.5 kilometers downstream of Union Street Dam is the James P. Price Trap-and-Transfer Facility, which is owned by Traverse City and operated by Michigan Department of Natural Resources (MIDNR). The MIDNR installs removable grates in the fall to direct migrating salmon into a fish ladder

where Coho and Chinook salmon are harvested and rainbow and brown trout are returned to the river upstream of the weir. When the weir is not installed, fish moving upstream from Grand Traverse Bay have unimpeded access up to Union Street Dam. Fish may also enter Kids (Hospital) Creek, the only tributary located between Union Street Dam and the Trap-and-Transfer Facility, but access is limited due to a perched culvert outlet structure.

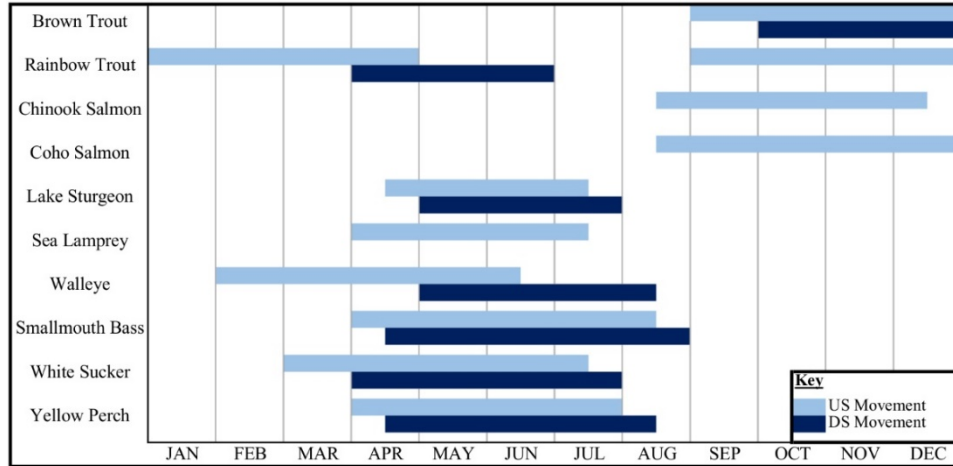


Figure 2-1. Migration timing of adults of select target species in the Boardman River. Timing data adapted from Goodyear et al. (1982), Biette et al. (1981) and Velez-Espino et al. (2011).

3. Existing assessment database

The Boardman River has been well studied, especially upstream of the Union Street Dam where a series of dam removals are underway. Monitoring programs established by the Boardman River Implementation Team and conducted by the Grand Traverse Band of Chippewa and Ottawa Indians (GTB), MIDNR, and non-government organizations (NGOs) have collected data on fish community, amphibian and reptile populations, macroinvertebrate community, and stream mechanics (i.e., flow and sediment transport). A preliminary FishPass database has compiled all available biological and environmental data on the Boardman River (Table 3-1) and efforts are currently underway to generate a relational database for long-term data management.

Table 3-1. List of existing assessment database items, years of record, and data source.

Description	Years of Record	Source
List of species present up- and down-stream of Union Street Dam	NA	MIDNR Boardman River Assessment 2014
Catch of salmon and trout at Boardman Weir (DS of Union Street Dam)	1987-current	MIDNR Boardman River Assessment 2014
Average total length-at-age and growth (relative to state average) for brook and brown trout	2005-2006	MIDNR Boardman River Assessment 2014
Chapman-Petersen population estimate for brown trout, brook trout, and rainbow trout	2005-2006	MIDNR Boardman River Assessment 2014
Boardman Lake walleye population estimate	2014	Little Traverse Bay Band of Odawa Indians
Boardman River creel survey data	NA	MIDNR
Sea lamprey trap catches and water temp by day and trap number	April - June 1980-current	U.S. Fish and Wildlife Service, Sea Lamprey Control Program
Lampricide treatment years	1963-2012	U.S. Fish and Wildlife Service, Sea Lamprey Control Program
Sea Lamprey Larval survey results (downstream of Union Street Dam)	1960-current	U.S. Fish and Wildlife Service, Sea Lamprey Control Program
Sea Lamprey Larval survey results (upstream of Union Street Dam)	1960-2014	U.S. Fish and Wildlife Service, Sea Lamprey Control Program
Mollusk documentation within the Boardman River watershed	1930-1998	MIDNR Boardman River Assessment 2014
Daily average flow	1952-current	USGS Gauge
Water surface elevation rating	NA	Stanley Consultants Hydraulic Analysis 2013

4. Monitoring program

The current monitoring program for FishPass is focused on establishing baseline data prior to construction. The program consists of five major projects: (1) long-term monitoring; (2) sea lamprey assessment; (3) fish movement and habitat use; (4) genetic population structure, species composition, and distributions; and (5) contaminant movement and transfer. Table 4-1 describes data collected for each project and specifies the project objectives and biological goals addressed. The project objectives are key to achieving the FishPass mission of providing bi-directional passage of desirable fishes while simultaneously blocking and/or removing undesirable fishes, while project goals refer to broad statements of anticipated project results of the biological elements of the project. Detailed descriptions of project objectives and biological goals are provided in the Project Overview document. Where individual study plans were previously developed, the study plan objectives and methods are provided.

Table 4-1. Monitoring program efforts, data collected, and corresponding project objectives and biological element goals.

Effort	Data Collected	Project Objectives	Biological Goals
Long-term monitoring	<ul style="list-style-type: none"> • Species composition, abundance, biomass, and size • Water quality • Habitat and stream morphology 	1, 2, 3	B2
Sea lamprey assessment	<ul style="list-style-type: none"> • Larval abundance through whole river • Adult abundance downstream of Union Street Dam • Spawning habitat 	1, 2, 3	B1
Movement studies	<ul style="list-style-type: none"> • Relative rates and patterns of fish approaching, entering, and exiting the FishPass site • When, where, why and how fish move 	1, 2	B1, B2
Genetic and eDNA sampling	<ul style="list-style-type: none"> • Genotype sequences • Presence of rare species • Species distributions 	2, 3	B1, B2
Contaminant transfer	<ul style="list-style-type: none"> • Type and amount of contaminants moving in system • Distribution of contaminants 	2, 3	B2

The following sections provide details on what monitoring techniques will be employed, by whom, when, and how the data collected will be evaluated with reference to project objectives and biological element goals. A summary of all fish sampling required for each monitoring effort is provided in Appendix Table I-1.

4.1. Long-term monitoring

Project team: H. Hettinger (PI), B. Fessell, J. Garavaglia, R. Swanson, D. Zielinski

Current funding status: In-kind

The purpose of a long-term monitoring (LTM) plan is to document changes in fish community and habitat over time at stationary sites located throughout the Boardman River. Six monitoring sites have been established, two downstream of Union Street Dam and four upstream (Fig. 4-1). Routine measurements of fish community, water quality, habitat, and stream morphology (Table 4-2) will be collected at each site over a period of 10 years. The upstream sites were selected based on existing MIDNR monitoring plans and monitoring efforts associated with dam removals. Specifically, the Brown Bridge Road site is a MIDNR index site and fisheries surveys have been conducted regularly since 1985. The downstream sites are new monitoring locations in the Boardman River. Monitoring techniques planned for each LTM site are provided in Table 4-2. A detailed summary of monitoring efforts organized by agency responsibility, data collected, and recording units is provided in the Appendix Table I-2.

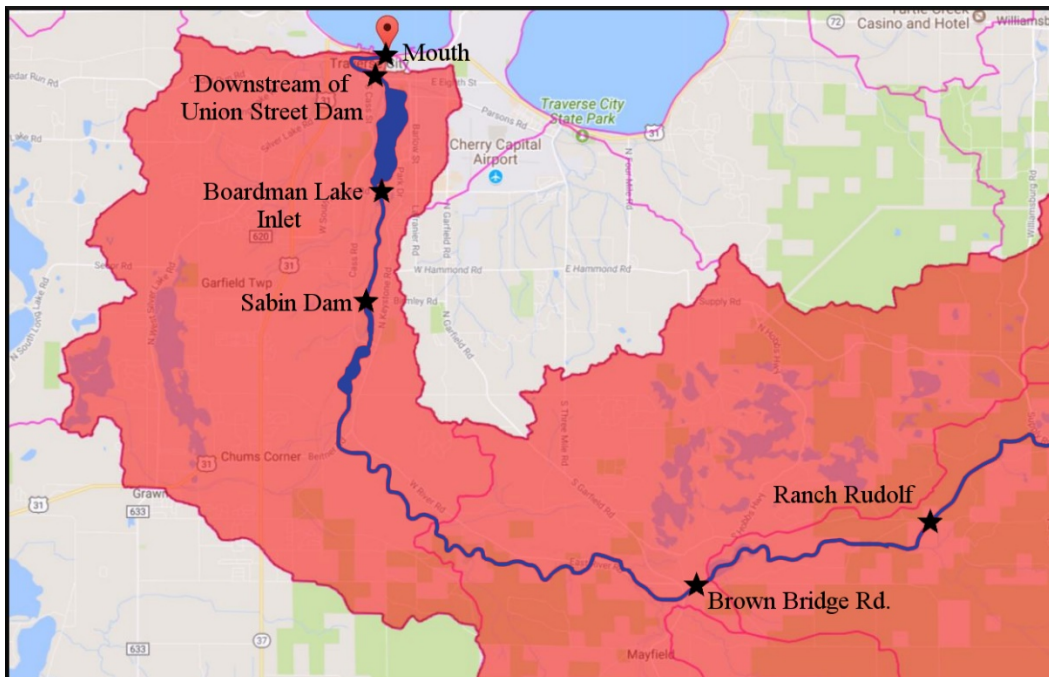


Figure 4-1. Locations of six long-term monitoring sites in the Boardman River.

Table 4-2. Monitoring techniques used at each LTM site. Agencies responsible for data collection are listed in parentheses.

LTM Site	Monitoring techniques
Mouth	<ul style="list-style-type: none"> • 3-4 electrofishing transects per year and habitat survey (MIDNR, GTB) • Water level gauge (TC)
Downstream of Union Street Dam	<ul style="list-style-type: none"> • Quarterly electrofishing transects and habitat survey (MIDNR, GTB) • Water level gauges up- and down-stream of dam (TC) • Water quality sensor at Trap-and-Transfer Facility (GLFC)
Boardman Lake Inlet/ S. Airport Road	<ul style="list-style-type: none"> • 2 Pass electrofishing survey (MIDNR) • Water quality sensor at YMCA (GLFC)
Sabin Dam	<ul style="list-style-type: none"> • 2 Pass electrofishing survey (MIDNR) • Water quality sensor at Beitner Rd. (GLFC) • Stream gauge (USGS)
Brown Bridge Road	<ul style="list-style-type: none"> • 1 Pass electrofishing survey and habitat survey (MIDNR) • Stream gauge (USGS)
Ranch Rudolf	<ul style="list-style-type: none"> • 1 Pass electrofishing survey and habitat survey (MIDNR)

Electrofishing Surveys: Electrofishing surveys are conducted by the GTB and MIDNR and will follow the MIDNR electrofishing sampling protocols (Wills et al., 2011). The frequency of surveys in Table 4-2 represent yearly goals, but the completion of all surveys will be subject to weather and stream conditions. Surveys downstream of Union Street Dam consist of a single pass with a boomshocking electrofishing boat and fish collection using 4.7mm (3/16 inch) delta mesh dip nets. Fish less than 25.4 mm (1-inch) total length are not surveyed. The reach between the Trap and Transfer facility and Union Street Dam is only accessible by boat when conditions allow the boat to be launched from the shoreline of Hannah Park. Backpack electrofishers are used in river reaches upstream of Union Street Dam that are inaccessible to a boomshocking electrofishing boat. The species, weight (pounds), and total length (inch) identification of all fish greater than 25.4 mm (1-inch) standard length are recorded and fish are released or collected for other monitoring projects. Fish identified for the movement study (Section 4.3) will be tagged prior to release. Electrofishing surveys provide data to identify species composition, relative abundance (number of fish per unit of sampling effort), biomass (pounds/acre), density (fish/acre), size structure (mean length in inches at age), and percent survival (%).

Habitat Survey: In conjunction with electrofishing surveys, measurements of local habitat are collected. Habitat surveys follow MIDNR habitat sampling protocols (Wills et al., 2011). Generally, habitat surveys will document: (1) riparian zone conditions (dominant vegetation within 30 feet of water’s edge);

(2) width (feet), depth (feet), and substrate type (classify as pool, riffle, or run); (3) large woody material (count of material >6 inches in diameter and > 6 feet long); and (4) discharge (ft^3/s).

Stream Gauge: Discharge in the Boardman River is continuously monitored by USGS gauges at Beitner Road (04127200) and Brown Bridge Road (04126970), and can be adjusted for sampling location through direct drainage area ratio adjustment.

Water level gauges: River stage (feet relative to sea level) data are collected from three HOBO data loggers (model: MX2001-04) installed on the US-31 Highway Bridge over the Boardman River mouth, Union Street Bridge (downstream of Union Street Dam), and Cass Street Bridge (upstream of Union Street Dam) and are maintained by Traverse City.

Water quality sensors: Continuous water quality data are collected using a YSI multiparameter water quality sondes (model: 6600V2-4) installed at the Trap-and-Transfer facility, near the YMCA (upstream of Boardman Lake inlet), and Beitner Road (between Sabin Dam and Brown Bridge Road) and are maintained by the Great Lakes Fishery Commission (GLFC). The sondes record hourly water temperature ($^{\circ}\text{C}$), conductivity ($\mu\text{S}/\text{cm}$), turbidity (NTU), and dissolved oxygen (mg/L).

4.1.1. Link to project objectives and goals

Overall the LTM efforts will provide measures of species composition and relative abundance, habitat type, and environmental data (e.g. water level and quality). Together, these measures address all project objectives and biological goal B2.

4.2. Sea lamprey assessment

Project team: A. Jubar (PI), J. Barber, B. Fessell, J. Garavaglia

Funding Status: In-kind

Assessment of sea lamprey populations downstream of the Union Street Dam are conducted by the GTB in collaboration with the U.S. Fish and Wildlife Service (USFWS) and larval assessments are conducted upstream of the dam by the USFWS. Two sea lamprey traps are installed on the downstream side of Union Street Dam during April-June to capture, enumerate, and remove migrating adult sea lamprey. A portion of trapped sea lamprey are tagged and released back downstream to provide an estimate of abundance (Mullett et al., 2003; GLFC, 2018). Upstream of the Union Street Dam, larval assessments are done annually (July-August) by backpack electrofishing at two LTM sites (Hansen and Jones, 2008). Upstream production potential of sea lamprey will be examined using quantitative assessment (Slade et al, 2003) of native lamprey (*Lethenteron appendix* and *Ichthyomyzon* spp.), used as surrogates, at each LTM site prior to construction of FishPass.

4.2.1. *Link to project objectives and goals*

Sea lamprey assessment efforts will determine the adult stream abundance of sea lamprey production potential of the Boardman River upstream of Union Street Dam and detect passage of sea lamprey upstream, which directly addresses all project objectives and biological goal B1.

4.3. Movement studies

Project Team: D. Zielinski (PI), R. Swanson, E. McCann, B. Fessell, H. Hettinger, C. Holbrook, T. Castro-Santos, R. Goodwin, F. Dituri, A. Muir

Funding Status: 2 years: \$140,000 (YR1), \$200,000 (total) from Great Lakes Restoration Initiative

Space-use patterns of fishes downstream of FishPass and movement (i.e., approach and passage) rates upstream are expected to change in response to increased connectivity provided by FishPass (once constructed). A fish movement monitoring plan has been created to assess if and how fish movement and space use change in response to selective fish passage. The monitoring plan specifies telemetry to (1) establish a baseline understanding of fish movement in the Boardman River, especially below Union Street Dam, and (2) identify changes in movement in response to selective passage. A baseline fish movement monitoring program will eventually help distinguish the relative effectiveness each selective fish passage treatment and identify ways to increase efficacy.

4.3.1. *Space use of resident and migratory fishes in the lower Boardman River before installation of a selective fish passage facility*

This plan will focus on monitoring movement of five species (i.e., white sucker, rainbow trout, smallmouth bass, walleye, common carp) representing the typical assemblage of large-bodied fishes downstream of the Union Street Dam and invasive sea lamprey using a combination of telemetry gear including radio and RFID (Radio-frequency identification) passive integrated transponder (PIT) tags. Radio telemetry systems are able to measure fish movement at coarse scales and are ideal for sites with shallow water, low conductivity, high turbidity, and turbulence. PIT tags have been used to automatically identify and track animals and/or objects since the early 1970's and are known as robust and reliable monitoring technique for fish passage evaluation (Castro-Santos et al., 1996). All fish collected during MIDNR and GTB fishing surveys below Union Street Dam will be implanted with a passive integrated transponder (PIT; 23-mm half duplex). PIT antennas will be installed at the entrance of the existing fishway at the Union St. Dam to document fishway encounter and entry rates and at the MIDNR trap-and-transfer weir (Fig. 4-2). There will be no need to monitor passage through the fishway because all passage will be blocked by a screen while Sabin Dam is being removed (2018-2019) and FishPass construction (2019-2020) and optimization of sorting (2021-2030).



Figure 4-2. Locations of radio receivers with aerial directional Yagi and underwater omnidirectional dipole radio antennas, and PIT antennas in the existing Union St. Dam fishway and trap-and-transfer facility weir.

All fish collected by GTB and MIDNR during LTM sampling downstream of Union Street Dam or collected during operation of the trap-and-transfer facility will be scanned for PITs. A sample of each target species ($n=10$ of each fish in Year 1, 60 total; $n=40$ in Year 2) will also be anesthetized (AQUI-S 20E per MIDNR INAD), implanted with a coded radio transmitter, measured, sexed (when possible), and released. Radio tags will be monitored by an Orion Broadband Receiver/Datalogger system (SigmaEight, Ontario CAN). The system selected for the Boardman River operates at 164.48 MHz. A radio receiver with two directional Yagi antennas (one pointed upstream, one pointed downstream) is installed at the river mouth (E. Grandview Parkway Bridge) to document movement in and out of the river. Two radio receivers with an underwater dipole antenna array will be placed along the river width at the S. Union St. Bridge to document entry into the future project area and cross-channel distribution (north vs. south bank). The study area (between E. Grandview Parkway and Union Street Dam) will be searched from a small boat or shore once every two weeks using a handheld radio receiver and Yagi antenna. Location (long., lat.), signal strength (dB), and bearing ($^{\circ}$) of each detection will be recorded and approximate location of fish detected during each survey will be estimated. Movement data will be compiled with daily measurements of streamflow (ft^3/s), water level (ft, above dam, below dam, and at river mouth), and water quality (see LTM water quality data). Water velocity profiles at each monitoring site will also be characterized (across a range of river discharges) using ADV or ADCP. A camera installed near the

Union Street Bridge will collect time-lapse images of recreational boating and fishing activity at the Union Street Dam to evaluate potential angler efforts on fish movement.

The fish space use study will be conducted in two phases:

- Phase 1 (Summer 2018) will be a pilot level study with the goal of installing the telemetry systems and troubleshooting any unexpected issues. A small number of radio tags will be deployed ($n=10$ of each fish, 60 total). All fish weighing more than 30 g (no more than 50 per species) captured during LTM surveys or trap-and-transfer facility operation (e.g., rainbow trout and brown trout) will be implanted with PIT tags. Manual tracking efforts will help determine whether additional telemetry gear is required to monitor fish movement between Kids Creek and Boardman River.
- Phase 2 (Fall 2018 – Spring 2020) will expand the study plan. New research questions will be identified as part of the FishPass research plan and adjustments to the telemetry system will be made. More radio tags will be deployed ($n=40$), and PIT tagging will continue. The project team will develop a proposal in collaboration with the Great Lakes Acoustic Telemetry Observation System (GLATOS) to incorporate acoustic telemetry into the fish movement monitoring plan.

4.3.2. DIDSON survey plan

Dual-frequency Identification Sonar (DIDSON) cameras produce near-video quality data that allow for monitoring fish movement at much finer temporal and spatial scales than traditional monitoring techniques (Hateley and Gregory, 2006). DIDSON cameras (Sound Metrics, Seattle WA) have been proven an effective tool to view fish under low light and high turbidity. DIDSON imagery can be manually examined to quantify fishes as they swim through the field of view using DIDSON software. In general, fish species cannot be easily discerned from the footage, but lengths and behaviors can be documented.

A DIDSON was deployed in 2017 on the north bank of the Boardman River at the James P. Price Trap-and-Transfer Facility to capture video of fish movement and behavior in conjunction with environmental variables collected as part of the LTM plan. The DIDSON data is used to evaluate the environmental triggers for stream entry of three size classes of migratory fishes in the Lower Boardman River.

The DIDSON field of view is positioned horizontally and perpendicular to stream flow at a downward tilt of -4.7° with a viewing window spanning from 1.67 m to 11.67 m (Fig. 4-3). DIDSON data are manually examined to quantify fishes as they swim through the field of view using the DIDSON V5.25 software

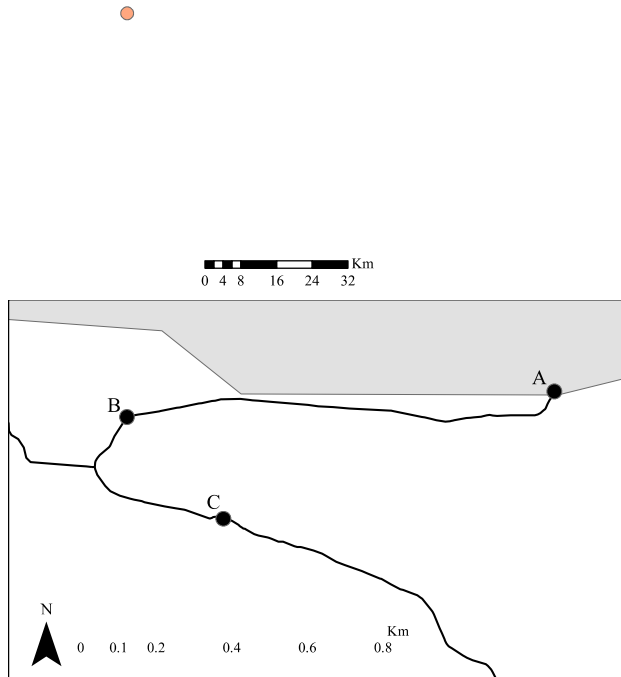


Figure 4-3. Panel A: Map of the Boardman River watershed; Panel B: Map of the Boardman River study site showing A) the Boardman River mouth, B) the location of the DIDSON, and C) the location of the Union Street Dam; Panel C: General diagram (aerial view) of the DIDSON setup.

(Sound Metrics Corps.). Fish are sorted into three size classes: small (< 30 cm), medium (>30 cm and < 50 cm), and large (> 50 cm). While fish species cannot be determined for most fusiform fish, sea lamprey can be identified because of their unique body shape and sinusoidal swimming behavior. Fish observed in the field of view are measured and assigned a general size range. Fish appearing on the cusp of a size class are measured using the measuring tool in the DIDSON software. Total counts of fish in the field of view are recorded, but due to the complex variability in swimming behaviors, direction of travel cannot be determined. Data are subsampled daily into four, two-minute DIDSON clips at sunrise, sunset, the midpoint between sunrise and sunset (i.e., mid-day) and the midpoint between sunset and sunrise (i.e., mid-night). All clips from all days are randomized prior to manual inspection. Increased subsampling may be required during peak sea lamprey migration to assess stream entry timing. Stream temperature, river stage, and stream discharge are measured as part of the LTM plan and used to determine their relative influence on the presence of the three size classes of fishes.

Daily passage and environmental conditions are assessed using a hazard analysis to evaluate environmental cues that predict passage of fishes of a given size class within the Lower Boardman River. A hazard analysis is used to estimate the “cumulative hazard” for each individual to determine what environmental trigger(s) most influence fish passage. The covariates used during the initial run of the hazard analysis on 2017 data included time of day, stream temperature (°C), river stage (feet), stream discharge (ft³/s), and 24-hour time delays of stream temperature (°C), river stage (feet), and stream

discharge (ft³/s). Akaike's information criterion (AIC) is then used to rank several subsets of the full model to determine which variables most influence fish movement for each size class. Results from the 2017 data concluded that stream temperature, 24-hour time delayed river stage, and time of day were the best predictors of stream entry for all three size classes of fish. The DIDSON was deployed in 2018 to collect a second year of data and an additional hazard analysis will be performed. Following the second year analysis, the data will be reviewed and options for future deployment considered.

4.3.3. Link to project objectives and goals

Overall, the telemetry plan will determine: (1) longitudinal space use (utilization distributions) of individuals in the lower Boardman River (i.e., between Lake Michigan and the Union Street Dam); (2) the rate that individuals enter the future project site (i.e., pass the S. Union St. bridge); (3) the lateral (cross-channel) distribution of individuals as they enter the future project site; (4) the rate that individuals encounter the existing Union St. Dam and fish ladder; and (5) if site approach rates, spatial distributions (lateral and longitudinal), dam encounter and passage, differ among species, life stages, season, recreational boating, fishing, or environmental conditions. The DIDSON survey also evaluates the environmental triggers for stream entry of migratory fishes in the Lower Boardman River without needing to tag fish. Combined, these outcomes directly address project objectives #1 and #2 and biological goals B1 and B2.

4.4. Genetic and eDNA sampling

Project Team: W. Larson (PI), W. Stott, H. Hettinger, F. Dituri, D. Zielinski, A. Muir

Funding Status: 2 years: \$125,000 (YR1), \$150,000 (total) from Great Lakes Restoration Initiative

Collecting baseline genetic data from species such as walleye, smallmouth bass, yellow perch, white sucker, and rock bass as well as eDNA water samples from sites up- and down-stream of the Union Street Dam will allow us to determine the potential genetic consequences and benefits of fish passage on the genetic diversity of fish populations upstream of FishPass.

4.4.1. Genetic assessment of Boardman River fish populations prior to dam removal

We hypothesize that differences in genetic diversity and significant population structure will exist between fish populations up- and down-stream of the Union Street Dam due to physical separation. We further hypothesize that decreases in genetic differentiation between fish populations separated by the Union Street Dam and increases in genetic diversity of populations upstream of the dam will be detectable 5-10 years after fish passage has been initiated. However, it is likely that these effects will vary substantially by species due to differences in life history. We also hypothesize that eDNA will be a useful tool for monitoring species diversity and distribution patterns.

The plan objectives are to: (1) characterize baseline genetic structure for five fish species up- and down-stream of the Union Street Dam to determine if these populations are significantly differentiated and/or show differences in diversity; and (2) determine the utility of eDNA for investigating species diversity and distribution patterns.

Objective 1: We will use restriction-site-associated DNA (RAD) sequencing to genotype thousands of genetic markers in five fish species sampled above and below the Union Street Dam as part of LTM efforts. RAD sequencing was selected because RAD data provides substantially more power to detect population structure compared to microsatellite data and also facilitates individual-based analyses and detection of markers that may be under selection. The five species that will be included in this project are white sucker, smallmouth bass, walleye, yellow perch, and rock bass. As part of a separate research project, the Larson lab also collected baseline genetic data from brook trout upstream of Union Street Dam. The brook trout data will be included in findings reports for this study. The species selected for this study display variation in a number of life history parameters that may influence their genetic response to habitat fragmentation including size, age at maturity, population size, propensity to migrate, spawning behavior, and fecundity. Baseline sampling will occur over two years, and 50 samples from up- and down-stream of the dam will be collected for each species in each year (200 total samples per species). Fin clips will be collected by GTB and MIDNR as part of LTM sampling. Biological data including species name, total length (inch), and sampling location will be recorded. Tissue samples will be stored in sample tubes (provided by the Larson Lab) filled with 95% ethanol. Samples will be shipped to the Larson Lab, where standard genetic analyses will be conducted to determine whether significant differentiation and differences in diversity exist between populations up- and down-stream of the dam.

Genetic diversity measures will be estimated for all sampled populations using Genetic Analysis in Excel v6.502 (GenAlEx; Peakall and Smouse, 2006). Conformation to Hardy-Weinberg equilibrium (HWE) for each sampled population will be estimated using the Hardy-Weinberg exact test to ensure the samples are representative of discrete populations. Population-specific measures of diversity including mean number of alleles/locus, effective number of alleles, expected and observed heterozygosity, and private alleles along with the standard error or standard deviation of each measure will be calculated. Genetic structure among the populations will be determined using three different statistical tests. Pairwise population F_{ST} estimates (θ ; Weir and Cockerham, 1984) based on the variance in allele frequencies and significance from a null of zero will be estimated for the pairwise population comparisons using GenAlEx with 9,999 permutations. The Weir-Cockerham adjusted F_{ST} statistic (θ) is commonly used to characterize genetic structure by comparing the genetic diversity both within and among the different populations. A second approach using Bayesian admixture analysis (STRUCTURE 2.3.4; Pritchard et al., 2000) will be

employed to determine if the two populations of each species represent a single genetic source or are multiple genetic units. This method assumes that all individuals are equally likely to be assigned to a given genetic unit regardless of population origin. The final test, Principal Coordinate Analysis (PCoA; Orloci, 1978) as employed in GenAEx, will be used to plot and visualize the major multivariate variation between the two populations of each species. Genetic differentiation between populations is likely to be low since populations up- and down-stream of the dam have only been isolated for 10 – 20 generations.

Objective 2: Aquatic species constantly slough DNA from skin cells, excrement, and other sources; this DNA, termed environmental or eDNA, can be collected and used to determine which species are present in a given section of river. A rapidly growing body of literature suggests that eDNA sampling can provide a cost-effective alternative to traditional sampling and can often detect species that may not be detectable with traditional methods. We will use eDNA metabarcoding to investigate species diversity at the LTM sites in the Boardman River. The MIDNR will collect water samples for eDNA analysis from these sites at the same time that they conduct traditional backpack electrofishing surveys for species diversity. Samples will be taken close (within 1 m) of each bank and in the river thalweg. At each location samples will be taken from the water column rather than at the surface or bottom. Three replicates will be taken within a few meters of each other for each of these three sampling areas (thalweg, and each bank). Samples will be collected using an unused conical tube (1 for each bank and the thalweg) containing 15 ml of water sample, 33 ml of ethanol, and 1.5 ml of sodium acetate. Water will be added to the conical tube containing the ethanol and sodium acetate, sealed with plastic, inverted once or twice, and sent to the PI within a 2-3 days. Samples will also be taken throughout the year to determine whether temporal differences in species composition exist. eDNA metabarcoding involves using polymerase chain reaction to amplify regions of the mitochondrial genome such as cytochrome oxidase subunit I (COI) that are commonly used to conduct species identification then sequencing those amplified fragments on a high-throughput sequencer. The Larson lab has developed primers from COI sequences that can be used to differentiate over 80 fish species; these primers should be useful for identifying nearly all the species found in the Boardman River. Once eDNA sampling is completed, we will compare the results to traditional field surveys to determine the strengths and weaknesses of each method.

4.4.2. Link to project objectives and goals

Overall genetic assessment of fish communities up- and down-stream of the Union Street Dam, and eventually the FishPass facility, will allow for evaluation of the genetic effects of fish passage on fish populations upstream of the dam and investigate distribution patterns of species that are being passed, including rare species. These outcomes directly address project objectives #2 and #3 and biological goal B2.

4.5. Contaminant transfer

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A suite of Lake Michigan migratory fishes will be measured for contaminants to assess risk of contaminant transfer to resident fishes prior to and following implementation of selective fish passage. The Boardman River, MI, Union Street Dam removal (2019-2020) and replacement with FishPass will be used as a case study to inform issues related to fish passage and contaminant biotransport.

4.5.1. Predicting contaminant transfer following re-establishment of controlled connectivity in the Boardman River

Legacy contaminants can accumulate in both fishes separated by dams and sediments retained upstream of dams, such that dam removal can inadvertently facilitate both contaminant transport upstream by migratory fishes and downstream via sediment flushing. Additionally, lake migrants, particularly semelparous fishes that spawn and die in rivers, could represent a novel influx of lake-derived contaminants once connectivity is re-established. Such biologically and physically transported contaminants can negatively impact ecosystems, fisheries, and human health. These risks must be evaluated in scope and weighed against the positive ecosystem benefits of re-establishing connectivity. Prior studies have shown that the contaminant burden of resident fishes in Great Lakes tributaries relates strongly to the flux of contaminants supplied by spawning non-native Pacific salmon (Gerig et al., 2016, Janetski et. Al., 2012). At present, little is known about contaminant transport by the other ~50 Great Lakes fish species that migrate with varied frequency to tributaries to spawn, including many historically found in the Boardman River. As such, future policy related to fish passage and potential effects on human consumption limits after re-establishing connectivity would be greatly informed by understanding which migrants pose the greatest risk for contaminant transfer. Further, knowledge of contaminant transport risks can help inform the Advisory Board on species and numbers of individuals of a given species to pass.

In this project, we propose to assess the potential for migratory fishes to act as a vector for contaminant transfer in the Boardman River watershed. The project objectives are to: (1) assess the contaminant burden of Great Lakes spawners to inform future fish passage decisions; (2) evaluate the background contaminant burdens of resident fishes prior to dam removal; (3) measure background contaminant levels of water within the Boardman River watershed; and (4) couple empirically collected diet data to a lifetime bioenergetics-bioaccumulation model to determine the impact of various fish passage scenarios on resident fish growth and bioaccumulation. Future research following fish passage will evaluate the

overlap in distribution between Great Lakes migrants and stream-resident fishes to further infer benefits of restored connectivity and risk of contaminant transfer.

Objective 1: To establish risk of contaminant transfer through selective passage, we will measure the contaminant burden of whole fish and egg samples for a subset of Great Lakes migrants including Chinook salmon, Coho salmon, lake trout, rainbow trout, brown trout, white sucker, longnose sucker, walleye, yellow perch, smallmouth bass, and rock bass. We will focus on migrants that are common to the Boardman River, which exhibit species-specific traits with respect to life history (semelparous, iteroparous), origin (native, introduced), spawning mode (broadcast, redd building), fecundity (gonadosomatic index), phenology (spring, summer, fall), abundance (run size), and biochemical makeup (lipid content, contaminant burden) that might mediate contaminant transfer. A total of 10 fish (5 male, 5 female) of each species and of similar size (> 20 g) will be collected. Eggs will also be collected from the 5 female migrants. All migrants will be measured for polychlorinated biphenyls (PCBs dichloro-diphenyl-trichloroethylene (DDT) metabolites, chlorinated pesticides, and total mercury (THg). We will relate contaminant burden to individual fish variables including stable isotope ratios of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$), lipid content, fish age, fish size, gonadosomatic index, and species traits to identify factors that increase the likelihood of contaminant transfer. Migratory fishes will be captured during their respective spawning run to the Boardman River as part of LTM.

Objective 2: Resident fishes will be sampled from the LTM stations throughout the Boardman River watershed (Fig. 4-1) along with one site in Boardman Lake. Stream-resident fishes, including brook and brown trout, and other species of interest will be measured for the same suite of contaminants and biological variables as Great Lakes migrants. A total of 10 fish of each species and of similar size (>20 g) will be collected. Fish diet composition will be measured using standard survey methods (Hyslop, 1980) including gastric lavage. These data will establish a baseline for background contaminant levels in the Boardman River prior to dam removal and FishPass construction.

Objective 3: Dissolved concentrations and potential sources of persistent organic contaminants (PCBs, DDT, chlorinated pesticides) will be characterized at the LTM stations throughout the Boardman River watershed (Fig. 4-1) along with one site in Boardman Lake using semi-permeable membrane passive sampling devices (SPMDs). SPMDs will also be used to determine contaminant concentrations in the Boardman River prior to and following the removal of the Union Street Dam and construction of FishPass. An additional SPMD deployment will be completed in Grand Traverse Bay to act as a reference location where atmospheric deposition is the predominant contaminant source. SPMDs will provide evidence of spatial variability in the sources of anthropogenic contaminants, which can increase bioaccumulation in resident fishes.

Objective 4: Empirical and literature data on migratory and resident fishes will be integrated to parameterize an individual-based bioenergetics-bioaccumulation model that has already been developed (Gerig et al., 2018) to predict the impacts of migratory fishes on resident fish growth and contaminant bioaccumulation under different fish passage scenarios. The model will be parameterized with system-specific empirical data based upon background resident fish contaminant concentrations, diet composition, prey contaminant concentration, and stream water temperature. The model will be validated using empirical data collected from sites without migratory fishes. After validation, a simulation will be run for multiple fish passage scenarios to predict the individual and combined impact of migratory spawners on growth and contaminant burden of resident fish species in the Boardman River watershed.

4.5.2. *Link to project objectives and goals*

The conceptual model, rooted in fish traits, will be used to evaluate the “axes of risk” which govern contaminant biotransport by migratory fishes. In addition, the bioenergetics-bioaccumulation model will disassemble the process of contaminant biotransport to address how background contamination and fish migrations interact to influence resident fish bioaccumulation. Further, legacy contaminants of concern (fat-bound PCBs and muscle-bound Hg) will be modeled simultaneously so potential differences in their fluxes from fatty (salmon, rainbow trout) vs. lean (suckers) species can be evaluated. The model will provide managers with a simple yet powerful decision-support tool to manage migratory fishes in the Boardman River. These outcomes directly address project objectives #2 and #3 and biological goal B2.

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Appendix I

Table I-1. Summary of fish sampling organized by location for FishPass monitoring efforts

Site/Study	Long Term Monitoring	Sea Lamprey Assessment	Telemetry	Genetics	Contaminant
River Mouth	Quarterly sampling all species (ID, length, weight, number)	None	<p>All samples DS of Union Street Dam</p> <p><i>Radio Tags (total of 10 individuals each, >30 g):</i></p> <ul style="list-style-type: none"> •Sea Lamprey •Rainbow Trout •Common Carp •White Sucker •Walleye •Smallmouth Bass 	<p>Samples DS of Union Street Dam (any site)</p> <p><i>Water samples for eDNA during each LTM sampling:</i></p> <ul style="list-style-type: none"> •3 x 15ml samples (left bank, right bank, thalweg) <p><i>Fin clips (50 individuals per species per year):</i></p> <ul style="list-style-type: none"> •White Sucker •Walleye •Smallmouth Bass •Yellow Perch •Rock Bass <p><i>Fin clips or scale (If encountered)</i></p> <ul style="list-style-type: none"> •Lake Sturgeon 	<p>Samples DS of Union Street Dam (any site)</p> <p><i>Whole fish samples (5 males, 5 females + eggs of each species, approx. same size, >20 g):</i></p> <ul style="list-style-type: none"> •Lake Trout •Sea Lamprey •Rainbow Trout •Chinook Salmon •Coho Salmon •Brown Trout •Walleye •Yellow Perch •White Sucker •Longnose Sucker •Smallmouth Bass
Downstream of Union Street Dam	Quarterly sampling all species (ID, length, weight, number)	Sea lamprey index trapping	<p><i>PIT tag (Max 50 per species, >30 g):</i></p> <ul style="list-style-type: none"> •Sea Lamprey •Rainbow Trout •Common Carp •White Sucker •Walleye •Smallmouth Bass •Lake Trout •Brown Trout •Yellow Perch •Rock Bass 		
S. Airport Road	2 Pass electrofishing survey all species (ID, length, weight, number)	None	None		<p>Samples US of Union Street Dam (Boardman Lake/ S. Airport Rd.)</p> <p><i>Whole fish samples (10 individuals of each species approx. same size >20 g):</i></p> <ul style="list-style-type: none"> •Brook Trout •Brown Trout •Walleye •Yellow Perch
Sabin Dam/Lone Pine	2 Pass electrofishing survey all species (ID, length, weight, number)	None	None	<p>Samples US of Union Street Dam (any site)</p> <p><i>Water samples for eDNA at each site:</i></p> <ul style="list-style-type: none"> •3 x 15ml samples (left bank, right bank, thalweg) 	<p>Samples US of Union Street Dam (Sabin Dam/Lone Pine)</p> <p><i>Whole fish samples (10 individuals of each species approx. same size >20 g):</i></p> <ul style="list-style-type: none"> •Brook Trout •Brown Trout
Brown Bridge	1 Pass electrofishing survey all species (ID, length, weight, number)	None	None	<p><i>Fin clips (50 individuals per species per year):</i></p> <ul style="list-style-type: none"> •White Sucker •Walleye •Smallmouth Bass •Yellow Perch •Rock Bass 	<p>Samples US of Union Street Dam (Brown Bridge)</p> <p><i>Whole fish samples (10 individuals of each species approx. same size >20 g):</i></p> <ul style="list-style-type: none"> •Brook Trout •Brown Trout
Ranch Rudolph	1 Pass electrofishing survey all species (ID, length, weight, number)	None	None		<p>Samples US of Union Street Dam (Ranch Rudolph)</p> <p><i>Whole fish samples (10 individuals of each species approx. same size >20 g):</i></p> <ul style="list-style-type: none"> •Brook Trout •Brown Trout

Table I-2. Monitoring techniques used at each LTM site with agency responsibility, data collected, and units.

Site/Study	Monitoring Technique	Agency	Data	Units
River Mouth	Quarterly electrofishing transects	MIDNR, GTB	•Fish ID •Total length •Weight •Abundance •3 x 15ml water sample	•Species name •inches •pounds •count •eDNA
	Quarterly habitat survey	MIDNR, GTB	•Riparian zone condition •Width, depth, substrate type •Large woody debris •Discharge	•Dominant veg. with 30ft of water's edge •feet, feet, classify as pool, riffle, or run •count on material >6 in dia. & > 6 ft long •ft ³ /s
	Water level gauge	Traverse City	•River stage	•feet relative to sea level
Downstream of Union Street Dam	Quarterly electrofishing transects	MIDNR, GTB	•Fish ID •Total length •Weight •Abundance •3 x 15ml water sample	•Species name •inches •pounds •count •eDNA
	Quarterly habitat survey	MIDNR, GTB	•Riparian zone condition •Width, depth, substrate type •Large woody debris •Discharge	•Dominant veg. with 30ft of water's edge •feet, feet, classify as pool, riffle, or run •count on material >6 in dia. & > 6 ft long •ft ³ /s
	Water level gauge (up- and down-stream of dam)	Traverse City	•River stage	•feet relative to sea level
	Water quality sensor at Trap-and-Transfer Facility	GLFC	•Temperature •Conductivity •Turbidity •Dissolved oxygen	•°C •µS/cm •NTU •mg/L
S. Airport Road	2 Pass electrofishing survey	MIDNR	•Fish ID •Total length •Weight •Abundance •3 x 15ml water sample	•Species name •inches •pounds •count •eDNA
	Water quality sensor at YMCA	GLFC	•Temperature •Conductivity •Turbidity •Dissolved oxygen	•°C •µS/cm •NTU •mg/L
Sabin Dam/ Lone Pine	2 Pass electrofishing survey	MIDNR	•Fish ID •Total length •Weight •Abundance •3 x 15ml water sample	•Species name •inches •pounds •count •eDNA
	Water quality sensor at Beitner Rd.	GLFC	•Temperature •Conductivity •Turbidity •Dissolved oxygen	•°C •µS/cm •NTU •mg/L
	Stream gauge (04127200)	USGS	•Discharge	•ft ³ /s
Brown Bridge	1 Pass electrofishing survey	MIDNR	•Fish ID •Total length •Weight •Abundance •3 x 15ml water sample	•Species name •inches •pounds •count •eDNA
	Habitat survey	MIDNR	•Riparian zone condition •Width, depth, substrate type •Large woody debris •Discharge	•Dominant veg. with 30ft of water's edge •feet, feet, classify as pool, riffle, or run •count on material >6 in dia. & > 6 ft long •ft ³ /s
	Stream gauge (04126970)	USGS	•Discharge	•ft ³ /s
Ranch Rudolph	1 Pass electrofishing survey	MIDNR	•Fish ID •Total length •Weight •Abundance •3 x 15ml water sample	•Species name •inches •pounds •count •eDNA
	Habitat survey	MIDNR	•Riparian zone condition •Width, depth, substrate type •Large woody debris •Discharge	•Dominant veg. with 30ft of water's edge •feet, feet, classify as pool, riffle, or run •count on material >6 in dia. & > 6 ft long •ft ³ /s