GREAT LAKES FISH AND WILDLIFE RESTORATION ACT FULL PROPOSAL FY 2010

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COVER PAGE

Title: CONSERVATION OF NATIVE FRESHWATER MUSSEL REFUGES IN GREAT LAKES COASTAL ZONES

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PROJECT PROPOSAL

TITLE: CONSERVATION OF NATIVE FRESHWATER MUSSEL REFUGES IN GREAT LAKES COASTAL ZONES

PROJECT APPLICANT(S) (see CVs for full contact info – **PIs in bold):**
Dr. D. Zanatta (Principal Applicant), Central Michigan University, Biology Dept., Brooks Hall 186, Mount Pleasant MI, 48858, 989-774-7829, zanat1d@cmich.edu; **Dr. L. Burlakova**, Buffalo State College, burlakle@buffalostate.edu; **Dr. A. Karatayev**, Buffalo State College, karataay@buffalostate.edu; **Dr. R. Krebs**, Cleveland State University, r.krebs@csuohio.edu; **Dr. M. Hoggarth**, Otterbein College, MHoggarth@otterbein.edu; **Dr. F. A. de Szalay**, Kent State University, fdeszala@kent.edu; **Dr. J. Bossenbroek**, University of Toledo, jbossen@utnet.utoledo.edu; **E. Meyer**, Pennsylvania Natural Heritage Program, EMeyer@paconserve.org; **M. Walsh**, Pennsylvania Natural Heritage Program, MWalsh@paconserve.org; Dr. M. Schlesinger, NY Natural Heritage Program mdschles@gw.dec.state.ny.us; R. Haas, Michigan DNR, haasr1@michigan.gov; T. Crail, U. of Toledo, todd.crail@utoledo.edu; P. Badra, Michigan Natural Features Inventory, badrap@michigan.gov; N. Welte, Pennsylvania Fish and Boat Commission, c-nwelte@state.pa.us; L. Holst, NY Department of Environmental Conservation, lkhost@gw.dec.state.ny.us; D. Schloesser, USGS – Great Lakes Science Center, dschloesser@usgs.gov

MANAGEMENT IMPLICATION OR APPLICATION:

Freshwater mussels (Unionidae) are often considered ideal sentinel organisms for degradation or improvement of aquatic systems and overall aquatic biodiversity given their sensitivities to habitat changes and their lifecycle dependence on host fish populations. Freshwater mussels have become the most imperiled faunal group in North America as a result of human impacts such as overharvest, pollution, impoundment of rivers, and the introduction of exotic species. The diverse native mussel communities have declined sharply since the introduction of dreissenid mussels into the Laurentian Great Lakes in the late-1980s. However, there have been several locales identified as refuges in coastal and nearshore areas. Although, these have existed with the ongoing threat of dreissenid mussels in nearby offshore waters for over 20 years, the long-term survival of unionids in these habitats remains in question. How unionids survive in these habitats is being studied, but we still do not know the key habitat characteristics of these refuges or how many species and populations remain in the Great Lakes. Moreover, another major issue that has (as yet) been overlooked for the Great Lakes, specifically: within site genetic diversity/ between site gene flow. Genetic analyses are needed to determine if existing populations need additional management efforts to prevent inbreeding. We propose to sample unionids in 23 known refuges in the lower Great Lakes and in addition investigate several possible refuges. We will examine their genetic diversity/isolation to determine if there is gene flow between coastal refuges and nearby riverine habitats. This information will help managers develop conservation strategies to sustain existing populations in these refuges. We will also sample key habitat attributes in these refuges to develop predictive models. This will provide managers with information to locate and protect additional unionid refuges and also to manage sites to promote unionid colonization and survival. We will also make management recommendations to agencies responsible for conservation of coastal zones and recovery of listed (Endangered and Threatened) species. Finally, this expansive project will also train undergraduate and graduate students, thereby creating a cadre of future scientists and managers who will work to protect this imperiled resource.

RATIONALE:

Freshwater pearly mussels (Family: Unionidae; also known as unionids or naiads) are large (up to 30 cm long) bivalves that live in the sediments of rivers, streams, and lakes worldwide [1]. About 1000 species are known, 300 of which live in North America [2] and more than 50 species are native to the Great Lakes watershed [3,4,5]. These long-lived molluscs – living for decades to over a century – have a unique life cycle (Figure 1). While most bivalves have free-living larvae, unionid larvae are obligate parasites on fish hosts. The larvae can be transported for great distances within watersheds when attached to their host fish. This is a useful adaptation because it helps maintain genetic diversity in spatially isolated unionid populations. Juvenile mussels are thought to live for a several years buried in sediments and pedal feeding (i.e., scooping food into mouth with foot). Adult mussels remain at the substrate surface and are suspension feeders but they will burrow during adverse environmental

conditions (e.g., ice scour, high water temperatures). Freshwater mussels are the most imperiled faunal group in North America [6] mostly as a result of many human impacts from pollution, to impoundment of rivers, to the introduction of exotic species (e.g., dreissenid mussels) [7,8].
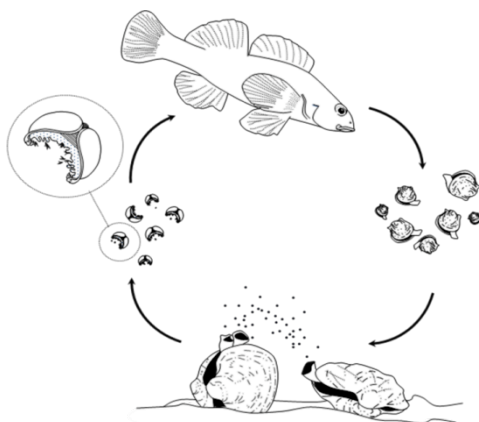
Figure 1: Lifecycle of freshwater unionid mussels. Male fertilizes female by releasing sperm into the water column (bottom right), the female takes sperm into her body through her incurrent siphons to fertilize eggs within her marsupial gills. The parasitic larvae are released into the water column and encyst on the gills of their specific fish host species. Once they develop to the juvenile stage they drop off and burrow into the sediments.

Native unionid mussels in the Laurentian Great Lakes have declined sharply since the introduction of dreissenid mussels [9,10], which attach to their shells and outcompete them for food [11,12,13]. Therefore, conservation programs for these species have become increasingly crucial [7,14]. However, there have been several locales identified as refuges in nearshore areas and coastal wetlands [15,16,17,18,19,20,21,22,23]. Many of these refuges have remnant populations of the diverse communities that were historically found in the lower Great Lakes [24,25]. Several species in refuges are listed as Endangered, Threatened or Special Concern in Great Lakes States (Table 1), with three federally listed species believed lost from the Great Lakes and connecting channels. These refuges have existed with the ongoing threat of dreissenid mussels infesting nearby offshore waters for approximately 20 years [26], but their long-term survival remains in question. Reasons about why these refuges exist are being studied [9,10,18,19,20,21,27,28], but we still do not know the key habitat characteristics that allow unionids to persist in the refuges or how many species and populations are left in the Great Lakes. This information is important to develop predictive models to locate other existing refuges and management strategies to provide additional habitat for unionids. Moreover, other major issues that have (as yet) been overlooked for the Great Lakes are specifically: within site genetic diversity and among site gene flow. This is needed to determine if existing populations need additional management efforts to prevent inbreeding.

The main foci for this proposed study are: (1) assessment of unionid populations and their habitat in unionid refuges from the nearshore and coastal zone of the lower Great Lakes, (2) investigating genetic diversity of unionid populations in coastal wetlands and gene flow between coastal wetlands and nearby riverine populations, (3) develop and verify models based on habitat characteristics in unionid refuges that identify addition refuge locations, and develop management strategies that enhance unionid habitats.

Focus 1: Assessment of Unionid Refuges
Although unionid refuges were discovered over ten years ago in Lake Erie [16] and Lake St. Clair [15], a critical question remains - what are the key biotic and abiotic factors that allow unionids to survive and sustain populations in these refuges? Important habitat parameters most probably include those that either inhibit dreissenid establishment (e.g. water level draw downs, access by zebra mussel predators), or/and allow unionids to remove attached dreissenids from their shells (e.g. burrowing into soft sediments). However, habitat conditions may not favor all unionid species. Therefore, high-priority research questions are: (1) What environmental parameters are specific to unionid refuges in the lower Great Lakes? (2) What are the diversity and densities of unionid populations in these refuges, and (3) Are these populations sustainable, or are they remnant (i.e., "sink")

populations? To answer these questions, we will survey 23 known and several potential (to be determined using predictive modeling) coastal refuges in Lake Erie, Lake St. Clair, and Lake Ontario, and document habitat parameters that may be important for the existence of these refuges. We will sample the demographics of unionid populations by documenting species diversity, density, and age distribution. Although presence of juvenile mussels will be the evidence of ongoing recruitment, genetic analysis (see Focus 2) is needed to determine if these are isolated populations with evidence of a genetic bottleneck.   All of the biotic and abiotic data we gather will be used to develop a GIS-based model of unionid refuges (see Focus 3). Finally, we will estimate current and potential threats for these refuges, including anthropogenic factors (e.g., sediment disturbance, pollution, watershed development, exotic species invasion, etc), including those that will be affected by climate change (e.g., potential lower water levels in the lower Great Lakes), and provide recommendations on sensitivity to disturbance and management options. The data will be provided to USFWS and state environmental agencies and will be extremely important to address priorities for conservation, management and recovery plans. Based on our data, we will also identify sites that should be monitored for unionid diversity and abundance in the future and make management recommendations to restore unionid habitat in additional coastal wetlands.

Table 1: Unionid species of greatest conservation need (Endangered, Threatened, Special Concern) in MI, OH, PA, NY (found in Great Lakes proper)

Alasmidonta marginata	OH, MI, NY	*Pleurobema sintoxia*	OH, MI, NY
Epioblasma t. rangiana	Fed E, OH, MI, PA, NY	*Ptychobranchus fasciolaris*	OH, MI, NY
Epioblasma triquetra	Fed C, OH, MI, PA, NY	*Simpsonaias ambigua*	Fed C, OH, MI, PA, NY
Lampsilis fasciola	OH, MI, NY	*Toxolasma parvus*	MI, NY
Ligumia recta	OH, MI, NY	*Truncilla donaciformis*	OH, MI, NY
Ligumia nasuta	OH, MI, NY	*Truncilla truncata*	OH, MI, NY
Obovaria subrotunda	MI, NY	*Villosa fabalis*	Fed C, OH, MI, NY
Obliquaria reflexa	OH, MI	*Villosa iris*	MI, NY
Pleurobema clava	Fed E, OH, MI, PA, NY	*Uniomerus tetralasmus*	OH

Focus 2: Genetics

For the genetics component of the study the research questions we pose are as follows: (1) Do unionids in the coastal and nearshore refuges show any evidence of genetic differentiation or recent gene flow among the refuges and/or nearby riverine populations? (2) Do unionids in coastal wetland refuges have similar genetic diversity to "healthy" riverine populations or is there evidence of a recent genetic bottleneck? We will compare the coastal refuge populations to riverine populations as most populations of native mussels in rivers (Great Lakes tributaries) are usually much less impacted by dreissenid mussels. The concern is that populations of native mussel species in the coastal wetlands are small, isolated, potentially inbred (genetically), have had a rapid decline in numbers (and genetic diversity) causing a genetic bottleneck (leading to inbreeding), and may not have the genetic diversity necessary or connection to larger healthy populations to sustain their numbers without human intervention (including population augmentation from outside sources).

Fortunately, discovery of additional refuges and new evidence that some mussels occur within the lakes today provides the opportunity to investigate conservation in situ ahead of augmentation techniques; required approaches, however, depend on knowing the history of these refugia and nearshore populations. Two hypotheses for their presence are that they either survived at low population sizes during the height of the zebra mussel infestation and are now starting to recover, or these mussels have recolonized the lake from an as yet unidentified source(s). The structure of their populations and predictions as to the sustainability of current populations vary greatly depending on which hypothesis is correct. By using a diverse array of genetic markers, we can separate sources of variation due to nuclear genes (microsatellite DNA markers), variation due to female migration based on a typical mitochondrial DNA haplotype analysis, and separately assess that variation due to male migration (potentially through sperm release) by taking advantage of the unique double uniparental inheritance (DUI) of mitochondria found in mussels [29,30]. Haplotype networks obtained in mitochondrial trees are particularly valuable for connecting populations from which migratory events have occurred, for example among different refuges and between the lower Great Lakes and their tributaries. Microsatellites, however, are better for quantifying inbreeding and population differentiation across continuous habitat, and they can better indicate levels of

migration or isolation of mussel populations along the nearshore and potentially within the lake proper.

As predicted climate changes disrupt water levels in the Great Lakes, coastal areas will be among the first to be affected. Without connections of suitable habitat to healthy populations, the fragmented unionid populations in coastal wetlands will not be able to shift and thus survive the habitat alterations initiated by climate change. Furthermore, if outside sources are deemed necessary for population augmentations, the genetic data collected will be useful in identifying the best sources (most similar genetically). We will seek to answer these questions about geneflow and genetic differentiation by using the three different types of molecular markers described above. If the genetic population structures of the unionid species show evidence of recent migrants from nearby watersheds, this would indicate movement provided by their host fish during the larval stage. The results and interpretations coming from this study will assist in managing these important and declining biological resources. This type of study will have direct relevance to understanding and teasing apart the effects of habitat fragmentation at both ancient (natural fragmentation) and more recent (anthropogenic) timescales.

By studying genetic divergence and dispersal in common species, like Giant Floater (*Pyganodon grandis*), the maximum level and limits of genetic exchange present across species can be studied. The glochidial life stage of the Giant Floater has been identified to utilize a greater number and range of host fish than any species known from Lake Erie. Therefore, genetic connectivity among populations (corresponding to migration rates) is expected to be as high or higher than in any of the other species (i.e., species of conservation concern). If this species shows inbreeding and or significant levels of isolation (by F_{ST} analyses), then more significant limitations with respect to mobility can be anticipated for the other species extant in coastal refuges of the lower Great Lakes. Additional species (Table 2) will also be assessed for comparison using a subset of these genetic approaches, but generally each will combine microsatellite markers and conventional mitochondrial gene sequencing.

Focus 3: Habitat Modeling

Since we will use uniform sampling methods in all sites that we survey, we can apply univariate, multivariate and spatial analyses to determine the set of habitat parameters necessary to sustain unionid populations. One of our primary goals is to predict the locations of as-yet undiscovered refuges in the Great Lakes. Based on data collected, we will develop a GIS-based model that will summarize all the important features of the refuges. Using the model together with existing hydrological, topographical, physical GIS lake layers, we will then predict the locations of other refuges in the lower Great Lakes. Next, we will sample some of the predicted locations to ground-truth the model and further fine-tune the model. Using this iterative process, we will refine the model to make it applicable across all of the lower Great Lakes. A secondary goal of habitat modeling is to develop management protocols to create new Unionid habitat in existing coastal wetlands that currently do not provide unionid habitat.

The existing and potential habitat or ecological niches of unionid mussels will be assessed at two scales, local and regional. Ecological niche modeling is a rapidly developing field aimed at answering a wide range of environmental questions [31] including, 'where are potential conservation zones or areas for the reintroduction of threatened and endangered species?' [32], 'where is there suitable habitat at which unsurveyed sites of rare species might likely occur?' [33,34] and 'what is the potential distribution of invasive species?' [35,36]. The number of potential tools to use for ecological niche modeling include basic univariate analysis, multivariate analysis (CCA, NMDS), regression-based approaches (multivariate adaptive regression splines, GLMs, CARTs), and sophisticated modeling packages, such as MAXENT or GARP [36]. The local scale analysis will use species and habitat data collected in uniform fashion (from Focus 1), using compatible collection methods, to determine the set of habitat parameters necessary to sustain unionid populations. Niche modeling will then be conducted for mussel beds in general or for individual species. At the regional scale, our goal is to predict where other refuges could be found on the Great Lakes for future study and protection. These predictions will be based on regional scale data, such as existing hydrological, topographical, and physical GIS lake layers, as well as derived GIS layers that quantify potential driving factors of where unionids will occur, such as distance to rivers (which are potential source populations), proximity to dredging operations (which may affect sediment loads), etc. We will then predict the locations of other refuges in the lower Great Lakes. Next, we will sample the predicted locations to verify the predictions, and, if needed, will collect additional data that may be important for model adjustment and fine-

tuning. Using this field feedback data, we will refine and finalize the model to make it general and applicable for each of the studied lakes.

After interpreting the results of these projects, each of the collaborative members will – in addition to submitting manuscripts to peer-reviewed journals – make recommendations for unionid management to the appropriate agencies responsible for coastal zones and the conservation and recovery of listed (Endangered and Threatened) unionid species. Without scientifically sound guidelines for protecting, managing, and reestablishing native biodiversity many of these key indicator species will be doomed to extirpation from the Great Lakes. This project will also be used to train young biologists (undergraduate and graduate students at institutions involved with this expansive project) to further the region's knowledge of this imperiled resource in the future.

RELEVANCE:

Without scientifically sound guidelines to protect and reestablish native unionid biodiversity, many of the remaining species may be doomed to extirpation from the Great Lakes. The research we are proposing is consistent with the recommendations in the following documents: (1) **Non-indigenous Aquatic Nuisance Prevention and Control Act**: "to understand and minimize economic and *ecological impacts* of nonindigenous aquatic nuisance species that become established, including the zebra mussel." (2) **Great Lakes Regional Collaboration's "Strategy to Restore and Protect the Great Lakes"**: Our proposal will work to "Maintain widely distributed, self-sustaining populations where the species occurred historically basinwide" and "…in as many original habitats as is practical." (3) Our proposal focuses on gathering important information for the development of management plans for restoration of wildlife resources and their habitats in the Great Lakes Basin, and is consistent with the goals of the **Great Lakes Fish and Wildlife Restoration Act of 2006** in protecting and restoring habitat, as well as to bring Threatened and Endangered species back to viable, self-sustaining levels.

Ecological Benefits:

Unionid mussels are among the most imperiled groups of animals in North America. They play a major role in aquatic ecosystems by translocating nutrients by filter feeding, modifying sediment stability [37,38], increasing habitat physical complexity and providing food for other taxa [39,40]. This project will gather baseline data to make informed management decisions how to protect, conserve, and enhance remaining Great Lakes unionid communities, and it will develop management strategies to create additional unionid habitat. A major concern is if remnant unionid populations in the Great Lakes are inbred and have declining genetic diversity. If so, these populations may be genetically isolated from other "healthy" populations, and they will need management intervention to sustain themselves. If outside sources are used to augment populations, genetic data will be necessary to identify the best sources. Although targeting the lower Great Lakes, the results of our study will have basin-wide application to manage populations are threatened by extirpation due to anthropogenic stresses and invasive species.

Species and Habitats:

Numerous State and two Federal Threatened and Endangered (T&E) species (Northern Riffleshell and Clubshell) were historically found in the region, and some species of conservation concern (see Table 1) still continue to persist (at low densities). Thorough surveys or the Great Lakes coastal zones will provide valuable data for assessing the status of these species. When possible, our study will use common unionid species as surrogates for T&E species that are found in similar habitats, thus allowing for management decisions to be made for all unionids in the region. We will conduct surveys for unionids in nearshore areas, estuaries (drowned stream mouths) coastal wetlands, and tributary streams.

Benefits to Fish and Wildlife:

The results of this study will produce management guidelines for protecting and aiding recovery of unionids in the Great Lakes region. Freshwater mussels are often considered ideal *sentinel organisms* for degradation or improvement of aquatic systems and aquatic biodiversity [37,41] given their sensitivities to habitat changes and their lifecycle dependence on host fish populations. Improvements to the entire unionid community will result in an improved nutrient cycling system that has become severely depleted since dreissenid mussels invaded the lakes.

Negative Impacts:
None. We will work with USFWS and state agencies to develop a HACCP plan to reduce the risk of AIS or pathogens (e.g., VHS) being moved into new areas. For example, we will bleach field gear (waders and wetsuits) when traveling among refuges and watersheds to reduce the chance of moving AIS or pathogens among study areas. A minimal number of tissues samples will be taken and all samples will be collected non-lethally [42,43]. Limited numbers (<10% of total numbers collected) of common species (Giant Floater, Fat Mucket, Fragile Papershell) will be sacrificed for M-lineage mtDNA sequence analysis.

OBJECTIVES:

(1) Assess known coastal and nearshore unionid refuges in the lower Great Lakes to describe existing unionid diversity, habitat characteristics, and prioritize areas for conservation and management.
(2) Examine gene flow and differentiation among refuge sites and nearby riverine populations and examine their evolutionary significance.
(3) Model habitat characteristics to determine key environmental attributes and identify additional potential refuges.
(4) Develop management recommendations to state and federal agencies to protect and conserve unionid communities in coastal refuges.

METHODS:

We will conduct unionid surveys along an extensive range of nearshore habitats in Lake Erie/St. Clair and Lake Ontario and collect tissue samples from common unionid species. We will use existing molecular markers to determine population genetics and gene flow between habitats. Landscape (GIS) analyses will show current distributions and identify key habitat attributes of the best-quality unionid refuges. Using results from this study, we will create management recommendations and tools for enhancing unionid communities in coastal refuges.

Site Locations:
Coastal Refuges (those presently known or probable)**:**
The 23 known refuges in Lake St. Clair and Lake Erie will be surveyed. These refuges are known or suspected from several nearshore, coastal, estuarian (drowned stream mouths) and wetland (fringing, dyked, and undyked) areas. See Figure 2 for locations of known and suspected unionid refuges in the coastal zones of Lake St. Clair and Lake Erie. Unionid refuges are likely in Lake Ontario (New York), however no data are known to exist. Using the habitat models from Lake Erie, probable refuge sites will be identified in Lake Ontario. The models can then be tested (ground-truthed) to test their validity outside of Lake St. Clair and Lake Erie.

Refuge Assessment Surveys and Tissue Collections:
We will be surveying and collecting tissue samples from unionids in coastal and nearshore unionid refuges from the lower Great Lakes. We will compare the genetic diversity and assess genetic differentiation among unionids in the coastal refuges and adjacent riverine watersheds. Local experts and collaborators will carry out the unionid surveys and collections from coastal refuges and surrounding watersheds of Lake St. Clair, Lake Erie, and Lake Ontario, in Michigan, Ohio, Pennsylvania, and New York. Collections will be made with collaboration with graduate students who will travel from their home institutions to assist in field activities.

We will take advantage of decreased water levels in some refuges during seiche events, when strong westerly winds reduce the depth of the lake in its western-most edges. This "Seiche method" enables extensive visual survey of the lake bottom in areas where soft substrates otherwise reduce surveys to feeling for mussels. We will use time-searches and, if possible, area-searches during the time-restricted seiche events to describe the diversity, collect tissue samples for genetic analysis, and identify the most populated areas for consequent quantitative surveys (see above). As seiche events are uncommon and occur at somewhat unreliable intervals, detecting and quantifying unionid refuges in turbid waters >1.25 m, we will be limited to taking numerous mini-ponar/dredge samples. Should deep waters be clear enough, SCUBA surveys (similar to those described below) will be used.

In coastal refuges unionid sampling for population densities and species diversity will be based on the method

used by Zanatta et al.[15]. For each lacustrine (coastal refuge) site selected, which will include nearshore, deltaic, and coastal wetlands, an initial timed-search will be conducted to determine the presence of unionids. This will be followed by quantitative search of the substrate either visually [with mask and snorkel in shallow water (<1.25 m), and SCUBA or a surface supplied air diving system (Pioneer 275, Brownie's Third Lung) in deeper water] or by touch until a living unionid is found. A metal rebar stake will then mark the position of a unionid and a 4.55 m line will be attached to the stake. Using the 4.55 m line as a guide, a search for unionids will be conducted by swimming in concentric circles around the stake in an inward direction until the entire area (65 m^2) has been covered. This type of search will be conducted on up to three 200 m long transects selected at random locations in the area determined to have live unionids during an initial timed search (using a shore landmark as a waypoint), doing up five to ten "stake and rope" sampling plots on each transect. For areas that have very muddy substrates that could reduce visibility if they are disturbed by walking or swimming, an air mattress will be used as a float while unionids are probed for with a small diameter PVC pipe. In areas where a sufficient density of mussels is found (>0.1 m^{-2}), we will excavate and sift through a sieve at least ten (dependant on unionid densities and site area) 0.25 m^2 quadrats to reveal successful reproduction and recruitment. The survey data collected will be compared to previous survey data for monitoring purposes and changes in the unionid community (species diversity, densities, sizes, sex ratios) will be documented. This survey technique is reasonably effective in determining the presence of recruitment in coastal refuges and this will be especially important in determining if refuge populations are sources or sinks.
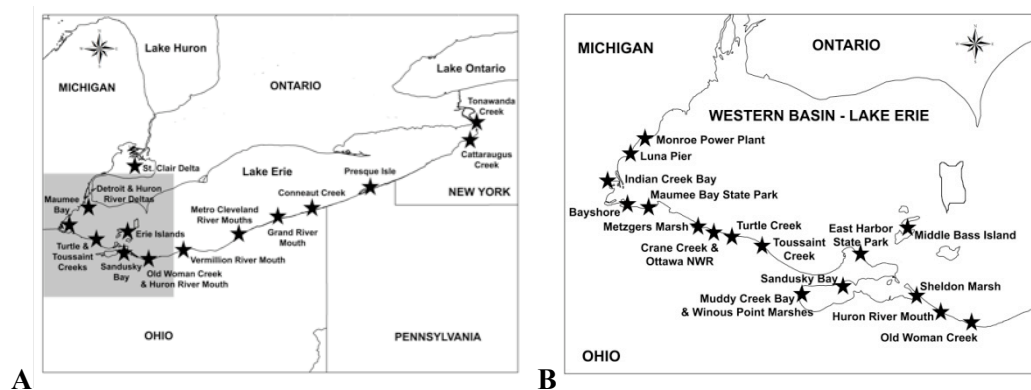
Figure 2. Known and suspected coastal refuges for unionid mussels in Lake St. Clair and Lake Erie (**A**) (Michigan, Ohio, Pennsylvania, and New York) and detail of refuge sites (**B**) in the western basin Lake Erie (shaded area in **A**).

In rivers, we will do targeted surveys and collections for species in common with those collected in nearby coastal refuges. We will use timed-search techniques of known or historical mussel populations. In areas where full surveys are deemed necessary (tributaries close to known refuges with probable, but un- or under-surveyed unionid populations) survey methods will be adapted from Metcalfe-Smith et al.[44] and Strayer and Smith[45], with modifications being made as needed for larger or smaller waterbodies. However, most tributary streams have been surveyed recently (last 10-15 years) and records exist of the species present and areas where mussels occur. The status of unionid communities in the surrounding watersheds is beyond the scope of this project.

All live mussels found will be identified to species and sexed where possible (some species are sexually dimorphic), females will be checked to determine if they are gravid (brooding larvae), and shell lengths will be measured to the nearest mm using calipers. Dreissenid mussels infesting the unionid shells will be counted and weighed, this can be used later to regress against the density of unionids with the degree of dreissenid overgrowth and dreissenids will be quantified by species. In addition, evidence of past dreissenid infestation (byssal threat cover) will be documented for each unionid collected. Animals will be returned to the water alive (with any infesting dreissenid mussel removed), and placed carefully into the substrate from which they were taken. Some individuals from some species will be targeted for genetic analysis (see below).

Abiotic parameters that we will record from areas inhabited by mussels will include: substrate type, depths, water temperature, water levels and variation in water levels, and location. To estimate current and potential threats for

these refuges we will use field observations, literature and other sources. These will include sediment disturbance (filling, grading, removal of vegetation, building construction and changes in water levels and drainage patterns), pollution, watershed development, exotic species invasion, and those that will be affected by climate change (e.g., water level alterations).

A subsample (at least 20 individuals of each target species at each site, to maximize statistical power) of all adult mussels will be sampled non-destructively using a modified 'swab' method for species of conservation concern [42] or a non-lethal mantle tissue biopsy for non-listed species [43]; adults will be temporarily removed from the water, swabbed to obtain epithelial cells or have a mantle clip taken, and returned to their original location (with any dreissenid mussels removed). For the three most common species in the region, *Lampsilis siliquoidea*, *Pyganodon grandis*, and *Leptodea fragilis* a small number of individuals will be collected (not to exceed 10% of discovered individuals) for extraction of the gonadal tissue necessary to assay male inherited mitochondria. This collection targets males, and as lampsiline mussels (*Lampilis* and *Leptodea*) are sexually dimorphic, males can be identified, as can mature breeding females of *Pyganodon*.

Genetics:
We will use two separate and complementary approaches to assess the genetic diversity, geneflow, and population structure of target unionid species in the lower Great Lakes refuges. Microsatellite DNA markers are only available for a select group of species (Table 2), but are extremely sensitive and can be used to detect very fine-scale and subtle differences among population due to their hypervariable nature. In contrast, using the unique genetics of unionid mussels called double uniparental inheritance (DUI) of mitochondrial DNA (mtDNA), a combination of female- and male-lineage mtDNA sequences can be used in a similar population genetic framework to microsatellite DNA markers [29,30]. We will apply this comparative approach using both mtDNA sequencing and microsatellite DNA genotyping to assess the population genetics of unionids in coastal refuges with respect to those in Great Lakes tributaries.

Table 2: Target species for genetic analyses and loci to be used.

Species	Microsatellite DNA	F-lineage mtDNA	M-lineage mtDNA
Fatmucket, *Lampsilis siliquoidea*	X[46,47]	X	X
Plain Pocketbook, *Lampsilis cardium*	X[46,47]	X	
Mapleleaf, *Q. quadrula*	X[48]	X	
Eastern Pondmussel, *Ligumia nasuta**	X[46,47]	X	
Giant Floater, *Pyganodon grandis*		X	X
Fragile Papershell, *Leptodea fragilis*		X	X
Threeridge, *Amblema plicata*		X	
Wabash Pigtoe, *Fusconaia flava*		X	

*State Listed Species

Genetic Methods – Microsatellite DNA genotyping:
Microsatellite DNA markers will be used to determine the genetic diversity and population structure of targeted mussel species. These markers are hypervariable and fast mutating, making them very powerful for research on evolutionary changes at contemporary (recent) timescales. The mussel species to be targeted for genetic study will be those that have had microsatellite DNA primers developed and published in the literature, thus saving time and money for developing new markers. For example, microsatellite markers developed for lampsiline unionids [46,47,49] and quadruline unionids [48] will be used on certain commonly occurring species in nearshore and coastal zones. *Lampsilis siliquoidea*, *L. cardium* [15], and *Quadrula quadrula* [20] are all found in abundance among several coastal refuges and the surrounding watersheds and have had already had primers optimized for many of the microsatellite DNA markers described above. The population genetics of these species will be used as surrogates for listed-species, with any recommendations stemming from the common species being best-case scenarios for species of conservation concern.

Genetic Methods – Mitochondrial DNA sequencing:
Direct sequencing of mitochondrial genes will provide a second measure of genetic diversity and population

structure of the targeted mussel species, and for the three most common species, data from both male and female lineages will be used to determine population structure following protocols in [29]. Standard mitochondrial approaches will complement microsatellite DNA marker analysis, as no comparison of these independent genetic tools has been made in unionid mussels, and only a single tissue sample is needed from each mussel, as well as DNA extraction protocol, which improves assay efficiency. The analysis of male-derived mitochondria unfortunately requires sacrificing individual mussels, which is why this approach will be restricted to Giant Floater (*P. grandis*), Fat Muckets (*L. siliquoidea*), and Fragile Papershell (*L. fragilis*). For both of these species a significant number of the individuals have already been collected from tributaries where both can be very common. The male-derived mitochondrial forms predominate sufficiently in testicular tissue to sequence using the same universal primers that amplify typical female-derived genes, in this case a variable region fragment of 16S rRNA gene and about half the CO1 gene. Once several sequences are obtained, the male and female forms, which differ at over 20% of bases (Krebs, 2004) can be aligned together, and gender-specific primers designed from non-variable regions in any mussel species. The primers necessary for studies on Fatmuckets and Giant Floaters for both of these genes have been developed and used successfully to amplify these gene regions in multiple individuals (Krebs, unpublished).

Statistical Analyses – Microsatellite DNA genotyping:
Comparisons of genetic diversity among sites within coastal wetlands and among coastal refuges and nearby riverine populations will be analyzed. Genetic diversity in each sample will be summarized as allelic richness, measured as the mean number of alleles per locus after correcting for sample size and expected heterozygosity. Allelic richness (A) will be calculated in FSTAT [50]. Detection of deviations from Hardy-Weinberg equilibrium and randomization tests for linkage disequilibrium will be conducted using GENEPOP. A hierarchical analysis of molecular variance (AMOVA) will be used to estimate the partitioning of genetic variance within and among populations. The AMOVA will be carried out in ARLEQUIN [51], using data pooled across loci. Among-population variation (F_{ST} [52]) will be calculated for all pairs of populations and drainages. Significance of F-statistics will be calculated to test the null hypothesis of panmictic populations. Genetic distances between populations will be estimated using Nei D_A genetic distance as implemented in the program DISPAN [53]. The resulting distance matrix will used to construct a neighbour-joining network in MEGA [54]. Genotype assignment tests, implemented in STRUCTURE [55], will be used to estimate the number of populations. A test to detect recent population bottlenecks (especially important for the remnant populations in the Great Lakes refuges) will be implemented in BOTTLENECK [56].

Statistical Analyses – Mitochondrial DNA sequencing:
Genetic diversity across mitochondrial sequences will similarly examine diversity indices like haplotype frequency, h, and nucleotide diversity, π, among sites using ARLEQUIN (as above [51]), with the relationship between h and π applied to infer demographic histories *sensu* Grant and Bowen [57]. The slower evolutionary change in these genes relative to microsatellites means that the variation assessed is assumed to be haplotypes that migrated into Lake Erie in the recent or distant past. A phylogeographic analysis will therefore follow Borden and Krebs [58] and begin with characterizing haplotype relationships using maximum likelihood (ML) and Bayesian (BY) criteria to identify evolutionary lineages based on substitution models of best fit implemented in MRMODELTEST [59]. ML analyses will be conducted in TREEFINDER [60] to identify significant nodes; two Bayesian analyses will be run in MRBAYES to examine convergence [61]. From these trees haplotype networks will be constructed and contrasted with the geographical distribution of the individuals within various nested clades [62] as a first step in identifying species demography. A second protocol to try is a multipopulation isolation-with-migration (IM) model [63], which, by using a full model of all populations, has the potential to reveal historical gene flow involving ancestral populations.

Most unionid mussels have significant genetic population structure among river drainages (even among Great Lakes drainages [64,65]), with many studies defining or having data suggesting the existence of multiple units for conservation [64,66,67]. Thus, understanding the genetic population structure of listed unionids is of extreme importance for conservation. Conservation geneticists have generally recommended that until genetic studies prove otherwise, conservation managers responsible for unionids should consider populations in separate drainages to be separate conservation units when preparing for augmentations or reintroductions through artificial

propagation and/or relocations. This approach will maintain the greatest amount of genetic diversity in imperiled unionids, and it will prevent the loss of genetic uniqueness, evolutionary potential and local adaptations. The data and analyses we are proposing to provide for Michigan and Ohio rivers and coastal wetlands will be of critical importance for future conservation planning of unionid mussels throughout the region.

Habitat Modeling:
Habitat parameters important for unionid protection from zebra mussels may include those that either inhibit the establishment of stable dreissenid populations, affecting their survival, or/and that allow unionids to burrow and thus remove attached dreissenids from their valves. Among these factors are prevalence of soft substrates [15,16,17,19,68] in large areas of shallow waters (protected bayous) with low flow and warmer temperatures that encourage unionids burrowing [16]; they are hydrologically connected to the lake [18,19]; wave action, water level fluctuations, and ice scouring that inhibit dreissenids [16,18,19]; remoteness from deep water and wind-driven currents delivering dreissenid veligers [10,15]. We will quantify the habitat/niche of mussel beds at both the local and regional scale. At the local scale, the parameters that we will record from areas inhabited by mussels will include: substrate type, depths, water temperature, water velocity and location. With data on environmental parameters, species richness and abundance, and sample location, we will use multivariate methods such as multiscaled ordination with CCA [69] (MSO-CCA) to define local scale habitat. MSO with CCA partitions the explained variance in community composition between environmental factors and geographic location. This is particularly important as unionids are known to be patchily distributed. MSO with CCA accounts for spatial autocorrelation and enables a more clear interpretation of what environmental factors are associated with the community structure of these communities.

At a regional scale, we will use ecological niche modeling to predict the potential presence or absence of mussel beds. The data required for ecological niche modeling depends on the method used, some such as CART (Classification and Regression Trees) require presence and absence data, while some only require presences data (e.g. MAXENT and GARP). We expect that with a high need to first focus on the local scale studies of known mussel beds, we will likely first use techniques that only require presence data. Along with presence data (i.e. location data), these techniques require environmental data that can be defined over regional areas and are amenable to analysis using a GIS. We will aggregate existing GIS data, such as water depth, wind-driven currents, and mean, maximum and minimum annual temperature. We will also developed GIS data layers that may aid in predicting the location of mussel communities, including turbidity (as measured for specific times of the year via satellite data), distance to deep-water, bay area and shape, bottom oxygen (modeled from fish survey data from state and provincial agencies), distance to rivers (which contain potential source populations), and human-related factors, such as distance to nearest dredging operation and distance to dams in upstream rivers, and land use

DELIVERABLES/PRODUCTS:
- We will provide the USFWS and state agencies with annual reports and a final report including delineation of refuge areas, genetic differentiation/characterization and management recommendations (Years 1-3).
- Detailed information on the current distribution, abundance and diversity of unionids populations, including species of greatest conservation need, in lakes Erie, St. Clair and Ontario. The species database with distribution, abundance and habitat data will be provided to USFWS and state environmental agencies to link into the Natural Heritage Databases, making all data readily available for conservation, monitoring and decision making (Year 3).
- A predictive model that can potentially be used to locate additional unionid refuges in the upper Great Lakes, and for refuge management to promote unionid colonization and survival.
- A web-page with results of the project to provide information on the distribution (exact locations masked) and diversity of unionids in the lower Great Lakes for government agencies, academic researchers and general public (Year 3).
- Estimate the threats and develop recommendations on sensitivity to disturbance, monitoring and management options that will aid in developing management or recovery plans for species of greatest conservation concern, and stepped-down watershed management plans. These data will be essential for local management agencies for developing plans for restructuring some of the nearshore to enhance unionid survival including

by reducing the impact of dreissenids (Year 3).
- Students will be trained, providing expertise in this area to the future workforce in the region (Years 1-3).
- The results will also be disseminated to the scientific community in peer-reviewed journals (e.g., *JGLR*) and at international conferences (*e.g.*, IAGLR, NABS, ASLO, etc.) (Year 3+).

SCHEDULE FOR COMPLETION:

This project is ambitious, but as all the participants are experts in the field of unionid conservation, excellent science and meaningful results are ensured, and the collaborative plan will likely spawn numerous additional research projects. Scientific and T&E collection permits are required for this project.

Month/year	Activities/Deliverables
Fall 2010	-Genetic analyses on tissues collected in summer 2010 -Survey nearshore areas during seiche events – collect tissues samples for genetic analysis
December 2010	-2 day organization meeting for collaborators @ Lake Erie Center (University of Toledo). Finalize sampling locations, logistics and standardize results
Winter 2011	-Apply for sampling permits for 2011
May 2011	-Complete genetic analyses on tissues collected in summer 2010 -Preliminary report to USFWS
June – September 2011	-Refuge and tributaries assessment and tissue collections throughout lower Great Lakes
Fall 2011, Winter 2012	-Genetic analyses on tissues collected in summer 2011 -Preliminary modeling of habitat data -Apply for sampling permits for 2012
May 2012	-Complete genetic analyses on tissues collected in summer 2011 -Interim report to USFWS
June – September 2012	-Visit sites predicted as unionid refuges using habitat models. Collect additional tissue samples for genetic analysis
October 2012 - September 2013	-Genetic analyses on tissues collected in summer 2012 -Refine of habitat models
by September 30, 2013	-Final report to USFWS -Preparation of manuscripts for peer-reviewed journals -Disseminate results at scientific conferences

BUDGET JUSTIFICATION:

Applicant Salary Justifications
- Zanatta (pre-tenure faculty), 10-month appointment (teaching release and summer salary). Teaching release (normal teaching load is 9 contact-hours/semester) will give Dr. Zanatta more time for research (field, lab, and writing) and mentoring students.
- Burlakova, soft-money faculty (salary)
- Western Pennsylvania Conservancy - Pennsylvania Natural Heritage Program – Contract-funded staff (salary)
- Michigan Natural Features Inventory – Contract-funded staff (salary)
- Student salaries (summer and academic year research assistantships) are required to have students conduct fieldwork, collect data (field and genetic), construct models, write manuscripts and reports, and be trained for their future roles in conservation research in the Great Lakes region.

Equipment Purchases:
- Thermocycler – CSU ($6500 for purchase). This unit is needed to amplify DNA for the genetic component of this study. Higher than normal laboratory throughput is expected due to the number of samples associated with this project, thus ensuring that DNA amplifications (PCR) is completed in a timely manner.

Conference Travel

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- $2000 for Zanatta and Graduate Student – travel to international conference to present results (maximum allowable under grant guidelines).

Planning Meeting
- $15,000 for planning meeting at Lake Erie Center (University of Toledo): 2 days in December 2010, travel (mileage, lodging, meals) for all collaborators and students (up to 20 people), and use of facility. Will discuss standardization of sampling methods (including training), site selection, background data.

Supplies
- Genetic supplies and services include: test tubes, PCR plates, pippetter tips, DNA primers, Taq polymerase, DNA tissue extraction kits, DNA purification kits, DNA sequencing costs (done at user-pays core-facility, off site), DNA genotyping costs (done at a user-pays core-facility, off-site).
- Field gear includes: waders, wetsuits, masks/snorkels, underwater viewers, Ponar dredges, Eckman dredges, rebar, nylon rope, quadrats, sieves

REQUIRED NON-FEDERAL MATCH:
- Non-federal match = $206,840.75 (35.18% of the total project cost)
- All partner institutions have agreed to a 5% maximum for indirect costs, the unclaimed indirect costs are acting as a significant part of the match ($123,031.52 of total match).
- Additional match:
  o Teaching release (salary and benefits equivalents) to: Zanatta from CMU (College of Science and Tech. – 1 term, Office of Research and Sponsored Programs – 1 term), Krebs from CSU (1 credit/year).
  o In-kind salary and benefits (for time on project): Krebs from CSU, Burlakova and Karateyev from BSC.
  o Graduate assistant salary and tuition (2010-2012) from KSU.
  o In-kind use of Zanatta lab equipment (provided by CMU); vehicles, boats (provided by CMU and WPC-PHNP).
  o Field work cost-share from BSC.

COSTS: Funds Requested: $381,167.70   **Non-Federal Partner Match: $206,840.75**
Total Project Cost: $588,008.45   **Percent Non-Federal Partner Match: 35.18%**
(institutional indirects <u>do not</u> exceed 5%)

Project costs and Value:
The costs for this project include student salary and stipends, salary for non-tenured faculty and non-permanent staff, a planning meeting, fieldwork costs, limited conference travel to disseminate results, and lab costs (genetic data, geospatial data). These costs are reasonable when compared to costs at larger institutions and are *outstanding* when compared to the costs for an environmental consultant. The reduction of indirects to 5% (e.g., from 41.5% at CMU) for this project makes it of *exceptional value* for a research project administered through an academic institution.

PAST GLFWRA FUNDED PROJECTS:
None

IDENTIFY OTHER FUNDING SOURCES
- DNEQ-Coastal Zone Management grant to **Zanatta**, $15,000 (NOAA funding administered by Michigan DNRE, 2010-2011). *Conservation of Freshwater Mussels in the St. Clair River Delta*. Acts as a small-scale test for larger scale projects throughout basin (current USFWS-GLFWRA proposal). A graduate student will start on this project in summer 2010.
- Research Excellence Fund to **Zanatta**, $60,000 (CMU – internal grant, 2009-2011). *Towards the Recovery of Michigan's Aquatic Biodiversity: Understanding Freshwater Mussel Population Structure Using Landscape Genetics*.
- Lake Erie Protection Fund to **Krebs** $15,000 (State of Ohio). *Protecting mussel habitat on Lake Erie's coast*. Predominantly for Summer 2010, to look for mussel populations along Lake Erie that are not in currently protected lands.

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