

Restoration assessment of nearshore reef habitat in inner Saginaw Bay

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

Date: March 3, 2024



A1. Title and Approval Page

Project Title: Restoration assessment of nearshore reef habitat in inner Saginaw Bay

Project Tracking Code:

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A2. Table of Contents

Title and Approval Page	2
Distribution List	5
Project/Task Organization	6
Problem Definition / Background	7
Project / Task Description	8
Quality Objectives and Criteria	11
Special Training and Certification	12
Documents and Records	12

A3. Distribution List

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A4. Project/Task Organization

This reef habitat restoration assessment project is conducted by Michigan Department of Natural Resources (DNR) and Purdue University (Figure 1). The work is broken into three study objectives; (1) determine habitat suitability of inner bay reef sites by assessing water quality, potential egg predators, and egg-incubation potential, (2) evaluation pre-construction reproductive usage by adult fish during both the spring and fall spawning periods, and (3) assess the potential for using environmental DNA as an index of reproductive utilization in comparison to traditional spawning assessments. These objectives will be implemented by Purdue University. Michigan DNR is the funding recipient and they in turn are contracting with Purdue University as a project partner. Besides the co-project managers already identified, a graduate student (still to be identified) will be brought on by Purdue University for the conduct of their portion of the study, overseen by the University's co-project manager's Dr. Tomas Höök, Dr. Paris Collingsworth, Dr. Peter Euclide, and Dominique Turney.

Grant management and financial oversight at the Michigan DNR is provided by Denise Elowsky. Contract financial (subgrant) management at Purdue University is overseen by the Office of Sponsored Program Services. Assisting project co-managers may be other Purdue research staff including Ben Szczygiel (likely Lead Technicians in Figure 1), a TBD graduate research assistant and student hourly research assistants. There may be additional technicians or students that will aid with field and lab work conducted by Purdue University.

The graduate student and field crews from Purdue University will be the principal data collectors. The quality of the data collected and adherence to study plans on a daily basis will be overseen by project co-managers. Data analysis will be performed by the graduate student and by the project co-managers. The subsequent report writing will be done by the graduate student and project co-managers. There is no specific management decision making made by the individuals conducting this project but reports may include conclusions on efficacy of the reef habitat restoration and recommendations on its continued management or similar projects in the future. Broader decision making about the application of these findings may include fishery managers and administrators in the Michigan DNR and others agencies in the Great Lakes area. The Project QA plan will be maintained by the project co-managers and coordinated and reported to the Quality Assurance Manager Bretton Joldersma. Expected are quarterly meetings or conference calls.

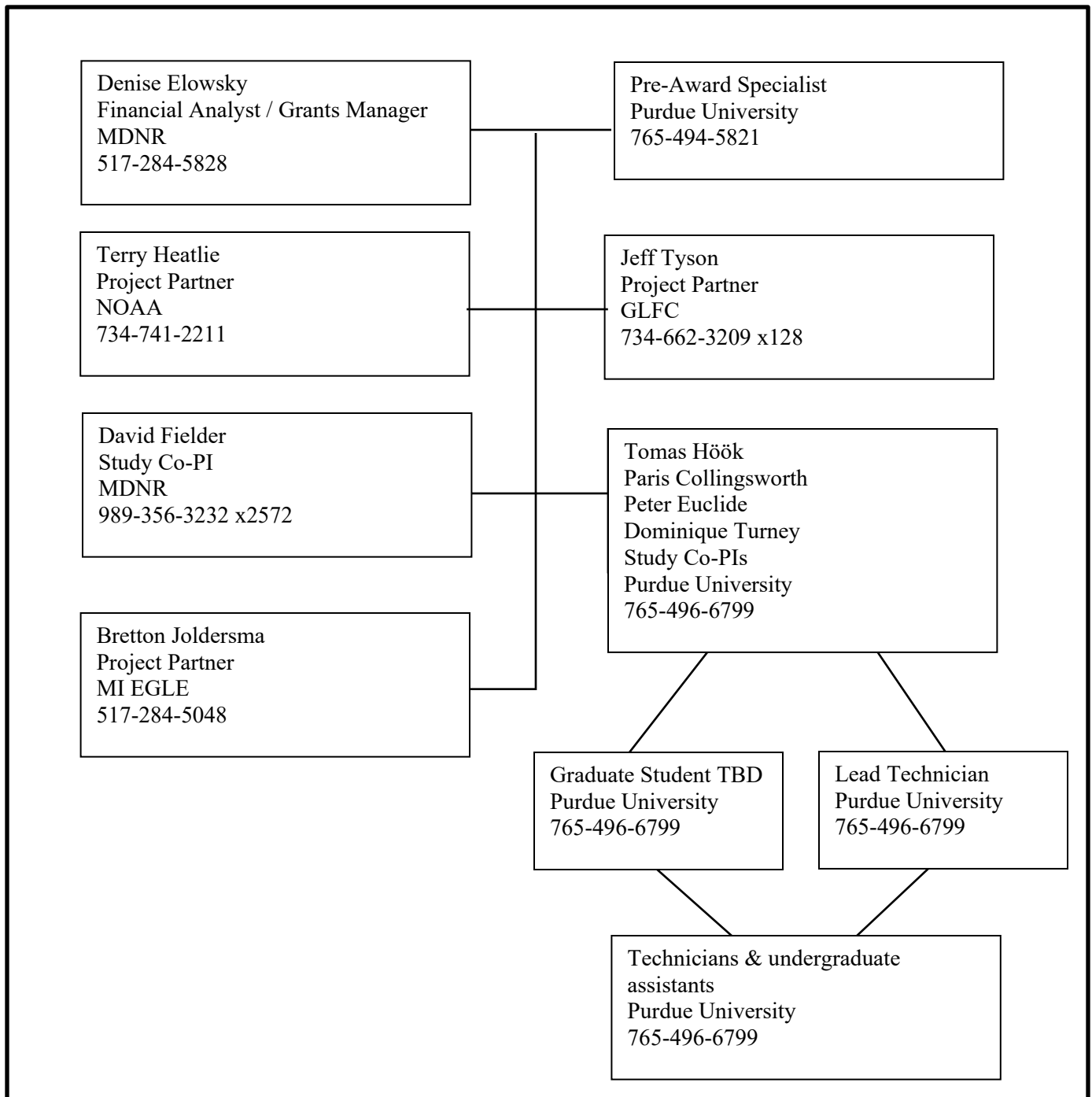


Figure 1. Project organization chart indicating roles and lines of responsibilities.

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

sedimentation, brought about by timber harvest and agricultural land-use in the Saginaw Bay watershed. The loss of this important spawning habitat contributed to the collapse of Walleye and impacted the local production of other species such as Lake Whitefish, Lake Trout, and Burbot. Motivated by recent successes of reef restoration and construction in other areas of the Great Lakes, an initiative was undertaken to restore an inner bay rock reef in Saginaw Bay in hopes of rescuing some of this historic spawning behaviors and diversifying reproductive sources. A pre-restoration assessment conducted by Purdue University is essential to establish a comprehensive baseline for physical habitat alterations and biotic responses, serving as a reference for subsequent post-restoration evaluations.

A6. Project / Task Description

We will survey two proposed restoration sites in inner Saginaw Bay (Spoils Island and Kawkawlin), along with one control site (-83.861061, 43.669264) over a multi-year period during the fall (targeting Lake Whitefish) and spring (targeting Walleye) spawning events (Table 1; Figure 2; Preliminary reef locations, -83.798169, 43.669094; -83.884203, 43.662967). Coordinates for proposed restoration and control sites may be slightly adjusted to accommodate for logistical and safety purposes. Outcomes of this project will provide important pre- and post-construction, baseline physicochemical and biological information. We will specifically assess conditions at the two planned reef restoration/construction sites and control site. Given the timeline of reef construction (late spring to summer 2025), we aim to assess pre-construction conditions during spring 2024, fall 2024, and spring 2025. The specific objectives for this construction assessment project are:

Table 1. Schedule of field sampling by objective. Corresponding laboratory work would occur between sampling periods. Analysis and reporting will take place in 2024 and beyond.

Objective	Activity	Entity	Season	Years
1	DO archival logging	Purdue	Year-round	2024 & 2025
1	DO profiles	Purdue	All occasions	2024 & 2025
1	Egg incubation	Purdue	Spring	2025
1	Egg predator gillnetting	Purdue	Fall & spring	2024 & 2025
2	Spawner abundance gillnetting	Purdue	Fall & spring	2024 & 2025
2	Spawning via egg mat placement	Purdue	Fall & spring	2024 & 2025
3	eDNA sampling	Purdue	Spring	2024 & 2025



Figure 2. Location of proposed 3-Reef System in Saginaw Bay. Image is courtesy 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 $<0.25 \text{ m s}^{-1}$) 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 anti-fouling 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 $\frac{1}{2}$ meter on a metal bar into the water column. The second duo unit will be

positioned below the water surface. In total, we will deploy a total of 6 duo units (2 per site). The data loggers will be programmed to record data every 10 minutes each day and retrieved in the spring, allowing for about 144 measurements per day per logger. The data will be uploaded, plotted, and analyzed, supplementing DO sonde readings to reflect year-round DO trends.

During the spring and fall seasons, Purdue University will survey potential egg predators, including both small-bodied and large-bodied fish. Specifically, we will use multi-mesh, microfilament experimental gillnets (50 m length; 5 cm, 10 cm, 15 cm, 20 cm, 25 cm, 30 cm, and 35 cm mesh sizes) to sample potential large-bodied egg predators at the potential restoration and control sites. These nets are identical to those used during previous restoration assessments. We will set the experimental gillnets overnight for at least 12 hours. We will use micro-mesh gillnets (28.5 m length; 1.25 cm, 2.00 cm, and 2.50 cm mesh sizes) to collect potential small-bodied egg predators. We will set the micromesh nets for two hours during daytime. We have found this is sufficient time to collect these more abundant, small fish. Moreover, the short duration of these sets should limit potential rapid digestion of eggs in these small fish. 1-2 experimental gillnets will be set overnight per survey site (restoration sites and control site) once per week during the fall spawning season (November through early December) and spring (April/May). Similarly, 1-2 micromesh gillnets will be set for two hours per survey site once per week during the fall spawning season (November through early December) and spring (April/May). The decision to deploy one or two gillnets will depend on the number of catches. Given annual differences in the timing of spawning, the predator survey period may vary some from year to year. We plan to survey 5-7 consecutive weeks during each fall and spring survey seasons.

Upon collection, we will place fish on dry ice to quickly freeze and slow digestive processes. In the laboratory, the graduate student assisted by university technicians, will identify and enumerate all fish eggs in fish stomachs. This will involve thawing fish stomachs and examining stomach contents under a dissecting microscope. There are limited numbers of species that spawn at the same time as Walleye and Lake Whitefish in Saginaw Bay and we do not anticipate difficulty in species identification of eggs. Egg species will be identified using diameter (mm), oil content, and coloration, using methods developed during the pre-construction evaluation.

We will also evaluate the suitability of egg incubation at the different sites during spring 2025 by deploying fertilized walleye eggs in egg incubation trays. We will check these trays after 2-3 weeks of incubation to evaluate successful hatching. (The intent is ultimately to compare egg incubation success pre-construction with incubation success post-construction).

Objective 2 - Evaluate pre- and post- construction reproductive usage by adult fish during both the spring and fall spawning periods: We will assess reproductive usage by a) collecting potential spawners and b) collecting eggs deposited onto sediment during both spring and fall spawning periods. We anticipate assessing reproductive utilization for 5-7 consecutive weeks during each spawning seasons and beginning assessments before spawning initiatives and continuing until spawning ceases. Gillnetting (using the same overnight sets of large-mesh experimental nets targeting large-bodied potential egg predators) will allow us to assess relative abundances (CPUEs), spawning condition, size structures and demographic (age, sex) composition of spawners. Specifically, the graduate student assisted by university technicians, will set overnight experimental gillnets at each survey site, and we will examine and measure individual fish either upon capture (if still alive at time of capture to allow live release) or in the laboratory (after freezing and transporting fish dead at time of capture). Tissue samples (fin clips) will be taken from walleye and lake whitefish for to facilitate potential future genetic analysis to identify the regional ancestry of reef-colonists.

Egg mats will be used to assess magnitude of spawning by walleye, lake whitefish, and other fishes at the proposed restored sites. Most eggs will be preserved in formalin and identified/enumerated through

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 JonahVentures (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.

Table 2. Study metrics, units and anticipated accuracy in measurement.

Metric	Units	Expected accuracy	Comments
Dissolved Oxygen	mg/l	+/- 0.3 mg/l – sonde +/- 0.2 mg/l logger	Manufacturers specifications
Temperature	°C	+/- 0.4°C	Manufacturers specifications
Conductivity	µS/cm	+/- 0.5% FS	Manufacturers specifications
GPS position	m	+/- 4.0 m	U.S. Government standards
Egg enumeration	# m ⁻³ d ⁻¹	100%	(Dense eggs mats will be counted in lab)
Fish identification	Species	100%	
Fish enumeration	Count	100%	

Statistical tests will make use of parametric statistics when assumptions of normality are met and nonparametric equivalent tests when unmet. We intend to use similar ANOVA statistical methods as used when evaluating pre-restoration data. Statistical significance of a Type I error will be made on a P-value of 0.05 or a 95% confidence. In some instances, means may be compared with two standard errors constituting the Wald Confidence Intervals and those values without overlapping bounds may be deemed statistically significantly different. Some data may be additionally graphically depicted for visual inspection as opposed to some numerical quantification.

A8. Special Training / Certification

All necessary skill sets to conduct this work are already possessed by the study co-investigators and their teams. Part of the graduate student experience is learning and any needed training or practice will be conducted to ensure fulfillment of methods to specified levels of accuracy before field work is attempted. Purdue University requires all employees and students working with vertebrates to have certified training (individual Q number). In addition, the project will fall under a general protocol approved by the Purdue Animal Care and Use Committee (protocol # 1112000400). And, Purdue's fish collection will be reviewed by a separate group within the Michigan Department of Natural Resources as part of obtaining an approved Collector's Permit.

A9. Documents and Records

All staff participating in this project will review this QAPP and be familiar with its provisions.

Data loggers will electronically store data and be uploaded to a computer once retrieved. Those data will stay in electronic form but be given unique file names and annotated as necessary. All other data recording will begin on paper and entered into a database at first opportunity upon returning to shore. Data entry will be reviewed for accuracy by one independent person (graduate student or university technician). Paper data sheets will be retained. Purdue will maintain master datasets for all objectives. Every attempt will be made to guard against duplication via date annotation in file names and adherence to master dataset integrity. Long term data preservation will lie with project co-managers and their institutions beyond their tenure. Upon completion of the study, we will publish (including DOI) all biotic assessment data from both pre-restoration and post-restoration assessments to the Purdue University Research Repository for long-term data storage and curation (<https://purr.purdue.edu/>). Within 2 years of project completion, all data will be shared with the Great Lakes Fishery Commission (GLFC) for its publicly accessible

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.

A9B5 Quality Control: See sections A6 and A7 for details on quality control. Most field operations are either achieved or not. The quality control (QAPP) auditor will also monitor study implementation to ensure fulfillment of data requirements.

A9B6 Instrument /Equipment Testing, Inspection and Maintenance: Data loggers will be calibrated and activated before deploying using manufacturer software. Initial spot checks on data quality in-situ will be made to ensure data is adequate. All other gear will be inspected before deployment.

A9B7 Instrument/Equipment calibration and frequency: Data loggers will be calibrated and activated before deploying using manufacturer software.

A9B8 Inspection/Acceptance of Supplies and Consumables: Respective project staff will ensure supplies and consumables are adequate before any field work. These include sufficient sample bags and jars, labeling pens, spare batteries for sondes, ice for biota collections, fuel for marine motors, and field datasheets and pencils. Gillnets will be examined after each use for tearing and either mended or replaced or mended if damage is detected. On the water staff will be encouraged to carry and use sun screen.

A9B9 Nondirect measurements: Data from the previous reef construction assessments are already in the possession of the study co-managers. Their quality is ensured by the same co-PIs conducting this study. Other datasets, if needed, will be obtained from published literature or by contacting those authors. Every effort will be made to validate their quality as well.

A9B10 Data Management: Data will be managed by the project co-managers or their designates. Imagery files (video footage and any stills) will be saved in common file formats such as mp4 format or jpeg file format in study folders maintained by study co-PIs. All files will be backed up off site including posting to the Alpena Fisheries Research Station's YouTube channel, accessible at: <https://www.youtube.com/channel/UCQC2S9tK6D82cd-Nu2UteJA> . Upon completion of the study, we will publish (including DOI) all biotic assessment data from both pre-restoration and post-restoration assessments to the Purdue University Research Repository for long-term data storage and curation (<https://purr.purdue.edu/>) and provide summary and/or raw data to the GLFC for posting on their publicly available Inner Saginaw Bay Reef Restoration webpage.

A9C1 Assessments and Response Actions: Teams will meet periodically to gauge project progress and adherence to data standards. External assessments of field work will be conducted either in simulated field conditions ahead of field work or on site, if necessary, conducted by study co-PIs. Peer review of resulting reports and manuscripts will be obtained as part of a publication process.

A9C2 Reports to Management: Progress reports to the NOAA will be available upon request. NOAA will be notified if there are any deviations from this QAPP as soon as possible after they occur. Anticipated are presentations on progress and preliminary findings at professional meetings during the study. Final reports will take the form of a graduate student thesis or dissertation. Any project work not covered by the student will be summarized in writing separately as a white paper or possibly a Michigan DNR Fisheries Report. Anticipated is at least one published article in a peer reviewed journal.

A9D1 Data review, verification and validation: Data will be examined for outliers and data entry errors. Erroneous data will be corrected if possible or removed if not. Handling of outliers will be decided based on the analytical judgement of the co-PIs. They may be removed in some instances or sensitivity analysis may be conducted to assess their effect on data analysis and statistical testing. Any outliers or substantial missing data will be detailed in the methods section of any resulting report or presentation.

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.