Scope of Work

Project title: *In situ* evaluation of spawning lake whitefish eDNA detection in Lake Michigan's Green Bay.

GLFT Proposal ID Number: 2022.1987.

Problem statement. Most lake whitefish (LWF) populations across the four lower Great Lakes have experienced recruitment declines. Southern reaches of Lake Michigan's Green Bay are one area countering this broader trend, with rebounding catches following decades of reduced abundances (Hansen, 2019). This recovery has been most apparent in the Bay's tributaries where reestablished spawning runs and larval production have been observed in recent years (Ransom *et al.*, 2021). However, the estimated tributary production (<725,000 fish annually; Ransom *et al.*, 2021) is not large enough to account for Green Bay LWF abundance estimates that averaged 45.9 million age-3+ fish over the three most recent assessments (2016-2018; Wisconsin DNR).

With reef spawning being the primary reproductive mode for LWF throughout the rest of the Great Lakes, it is reasonable to suspect that spawning in open waters could be a substantial contributor to southern Green Bay's rebounding LWF numbers. Recent research has supported this notion. For instance, Doerr *et al.* (2021) observed an absence of tributary signatures in otolith microchemistry of larval LWF collected from Green Bay, suggesting a likely open water origin. Preliminary results from larval sampling conducted in 2021 also indicates larval whitefish abundance in Wisconsin tributaries is low compared to beach habitats along Green Bay shorelines, some of which are located > 20 km from tributaries with known spawning runs. Furthermore, telemetry shows that some (≤ 10%) LWF tagged during spawning runs in Green Bay's largest inflowing tributaries, Fox and Menominee Rivers, did not return to rivers in subsequent spawning windows. While these telemetry-monitored fish were known to be alive, whether they skipped spawning or spawned at open-water locations cannot be determined.

The inability to identify precise locations of LWF open water spawning sites in Green Bay is an impediment to adequately assessing LWF stock dynamics and identifying critical habitats. Without knowledge of their locations, resource managers currently cannot know how much spawning habitat is available, or even what its characteristics are. Preventing degradation of this habitat cannot be achieved if the areas and habitat characteristics that need to be protected remain unknown. Identifying the attributes of LWF spawning habitat in the open waters of Green Bay also has implications throughout the Great Lakes because it may identify factors that are limiting successful recruitment elsewhere. Knowledge of discrete spawning locations would also enable fishery independent assessments of spawning populations.

Previous efforts have not been successful in identifying LWF spawning locations in southern Green Bay. In fall of 2019, the USFWS used hydroacoustics to survey the Bay side of Door County looking for suspected spawning aggregations of whitefish and were able to successfully capture ripe fish in gillnets in a location near Sturgeon Bay but were not able to locate any deposited eggs using divers. Egg mats have also been deployed in two years in suspected spawning areas in Sturgeon Bay and suction sampling for eggs was performed near Point Sable without conclusive results. Ongoing telemetry research provides a promising avenue for

identifying open-water spawning locations given the grid of receivers deployed in Green Bay (8-km spacing), but the spatial resolution of this information remains unknown and may only marginally reduce the overall area where additional searching for spawning locations is logical.

Environmental (e)DNA provides a means of surveying large bodies of water to determine the presence of specific fish species and is a promising tool for detecting LWF spawning aggregations in the open waters of Green Bay. eDNA field, laboratory, and analytical methods have matured over the past decade since finding initial applications as an early detection tool for several invasive carp species threatening to invade the Great Lakes (Mahon *et al.*, 2013). For instance, research has revealed the environmental conditions that affect eDNA detection probabilities (Barnes *et al.*, 2014; Goldberg *et al.*, 2016) and the sources of false positive eDNA detections, as well as how they can be minimized or otherwise accounted for (Darling *et al.*, 2021; Lahoz-Monfort *et al.*, 2016; Tingley *et al.*, 2021). Because eDNA concentrations in water are largely a function of individual-level shedding rates, eDNA has also been repeatedly validated as a means of estimating species' relative biomass (Di Muri *et al.*, 2020; Rourke *et al.*, 2021; Sard *et al.*, 2019; Tillotson *et al.*, 2018). This connection between localized species biomass and eDNA concentrations has allowed the technique to be used to identify timing and locations fish spawning, a processes aided by spikes in eDNA concentrations generated by the act of spawning itself (Bracken *et al.*, 2019; Di Muri *et al.*, 2022). However, accuracy in identifying the origination points of eDNA can be greatly improved through hydrologic modeling of eDNA dispersal dynamics (Andruszkiewicz *et al.*, 2019; Wood *et al.*, 2021).

eDNA dispersal dynamics are complex, but once described, they can be incorporated into models that are useful for determining fish location and estimating biomass. For instance, Akatsuka et al. (2018) found correlations between modeled and measured eDNA quantities in an estuarine system through numerical modeling of flow fields, demonstrating applicability of physical models for refinement of eDNA studies. Like other molecules dispersing in water, movement of eDNA particles can best be thought of as a plume that is dispersing outward from a relatively high concertation source. This plume expands and dilutes over time due to eDNA transport (Deiner & Altermatt, 2014), mixing and diffusion (Jane *et al.*, 2015), physical degradation of eDNA molecules (Strickler *et al.*, 2015), and settling of those molecules onto and into substrates (Dejean *et al.*, 2012). Such eDNA dispersal processes are highly predictable in areas with known hydrodynamics (Fukaya *et al.*, 2021), and can be used to hindcast the plume origin, *i.e.*, the location of fish (Andruszkiewicz *et al.*, 2019; Wood *et al.*, 2021). When properly accounting for transport, mixing and loss, even small numbers of aggregating fish generate plumes of eDNA which are readily detectible above ambient levels (Wood *et al.*, 2021). Hydrodynamic particle transport models already exist for southern Green Bay that could be modified to model LWF eDNA dispersal dynamics given proper parameterization of dispersal and decay rates.

The core premise of our proposed research is that measured eDNA concentrations coupled with models that account for known species- and habitat-specific eDNA dispersal dynamics provide a powerful means for detecting spawning aggregations of LWF. eDNA alone cannot currently differentiate between an aggregation forming for spawning versus one forming for some other purpose. However, known aggregation locations could be verified as spawning sites through

direct observation techniques such as egg sampling. In this way, using eDNA to identify potential spawning locations will reduce the number of potential sites for traditional sampling by orders of magnitude, allowing managers to search for spawning with lower effort and a higher success rate. Knowledge of where LWF aggregate during the spawning window would represent a remarkable step forward in understanding where open water LWF spawning is occurring in Green Bay, a prerequisite to protecting those critical habitat features.

Prior to mounting a full-scale survey for LWF spawning locations throughout southern Green Bay using eDNA, we must address two knowledge gaps related to development of properly calibrated eDNA particle transport models. First, an ability to distinguish peaks in eDNA concentration from ambient levels is necessary for differentiating aggregations of spawning LWF from typical densities. Second, habitat-specific spatial and temporal eDNA degradation rates and dispersal dynamics must be determined prior to parameterizing existing hydrodynamic models to account for attributes unique to LWF eDNA particles in southern Green Bay. Here we propose addressing both these information needs to determine the feasibility of using spatially and temporally explicit eDNA concentrations paired with hydrodynamic models to determine likely LWF spawning sites in southern Green Bay.

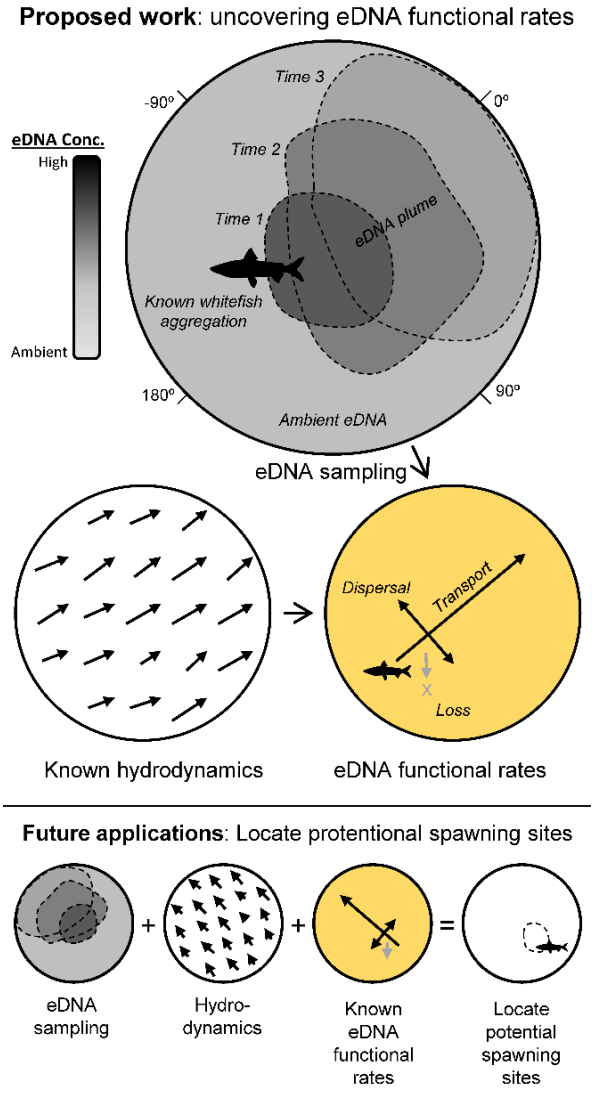

Figure 1. Conceptual workflow of whitefish eDNA calibration for spawning site identification. In our proposed work, we will conduct intense spatial and temporal sampling at known whitefish aggregations. We will combine our eDNA data with hydrodynamical models of Green Bay to solve for eDNA functional rates, including eDNA shedding, transport with currents, dispersal, and loss. In future work, these eDNA functional rates can be used—along with hydrological models—to hindcast eDNA sample data and reveal potential whitefish spawning sites, which could be confirmed with direct observation sampling techniques.

To be clear, the work described here is designed to serve as a proof-of-concept and calibration exercise for using eDNA to detect LWF aggregations in Green Bay that are likely a result of spawning activity. Knowledge gained from addressing our current research questions will directly inform further model development and implementation of a survey for LWF spawning sites throughout southern Green Bay. This pilot project will use observational techniques and field sampling to evaluate hypotheses pertaining to LWF eDNA detection and dispersal in Green Bay, providing a baseline for subsequent development of a coupled biological-physical hydrodynamic model for eDNA transport in the bay. A reliable 3D hydrodynamic model that can predict eDNA mass transport with accuracy offers capabilities to locate LWF aggregations in open waters of Green Bay with high spatial and temporal resolution beyond restrictions of costly and season-limited point observations. In other words, a hydrodynamic model can provide a bay-wide assessment of eDNA origins and dispersion patterns and enables spatial visualization of the extent that eDNA can be transported. Additionally, a transport model provides opportunity for eDNA assays under different hydrological conditions (flows and mixing) and tributary loading scenarios, as well as different meteorological conditions and climate stressors in the region. The hydrodynamic model can also help in closing the knowledge gaps identified in the 2018 GLFT-GLFC LWF workshop on better understanding the relevant physical processes and their impacts on fish early life movement patterns and recruitment for Green Bay. An added advantage of having a physically-based eDNA transport model is that such a platform will set the stage to implement the framework in other areas of Lake Michigan and the Great Lakes, as well as coupling with other biogeochemical models of the system.

Hypothesis to be tested. We propose a pilot project that will be an empirical *in situ* assessment of the feasibility of using eDNA to detect LWF spawning aggregations in southern Green Bay. eDNA concentrations have been demonstrated to be correlated with fish biomass and have been used to determine the location and timing of spawning in other species (Bylemans *et al.*, 2017; Wood *et al.*, 2021). Such properties should make the technique a powerful means of identifying LWF open water spawning locations, especially when leveraged alongside ongoing or planned projects by co-PIs that use telemetry, microchemistry, and egg sampling in attempts to achieve the same goal. Our proposed pilot project will address two questions on the suitability of eDNA to detect LWF spawning aggregations in Green Bay. **Question 1.** What is the ambient (background) concentration of LWF eDNA in southern Green Bay? We hypothesize that LWF eDNA will be spatially variable but present throughout southern Green Bay, however at consistently lower concentrations than those measured near aggregations. The null hypothesis being evaluated by question 1 is that eDNA cannot be used to detect LWF aggregations because aggregations do not produce eDNA concentrations high enough to rise above ambient levels. We would reject this null hypothesis if we could, as expected, detect whitefish aggregation eDNA over ambient eDNA concentrations. **Question 2.** How does LWF eDNA disperse in southern Green Bay through space and time? We hypothesize LWF eDNA concentrations will be a function of LWF biomass at the point of origin, with eDNA dispersing in a detectible plume in the direction of water currents and concentrations gradually reducing to ambient levels within 2 km of the LWF aggregation. The null hypothesis being evaluated by question 2 is that eDNA dynamics at aggregations do not resolve to an intelligible plume, with the alternative hypothesis being that plume parameters are readily calculable from whitefish aggregation eDNA data.

Answering these two key component questions will in turn permit the two key outcomes of the project: 1) a proof-of-concept for detecting spatial and temporal peaks in actual LWF eDNA concentration in southern Green Bay and 2) parameterization of an applicable eDNA plume model specific to LWF eDNA dispersal dynamics in southern Green Bay.

Methods and/or Activities. eDNA sampling will be conducted at 1-2 meters from the lake bottom using a weighted tube attached to a Smith-Root eDNA sampler that allows the volume of water filtered to be precisely monitored (Thomas *et al.*, 2018). Sampling near the lake bottom, where spawning would be occurring, will allow us to assess eDNA concentrations at the depth that we would sample for a follow-up survey that intends to identify LWF spawning locations. We will pass one liter of water through a filter with a 1.2 µm pore size contained in a cartridge (Thomas *et al.*, 2018) that has been demonstrated to be watertight at depths up to 30 meters (A. Thomas, Smith-Root, personal communication). One liter of water is a common filtering volume for eDNA studies (e.g., Sard *et al.*, 2019; Wood *et al.*, 2020) and a 1.2 µm pore size provides a reasonable trade-off between maximizing detection probabilities and minimizing filter clogging that can occur at finer sizes (Lacoursière-Roussel *et al.*, 2016; Turner *et al.*, 2014). The presence of any potential contamination accrued during sampling will be assessed by collecting negative control samples prior to and at the end of each day's sampling. Negative controls will consist of a filtered one-liter volume of distilled water brought into the field and filtered while aboard the vessel used during sampling. Negative controls will be processed in the laboratory in the exact manner as samples collected for analysis. The eDNA sampling unit and research vessels will be provided by the USGS Wisconsin Cooperative Fishery Research Unit where co-PI Isermann is Unit Leader and PI Homola is Assistant Unit Leader.

All eDNA analyses will be performed at the Molecular Conservation Genetics Lab at the University of Wisconsin-Stevens Point, where PI Homola serves as director. eDNA from all filters will be extracted using the protocol described by Laramie *et al.*, (2015). The protocol includes a combination of a QIAshredder homogenization kit (QIAGEN, Inc.), DNeasy Blood and Tissue extraction kit (QIAGEN), and a OneStep PCR inhibitor removal kit (Zymo Research). eDNA extractions will be carried out inside of a sterile enclosure in a separate room away from the laboratory where genomic DNA is handled. All necessary materials and bench spaces will be cleaned prior to use with UV light and bleach. All pipetting will be done with pipets only used for eDNA samples and with sterile filter tips. A DNA extraction negative will be processed during each extraction day to test for contamination during the DNA extraction procedure. eDNA concentrations will be quantified using quantitative polymerase chain reaction (qPCR) with previously published primers and TaqMan probes that do not cross-amplify cisco (Hernandez *et al.*, 2020), a congener that may be present in Green Bay. We have screened these primers and verified both amplification of LWF and non-amplification of cisco tissues that were collected from Green Bay. qPCR amplifications will be carried out in duplicate using the TaqMan Environmental Mastermix 2.0 (Thermo Fischer Scientific).

Obj 1. Using eDNA to detect spawning aggregations of LWF is contingent upon differentiating locations of high eDNA concentration from ambient concentrations (i.e., background noise). We will determine the range of LWF eDNA concentrations present throughout southern Green Bay

by sampling 50 randomly selected sites throughout our geographic focus area during one week in spring 2023. Although LWF spawn in the fall, spring sampling is preferred for this work because it will allow for improved access to commercial trap nets that we will use as a proxy for spawning aggregations (see Obj. 2), while still ensuring water temperatures that may influence eDNA dispersal dynamics are similar to those observed during fall spawning. We will calculate the mean and variance in observed eDNA concentrations to generate a baseline distribution for use in detecting outlier concentration peaks that may be associated with aggregations of spawning LWF. We acknowledge that there will likely be spatial and temporal variation in ambient eDNA distributions in the Great Lakes, and this variation could affect the detectability of a whitefish eDNA plume elsewhere. While quantifying this variation is beyond the scope of this project, we will conduct a power analysis that evaluates the potential to detect an eDNA plume under differing ambient eDNA conditions (e.g., higher mean and variation). This power analysis will inform the expansion of our work outside of southern Green Bay in future projects. The GLFT Science Advisory Team suggested consideration of a control site that is free of LWF eDNA. Because our project is not focused on presence or absence of eDNA, but rather the concentrations, we have designed Obj. 1 to achieve the purpose of a control site by empirically demonstrating the null expectation, in this case being the ambient distribution of eDNA concentrations present throughout our study location. That ambient concentration distribution will serve as the baseline that peaks in eDNA concentration will be judged against, therefore achieving the goal intended by the suggestion of collecting data from a control site. We will use control and experimental environmental samples to evaluate the specificity of the LWF eDNA qPCR primers by analyzing five samples each from water known to contain LWF eDNA (e.g., a holding tank used during LWF telemetry tagging) and water known to be absent of LWF (e.g., McDill Pond near Stevens Point, WI).

Obj 2. Quantifying LWF eDNA spatial diffusion and retention rates is necessary to develop models to hindcast the origin of detected eDNA peaks associated with spawning. This project will complete the first phase of that modeling by constructing a habitat- and species-specific eDNA plume model. To implement our model, we will determine four fundamental eDNA

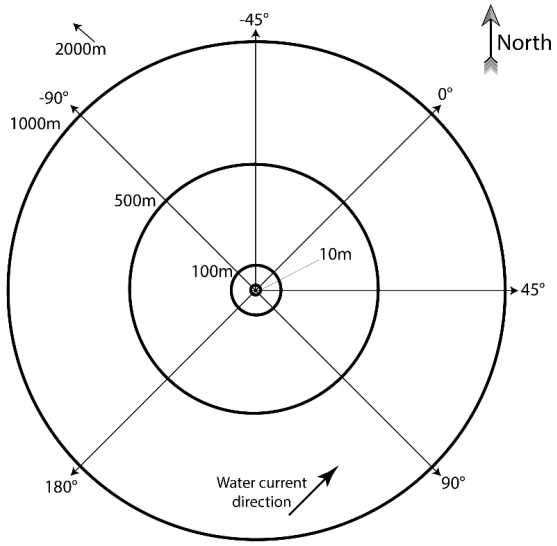

Figure 2. eDNA sampling point for Obj. 2. The diagram is centered on the location of a commercial lake whitefish trap net that will serve as a surrogate for a spawning aggregation of lake whitefish. Sampling will be performed along size transects whose directionality is determined by the water current direction. Each transect will be sampled at 10m, 100m, 500m, 1000m, and 2000m from the trap net, as indicated by the concentric circles (2000m distance not shown to improve figure clarity).

parameters: 1. individual-specific eDNA shedding rate – the rate of eDNA output by individual fish, 2. eDNA transport rate – the degree to which eDNA moves in the direction of the prevailing current, 3. eDNA dispersal rate – the degree to which eDNA disperses in space, regardless of the prevailing current, and 4. eDNA loss rate – the rate at which eDNA is lost from the water column (through degradation and settling).

To characterize the above plume dynamics, we will systematically quantify eDNA concentrations around LWF commercial trap nets (Figure 2). Trap nets simulate high-concentration fish aggregations, while also providing a biomass estimate that can be used as a predictor variable when modeling eDNA concentrations. We have support from two Green Bay commercial LWF fishing operators for sampling around their nets and for them to provide information on the biomass of LWF contained in each net during sampling. After determining the water current direction near the net using an acoustic doppler current profiler, we will sample outward from each net along six transects situated in the direction of the water current (0°), 45° and -45° from the water current, 90° and -90° from the water current, and directly opposite from the water current (180°; Figure 2). We will sample the transects at 10m, 100m, 500m, 1000m, and 2000m from the trap net to characterize fine-scale mixing dynamics and long-distance transport rates (Figure 2). Sampling will be repeated at four weekly time points at each of three trap nets. All sampling will occur in April-May of 2023. The exact timing of sampling will depend on springtime warming; however, it will be completed prior to stratification of the bay to ensure water temperature distributions are analogous to fall conditions.

We will use a nested, zero-inflated generalized linear mixed model to predict observed eDNA concentrations given known hydrological conditions and unknown fundamental eDNA plume parameters. The unknown plume parameters will be determined during this model-fitting process as the set of parameters that minimize Akaike information criterion that has been corrected for small samples sizes (AICc) of the model predicting observed eDNA concentrations. This model will have a binomial distribution for detection and a log-normal distribution for eDNA concentration, given detection (Wood et al. 2021). The fixed effects of our statistical model will be a function of our plume model fundamental parameters (see above) and hydrological predictions (i.e., water current and direction). The random effects will include technical replicate, nested within sampling site. By collecting eDNA samples around a LWF aggregation of known size in each net, we will be able to assess model predictions in light known relative biomass. Using our eDNA plume dynamic parameters, along with our understanding of eDNA distributions and fold effects against baselines, we will simulate hypothetical alternative eDNA survey designs for detecting whitefish spawning aggregations in Green Bay, to identify designs that balance survey power with alternate levels of effort (field and lab resources).

Potential Management Benefits and Outcomes of Proposed Project. Researchers and agency biologists have spent decades trying to determine which stocks or spawning locations are important to whitefish fisheries in Lake Michigan using a variety of tagging methods (Ebener and Copes 1982, Ebener et al. 2010a, 2010b), genetics (Andvik et al. 2016), and otolith microchemistry (Doerr 2019). Identifying sources of recruitment has become increasingly relevant for southern Green Bay, which represents one of the few bright spots for whitefish

recruitment in the Great Lakes. As such, southern Green Bay provides a unique opportunity to identify habitat characteristics that contribute to successful whitefish recruitment in a contemporary context. LWF stocks that are in recent decline were largely supported by spawning that occurred in open water habitats. Hence, identifying the location of and the attributes of open water spawning habitat in Green Bay could help managers determine whether this formula for success can be replicated in other areas via restoration or other strategies. To do this, we first must locate these open water spawning areas in southern Green Bay. Identifying contributions of individual tributaries to LWF fisheries is a specific need highlighted in the WDNR Integrated Fisheries Management Plan for Lake Michigan (WDNR 2017) and this need extends to open-water spawning locations as well. Although still in draft form, the 2022 Wisconsin DNR Lake Michigan/Green Bay research priority inventory states a research need of "Green Bay spawning potential – determine presence and abundance of Green Bay (e.g. Sturgeon Bay) spawning population(s)" which our proposed research will help address.

The key management implication of this work will be advancing eDNA as a tool to identify potential whitefish spawning aggregation sites. We will provide proof-of-concept that aggregating LWF generate a spike in eDNA concentrations as well as develop a plume model that together will provide the foundation for development of a coupled biological physical model that can be used to perform hindcasting to identify LWF aggregations in Green Bay. The positions of those aggregations will reflect the most likely locations of spawning activity as well as the relative abundance of fish at those sites, thereby massively reducing the spatial scope for confirmatory studies with methods such as egg mats. The developed model will be adaptable to other processes such as better understanding transport patterns of larval fish and zooplankton.

Our modeling exercise, above, will be expandable for broad use in detecting whitefish aggregations in Green Bay. Currently, there are two unknowns in modeling whitefish eDNA: the physical locations of the fish and the fundamental eDNA parameters of whitefish in Green Bay. Our proposed here leverages *known* locations of whitefish aggregations to solve for the *unknown* fundamental eDNA parameters. Once these fundamental eDNA parameters are revealed by our work here, the same modeling approach can be implemented—but partially inverted—to use the now *known* eDNA parameters to solve for *unknown* potential fish spawning locations.

This pilot project is foundational for a broader survey to identify LWF spawning locations in Green Bay. That follow-up work will support objectives outlined by the Council of the Lake Committees pertaining to understanding drivers of LWF recruitment variability. Research on LWF spawning, including identifying spawning locations, has been repeatedly advocated for by fishery managers. Locating open water spawning locations is a prerequisite for addressing knowledge gaps pertaining to LWF early life history and recruiting identified in the 2018 GLFT-GLFC LWF workshop. The workshop proceedings further state that "Protecting or improving spawning and nursery habitat is important, but would require knowledge of critical habitat locations…", which our follow up project will identify. More generally, an inability to gauge the relative importance of open water spawning relative to tributary spawning for Green Bay LWF is a barrier to characterizing stock dynamics across this commercially and recreationally important fishery. Identifying the spawning locations that contribute the most to the fishery would be

invaluable for directing management and restoration resources to protecting habitats most relevant to the recovery of the LWF fishery in Green Bay and throughout the Great Lakes.

Geographic Focus Area. This work will occur in southern Green Bay, defined here as the region south of a line between the Peshtigo River mouth and Sturgeon Bay (area approx. 55km x 15 km). Knowledge gained from this project would aid in use of eDNA to detect LWF spawning sites throughout the Great Lakes following site-specific biophysical model parameterization.

Communication of Findings. This project is targeted toward fisheries managers responsible for LWF management in Green Bay and throughout the Great Lakes. This includes management biologists from state agencies such as the Wisconsin and Michigan Departments of Natural Resources and tribal agencies such as the Sault Ste. Marie Tribe of Chippewa Indians and the Chippewa-Ottawa Resource Authority. Our work is also targeted toward commercial fishers who would benefit from protection of currently unknown LWF open water spawning areas. An ability to identify and subsequently protect and restore LWF critical open water spawning habitats would ultimately improve sustainability of the fishery

This work will generate one peer-reviewed publication in a fisheries-oriented journal such as Canadian Journal of Fisheries and Aquatic Sciences or Transactions of the American Fisheries Society. We will provide reports as requested to the GLFT. Our research will be presented to managers and biologists at a professional conference such as the International Association for Great Lakes Research Annual Conference. We will also present our findings at meetings of the Lake Michigan management teams in Michigan and Wisconsin and would offer to provide a presentation to the Lake Michigan Technical Committee and groups such as the Lake Michigan Commercial Fishing Board. Data and computational code used for this work will be housed in a public repository, such as GitHub or ScienceBase.

Relationship to Ongoing Activities. Identifying LWF reef spawning locations in Green Bay is a significant challenge that will be best met using multiple research methods. Projects with this shared goal are either in development or underway using telemetry (led by co-PI Isermann; GLFWRA funded), microchemistry (led by co-PI Forsythe, funded GLFT pilot study), and egg sampling (led by co-PI Hansen; WDNR supported). Relative to these other approaches, eDNA methods allow for surveying a very large area in a short time without the need to physically capture LWF. Consequently, eDNA-based inferences are valuable for focusing other more time-intensive strategies (e.g., egg sampling) on locations with a high probability of open water spawning. This proposed pilot project would inform a follow-up project that would adapt currently available hydrodynamic models to account for eDNA diffusion to identify LWF spawning locations. Coordination of these projects with a shared goal and complementary strengths is an inherent benefit of our proposal's team of co-PIs.

Our proposed work complements several ongoing Lake Michigan LWF research projects. This includes a project assessing variation in larval and post-larval whitefish abundance and fitness around Lake Michigan in relation to the availability of zooplankton prey. In the first year of sampling for this project, larval and post-larval LWF were collected from many beach locations along the shore of Green Bay, but the source (i.e., hatching locations) of these fish remain

unknown and would be valuable in understanding how physical processes (e.g., wind and currents) influence the distribution of age-0 whitefish. Otolith microchemistry could be used to assign age-0 LWF to different hatching locations (Doerr et al. 2021). However, spawning/hatching locations and their respective otolith chemical signatures must be identified to determine the spatial scale at which microchemistry will be applicable, which is part of ongoing work being conducted at the University of Wisconsin-Green Bay. As noted previously, ongoing telemetry using a grid of receivers deployed in Green Bay (8-km spacing) will provide coarse information regarding the locations of open-water spawning locations, but the resolution of this information remains unknown. Combining the use of telemetry and eDNA might provide an effective and efficient method for locating spawning locations as telemetry might provide guidance as to where eDNA sampling should be conducted. Alternatively, eDNA sampling could help guide acoustic receiver placement in the future, allowing for strategic collection of LWF movement data at finer scales than provided by the current 8-km grid.

Applicant Capacity. The project team has many years of experience in eDNA analyses, LWF biology and management, and hydrodynamic modeling. Jared Homola (principal investigator) is the Assistant Unit Leader of the USGS Wisconsin Cooperative Fishery Research Unit and Director of the Molecular Conservation Genetics Laboratory at University of Wisconsin-Stevens Point. Homola is PI on a current GLFC-funded Lake Michigan LWF genomics project and co-PI on a current GLFWRA-funded eDNA-based biodiversity assessment of Great Lakes region wetlands. Scott Hansen, Ted Treska, and Dan Isermann are directly involved in whitefish research or management in Lake Michigan and have extensive experience studying whitefish ecology and population dynamics. Isermann has served as PI on LWF telemetry research projects, including one currently underway that aims to identify spawning aggregations in Green Bay. Bahram Khazaei is a physical research scientist with NOAA/UCAR who specializes in hydrological and hydrodynamics modeling, including publishing transport models specifically for Green Bay (Khazaei *et al.*, 2021). Co-PI Kinnison directs the Maine Center for Genetics in the Environment and research lead of the NSF EPSCoR RII Track 1 Maine-eDNA program that supports over 50 researchers applying eDNA for aquatic ecosystem inference, including fisheries restoration assessment. Co-PI Wood is a leader in the analytical methods necessary to characterize eDNA plumes and use them to determine fish location and abundance (Wood *et al.*, 2021, 2020). Wood collaborates with agencies in Maine on eDNA projects, serves as a collaborator for the Maine-eDNA program, and has an ongoing role in Division of Fisheries and Oceans Canada's integration of eDNA into fisheries management.

Budget Narrative. Our budget includes funds for two limited-term employees to collect and analyze samples at $15/hr for 600 total hours and University of Wisconsin – Stevens Point (UWSP) standard fringe rates of 17.3% for FY23 and 22.3% for FY24. Material/supplies includes eDNA filters (322 at $15.95/sample), Zymo PCR inhibitor clean-up reagents (322 at $4/sample), DNA extraction reagents (322 at $6/sample), and a double assay PCR (322 at $15/sample). We have also budgeted $500 for conference travel and $2500 for field travel for repeated trips from UWSP to Green Bay. Indirect costs are capped at 10% of salary and fringe. Matching funds consistent of waived indirect costs based on the standard UWSP 34% rate.

Literature Cited

Andruszkiewicz, E. A., Koseff, J. R., Fringer, O. B., Ouellette, N. T., Lowe, A. B., Edwards, C. A., & Boehm, A. B. (2019). Modeling environmental DNA transport in the coastal ocean using Lagrangian particle tracking. *Frontiers in Marine Science*, *6*, 1–14.

Barnes, M. A., Turner, C. R., Jerde, C. L., Renshaw, M. A., Chadderton, W. L., & Lodge, D. M. (2014). Environmental conditions influence eDNA persistence in aquatic systems. *Environmental Science and Technology*, *48*, 1819–1827.

Bracken, F. S. A., Rooney, S. M., Kelly-Quinn, M., King, J. J., & Carlsson, J. (2019). Identifying spawning sites and other critical habitat in lotic systems using eDNA "snapshots": A case study using the sea lamprey Petromyzon marinus L. *Ecology and Evolution*, *9*, 553–567.

Bylemans, J., Furlan, E. M., Hardy, C. M., McGuffie, P., Lintermans, M., & Gleeson, D. M. (2017). An environmental DNA-based method for monitoring spawning activity: a case study, using the endangered Macquarie perch (Macquaria australasica). *Methods in Ecology and Evolution*, *8*, 646–655.

Darling, J. A., Jerde, C. L., & Sepulveda, A. J. (2021). What do you mean by false positive? *Environmental DNA*, *3*, 879–883.

Deiner, K., & Altermatt, F. (2014). Transport distance of invertebrate environmental DNA in a natural river. *PLoS ONE*, *9*.

Dejean, T., Valentini, A., Miquel, C., Taberlet, P., Bellemain, E., & Miaud, C. (2012). Improved detection of an alien invasive species through environmental DNA barcoding: The example of the American bullfrog Lithobates catesbeianus. *Journal of Applied Ecology*, *49*, 953–959.

Fukaya, K., Murakami, H., Yoon, S., Minami, K., Osada, Y., Yamamoto, S., … Kondoh, M. (2021). Estimating fish population abundance by integrating quantitative data on environmental DNA and hydrodynamic modelling. *Molecular Ecology*, *30*, 3057–3067.

Goldberg, C. S., Turner, C. R., Deiner, K., Klymus, K. E., Thomsen, P. F., Murphy, M. A., … Taberlet, P. (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods in Ecology and Evolution*, *7*, 1299–1307.

Hansen, S. (2019). Lake Whitefish. In Wisconsin Lake Michigan Fisheries Team (Ed.), *Lake Michigan Management Reports* (pp. 37–43). Ann Arbor, MI.

Hernandez, C., Bougas, B., Perreault-Payette, A., Simard, A., Côté, G., & Bernatchez, L. (2020). 60 specific eDNA qPCR assays to detect invasive, threatened, and exploited freshwater vertebrates and invertebrates in Eastern Canada. *Environmental DNA*, *2*, 373–386.

Jane, S. F., Wilcox, T. M., Mckelvey, K. S., Young, M. K., Schwartz, M. K., Lowe, W. H., … Whiteley, A. R. (2015). Distance, flow and PCR inhibition: EDNA dynamics in two headwater streams. *Molecular Ecology Resources*, *15*, 216–227.

Khazaei, B., Bravo, H. R., Anderson, E. J., & Klump, J. V. (2021). Development of a Physically Based Sediment Transport Model for Green Bay, Lake Michigan. *Journal of Geophysical Research: Oceans*, *126*, 1–35.

Lacoursière-Roussel, A., Rosabal, M., & Bernatchez, L. (2016). Estimating fish abundance and biomass from eDNA concentrations: variability among capture methods and environmental conditions. *Molecular Ecology Resources*, *16*, 1401–1414.

Lahoz-Monfort, J. J., Guillera-Arroita, G., & Tingley, R. (2016). Statistical approaches to account for false-positive errors in environmental DNA samples. *Molecular Ecology Resources*, *16*, 673–685.

Laramie, M. B., Pilliod, D. S., & Goldberg, C. S. (2015). Characterizing the distribution of an endangered salmonid using environmental DNA analysis. *Biological Conservation*, *183*, 29–37.

Mahon, A. R., Jerde, C. L., Galaska, M., Bergner, J. L., Chadderton, W. L., Lodge, D. M., … Nico, L. G. (2013). Validation of eDNA Surveillance Sensitivity for Detection of Asian Carps in Controlled and Field Experiments. *PLoS ONE*, *8*, 1–6.

Di Muri, C., Lawson, H. L., Bean, C. W., Benucci, M., Harper, L. R., James, B., … Hanfling, B. (2022). Spatio-temporal monitoring of lake fish spawning activity using environmental DNA metabarcoding. *BioRxiv (Preprint)*, 1–26.

Di Muri, C., Handley, L. L., Bean, C. W., Li, J., Peirson, G., Sellers, G. S., … Hänfling, B. (2020). Read counts from environmental DNA (eDNA) metabarcoding reflect fish abundance and biomass in drained ponds. *Metabarcoding and Metagenomics*, *4*, 97–112.

Ransom, A. L., Houghton, C. J., Hanson, S. D., Hansen, S. P., Doerr, L. R., & Forsythe, P. S. (2021). Recolonization of lake whitefish river spawning ecotypes and estimates of riverine larval production in Green Bay, Lake Michigan. *Journal of Great Lakes Research*, *47*, 213–225.

Rourke, M. L., Fowler, A. M., Hughes, J. M., Broadhurst, M. K., DiBattista, J. D., Fielder, S., … Furlan, E. M. (2021). Environmental DNA (eDNA) as a tool for assessing fish biomass: A review of approaches and future considerations for resource surveys. *Environmental DNA*, 1–25.

Sard, N., Scribner, K. T., Robinson, J. D., Herbst, S., & Kanefsky, J. (2019). Comparison of fish detections, community diversity and relative abundance using environmental DNA metabarcoding and traditional gears. *Environmental DNA*, 1–31.

Strickler, K. M., Fremier, A. K., & Goldberg, C. S. (2015). Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. *Biological Conservation*, *183*, 85–92.

Thomas, A. C., Howard, J., Nguyen, P. L., Seimon, T. A., & Goldberg, C. S. (2018). ANDe^{TM}: A fully integrated environmental DNA sampling system. *Methods in Ecology and Evolution*, *9*, 1379–1385.

Tillotson, M. D., Kelly, R. P., Duda, J. J., Hoy, M., Kralj, J., & Quinn, T. P. (2018). Concentrations of environmental DNA (eDNA) reflect spawning salmon abundance at fine spatial and temporal scales. *Biological Conservation*, *220*, 1–11.

Tingley, R., Coleman, R., Gecse, N., van Rooyen, A., & R. Weeks, A. (2021). Accounting for false positive detections in occupancy studies based on environmental DNA: A case study

of a threatened freshwater fish (Galaxiella pusilla). *Environmental DNA*, *3*, 388–397.

Turner, C. R., Barnes, M. A., Xu, C. C. Y., Jones, S. E., Jerde, C. L., & Lodge, D. M. (2014). Particle size distribution and optimal capture of aqueous macrobial eDNA. *Methods in Ecology and Evolution*, *5*, 676–684.

Wood, Z. T., Lacoursière-Roussel, A., LeBlanc, F., Trudel, M., Kinnison, M. T., Garry McBrine, C., … Gagné, N. (2021). Spatial Heterogeneity of eDNA Transport Improves Stream Assessment of Threatened Salmon Presence, Abundance, and Location. *Frontiers in Ecology and Evolution*, *9*.

Wood, Z. T., Erdman, B. F., York, G., Trial, J. G., & Kinnison, M. T. (2020). Experimental assessment of optimal lotic eDNA sampling and assay multiplexing for a critically endangered fish. *Environmental DNA*, 1–11.