# Restoration assessment of nearshore reef habitat in inner Saginaw Bay

## **Quality Assurance Project Plan for Assessing Use of a Restored Reef by Spawning Fishes**

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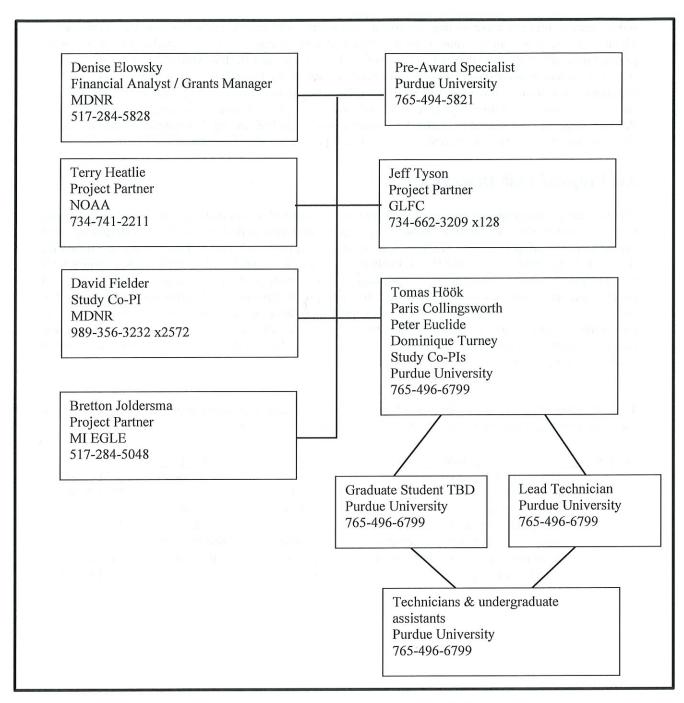


Figure 1. Project organization chart indicating roles and lines of responsibilities.

### A5. Problem Definition / Background

Historically, Saginaw Bay contained an inner bay rock reef complex that provided critical spawning habitat for a diversity of native fishes during both spring (Walleye, Smallmouth Bass) and fall (Lake Whitefish, Cisco, Lake Trout and Burbot). However, this habitat complex was largely lost due to



Figure 2. Location of proposed 3-Reef System in Saginaw Bay. Image is curtesy of Michigan EGLE.

Objective 1 - Determine pre- and post-construction habitat suitability of inner bay reef sites by assessing water quality, potential egg predators and egg-incubation potential: During surveys, we will monitor water quality parameters (e.g., dissolved oxygen, turbidity, temperature, conductivity). In addition, we will monitor winter and spring, near-sediment oxygen concentrations using archival dissolved oxygen data-loggers.

During every survey occasion (site visit), Purdue University will measure dissolved oxygen (DO) at the surface and at the bottom substrate/water interface using a YSI model 85 multiparameter DO sonde. In addition, during occasions of biological sampling, Purdue will also measure additional water quality parameters (e.g., turbidity, temperature, and conductivity). During occasions of biological sampling, the sonde will be lowered to the substrate (rate <0.25 m s<sup>-1</sup>) and held immediately above the bottom for 30 sec before retrieval to construct a profile of the water quality parameters. Sondes will be freshly calibrated before each occasion (using manufacturer calibration instructions). We will make multiple such casts upon each biological sampling occasion. The disposition of the reef relative to sedimentation and Dreissenid mussels (e.g., quagga and zebra mussels) and Cladophora algae colonization will be monitored via drop camera during surveys.

To assess how reef restoration may alter oxygen conditions, in-situ dissolved oxygen and temperature archival dataloggers (PME miniDOT Temperature and Oxygen Logger, Precision Measurement Engineering) will track seasonal oxygen and temperature conditions in the proposed restoration and control sites. Each miniDOT logger will be equipped with a miniWIPER that will be used as an antifouling device to reduce the growth of various organisms on the miniDOT logger sensor. We will deploy two duo units (miniDOT and miniWIPER) per site. One duo unit will be positioned at the water-substrate interface and elevated about ½ meter on a metal bar into the water column. The second duo unit will be

microscopic assessment in the laboratory. Identical egg mats (a 50.8x76.2x2.54 cm furnace filter wrapped around a steel frame, anchored on benthic substrate in gangs of three spaced 3 m apart), will be used to assess the magnitude of spawning by Walleye, Lake Whitefish, and other fishes at the restored reef and reference site. We will deploy 3 gangs of egg mats per site (i.e., 9 mats per site total). Comparisons will also be made to similar assessments previously performed in other areas of the Great Lakes (i.e., Lake Erie Walleye, Thundery Bay Lake Trout and Whitefish). Egg mats will be checked weekly, and eggs captured during each approximately weeklong set will be removed. If egg deposition rates are very high (i.e., clearly greater than 500 eggs per mat), entire furnace filters will be removed (and replaced). Furnace filters will then be placed in large plastic bags and kept on ice for transport to the laboratory. In the laboratory, eggs will be counted, and identified to species. Egg species will be identified in the lab using diameter (mm), oil content, coloration, and season.

Objective 3 - Assess the potential for using environmental DNA as an index of reproductive utilization: Environmental DNA can be an efficient and non-invasive method to estimate fish spawning activity and biomass (Yates et al, 2019; Tsuji and Shibata 2020); Especially when paired with conventional sampling (Spear et al., 2021). As a complementary estimate of spawning behavior and biomass, we will collect water samples during the spring in 2024 and 2025 at the two proposed reef sites, one control site, and in the Tittabawassee River where hatchery broodstock were historically collected. Given cost limitations, we will focus this analysis on walleye spawning in the spring. Three 1-liter water samples will be collected from the surface prior to setting gillnets each sampling week. One field blank of reverse osmosis water and one positive control will also be collected per site. The three samples will be spaced approximately evenly apart to cover the proposed reef area. Following collection, up to 1-L of water will be filtered using Jonah Ventures (Boulder, CO) sample kits and stored at 4°C for DNA extraction. In total, we expect to collect 240 samples. The quantity of walleye DNA will be assessed using quantitative PCR (Dysthe et al., 2017; Klymus et al., 2020; Spear et al., 2021). In addition to collecting data from existing qPCR assays outlined in Spear et al., (2021), we will develop and amplify a second nuclear rDNA marker assay. Once developed, use of the combined assay set could be used to help distinguish between fish presence and active spawning by tracking the relative concentrations of both nuclear and mitochondrial eDNA of walleye in future post-reef construction assessments. (Vasemägi, personal communication).

## A7. Quality Objectives and Criteria

The objectives of the survey are to obtain samples that can be expressed as data of sufficient sample size and representation so as to accurately characterize the measured parameters and allow for statistical contrasting with other data such as those from the pre-construction evaluation. Sample sizes based on effort (number of data loggers and net sets for example) can be and have been defined, but the resulting sample size of biota captured cannot necessarily be anticipated. Zeros are important values and constitute informative data when they occur. In some instances, sample effort may be expanded through the course of the study if the resulting data exhibits wide ranging variability. Generally, one basis of judgement may be coefficients of variation that are within 30% of the mean as sufficiently described representations of central tendency. We may discover with initial sampling that collection efforts vary considerably on the reef and the two-acre area requires additional stratification to fully characterize. Generally, we believe the biota will colonize and use the reef uniformly and this is largely supported by observations from similar reef restoration project. However, environmental assessment in field locations can be difficult to predict and some in-situ adjustment may be necessary. Replication will take place via multiple gear sets (data loggers, gillnet sets, egg mats, etc.). Temporal replication will be achieved by sampling for two years (two spring spawning events and one fall spawning event), as well as sampling during multiple weeks during each spawning season. Parameter accuracy is summarized in Table 2.

project page as a part of the data management plan. The Project Manager will oversee all data handling and storage and will prepare any summary reports, which will include the following NOAA disclaimer:

"These data and related items of information have not been formally disseminated by NOAA and do not represent any agency determination, view, or policy."

**A9B1 Sampling Process Design (Experimental Design):** The experimental design is to sample potential reef restoration sites during periods of biological activity that were the objective of the restoration effort. Expected sample sizes are summarized in Table 3.

Table 3. Expected (target) sample sizes for this study. Actual may be affected by weather (winter freeze up and spring thaw).

Measure	Season	Minimum Number	Study total
DO archival logger	Year-round	6 loggers, two springs, one summer one spring, 10 min intervals, 864 obs/day	~315,000
DO sonde measures	Each sample occasion	4/week for 5-7 weeks per season	80+
Egg mat collections	Spring / fall	162 each season, 2 years, examined weekly	162+
Egg predator micromesh gillnets	Spring / fall	1-2/ week per 3 sites for 5-7 weeks each season	80+
Spawner and predator experimental gillnets	Spring / fall	1-2/ week per 3 sites for 5-7 weeks each season	80+
eDNA sampling	Spring	5 1-L surface samples per 4 sites for 5-7 weeks each season	~240

A9B2 Sampling Methods: Sampling methods will use a variety of gears, technology and instrumentation. See A6 Project Task description for more detail. The project graduate student will be responsible for corrective actions for any biofield collection errors or mishaps for the biological sampling. Dave Fielder (co-PI) or his designee will be responsible for physical habitat measurements.

A9B3 Sample Handling and custody: Samples will be uniquely labeled and kept together. Specimen containers will either be ziplock plastic bags labeled with indelible ink (e.g., Sharpie pen) or in plastic jars with lids and similarly labeled. Specimens requiring preservation will be packed on ice until refrigeration or freezing. Live eggs will be retained in plastic jars with lids in oxygenated water and kept cool until delivery to incubation chambers. Gillnet collections will be recorded on a paper form likely using write-in-rain paper and pencil. Data sheets will be kept protected in a closable clipboard until return to shore and filed. Samples and datasheets will be managed by Purdue graduate student.

**A9B4 Analytical Methods:** See sections A6 and A7 for details on analytical methods. Gillnets will always be lifted after one overnight set. Specimens will be processed and recorded immediately after collection. Egg laboratory identification will be performed within 72 hours of collection or preserved if identification has to be delayed. Live egg specimens will be delivered to incubation chambers within 8 hours and reared for as long as necessary to obtain hatch.

**A9D2 Verification and Validation Methods:** Project co-managers or their designees will ensure data validation and verification as needed through read-backs on data already entered or inspection for outliers.

**A9D3 Reconciliation with User Requirements:** There are no immediate decisions pending on the findings of this work. Instead, recommendations and conclusions may be made in final reports. Broader decision making will hopefully utilize the findings of this work, but no subsequent interaction is planned for decision makers beyond the written reports.