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POPULATION GENETICS STRUCTURE AND MORPHOMETRIC ANALYSES OF ROUND GOBY *NEOGOBIUS MELANOSTOMUS* COLLECTIONS FROM LAKE ERIE, PRESQUE ISLE BAY, AND THREE ERIE COUNTY, PENNSYLVANIA, STREAMS

A Thesis in

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by

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ABSTRACT

The Laurentian Great Lakes represent one of the world's most invaded freshwater systems primarily due to decades of transatlantic ships purging ballast tanks containing exotic flora and fauna. The Round Goby, *Neogobius melanostomus,* a benthic, Eurasian fish native to the Ponto-Caspian region of the Black and Caspian seas, was first reported in North America in the St. Claire River of Michigan in 1990 and made its way to the eastern basin of Lake Erie by the mid-1990s. The present-day distribution of Round Goby includes all five Great Lakes and many of their tributaries. The tributary invasion success of Round Goby raises the question whether these fish are exhibiting site fidelity for these systems solely for spawning purposes or spending their entire life cycle within the same stream. If the latter is true, Round Goby may be exhibiting evolutionary adaptability to localized environments and functioning as discrete populations. Studying patterns of genetic variability of Round Goby may aid in predicting future invasion success. Identifying post-invasion dispersal of Round Goby can prove to be an important management tool for predicting range expansion capabilities; moreover knowing their population genetic structure, thus promoting a better understanding of evolutionary change and mechanisms of species adaptation. The purpose of this study was to determine if lake and tributary collections of Round Gobies are distinct by comparing tissue and whole specimen samples genetically and morphologically, respectively. Using tissue samples collected from 335 individual Round Gobies obtained from 12 interspersed sample locations (tributaries [n=3], Presque Isle Bay [n=3], offshore trawls [n=4], and tributary embayments [n=3]), an initial suite of 21 novel microsatellites were developed to enable detailed population genetic analyses. Moreover, these microsatellite markers complement the limited suite of existing microsatellites and will aid in determining source locations (founder effect) for future collections of Round Goby from new invasions and/or introduction. Here I report on variation at 12 microsatellite DNA markers for

314 Round Gobies (originally 335 specimens; 21 were later removed due to extraction error and various constraints). Levels of genetic diversity were low in all collections (with 2 to 10 alleles per locus), and heterozygosity ranged from H_e=0.628 to H_e=0.703. Overall tributary collections were no more diverse than Lake Erie and Presque Isle Bay populations, genetically. Tests of population differentiation among all collections (overall F_{ST}=0.036) suggest a low level of genetic differentiation and an overall panmictic population. This result was supported by Bayesian clustering analyses in STRUCTURE, which suggested K = 1 cluster or populations.

In addition, morphometric and meristic analysis were conducted on a subsample of Round Gobies (n=90) collected for genetic assays. Principle component analysis (PCA) and analysis of variance (ANOVA) were performed in order to determine whether unique morphotypes exist according to habitat occupancy. While ANOVA results suggest statistically significant phenotypic differentiation ($p<0.05$), these data are functions of phenotypic plasticity seen through habitat occupancy and available food source. These results implicitly support genetic analysis results as to the presence of one large panmictic, interbreeding population of Round Goby in and around the Presque Isle, Pennsylvania portion of Lake Erie and tributaries.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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CHAPTER 1

Biology of Round Goby in native and invasive environments

The Round Goby

The Round Goby, *Neogobius melanostomus* (Pallas, 1814) has been a species of interest for both biologists and ecologists in North America and portions of Europe for the past two decades. *N. melanostomus* is a small (<290 mm) benthic dwelling euryhaline fish native to Eurasian waterways of the Azov, Black and Caspian seas. Within the aforementioned seas, Round Gobies occupy the following rivers: The Don River, draining into the Azov; the Danube, Dniester, and Dnieper Rivers draining into the Black Sea; and the Volga River, which flows into the Caspian Sea (Brown and Stepien, 2009).

Within the Black and Caspian seas, the Round Goby is one of the most prolific near-shore benthic fish and currently holds the same distinction in Lake Erie (Brown and Stepien, 2008). Round Goby have become aquatic invaders in areas greatly removed from their native range such as all five Great Lakes in North America. In North America, *N. melanostomus* have displaced native fishes (Jansen and Jude 2001; Lauer 2004) as well as caused shifts in stream macroinvertebrate abundance (Krakowiak and Pennuto, 2008). In areas where they have been introduced such as Lake Erie, managers are concerned with the detrimental effects invasive Round Gobies have with native species (Grant et al. 2012).

In the United States, the Round Goby has been a competitor for food resources and site occupancy of native fishes (Krakowiak and Pennuto, 2008). Additionally, the few predators the Round Goby has encountered, such as Smallmouth Bass *Micropterus dolomieu* and Lake Trout *Salvelinus namaycush*, have failed to slow their expansion (Jansen and Jude 2001). A similar gobiid, the Tubenose Goby, *Proterorhinus semilunaris*, (Heckel, 1837) has also been observed

and reported in Lake Erie and Erie County Pennsylvania's Presque Isle Bay (Grant et al., 2013), but has yet to claim as much territory as the Round Goby.

Gobiidae

Gobiid fishes represent the most species-rich family of fishes in the North-Eastern Atlantic Ocean, Mediterranean and Black seas (Kovacic and Patzner, 2011). To date, 93-species of gobioids have been described in this region representing 39-genera; with five listed as exotic and 34 native (Kovacic and Patzner, pg. 182, 2011). *Neogobius melanostomus* and *P. semilunaris* have experienced the greatest expansion outside their native range in the recent decade. Invasion success can be attributed to its ability to spawn several times within a season, aggressive behavior and cavity nesting (Phillips et al. 2003). Depending on the size of the female, water temperature, and photoperiod, Round Goby can spawn every 20-days, up to six-times per year and produce 100-5,000 eggs per female (Jude 1997, Corkum et al. 1998, Phillips et al. 2003). Both species were introduced to North America through the trans-Atlantic shipping trade, whereas in Europe, the construction of canals for shipping vectored these benthic fishes into river systems previously uninhabited by gobies. Neilson and Stepien (2011:689) aptly summarized Wilson *et al.* (2009) by stating the above-listed invasion mechanisms resulted from 'jump dispersal' and 'corridor expansion' "with each (species) having considerably different ecological and evolutionary trajectories."

Round Goby became a species of intense study during the past decade, with the advancement in Next Gen molecular sequencing tools. These technologies have allowed scientists to quickly determine point of origin and dispersal techniques from tissue collected from individual specimens. New taxonomic classifications have also been made for certain species through Next Gen sequencing (Neilson and Stepien, 2009), although acceptance in the scientific community has not always been receptive (Sorokin et al, 2011).

Invasive Range

The Round Goby is the most widespread and successful nonindigenous gobiid in the World (Brown and Stepien, 2008). Its success lies in its adaptability to new environments and wide-ranging dietetic plasticity. In the Ponto-Caspian region, the Round Goby has expanded its range into the North and Baltic sea basins via artificial waterways (Freyhof, 2011). Introductions in North America were the result of ballast water purging from trans-Atlantic shipping freighters. *Neogobius melanostomus* invasion was first reported on the continent in 1990 at the St. Clair River, Michigan (Jude et al. 1992). Within the next decade, *N. melanostomus* spread to all five Laurentian Great Lakes and has been considered a species of high concern for several state and federal agencies whose jurisdictions adjoin river systems draining or feeding the Great Lakes. Poulos et al. (2012) predicted that Round Goby might become an invader in the Illinois and Missouri rivers, as well as the Connecticut River in New England. Kornis et al. (2012) noted that inland spread of *N. melanostomus* from the Great Lakes has included not only tributaries, but marsh and estuary habitats as well.

As with other species of gobiids, *N. melanostomus* individuals typically move little geographically during their lives except for seasonal offshore migrations (Brown and Stepien, 2008) or larval dispersal (Kocovsky et al. 2011). In more localized events, *N. melanostomus* have been transferred between waterways by bait-bucket introductions which likely explains their presence in streams such as French and LeBoeuf creeks, both flowing through portions of Erie County, PA, neither of which is a tributary of Lake Erie.

Round Goby expansion and invasion of the Great Lakes

The Laurentian Great Lakes represent one of the world's most invaded freshwater systems (Jude et al. 1992; Corkum et al. 2004; Brown and Stepien 2009). The Round Goby was

first reported in Lake Erie in 1995 (Clapp et al 2001). Stauffer (pers. comm. 2013) noted that the Pennsylvania Fish and Boat Commission first reported Round Goby in the Pennsylvania waters of Lake Erie (eastern basin) during a trawl in 1996 (C. Murray, pers. comm.). Since then, *N. melanostomus* has spread to all five Great Lakes and many of their tributaries. In the Pennsylvania portion of Lake Erie, Round Goby are considered the dominant benthic fish of some tributary streams (Stauffer 2012). Krakowiak and Pennuto (2008) compared New York tributaries of Lake Erie containing Round Goby with neighboring tributaries without Round Goby. Their findings indicated native darter populations had been extirpated in those tributaries invaded by Round Goby due to competition for resources (Krakowiak and Pennuto 2008).

Identifying post-invasion dispersal of Round Gobies has proven to be an important management tool for predicting range expansion (LaRue et. al, 2011). While Round Goby have become more abundant in lake habitats, there has been an expansion of their populations into tributary streams and rivers (Krakowiak and Pennuto, 2008), prompting several US States not adjoining the Great Lakes (e.g., Tennessee) to list the Round Goby as a potential aquatic invader. Irons et al. (2006) noted that from 2003-2004, some individual Round Goby traveled 48-192 km in the Illinois Waterway south of Lake Michigan; suggesting Round Goby have the capacity to invade tributary systems at a great spatial distance given no significant barriers. Additionally, total length (TL) differences have been reported between age-0 Round Goby populations in Elk Creek, Walnut Creek, and Twenty Mile Creek (all located in Erie County, PA) at the same time of year (Stauffer, pers. comm. 2013) suggesting Round Goby may be exhibiting evolutionary or phenotypic plastic adaptability to their non-native environment. Allendorf and Luikart (2007:496) noted "Invasive species can undergo rapid adaptive evolution during the process of range expansion." Stepien and Tumeo (2004) added that studies applied to the patterns of genetic variability of Round Goby aid in predicting invasive success.

Habitat Preference

The Round Goby likely owes its success as an invader to its ability to occupy a wide range of habitats. *Neogobius melanostomus* has been found in the same macrophyte dominated habitats as *P. semilunaris*, but does not appear to be relegated to those near-shore littoral zones. In fact, Poulos et al. (2012) noted that the Round Goby, like other non-native invaders to the Great Lakes, has the ability to rapidly and repeatedly adapt to newly colonized environments. I have also observed Round Goby occupying cobble/slate rock substrates in small streams (<1m depth) and are commonly found in riffle/run habitats. In the Trent-Severn Waterway, Brownscombe and Fox (2012) noted that Round Gobies exhibited greater habitat selectivity for rocky substrates at range edges than in the longer established area. I have observed biologists with the Pennsylvania Fish and Boat Commission capture Round Gobies in offshore locations of Lake Erie from benthic trawls at depths ranging from 10-20m. Conversely, I have seined Round Gobies at depths less than 0.5 m, thus displaying that while they are benthic, their depth ranges are quite variable.

Diet

The diet of the Round Goby is broad. Brander et al. (2013:2064) characterized the Round Goby as "A predacious omnivore with high dietary overlap and generalistic feeding strategies." *Neogobius melanostomus* appears to be an opportunistic feeder. In fact, while they feed primarily on dreissenids in their native range, they have been observed to cause a negative shift in macroinvertebrate abundance in Lake Eire tributaries (New York Portion) within the past decade (Krakowiak and Pennuto, 2008). Seasonality plays a role in feeding of *N. melanostomus* in the Danube River system according to Brander et al. (2013) who noted that in early summer, chironomids comprised 33-percent of the diet while in late summer it only comprised 5-percent. Amphipods, however, comprised more than 70% of their diet in the Danube River (Brander et al.

2013). Two food sources on which Round Goby predate in their native habitat are also invasive in Lake Erie are the Zebra *Dreissena polymorpha* and Quagga *Dreissena rostriforis bugensis* mussels.

Research has indicated the presence of gobiids in their non-native ranges in North America and parts of Europe as originating from shipping and construction of canals to connect naturally separated river systems. The Round Goby has obviously held sway over conspecifics that have not been as widely distributed or adaptable to changing habitats. Tubenose Goby, while less successful in occupancy, has established residency in North America and portions of Europe previously unoccupied (Brown and Stepien, 2008). In small tributaries, it may be possible to block immigration of both species with lowhead dams and/or the use of piscicides such as Antimycin-A (Kulp and Moore, 2008).

In the past several decades, there have been increasing rates of introductions of non-native species throughout the world as international travel of humans has become more commonplace (Baskin 1998; Gilg, et al. 2012). Introduced species have caused both environmental (Williamson 1996;) and economic stress (Pimentel et al. 2000; Colautti et al. 2005; Gilg et al. 2012) and invasive species can outcompete or extirpate native species from their respective habitat (Janssen and Jude 2001; Lauer et al. 2004; Kornis et al. 2013). Krakowiak and Pennuto (2008) observed the threat Round Goby pose to native Smallmouth Bass, an environmentally and economically important game fish that spawns in Lake Erie tributaries as well as the lake itself. The Round Goby has also been linked to decreased spawning success in Lake Trout (*Salvelinus* namaycush) (Dufour et al. 2007, Chotkowski & Marsden 1999). As previously noted, the Round Goby has caused extirpations of native North American benthic fishes (Jansen and Jude 2001; Lauer 2004) and declines in macroinvertebrate abundance of tributaries (Balshine et al. 2005; Lederer et al. 2008). Native Mottled Sculpin (*Cottus bairdi*) and Johnny Darter (*Etheostoma nigrum*) populations have declined in areas of Lake Michigan since

Round Goby were first discovered in the late 1990s (Lauer 2004; Krakowiak and Pennuto 2008). Similarly, Phillips et al. (2003) found Mottled Sculpin were absent from or found in low densities in Elk Creek, Walnut Creek and Twenty Mile Creek (all located in Erie County, PA) post Round Goby invasion. Darter species such as Rainbow Darter, *Etheostoma caeruleum*, Fantail Darter, *Etheostoma flabellare*, and Logperch, *Percina caprodes*, all of which are native to Erie County tributaries, and have experienced adverse diet competition when food sources are limited in the presence of *N. melanostomus* (Carman et al. 2006, Abbett et al. 2013).

While the potential for impact on native ichthyofauna in Round Goby invaded tributaries is large, this impact may not be immediately detected (Poos et al. 2010; Kornis et al. 2013). From a public perspective, the Round Goby may not appear to be as destructive as more highly publicized invasive fish despite being one of the most abundant nearshore fishes in the lower Great Lakes with an estimated 90 individuals/m^3 (Ray and Corkum 2001; Jonson et al. 2005; Brown and Stepien 2009). Krakowiak and Pennuto (2008) noted reduced macroinvertebrate diversity in four nearshore tributaries of Lake Erie (New York portion) compared with four streams where gobies were absent (Kornis et al. 2013). These findings suggest a reduction in available diet to native fishes. Invasive dreissenid establishment preceded Round Goby invasions, which likely aided the adaptability of the Round Goby in the Great Lakes. The continued expansion of Round Goby inland both from natural dispersion and possible bait-bucket transfers, may impact the suitability of these tributaries as spawning and nursery habitat for native species.

The ability of Round Goby to adapt to a variety of habitats and environmental conditions poses threats to the biota of tributary systems and inland lakes (Krakowiak and Pennuto 2008). Moreover, Krakowiak and Pennuto (2008) also believed further knowledge, not just presence and/or inventory and monitoring data, is needed to better understand their potential impacts on Eastern Lake Erie tributaries. Genetic methods have allowed researchers to characterize mechanisms of dispersal during colonization which has led to an increase in studies reporting

stratified dispersal as a mechanism facilitating secondary range expansion and adaptation/speciation (Colautti et al. 2005; Parisod and Bonvin 2008; Darling and Folina-Rorem 2009; Bronnenhuber et al. 2011).

The genetic diversity of populations can respond to environmental heterogeneity via alterations in the relative strengths of the four opposing genetic forces: mutation, migration, selection, and genetic drift (Bagely et al. 2002). The resolution and sensitivity of measurements of genetic diversity have steadily increased with advances in molecular marker technologies. Measures of gene flow help identify evolutionary connectivity of populations and effective population size. Populations that have low connectivity with others have the potential to become genetically differentiated and unique (Bagely et al. 2002). While Round Gobies are invasive and pose threats to native biodiversity, they represent valuable natural experiments in species colonization and range expansion (Dufour et al. 2007). Furthermore, the use of microsatellite markers can be a powerful tool to provide insight into population structure and dispersal in tributaries (Dufour et al. 2007).

Thesis Purpose and Objectives

In the absence of empirical information, it was assumed that Round Goby inhabiting the eastern portion of Lake Erie exist as a metapopulation(s) consisting of subpopulations inhabiting each tributary connected by migration from neighboring streams or from the lake-resident population. If a control strategy were ever developed to mitigate or predict further spread of *N. melanostomus* it would require the characterization of the associated migration, colonization, and extinction processes among emerging populations. No detailed genetic information existed however on population structure, levels of effective movement, or relatedness among lake resident and tributary populations of the project study area.

The purpose of this study was to determine whether lake and tributary populations of Round Goby were morphologically and genetically differentiated. A second purpose of the study

was to develop additional genetic markers for Round Goby. Population genetic studies and genotyping of Round Goby promote a better understanding of evolutionary change and mechanisms of species adaptation (Salmenkova 2008). Additionally, by developing genetic markers for Round Goby collected from the above-listed tributaries and lake habitats versus a limited number of existing published primers (e.g., Dufour et al., 2007), greater levels of specificity per tributary and individual may possibly be established.

The objectives of this study were to 1) determine whether Round Goby within the collection region have similar morphology; 2) develop additional microsatellite DNA markers from massively parallel sequencing data; 3) utilize unique microsatellite DNA markers to determine if Round Goby in the Lake Erie drainage have established unique, detectable, reproductively isolated populations in the tributaries, Presque Isle Bay, and the open waters of Lake Erie that can be delineated with morphological and genetic data; and 4) provide additional genomic sequences of the Round Goby, which will be needed to produce specific primer sequences that may be used in future developments of environmental (e) DNA kits for rapid detection of this invasive species and serve as a model kit that can be developed for additional species.

CHAPTER 2

Study Area and Collection Methods for Round Goby specimens collected from Lake, Bay, and Tributary Habitats of Erie County, Pennsylvania

INTRODUCTION

The first reports of Round Goby in the eastern basin of Lake Erie were made by the Pennsylvania Fish and Boat Commission (PAFBC) in 1996 (C. Murray, pers. comm.). The non-native goby is currently found in all five of the Great Lakes where it has been documented displacing native benthic fishes (Jansen and Jude 2001). Invasion success can be attributed to its ability to spawn several times within a season, aggressive behavior and cavity nesting (Phillips et al. 2003). Depending on the size of the female, water temperature, and photoperiod, Round Goby can spawn every 20-days, up to six-times per year and produce 100 to 5,000 eggs per female (Jude 1997, Corkum et al. 1998, Phillips et al. 2003).

The objective of this portion of the study was to determine whether various collections of lake, bay and tributary populations of Round Goby were morphologically distinguishable. Morphometric differences between neighboring populations of fish (e.g., African cichlids in the genus *Metriaclima*) have been used to determine whether sufficient differences in morphology existed (Stauffer et al. 2013).

METHODS

Round Goby collections

All Round Goby collections were made in compliance with the PAFBC using a No. 736 Type I Scientific Collector's Permit issued to Sidney C. Abramson, PA Fishing License #032184517 for Calendar Years 2013-2014. This permit was obtained through the PAFBC Bureau of Fisheries - Environmental Services Division - Natural Diversity Section, 450 Robinson Lane, Bellefonte, PA, 16823. Additionally, all collections were made upholding the standards set

forth by the Institutional Animal Care and Use Committees (IACUC#44331) approval guidelines, which were completed through the Pennsylvania State University in April, 2013.

Study Area

In years 2013 and 2014, Round Goby (n=335) were collected from 15 locations in Erie County, Pennsylvania, and the Pennsylvania waters of the eastern basin of Lake Erie. Starting May 6, 2013, Round Goby were collected from Misery Bay, an embayment within Presque Isle State Park (n=36); May 7, 2013, the Twenty Mile Creek embayment of Lake Eire (n=7) as well as Twenty Mile Creek proper (n=30); May 8, 2013, Elk Creek Embayment of Lake Erie (n=11) in addition to Elk Creek proper (n=40); Walnut Creek Embayment of Lake Erie (n=15) and Walnut Creek proper (n=1). Offshore trawls of Lake Erie were made on July 23, 2013 and yielded four (n=4) Round Goby. On July 24, 2013, eleven (n=11) Round Goby were collected from the pier at the Port of Erie Terminal, the causeway between Lake Erie and the inlet of Presque Isle Bay; fifteen (n=15) Round Goby were collected from Marina Lake; on July 25, 2013, thirty-one (n=31) Round Goby were collected from Elk Creek proper. Offshore trawling of Lake Erie on October 28, 2013, produced twenty-five (n=25) Round Goby and forty-nine (n=49) Round Goby on October 29, 2013 (See Table 2-1 for waypoint locations).

Two Round Goby collections were made May 12, 2014, with thirty (n=30) specimens taken from Marina Lake (Presque Isle Bay State Park) plus an additional thirty (n=30) taken from the Elk Creek embayment of Lake Erie.

Table 2-1. The following list displays Round Goby *Neogobius melanostomus* collections from May 2013 to May 2014 through the Pennsylvania State University, Pennsylvania Fish and Boat Commission, and the United States Geological Survey.

2013 Collections

Sample	State	Site Name	Samples Collected	Date Collected	Location
CA13_001-CA13_036	PA	Misery Bay	36	5/6/13	N 42.09620, W 080.05666
CA13_037-CA13_043	PA	Twenty Mile Creek Embayment	7	5/7/13	N 42.26030, W 079.78141
CA13_044-CA13_073	PA	Twenty Mile Creek Seining	30	5/7/13	N 42.23982, W 079.77242
CA13_074-CA13_113	PA	Elk Creek Seining	40	5/8/13	N 42.02006, W 080.36809
CA13_114-CA13_124	PA	Elk Creek Embayment	11	5/8/13	N 42.02095, W 080.36809
CA13_125	PA	Walnut Creek Seining	1	5/8/13	N 42.07531, W 080.23811
CA13_126-CA13_140	PA	Walnut Creek Embayment	15	5/8/13	N 42.07531, W 080.23811
CA13_291-CA13_301	PA	Port of Erie Terminal	11	7/24/13	N 42.092412, W 080.04141
CA13_302-CA13_332	PA	Elk Creek Seining	31	7/25/13	N, 42.02006 W 080.36809
CA13_341-CA13_355	PA	Marina Lake Seining	15	7/24/13	N 42.15377, W 080.11347
CA13_141-CA13_144	PA	Lake Erie Trawl (Offshore)	4	7/23/13	Multiple locations, Table 2-2
CA13_145-CA13_169	PA	Lake Erie Trawl (Offshore)	25	10/28/13	Multiple locations, Table 2-2
CA13_170-CA13_218	PA	Lake Erie Trawl (Offshore)	49	10/29/13	Multiple locations, Table 2-2

2014 Collections

Sample	State	Site Name	Samples Collected	Date Collected	Location
CA14_001-CA14_030	PA	Marina Lake Seining	30	5/12/14	N 42.15377, W 080.11347
CA14_031-CA14_060	PA	Elk Creek Embayment	30	5/12/14	N 42.02095, W 080.36809

Figure **2-1**: Overview of the Round Goby collection sites in Erie County, Pennsylvania.

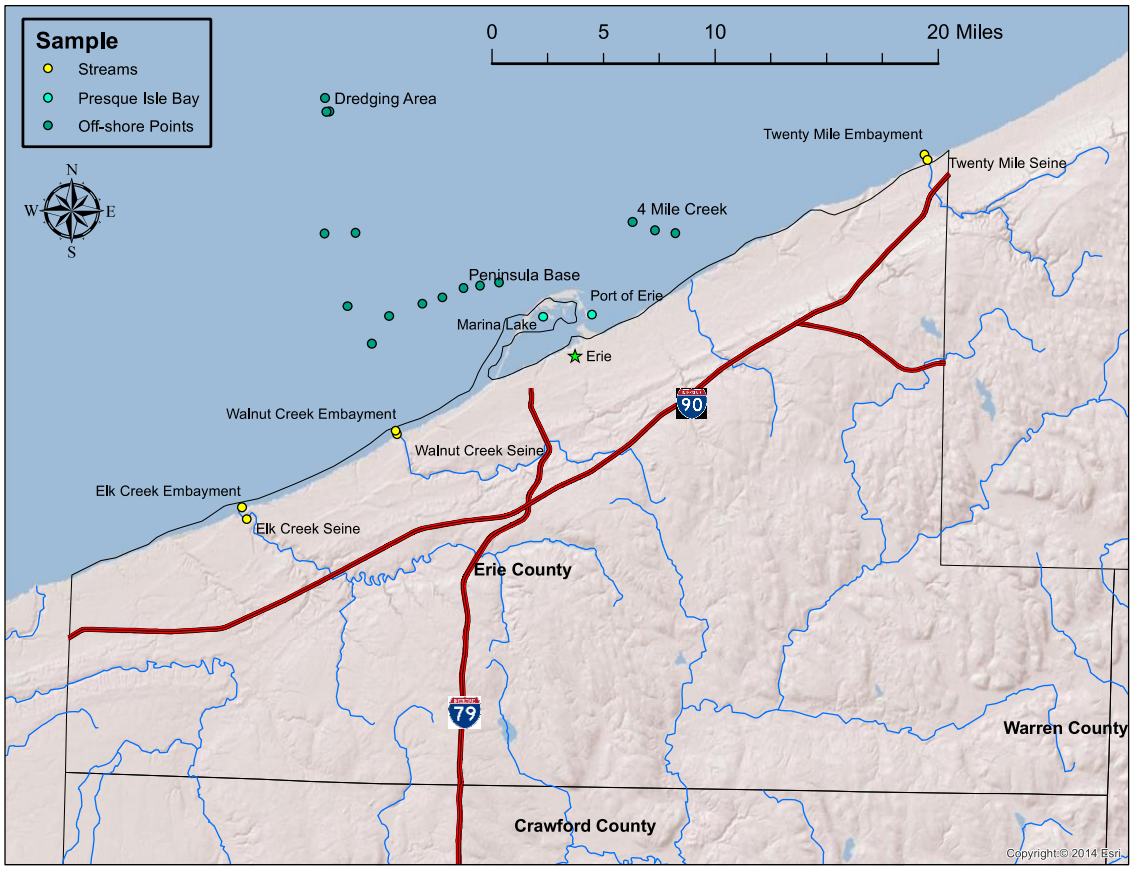


Figure**2-1:** Three stream collection sites, Presque Isle Bay and three offshore locations are listed in the above map. Collections were made in 2013 and 2014. The three offshore site names, provided by the Pennsylvania Fish and Boat Commission, represented multiple trawls at those locales.

Figure **2-2**: Overview of Twenty Mile Creek and Embayment in Erie County, Pennsylvania.

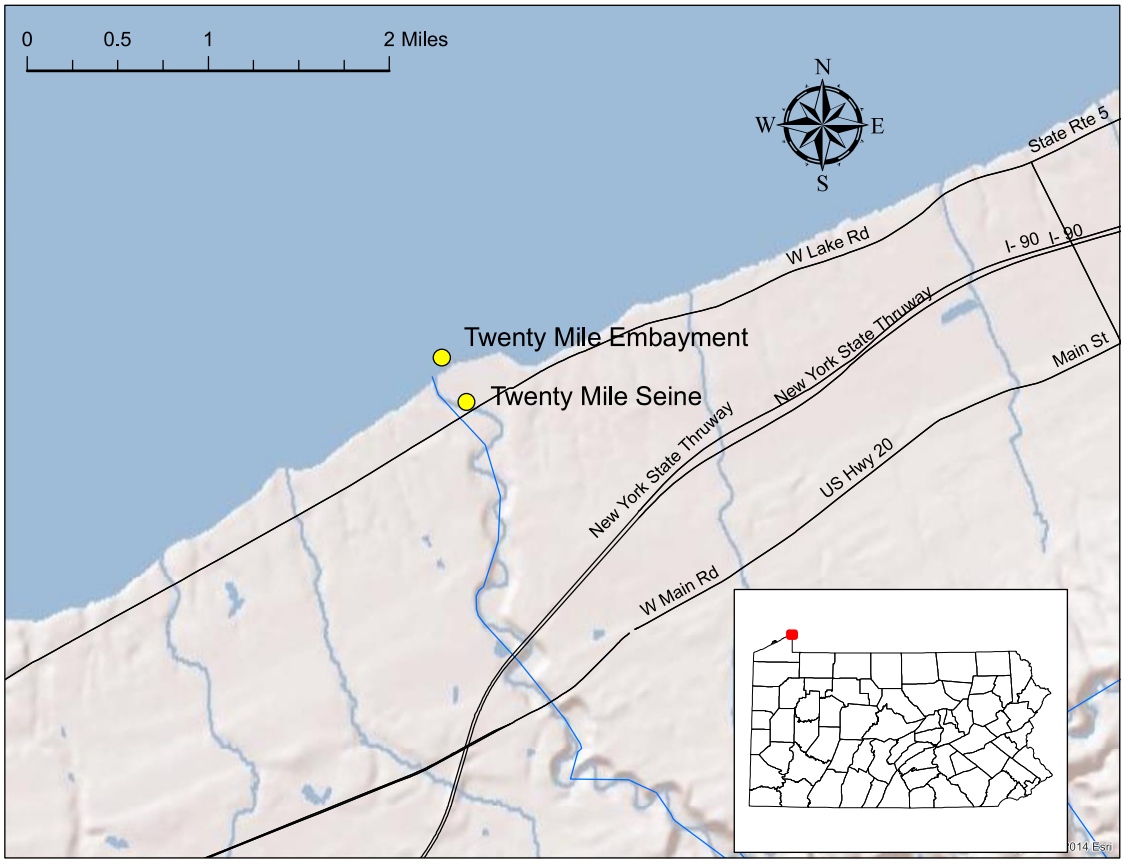


Figure **2-2**: Thirty-seven specimens of Round Goby were collected from Twenty Mile Creek embayment and stream in May, 2014. The embayment collection proved difficult using the PSU johnboat due to very shallow (<1m) and rocky substrates.

Figure **2-3**: Overview of Walnut Creek and Embayment in Erie County, Pennsylvania.

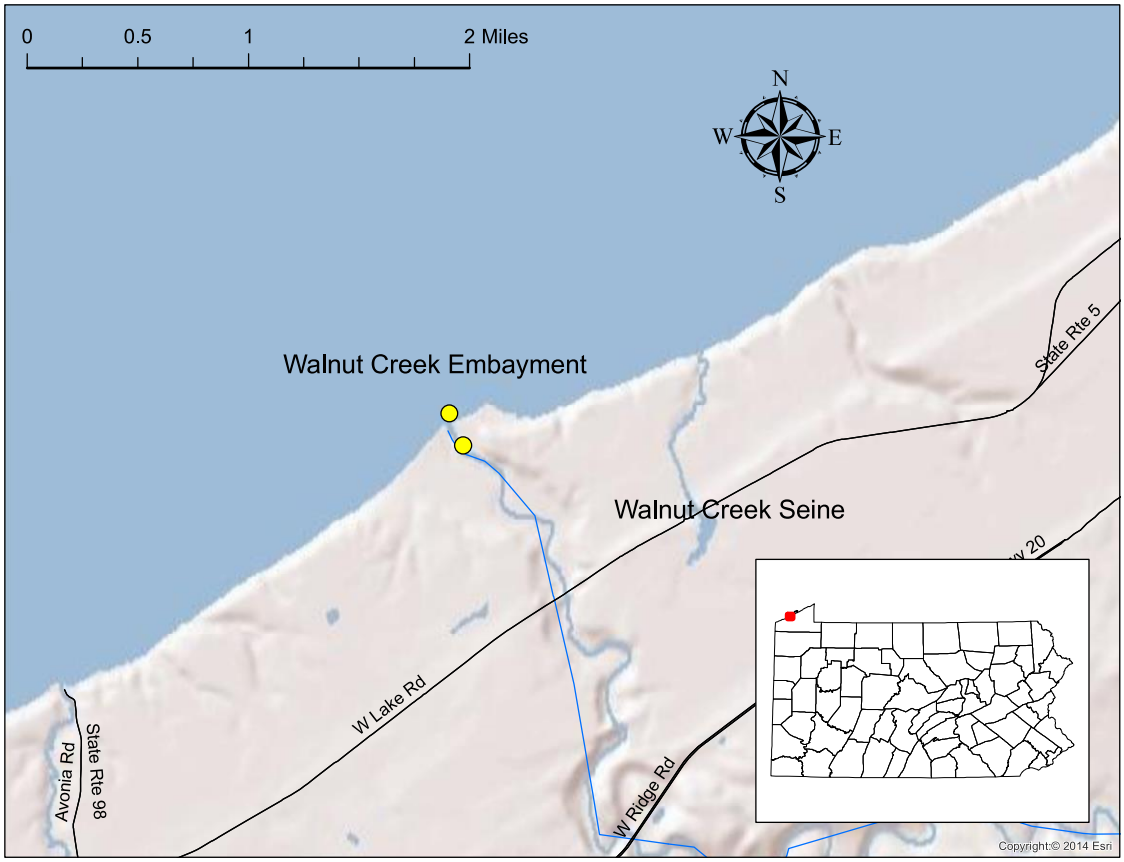


Figure **2-3**: In May 2013, fifteen Round Goby were collected from Walnut Creek embayment and one specimen from Elk Creek proper. Extensive kick seining of the stream substrate yielded only one-specimen.

Figure **2-4**: Overview of Elk Creek and Elk Creek Embayment, Erie County, Pennsylvania.

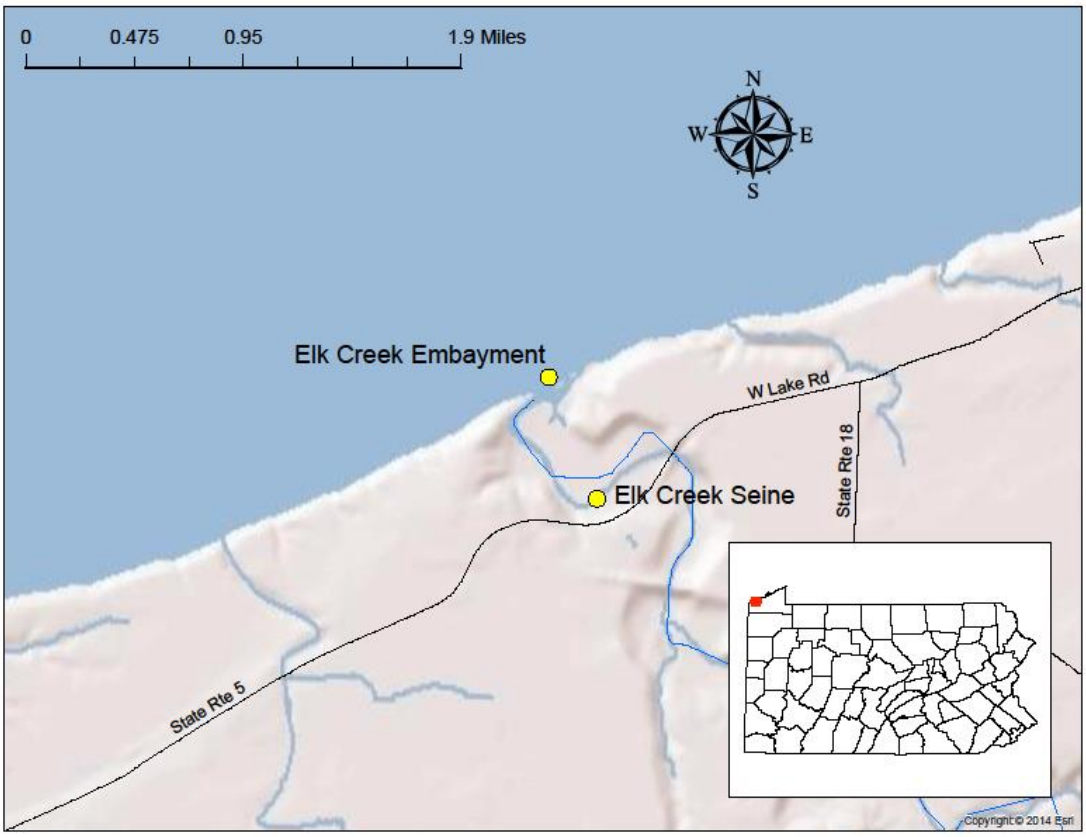


Figure **2-4**: Elk Creek provided the largest collection of Round Goby in 2013 and 2014. In May 2013, forty specimens were collected via kick seining while 11 were collected from the benthic trawl. In 2014, thirty specimens were trawled from the embayment.

Figure **2-5**: Overview of Presque Isle Bay, Erie County, Pennsylvania.

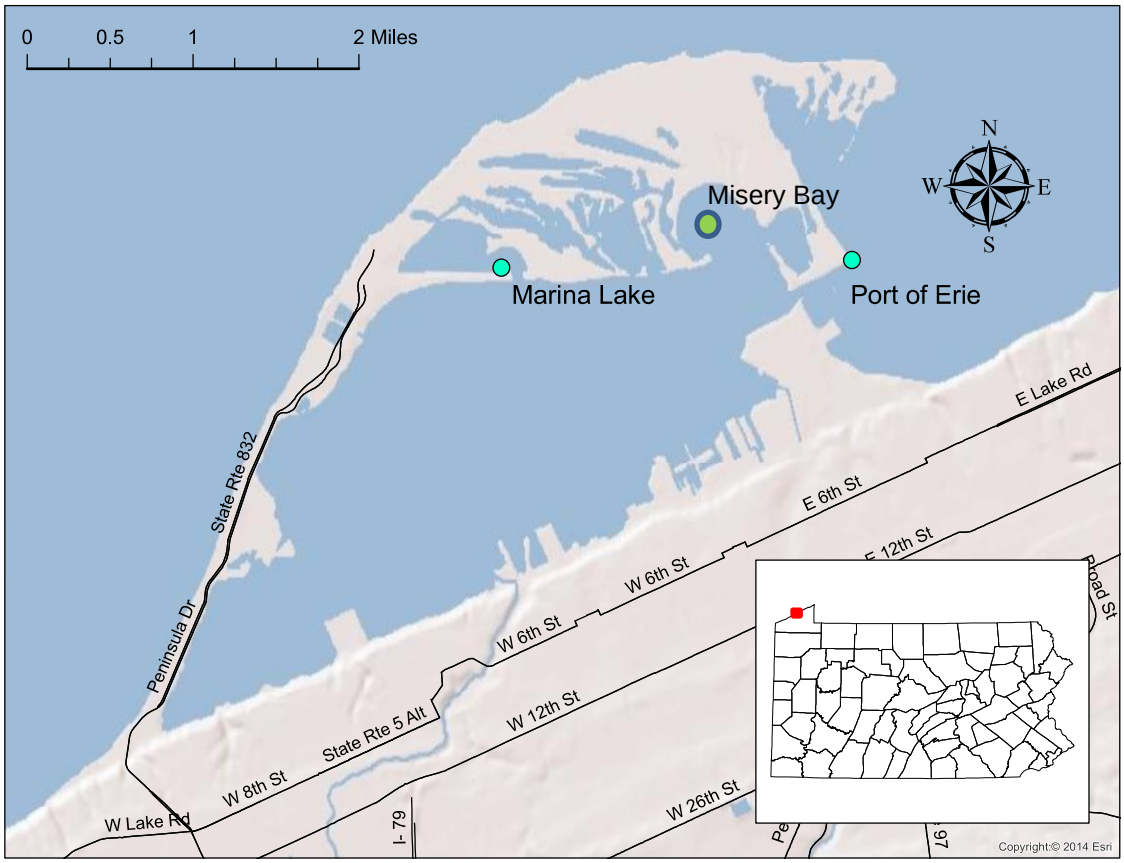


Figure **2-5**: Round Goby collections from Presque Isle Bay were taken from Misery Bay, the Port of Erie Terminal, and Marina Lake in 2013 and 2014. Thirsty-six specimens were collected from Misery Bay, 11 were caught using hook and line techniques from the Port of Erie Terminal, and 45-specimens were seined from Marina Lake.

Specimen Collection Methods

I accompanied the Pennsylvania Fish and Boat Commission (PAFBC) while they performed offshore benthic otter trawls in the Pennsylvania waters of Lake Erie on 23 July, 28 October, and 29 October, 2013. Trawls were conducted from the vessel, *PERCA*, which has been

in service for the commission since 1959 and served as the primary offshore collection vessel for the PAFBC in Erie County, PA. Dimensions for the trawl were as follows: headrope length, 9.93m; footrope length, 13.13m; and sidelines, 1.34m. Trawls were conducted at 10-minute intervals before retrieval and inspection (See Table 2-2). Trawls were not conducted for *N. melanostomus*, specifically, but Round Goby were collected as bycatch during an abundance assessment for Yellow Perch, *Perca flavescens*. This provided a comparison of the fish residing in Lake Erie versus those captured in Presque Isle Bay, Twenty Mile, Walnut, and Elk creeks.

Collections of *N. melanostomus* from Elk, Walnut, and Twenty Mile creeks' embayments were made using the Penn State University benthic electrified trawl (a modified Missouri trawl) using the methods described by Freedman et al. (2009). The PSU boat used was a 5.3m johnboat powered by a 25-hp outboard motor, while the PSU electrified trawl was powered by a Honda 3500-W generator (Freedman et al. 2009). Trawl times varied from 3 to 5-minutes per location.

Shoreline seines and tributary kick seine collections for *N. melanostomus* were conducted in May and July 2013 as well as May 2014. A 15-second kick technique was used to drive benthic fish toward a 3m seine for capturing *N. melanostomus* from riffle/cobble substrates at depths <1m until as many specimens as possible of the targeted species were collected per site. Twenty Mile Creek, Elk Creek and Walnut Creek were each seined at distances > 100-150m above their respective Lake Erie embayment. A 10m seine was used for shoreline collections in Marina Lake, which were taken by having one individual close to shore acting as a 'pivot' while the individual on the other end of the seine would swing 180-degrees until back in parallel line formation with both the shoreline and 'pivot' individual. When both individuals were parallel with the shoreline, additional students would kick from the shoreline toward the net while both net handlers seined shoreward. The 'kickers' then grabbed the base of the shoreline seine, pulled it ashore, and beached the contents. Maximum shoreline seining depth was no greater than 1m.

Table 2-2. The table lists offshore benthic trawling locations performed by the Pennsylvania Fish and Boat Commission in 2013 using the PERCA trawling vessel. Depth and speed are listed in meters and nautical knots, respectively.

Date	Depth	Speed	Start Lat/Lon		Finish Lat/Lon		Time Start	Time Finish	Course
7/23/13	15.2/14.6	3.0	42.28355	-80.30454	42.29332	-80.30795	10:21	10:31	NW
	15.5/15.8	2.9	42.29229	-80.30878	42.28537	-80.30582	10:54	11:04	S
	16.1/16.7	2.6	42.28325	-80.30714	42.28427	-80.31580	11:20	11:31	W
	20.7/20.4	2.9	42.20416	-80.30599	42.20495	-80.29696	12:27	12:37	ExS
	19.8/18.3	2.9	42.20522	-80.27887	42.20569	-80.26991	12:56	13:06	ExS
10/28/13	17.4/17.3	2.9	42.21666	-80.03744	42.21648	-80.02878	11:40	11:50	E
	16.6/15.8	2.9	42.21165	-80.01754	42.20942	-80.00878	12:13	12:23	ExS
	16.1/15.2	2.9	42.21003	-79.99970	42.21277	-79.99187	12:42	12:52	ExN
10/29/13	15.1/16.0	2.9	42.17119	-80.18331	42.17036	-80.19272	10:10	10:20	W
	15.7/14.9	3.0	42.16466	-80.20174	42.15910	-80.20732	10:36	10:46	SW
	16.0/17.8	3.1	42.16014	-80.21908	42.16486	-80.22637	11:05	11:15	NW
	16.6/16.0	2.9	42.15166	-80.24790	42.14586	-80.25316	11:38	11:48	SW
	14.0/12.7	2.8	42.13357	-80.26225	42.12741	-80.26612	12:05	12:15	SWxS
	22.5/24.4	3.0	42.15729	-80.28416	42.16414	-80.78751	12:49	12:59	N
	14.04/13.6	2.8	42.17295	-80.16893	42.17374	-80.15983	13:46	13:56	E
	12.4/14.8	2.9	42.17550	-80.15234	42.18060	-80.14599	14:09	14:19	ENE

On 24 July, 2013, eleven (N=11) specimens of Round Goby were caught from the North Pier Lighthouse of the Port of Erie terminal separating the open waters of Lake Erie from Presque Isle Bay. Those Round Goby specimens were caught by high school students enrolled in a week-long field ichthyology course taught by Dr. Jay R. Stauffer, Jr., Distinguished Professor of Ichthyology, Penn State University. A piece of live earthworm was cut to approximately 2.54-3.81cm in length and impaled on a #10 curved Mustad brand bait hook. Lead weight in the form of split-shot was fastened approximately 30.48 cm above the baited hook. Using closed-face spinning rods, the students cast their bait near the concrete pier and allowed it to sink to the substrate. When the students felt fish on the line, they set the hook and reeled in their catch. The collected Round Goby were then anesthetized using MS-222, fin-clipped and pinned for morphometric and meristic analysis.

Results of Specimen Collections

Offshore benthic trawls conducted by the PAFBC yielded (N=78) Round Goby. Additional fish species caught during the PAFBC benthic otter trawling included Yellow Perch, *Perca flavescens*; White Perch, *Morone americana*; Burbot, *Lota lota*; Emerald Shiner, *Notropis atherinoides*; Rainbow Smelt, *Osmerus mordax*; Freshwater Drum, *Aplodinotus grunniens*; Walleye, *Sander vitreus* and Common Carp, *Cyprinus carpio*. Invasive Zebra and Quagga mussels were also found in trawl nets. Biologists with PAFBC noted the higher prevalence of Quagga rather than Zebra Mussel and lower observed Round Goby catch rates than those found in the early and mid-2000s (Chuck Murray, personal communication, 2013).

Electrified benthic PSU trawling utilizing the PSU johnboat and equipment in years 2013-2014 yielded (N=99) Round Goby. Additional species collected while benthic trawling for Round Goby included Largemouth Bass, Smallmouth Bass, and Bluegill.

Kick and shoreline seining in years 2013-2014 yielded (N=147) Round Goby. Non-target species captured from the Marina Lake site included Blacknose Dace, *Rhinicnthys atratulus*; Northern Pike, *Esox lucius*; Bluegill, Black Crappie, *Pomoxis nigromaculatus*; Smallmouth Bass, and Yellow Perch. All non-target species were released without harm.

Hook-and-line collections yielded (N=11) Round Goby at the North Pier Lighthouse at the Port of Erie terminal, which connects the open waters of Lake Erie to Presque Isle Bay. Non-target species that were also caught during this collection method included Bluegill and Freshwater Drum.

I clipped the right pectoral fin of each Round Goby and stored it a 2 mL screw top vial correspondingly labeled with my initials, collection number, and collection year. Each vial contained 95% ETOH for preservation of tissue for DNA extraction. Vials were then placed in a refrigerator at 0ºC for 24-hours. After tissue samples were collected, 10 of the largest specimens (per sample site) were anesthetized using MS222 and placed on their right side in a rubber padded aluminum collection tray for measurements adhering to methods described by Hubbs and Lagler (1958) and Stauffer (1991, 1994). The first and second dorsal, caudal, and anal fins were pinned fully splayed for ease of counting fin rays. Similarly, each specimen also had additional pins set flush to both the dorsal and ventral portions of the snout to ensure it would remain set in place. Subsequently, a solution of 10% formalin was poured over them until they were completely submerged. Specimens remained in the trays for approximately 15-20 minutes until rigid, at which point they were removed, placed in a cheesecloth bag containing a PSU Fish Collection ID, and stored in 19.375L screw top buckets containing 10% formalin to be later processed in the laboratory.

Figure **2-6**. Round Goby pinned for morphometric measurements and meristic counts

After a period of one week in preservation, I transferred Round Goby from 10% formalin into wash basins and rinsed for a period of three days. All used formalin was labeled accordingly and collected by Environmental Health and Safety Penn State. Once rinsed, Round Goby were transferred to 70% ETOH for permanent storage in the Penn State University Fish Museum. All morphometric characters used as landmarks, the character abbreviations, and descriptions are recorded in Table 2-3.

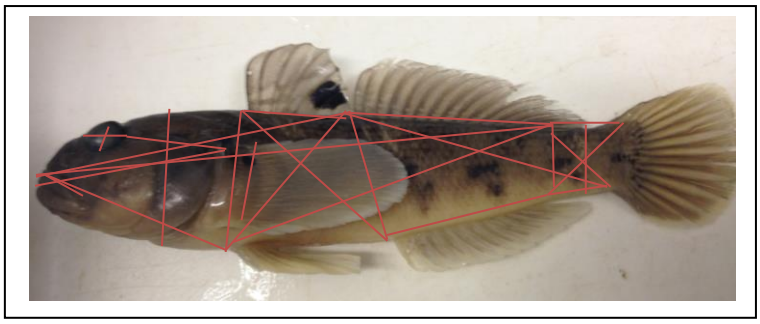
Figure **2-7**. A Round Goby specimen collected after kick-seining sample of Elk Creek, Erie County, PA. Tissue samples were collected from the right pectoral fin of each specimen (See **CHAPTER 4**).

Table 2-3. Morphometric and meristic measurement list for Round Goby *Neogobius melanostomus*. Thirty-five (n=35) morphometric measurements and nine (n=9) meristic counts are listed along with their acronym code.

Morphometric Characteristic (as % of SL)	Code
Standard length	SL
Head length	HL
Head width	HW
Head diameter	HD
Upper jaw length	UJL
Lower jaw length	LJL
Snout length	SNL
Post-orbital head length	POHL
Horizontal eye diameter	HED
Vertical eye diameter	VED
Body depth	BD
Caudal peduncle length	CPL
Least caudal peduncle length	LCPD
Snout to anterior first dorsal fin	SAFD
Snout to posterior first dorsal fin	SPFD
Snout to posterior second dorsal fin	SPSD
Snout to pelvic fin (ventral origin)	SP2
Anterior first dorsal fin to pelvic fin insertion	AFDP2
Posterior first dorsal fin to pelvic fin insertion	PFDP2
Posterior second dorsal fin to pelvic origin	PSDP2
Anterior first dorsal fin to anterior anal fin	AFDAA
Anterior first dorsal fin to posterior anal fin	AFDPA
Posterior second dorsal fin to posterior anal fin	PSDPA
Posterior first dorsal fin to anterior anal fin	PFDAA
Posterior first dorsal fin to posterior anal fin	PFDPA
Posterior second dorsal fin to anterior anal fin	PSDAA
Posterior second dorsal fin to posterior anal fin	PSDPA
Posterior first dorsal fin to ventral caudal fin	PFDVC
Posterior second dorsal fin to ventral caudal fin	PSDVC
Posterior anal fin to dorsal caudal fin insertion	PADC
Pelvic fin base length	P2BL
Anal fin length	AFL
First dorsal fin length	FDL
Second dorsal fin length	SDL
Pectoral fin base length	P1BL

Meristic Counts	Code
First dorsal fin rays	FDRAYS
Second dorsal fin rays	SDRAYS
Anal fin rays	ARAYS
Pectoral fin rays	P2RAYS
Pelvic fin rays	P1RAYS
Head canal pores	HCP
Gill raker lower	GRLOW
Gill raker upper	GRUP
Gill raker preopercular	GRPO

Fig. 2-8 Linear arrangement of morphometric measurements of *Neogobius melanostomus*



Chapter 3

Rapid isolation of microsatellite DNAs in the Round Goby collected from Lake, Bay, and Tributary Habitats of Erie County, Pennsylvania

INTRODUCTION

Invasive species pose one of the greatest contemporary threats to global biodiversity and ecosystem sustainability (Provan et al. 2005); the effects are often irreversible. The Laurentian Great Lakes represent one of the World's most invaded freshwater systems (Jude et al. 1992; Corkum et al. 2004; Brown and Stepien 2009). The Round Goby, *Neogobius melanostomus,* a benthic fish native to the Ponto-Caspian region of the Black and Caspian seas, was first reported in the St. Clair River of North America in 1990 (Jude et al. 1992) and in Eastern Lake Erie in 1995 (Clapp et al 2001). Since then, the Round Goby has spread to all five Great Lakes and many of their tributaries. *Neogobius melanostomus* has caused extirpations of native benthic fishes (Jansen and Jude 2001; Lauer et al. 2004) and declines in macroinvertebrate abundance of tributaries (Balshine et al. 2005; Lederer et al. 2008). In the absence of empirical information, it is assumed that this species exists as a metapopulation(s) consisting of subpopulations inhabiting each tributary connected by migration from neighboring streams or from a lake-resident population. The development of a control strategy to prevent further spread of *N. melanostomus* will require characterization of the associated migration, colonization, and extinction processes among nascent populations. No detailed genetic information exists however on population structure, levels of effective movement, or relatedness among lake resident and tributary populations. To address these information needs, we have developed a suite of polymorphic microsatellite DNA markers for *N. melanostomus* utilizing massively parallel genomic shotgun sequencing reads. Here we describe the isolation and characterization of 24 tri-, tetra, and penta-nucleotide microsatellite markers, ascertain the levels of diversity and heterozygosity among

individuals from a single collection from Lake Erie and demonstrate the unique utility this class of marker provides for assessing population demographic status for this species.

Polymorphic microsatellite DNA markers for *N. melanostomus* were developed at the USGS Leetown Science Center, Kearneysville, WV. As opposed to traditional methods of microsatellite isolation such as, cloning (Glenn and Schable 2005), massively parallel genomic shotgun sequencing was used to generate thousands of microsatellite containing sequences at modest cost and in a short period of time. The following is a description of the isolation and characterization of 24 tri-, tetra, and penta-nucleotide microsatellite markers used to determine the levels of diversity and heterozygosity among individuals from a single collection of *N. melanostomus* from Lake Erie. A subset of these markers were then used for assessing population genetic characteristics of this species from collections throughout the study area (see CHAPTER 4).

METHODS and MATERIALS

Tissue and DNA Processing

I collected fin clips from 3 individuals of *N. melanostomus* sampled from each of six localities (Table 1-3) from Pennsylvania's portion of Lake Erie encompassing the southeastern portion of the Central Basin and southwestern portion of the Eastern Basin. I extracted genomic DNA from fin clips using the Omega Bio Tek DNA extraction kit (Norcross, GA) in a 96-well plate format. The collection used to characterize the microsatellite loci was from Marina Lake, Presque Isle Bay, Lake Erie. I determined DNA concentrations and integrity as described in King et al. (2006) and above.

Table 3-1. General collection localities and number of individuals sampled of Round Goby (*Neogobius melanostomus*) sampled from Pennsylvania's portion of Lake Erie encompassing the southeastern portion of the central basin and southwestern portion of the eastern basin. Three fish from each location were pooled to generate a genomic DNA shotgun library for sequencing on the Ion Torrent Personal Genome Machine (Thermo Fisher Scientific, Waltham, MA).

Collection	Latitude	Longitude
Lake Erie Location		
20.5 km NNW of Presque Isle Bay	42°17’0.78"N	80°18’16.34"W
Tributary Locations		
Marina Lake, Presque Isle Bay	42°9’13.57"N	80°6’48.492"W
Elk Creek	42°1’10.65"N	80°22’17.09"W
Misery Bay	42°09’28.10"N	80°05’19.37"W
Twentymile Creek	42°15’38.71”N	79°46’49.33”W
Walnut Creek	42°4’31.12"N	80°14’17.20"W

Ion Torrent Library Preparation

I chose the Ion Torrent PGM (Grand Island, NY) as a sequencing platform. The Ion Xpress Plus Fragment Library Preparation Kit (Life Technologies) was used to prepare the *N. melanostomus* DNA shotgun library for sequencing. The whole genomic DNA library was size-selected for eventual PGM 400 base pair (bp) sequencing reads using the *E-gel*^{R} size-select 2% gel system (www.invitrogen.com). To allow for the increased size from linker and adaptor ligations, 25µl of the 420bp fragment were captured. The library was characterized for proper size (base pairs), quality, and concentration by means of both High Sensitivity DNA Chip visualization on the Agilent 2100 Bioanalyzer (Agilent Technologies) and Quantitative PCR (qPCR) using the Ion Library TaqMan^{R} Quantitation Kit (Life Technologies). The determined template dilution factor was used for the preparation of each individual library and fell within the optimized concentration range (~26pM) for downstream amplification of clonal library templates

on Ion Sphere™ particles. From the diluted library, 20 µl were used as the aqueous phase input for amplification using the OneTouch™ emulsion system (Thermo Fisher Scientific). The percentage of pre-enriched Ion spheres was determined by Qubit^{R} 2.0 fluorometic analysis and the IonSphere™ Quality Control Kit. The library was then enriched using the Ion-Torrent ES system utilizing Dynabeads^{R} MYONE^{TM} streptavidin C1 beads to capture the templated ion-spheres. All sequencing was performed on the Ion Torrent PGM using the Ion PGM^{TM} 400 Sequencing kit and a 318 semiconductor chip following manufacturer recommendations.

Bioinformatics and Microsatellite DNA Marker Development and Characterization

Sequence read processing consisted of all sequence reads being trimmed for length (>30 bp) and quality (≥20 PHRED), and subjected to duplicate removal, using CLC Genomics Workbench (version 7.0; Qiagen, Aarhus, Denmark). The program QDD version 3 (Meglécz et al. 2010) was used to screen the individual trimmed reads from the Ion Torrent genomic DNA libraries for microsatellite containing sequences. I developed primers for these microsatellites using the integrated Primer 3 code (Rozen and Skaletsky 2000) within QDD using the default settings. A 19 bp universal M13 tail sequence (Boutin-Ganache et al. 2001) was added to the forward or reverse primer of selected primer pairs to facilitate initial marker screening by fluorescent genotyping with M13 labeled FAM, NED, HEX or PET (Applied Biosystems). Only primers amplifying100-350 base pair (bp) fragments were selected for testing.

Each PCR consisted of 150 ng of genomic DNA, 1X buffer (Promega "Flexi"), 2mM $MgCl_2$, 0.20 mM dNTPs, o.2µM forward and reverse primer, and o.08 U/µl Taq DNA polymerase (Promega, Madison, WI, USA) in a total volume of 15 µl. I used either a PTC-200 or PTC-225 Thermal Cycler (MJ Research) for PCR amplifications using the following cycling procedure: initial denaturing at 95°C for 15 min; 29 cycles of 95 °C for 1 min, 60°C for 45 sec, 72 °C for 45 sec; 10 cycles of 95 °C for 1 min, 53°C for 45 sec, 72 °C for 45 sec; and a final

extension at 72°C for 10 min. Fragment electrophoresis and scoring were performed according to protocols described by King et al. (2006).

Data Analyses

I used GenAlEx 6.5 (Peakall and Smouse 2006) to quantify allelic diversity and heterozygosity. I used exact tests in GENEPOP (Raymond and Rousset 1995) to determine if genotypes at each locus conformed to Hardy-Weinberg equilibrium (HWE). I completed multi-locus tests of conformance to HWE using Fisher's method output by GENEPOP. I also tested linkage disequilibrium (LD) for all pairs of loci using contingency tables in GENEPOP. I used the default Markov chain parameters for all tests of HWE and LD tests in GENEPOP. Significance levels for HWE and LD tests were adjusted using the sequential Bonferroni correction (Rice 1989).

I used multiple techniques to describe the genetic and demographic status of the Presque Isle collection. All multilocus genotypes within the Marina Lake, Presque Isle Bay collection were subjected to analysis via GENECAP (Wilberg and Dreher 2004) to identify matching samples, calculate match probabilities, and estimations of the sibling probability of identity (PI_{sibs}; Evett and Weir 1998). To determine the randomness of the collection (e.g., to insure the collection did not consist of a small number of families), we analyzed for the presence of full-sibling families using the program COLONY v2.0 (Wang and Santure 2009). Settings for COLONY analyses included the assumption of male and female polygamy, no per locus genotyping error information, no inbreeding, long run length with full likelihood analysis, high likelihood precision, no allele frequency updates, and no sibship prior. Individual fish were analyzed as offspring without assignment of individuals as candidate males (fathers) or females (mothers), as these data were not available. While the inference of family relationships is weakened in this situation with no sex, age, relationship information, and the assumption of polygamy for both sexes, COLONY is predicted to be more accurate than pairwise estimates of

relationships (Wang and Santure 2009). Within a sample of individuals taken at random (with respect to kin) from a population, the frequencies of full and half sib dyads can be used to estimate the current effective size (N_e) of the population. Therefore, COLONY was also used to estimate N_e utilizing the estimates from the sibship assignment full likelihood method. To estimate whether the N_e for the Marina Lake collection has remained constant (i.e., achieved mutation-drift equilibrium; see Davies et al. 1999), BOTTLENECK (Piry et al. 1999), implementing a two-phased model of mutation (5% IAM; 95% SMM; Cornuet and Luikart 1996), was used.

RESULTS and DISCUSSION

Microsatellite DNA variation

The Ion Torrent PGM sequencing run generated 6.7 million raw reads, averaging 266 bp in length with an average GC content of 41%, and an average sequence quality score (PHRED) of 29. Initial bioinformatic processing applying the chosen trimming parameters in CLC (see methods) resulted in 6,477,119 reads with modal length of 345 bp and averaging 220 bp. In an initial screening, 1,432 microsatellite DNAs were identified among sequences; 50 were randomly chosen for detailed assessment for marker development. Of this number, 30 were deemed unique, of sufficient length (repeats), and possessed adequate flanking regions for primer development.

Microsatellite marker characterization is summarized in Table 3-2. Allelic diversity in the Marina Lake collection ranged from 2 (NmeQ13) to 10 (NmeQ20, NmeQ22, and NmeQ23) and averaged 5.8 alleles/locus and 3.2 effective alleles/locus. These diversity levels were sufficient to produce unique multilocus genotypes. The probability that two siblings would have identical genotypes was PI_{sibs}= 9.3 x 10^{-9} (Marina Lake) (Taberlet & Luikart 1999). Expected average individual heterozygosity (H_E) varied greatly among loci ranging from 15.6% (NmeQ13) to 85.1% (NmeQ22) and averaged 66.2% for Marina Lake *Neogobius melanostomus.* No

statistically significant linkage disequilibrium (GENEPOP) was detected within the collection (overall α=0.05, P<0.002; Rice 1989).

Table 3-2. Characteristics of 24 microsatellite DNA loci developed for the Round Goby (*Neogobius melanostomus*) from genomic shotgun sequences generated by the Ion Torrent PGM. The table includes locus designation, number of alleles (Na), effective number of alleles (Ae), allele size ranges, observed average heterozygosity (H_O), and expected average heterozygosity (H_E) for 24 fish genotyped from Marina Lake, Presque Isle Bay, Lake Erie (see details in Table 1).

Locus	Primer sequences (5'-3')	Repeat motif	N_a	A_e	Allele sizes	H_O	uH_E
NmeQ1	F:5'- GAG TTT TCC CAG TCA CGA CAT GAC TCC AGT GGG ATC CAG -3' R:3-' TAG TCC GCT GAC GAA GCC -3'	$(AAG)_{15}$	3	2.743	173-216	0.458	0.649
NmeQ2	F:5'- GAG TTT TCC CAG TCA CGA CAG AAG AAG AAA TGT GTT GGT CA -3' R:5'- TGT TCA TTA ACA TGC ACC CAA -3'	$(AAG)_{15}$	7	3.815	133-197	0.542	0.754
NmeQ3	F:5'- GAG TTT TCC CAG TCA CGA CGG GAG CAG TTT CAA TAA CCA GT -3' R:5'- ATT TGC ACA GGG CTG TGT TT -3'	$(ATC)_{14}$	6	4.028	148-172	0.792	0.768
NmeQ4	F:5'- GAG TTT TCC CAG TCA CGA CAA GAC TAA CAC GTC TAA TAC ATC ATC A -3' R:5'- GCG CGT CTC TGA ATA AAT GC -3'	$(ATC)_{13}$	3	1.882	217-229	0.417	0.479
NmeQ5	F:5'- GAG TTT TCC CAG TCA CGA CTG TAC GAG GAC TAT GGA TGA AA -3' R:5'- AAT ATT AAT GGA CAC TCA GTA GTC TGC -3'	$(ATCC)_{13}$	5	2.730	147-179	0.417	0.647
NmeQ6	F:5'- TGA AAG CTT TGT GTA ATC GCA-3' R:5'- GAG TTT TCC CAG TCA CGA CAT TTG CTG CCT CCA TTG TC -3'	$(ACT)_{13}$	3	2.661	134-143	0.625	0.637
NmeQ7	F:5'- GAG TTT TCC CAG TCA CGA CTC TTC ACA GCT TCT GTT CGG -3' R:5'- GCG CCA ATG AGA CGA TTT AT -3'	$(AAAG)_{13}$	6	1.648	143-185	0.375	0.402
NmeQ8	F:5'- GAG TTT TCC CAG TCA CGA CAA AGT GGA	$(AGC)_{12}$	3	2.880	199-205	0.750	0.667

Table 3-2 Extended

	AAC GTG ATC GGA -3’ R:5’- TCG CGA ATT GTG TTA CAT CC -3’						
NmeQ9	F:5’- CTTCGCTGTGCAGCTGTTT-3’ R:5’- GAG TTT TCC CAG TCA CGA CCC TGG AGA GAG ACA GAC GA -3’	$(AAG)_{12}$	7	4.397	239-291	0.833	0.789
NmeQ10	F:5’- GAG TTT TCC CAG TCA CGA CTT GTT AGT TAG CCC AGC GG -3’ R:5’- GAT TCA ACT ACA GCC TAC CCG -3’	$(ACT)_{10}$	3	2.246	162-168	0.500	0.566
NmeQ11	F:5’- GAG TTT TCC CAG TCA CGA CTC AAT TAA CCC AGT CCA GTC G -3’ R:5’- GAA GCC CTG CAG TTG TCC TA -3’	$(ATC)_{10}$	5	4.114	159-170	0.833	0.773
NmeQ12	F:5’- GGC TAA TTT ACA ATG TCC GTC C -3’ R:5’- GAG TTT TCC CAG TCA CGA CGC TTC GTT CCT GAT CAC TTT G -3’	$(AAT)_{10}$	6	2.645	216-252	0.391	0.636
NmeQ13	F:5’- TGG ACA ACT CCT GTA CGA CTG -3’ R:5’- GAG TTT TCC CAG TCA CGA CTG TAC AAG GGA CCT TAT GAA ACA -3’	$(AAT)_{10}$	2	1.180	259-265	0.167	0.156
NmeQ14	F:5’- GAG TTT TCC CAG TCA CGA CTC AAC CAA ACC CAG TCC AGT -3’ R:5’- CGC AGT TGA GCA CCA ATA AC -3’	$(AAT)_{10}$	5	3.263	218-238	0.833	0.708
NmeQ15	F:5’- GAG TTT TCC CAG TCA CGA CTT CCA TAC AAG CCT CCT GCA -3’ R:5’- TGT ACA AAG ACA CAG ATG C -3’	$(AAT)_{9}$	7	2.977	242-263	0.708	0.678
NmeQ16	F:5’- ATG ACT CAT GTC GGG ATG GC -3’ R:5’- GAG TTT TCC CAG TCA CGA CTC AGA TGG TTA CCA ATG CCA GA	$(ACTAT)_{10}$	6	3.905	218-253	0.583	0.760
NmeQ17	F:5’- GAG TTT TCC CAG TCA CGA CAC TTT CGG ACG CTT CTG GTT -3’ R:5’- TCT GAC AGC AGA GAG TCG CT	$(AAG)_{9}$	5	2.673	197-212	0.500	0.639
NmeQ18	F:5’- TGT ATG TGA ATA TGT ACA TGA TCC GA -3’ R:5’- GAG TTT TCC CAG TCA CGA CAG GGA GCA TGA GAC GTC ATT -3’	$(AATC)_{12}$	6	2.165	189-221	0.625	0.550
NmeQ19	F:5’- ATG TCA GAA CTA AAT CAC TTT GCA -3’	$(AATC)_{12}$	7	4.056	207-247	0.625	0.770

Table 3-2 Extended

	R:5'- GAG TTT TCC CAG TCA CGA CAA GAC AGG GAG GAC AGC AT -3'						
NmeQ20	F:5'- GAG TTT TCC CAG TCA CGA CGG CTT TGT CCT AAG GAG AGG T -3' R:5'- GCC AAG AGA TAC TTT CCT TGT CA -3'	$(AGAT)_{23}$	10	3.728	123-167	0.833	0.747
NmeQ21	F:5'- GAG TTT TCC CAG TCA CGA CAT GAC CAT GTC TGT GAA AGG C -3' R:5'- GGA ATA AAG AAG CTA TCA TTT GCA T -3'	$(AAG)_{15}$	8	2.954	152-234	0.667	0.676
NmeQ22	F:5'- GAG TTT TCC CAG TCA CGA CGG GCC ATA ATA GAG GAT GGG -3' R:5'- TCT ACT CCC TTT GAG CTT CCA -3'	$(ACT)_{20}$	10	6.000	195-240	0.875	0.851
NmeQ23	F:5'- GAG TTT TCC CAG TCA CGA CTG CTG ACC TGT TGC CCT A -3' R:5'- GCA ACA TTT CAT CAA ACA GAG G -3'	$(ACT)_{19}$	10	5.143	194-239	0.875	0.823
NmeQ24	F:5'- GAG TTT TCC CAG TCA CGA CTT TGG CTT CTT ATC AAC CGC -3' R:5'- GGC GCT AGC AGA GGG TAA AT -3'	$(AAG)_{19}$	7	3.932	126-243	0.583	0.762

Analyses of family structure in COLONY suggested that the Marina Lake collection was sampled randomly as it was not dominated by a small number of families. No full sibling dyads were observed, thus all individuals from the collection were retained for subsequent analyses. COLONY estimated the N_e (and 95% confidence limits) for this collection to be 79 (18-55). It should be noted that COLONY's sibship assignment method makes the critical assumption that the sample of individuals is taken at random (with respect to kinship) from a single cohort of the population. If there are several cohorts in the sample, then it is possible that some sampled individuals are actually parents of other sampled individuals. Without knowing the parent-offspring (PO) relationship, it is difficult to infer full-sibship (FS) reliably as PO and FS dyads are very similar due to identity by descent. Given that no FS relationships were observed, it is likely that no PO relationships were present either. BOTTLENECK indicated no statistically significant heterozygote excesses or deficiencies for the Marina Lake collection (α=0.05, P>0.295; Wilcoxon signed-rank test, Luikart et al., 1998). This finding indicates the effective size has remained constant suggesting the population has achieved mutation-drift equilibrium (Davies et al. 1999).

While partial or complete sequencing of large genomes of non-model organisms remains costly, it is clearly feasible to rapidly identify and develop multiple genetic marker types for such organisms. This study demonstrates that for a fraction of the cost of traditional clone-based sequencing for microsatellites the Ion Torrent PGM platform provided sufficient genome coverage and sequencing depth suitable for the identification of thousands of candidate microsatellite DNA markers. In addition to microsatellite recovery, the single Ion Torrent run allowed recovery and extensive coverage of the entire mitochondrial DNA genome (not shown). *Neogobius melanostomus* microsatellite markers isolated from sequence reads generated on the benchtop Ion Torrent PGM platform in a single workday yielded sufficient genetic diversity to: (i) produce unique multilocus genotypes and (ii) provide unique perspectives of population sizes

and historical demographics. This preliminary investigation suggests that read quantity and quality generated by genomic shotgun sequencing on the Ion Torrent PGM platform is sufficient for use in phylogeographic comparisons and could make a valuable contribution to understanding the evolutionary and ecological dynamics among populations of *N. melanostomus* and ultimately to quantifying the ecosystem impacts of this invasive species.

CHAPTER 4

Population Genetics of Round Goby in Lake, Bay and Tributary Habitats of Erie County, Pennsylvania

INTRODUCTION

The ability of Round Goby to adapt to a variety of habitats and environmental conditions poses threats to the biota of tributary systems and inland lakes (Krakowiak and Pennuto 2008). Moreover, Krakowiak and Pennuto (2008) also believed further knowledge not just presence and/or inventory and monitoring data, is needed to better understand their potential impacts on Eastern Lake Erie tributaries. Contemporary genetic methods have allowed researchers to characterize mechanisms of dispersal during colonization which has led to an increase in studies reporting stratified dispersal as a mechanism facilitating secondary range expansion and adaptation/speciation (Colautti et al. 2005; Parisod and Bonvin 2008; Darling and Folina-Rorem 2009; Bronnenhuber et al. 2011).

The genetic diversity of populations can allow adaptation to environmental heterogeneity via alterations in the relative strengths of the four opposing genetic forces: mutation, migration, selection, and genetic drift (Bagely et al. 2002). The accuracy and sensitivity of measurements of genetic diversity have steadily increased with advances in molecular marker technologies. Measures of gene flow help identify evolutionary connectivity of populations and effective population size. Populations that have low connectivity with others have the potential to become genetically differentiated and unique. While Round Gobies are invasive and pose threats to native biodiversity, they represent valuable natural experiments in species colonization and range expansion (Dufour et al. 2007). Furthermore, the use of microsatellite markers can be a powerful tool to provide insight into population structure and dispersal in tributaries (Dufour et al. 2007).

Population genetics studies explore the variations of allele frequencies between and within populations (Evanno et al. 2005). Wright's *F* statistics (Wright 1931) are the most widely used measures of population structure (Evanno et al. 2005). Evanno et al. (2005) noted that to calculate those previously noted indices, groups of individuals must be defined and then use their genotypes to compute variance in allele frequencies. Simple-sequence repeat loci, often referred to as microsatellites, are found primarily in nuclear DNA, which makes them useful to examine population characteristics as they represent DNA inherited from both the maternal and paternal lineage (Allendorf, et al. 2013). Microsatellites have become the standard markers for identifying population structure due to their hypervariablity and codominant expression (Ellegren 2004, King et al. 2014). Twelve (N=12) of 24 microsatellite loci developed in this study were used to genotype 12 collections of Round Goby in 2013 and 2014.

If a control strategy were ever implemented to mitigate or prevent the further spread of *N. melanostomus* it would require the characterization of the associated migration, colonization, and extinction processes among emerging populations. No detailed genetic information existed however on population structure, levels of effective movement, or relatedness among lake resident and tributary populations of the project study area. In the absence of empirical information, it is assumed that Round Goby exists as a metapopulation(s) consisting of subpopulations inhabiting each tributary connected by migration from neighboring streams or from the lake-resident population.

To address this research need, the objective of this study was to determine if Round Goby in the Lake Erie drainage has established unique, detectable, reproductively isolated populations in the tributaries, Presque Isle Bay, and the open waters of Lake Erie that can be delineated using polymorphic microsatellite DNA loci. The results of this study should promote a better understanding of the ecological and evolutionary processes acting on the Round Goby in this

portion of its range and provide insights into the mechanisms of this species' adaptive potential in invaded aquatic habitats (Salmenkova 2008).

MATERIALS AND METHODS

Tissue collection and Preservation

Tissue samples were taken from each specimen (n=335) collected in 2013-2014 for downstream genetic marker development and microsatellite analysis of *N. melanostomus* DNA (See **CHAPTER 2** for detailed collection methods). Since whole specimens were kept for morphometric and meristic analysis, consistency in tissue removal location was kept to the right pectoral fin of *N. melanostomus.* Tissue collections were made immediately after specimen capture. After Round Goby were collected, they were anesthetized using 15mg/L MS222 buffered to a pH of 7.0 prior to clipping the right pectoral fin from each individual. Fin tissue was then placed in a 1.5ml screw top vial filled with 95% ETOH. After fin tissue was collected, the fish were categorized by site location and were pinned to a collection tray containing a 10% formalin mixture for preservation (see **CHAPTER 2** collection methods for specific details).

Figure **4-1**. A Round Goby specimen collected after kick-seining sample of Elk Creek, Erie County, PA. Tissue samples were collected from the right pectoral fin of each specimen.

All wet-bench laboratory work (DNA extraction, primer development and genotyping) for Round Goby was performed at the USGS Leetown Science Center in Kearneysville, WV (See **CHAPTER 3**). Fin tissue was cut to approximately 20mg in weight before DNA was extracted using an OMEGA Bio-Tek E-Z 96 Tissue DNA Kit. This kit provides a high-throughput method to purify genomic DNA from animal tissues in a 96-well plate format (manufacturer description). The E-Z 96 Tissue DNA Kit Protocols (pgs. 8-11, April 2012 edition) were used for extracting all Round Goby fin clips collected in 2013 and 2014. The final elution and centrifugation steps in the above-listed protocol resulted in approximately 100-150 µL of stock DNA solution that was stored at -20ºC.

Quantification of stock DNA Solution

DNA from each individual sample of Round Goby was quantified to determine the DNA concentration (ng/µL) using a Thermo Scientific© NanoDrop 1000 and NanoDrop 8000 UV-Vis Spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE). Using either a single or eight-channel 2µL Eppendorf manual pipette, exactly 1 µL of stock, DNA was pipetted and placed on the NanoDrop. This step was repeated, individually, for all 335 stock DNA solutions. All DNA concentrations were recorded both manually and digitally. When the concentrations were determined, they were diluted using autoclaved water. Prior to Polymerase Chain Reaction (PCR), stock dilutions of DNA were made and stored similarly to the extracted DNA. Diluted stocks were stored for downstream use for all master mixes.

Genotyping

Genotyping populations of Round Goby consisted of Polymerase Chain Reaction (PCR) amplification in two master mixes. The primer master mix concentrations were the same volume (15µL for all 12 primers used; six primers per multiplex) to screen the collection populations (See **CHAPTER 3** for primer development). Each PCR master mix (calculated for 100-samples for insurance of filling all 96 wells in a 96-well PCR plate) consisted of 150 ng of genomic DNA, 1X

PCR buffer (Promega "Flexi"), 2mM $MgCl_2$, 0.20 mM dNTPs, 0.2 µM forward and reverse primer, 0.25 U/µl BSA, and 0.08 U/µl Taq DNA polymerase (Promega, Madison, WI, USA) in a total volume of 15 µl per sample. Primers in Multiplex I included NmeQ11, NmeQ14, NmeQ13, NmeQ6, NmeQ24, and NmeQ4. Primers included in Multiplex II were NmeQ22, NmeQ3, NmeQ17, NmeQ15, NmeQ16, and NmeQ23 (See Table 3-2 for full description of all 21 loci developed and the 12 used for genotyping). Exactly 13.5 µL of master mix solution and 1.5 µL of diluted DNA stock was then pipetted into the collection wells of a 96-well PCR plate prior to amplification. PCR amplification was conducted on a MJ Research PTC-200 Thermal Cycler under the following cycling conditions: 94ºC for 3-minutes; 94 ºC for 30-seconds (denaturation); 58 ºC for 1-minute (annealing); 72 ºC for 1-minute (extension) for 34 cycles. At the end of the PCR cycles, the plate was refrigerated at 4ºC until subjected to fragment analysis.

All Round Goby samples were genotyped at 12 microsatellite loci developed for this study (Table 3-2). Prior to electrophoresis, 1.5 µL of a 1:100 dilution of PCR product was mixed with a 12.2 µL solution containing 97% formamide and 3% Genescan LIZ 500 size standard (Applied Biosystems, Inc.) in a 96-well SEPTA plate, which was denatured for one cycle at three-minutes. Fragment analysis was performed on an ABI 3130 XL Genetic Analyzer (Applied Biosystems, Inc.) using fluorescently labeled forward primers and analyzed using GeneMapper software v3.7 (Applied Biosystems, Inc.).

Statistical Analyses

Genetic diversity of all Round Goby collections was assessed using GenAlEx (Peakall and Smouse 2006, 2012) to calculate allelic frequencies, number of alleles per locus (N_A), effective number of alleles (A_E), observed heterozygosity (H_O), unbiased expected heterozygosity (uH_E), and the average (across loci) inbreeding coefficient (F_{IS}) (Table 4-7) (King et al. 2014). Observed genotype frequencies were tested for conformance to Hardy-Weinberg and linkage equilibrium (Table 4-5) expectations using randomization tests implemented by GENEPOP 4.3

(Raymond and Rousset 1995, King et al. 2014). The Markov chain randomization test of Guo and Thompson (1992) for the Hardy-Weinberg test was used to estimate exact two-tailed *p*-values for each locus (Table 4-14) in each collection. Global tests combined these results over loci and sampling locations using Fisher's method (Sokal and Rohlf 1994, King et al. 2014). Linkage disequilibrium tests used the randomization method of Raymond and Rousset (1995) for all pairs of loci. Sequential Bonferroni adjustments (Rice 1989) were used to determine statistical significance for these and all other multiple tests.

Although the Round Goby populations are seemingly intact, bottlenecks are important to detect in conservation biology because they can increase the risk of population extinction (Piry et al. 1999). I performed a bottleneck test for samples of Round Goby to determine whether the collections have experienced any recent reduction in effective population size (N_e). The software tool BOTTLENECK 1.2.02 is a population genetics computer program that conducts four tests (Sign Test, Standardized Differences Test, Wilcoxon Test, and Mode-Shift) for identifying populations that have recently experienced substantial reductions in effective population size (N_e) (Piry et al. 1999). BOTTLENECK computes for each population sample and for each locus the distribution of the heterozygosity (H_{eq}) expected from the observed number of alleles (k), given the sample size (n) under the assumption of mutation-drift equilibrium (Piry et al. 1999). The Wilcoxon's test is the most useful of the four tests because it is the most powerful when used with few (<20) polymorphic loci (12 were used in this study) (Piry et al. 1999).

Genetics methods are increasingly being used to estimate effective population size (N_e) in natural populations (Waples and Do 2008). Coalescent theory provides a powerful framework to study effects of genetic drift, natural selection, mutation, and gene flow (the four fundamental mechanisms driving evolution) in natural populations (Rosenberg and Nordborg 2002, Cenik and Wakely 2010, and Allendorf et al. 2013). Migration rate and population size estimation was determined using the coalescent and maximum likelihood or Bayesian inference through the

computer program MIGRATE-N version 3.6.6. MIGRATE-N is used to estimate effective population sizes (N_e) and historic migration between populations using a migration matrix model that includes asymmetric migration rates and different subpopulation sizes (Beerli 2006). This approach can be used to study effective populations sizes (N_e) over long spans of time (Allendorf et al. 2013). To quantify long-term rates of genetic exchange among the most distal populations (Elk Creek and Twenty Mile Creek), a Bayesian coalescent model was implemented in MIGRATE-N and estimated θ and M, where θ represents mutation-scaled effective population size and M represents the mutation-scaled immigration rate (Beerli 2006, Beerli and Felsenstein 2001). Migration rates were allowed to be asymmetric and to vary between the populations. Distributions for θ and M were uniform between minimum and maximum values sets as 0-200 and 0-2000 respectively. The initial burn-in of this model ran with 100,000 trees, followed by data collection for 250,000 MCMC sweeps every 50 steps. Static heating was used (four chains) and the chains were allowed to swap. To calculate long-term genetic exchange rates in units of effective migrants from group *j* to group *I*, the relationship described by Beerli (1998; Equation 1) was used. The Skyline plots produced by MIGRATE-N were also examined to determine what, if any, observed genetic differences were the result of recent divergence or historic departure without genetic exchange (Beerli 1998). It should be noted that in the MIGRATE-N program used for this study, Twenty Mile Creek was considered Population 1 while Elk Creek was labeled Population 2.

The computer program LDNe uses a Visual Basic interface that implements a bias correction for estimates of effective population size (N_e) based on linkage disequilibrium data (Waples and Do 2008). LDNe reads genotypic data in standard formats and can accommodate an arbitrary number of samples, individuals, loci, and alleles, as well as random and lifetime monogamy mating scenarios (Waples and Do 2008). LDNe calculates separate estimates using different criteria for excluding rare alleles, which, according to Waples and Do (2008), facilitates

evaluation of data for highly polymorphic markers such as microsatellites. Additionally, LDNe utilizes a jackknife method for obtaining confidence intervals (Waples and Do 2008). LDNe facilitates the evaluation of the effects of allele frequency and, under default, the program returns separate estimates after excluding all alleles with frequencies less than three critical values (P_{crit}=0.05, 0.02, 0.01)(Waples and Do 2008).

Identifying genetically homogenous groups of individuals has been a long standing issue in population genetics studies (Waples and Gaggiotti 2006) and the Bayesian (MCMC: Markov Chain Monte Carlo) algorithm implemented in the software STRUCTURE 2.3.4 allows the identification of such groups and the detection of the true number of clusters (K) in a sample of individuals (Evanno et al. 2005). STRUCTURE uses an ad hoc statistic ΔK based on the rate of change in the log probability of data between successive K values (Evanno et al. 2005). I utilized the program to assign individuals to populations based on their genotypes while also estimating progenitor population allele frequencies (Zewdu et al. 2013). According to Evanno et al. (2005), STRUCTURE results are dependent to the type of markers used (microsatellites), number of loci scored, number of populations sampled, and the number of individuals typed in each sample. An MCMC method was used to estimate allele frequencies in each of the K populations and the degree of admixture for each Round Goby (Zewdu et al. 2013). Initial K was K=1-6, using 6 inferred clusters with each K being replicated three times. One-population assumed at a 100,000 Burn-in period, with 200,000 Reps; 4-populations assumed at a 100,000 Burn-in period, with 200,000 Reps; and 6-populations assumed at 10,000 Burn-in period, with 20,000 Reps. The above-listed Run parameters were all performed on 314 individual Round Goby at 12-loci.

STRUCTURE HARVESTER (http://taylor0.biology.cula.edu/structureHarvester/) was used for collating data outputted from the program STRUCTURE (Pritchard et al. 2000, Earl and vonHoldt 2012). The results of STRUCTURE HARVESTER are used to assess the level of genetic stratification in a multi-locus data set.

RESULTS

Basic Population Genetics Results

Genotype data were collected at 12 microsatellite DNA markers for 314 Round Gobies (originally 335 specimens; 21 were later removed due to extraction error and various constraints). Levels of genetic diversity were low in all collections (with 2 to 10 alleles per locus), and heterozygosity ranged from H_e=0.628 to H_e=0.703 (Table 4-1). Randomization tests showed that genotypes form most collections and most loci surveyed for this study were consistent with Hardy-Weinberg expectations. When *p*-values were combined over loci and analyzed for significance using Fisher's method, three collections deviated from HWE expectations (Elk Creek Embayment 14, Elk Creek Seine, and Lake Erie Trawl). Overall tributary collections were no more diverse than Lake Erie and Presque Isle Bay populations, genetically. Tests of population differentiation among all collections (overall F_{ST}=0.036) suggest a low level of genetic differentiation and an overall panmictic population. This result was supported by Bayesian clustering analyses in STRUCTURE, which suggested K = 1 number of clusters or populations.

In **Table 4-1**, the results of the BOTTLENECK bottleneck test are listed for all 12 populations at 12 loci. The Wilcoxon test revealed that with the assumption that all loci fit the Infinite Allele Model (a mathematical model for calculating genetic mutations in which each mutation leads to a completely new allele in the population), the probability (two tails for H excess and deficiency) of mutation-drift equilibrium is 0.00171. The probability for the assumption that all loci fit the Stepwise Mutation Model (distribution of allelic frequencies in a finite population when selectively neutral alleles are produced in stepwise fashion, (Kimura and Ohta 1978)) is 0.10986. According to the BOTTLENECK v 1.2.02 output, all populations appear to have reached equilibrium.

Despite running STRUCTURE v 2.3.4 with more randomization, e.g., 100,000 Burn-In with 200,000 Reps after Burn-in, the overall determination was that Round Goby collected for this study were experiencing true panmixing (King 2015, pers. comm.), as the frequency data as well as the L(K) and Delta(K) plots indicate one large randomly mating panmictic population.

This model was chosen to understand gene flow and connectivity between the most distal populations represented. Since Round Goby are a recent invader (Jude 1992), the observation of genetic differences resulting from recent divergence or historic departure are likely low. The Bayesian analysis data output from MIGRATE-N's Posterior distribution table mode values for all 12 loci at the two distal sites were θ_1=5.66667; θ_2=5.93333; $M_{2->1}$=20.667, and $M_{1->2}$=6.000 (Table 4-3). The formula for estimating the number of immigrants migrating from one population to the other per generation is: (theta1*M2->1)/4 = immigrants per generation. The product of (θ_1=5.66667)($M_{2->1}$=20.667) / 4 = 29.27 fish moving from Twenty Mile Creek to Elk Creek per generation, i.e., the rate of fish in Population 2 immigrating to Population 1. The number of fish immigrating from Population 1 (Twenty Mile Creek) to Population 2 (Elk Creek) is (θ_2=5.93333)($M_{1->2}$=6.000) / 4= 8.89. These results suggest the populations appear to be unimodal and relatively compact. Additionally, summary results of parameter values through time over all loci [Time [scaled by mutation rate / generation (DNA: per site, other: per locus)] suggest little if no evidence of the effective population size being higher during the period of invasion. The data suggest there has yet to be a time period at which the effective population (N_e) experienced a crash and that a large number of individuals founded the invasive population.

Table 4-1. Results from all populations in the computer program BOTTLENECK v 1.2.02. The Wilcoxon's test for all populations showed the probability (two tails for H excess and deficiency) to be 0.00171 for the assumption of all loci fitting I.A.M. mutation-drift equilibrium and a probability (two tails for H excess or deficiency) of 0.10986 for the assumption of all loci fitting S.M.M. mutation-drift equilibrium.

BOTTLENECK															
		Observed		under the I.A.M.				under the T.P.M.				under the S.M.M.			
Locus	**n**	**ko**	**He**	**Heq**	**S.D.**	**DH/sd**	**Prob**	**Heq**	**S.D.**	**DH/sd**	**Prob**	**Heq**	**S.D.**	**DH/sd**	**Prob**
NmeQ3	624	7	0.765	0.522	0.175	1.389	0.029	0.632	0.122	1.092	0.088	0.74	0.062	0.394	0.412
NmeQ17	610	6	0.503	0.468	0.186	0.19	0.474	0.584	0.126	-0.638	0.227	0.694	0.078	-2.435	0.027
NmeQ15	608	10	0.764	0.625	0.145	0.961	0.15	0.742	0.076	0.299	0.459	0.823	0.039	-1.515	0.077
NmeQ16	622	8	0.746	0.564	0.161	1.135	0.092	0.679	0.099	0.676	0.282	0.779	0.048	-0.679	0.219
NmeQ23	576	15	0.845	0.731	0.112	1.024	0.086	0.833	0.045	0.281	0.483	0.887	0.022	-1.911	0.056
NmeQ22	624	10	0.771	0.627	0.146	0.991	0.131	0.74	0.079	0.387	0.42	0.825	0.036	-1.484	0.079
NmeQ6	620	4	0.636	0.335	0.199	1.507	0.047	0.434	0.165	1.223	0.085	0.558	0.113	0.682	0.283
NmeQ11	586	8	0.809	0.557	0.166	1.523	0.007	0.677	0.102	1.302	0.035	0.778	0.049	0.633	0.292
NmeQ14	586	6	0.697	0.46	0.189	1.257	0.076	0.588	0.134	0.81	0.221	0.699	0.073	-0.031	0.418
NmeQ24	624	16	0.786	0.748	0.094	0.402	0.423	0.838	0.045	-1.16	0.118	0.893	0.02	-5.32	0.001
NmeQ4	612	7	0.629	0.518	0.175	0.634	0.423	0.639	0.112	-0.092	0.374	0.74	0.062	-1.791	0.06
NmeQ13	626	2	0.456	0.141	0.164	1.922	0.086	0.164	0.17	1.718	0.106	0.169	0.163	1.754	0.087
WILCOXON TEST															
Assumptions: all loci fit I.A.M., mutation-drift equilibrium.															
Probability (one tail for H deficiency): 1.00000															
Probability (one tail for H excess): 0.00012															
Probability (two tails for H excess and deficiency): 0.00024															
Assumptions: all loci fit T.P.M., mutation-drift equilibrium.															
Probability (one tail for H deficiency): 0.97388															
Probability (one tail for H excess): 0.03198															
Probability (two tails for H excess or deficiency): 0.06396															
Assumptions: all loci fit S.M.M., mutation-drift equilibrium.															
Probability (one tail for H deficiency): 0.05493															
Probability (one tail for H excess): 0.95386															
Probability (two tails for H excess or deficiency): 0.10986															

Table 4-2. Table output of the Evanno method results. Yellow highlight is performed dynamically on the website and shows the largest value in the Delta *K* column (Earl and vonHoldt 2012).

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	[Ln''(K)]	Delta K
1	3	-10846.8	0.6083	NA	NA	NA
2	3	-10954.83333	61.1098	-108.033	211.4333	3.4598
3	3	-11274.3	132.101	-319.467	402	3.043126
4	3	-11191.7667	102.3407	82.5333	174.0333	1.700529
5	3	-11283.2667	229.0033	-91.5	637.2	2.782493
6	3	-12011.9667	447.3519	-728.7	NA	NA

Table 4-3. Results of MIGRATE-N Posterior distribution table for 12 loci

Population	θ_1	$M_{j \to i}$	Effective immigrants/generation
Twenty Mile Creek	5.66667	20.667	29.27
	θ_2		
Elk Creek	5.93333	6	8.89

Using a P_{crit} of 0.02, the Harmonic Mean sample size for all 12 Round Goby populations is 292.7 after utilizing 2,049 independent comparisons. Within the same (0.02) critical value P_{crit} the estimated effective population size (N_e) is 637. The percentage of putative 95% confidence intervals that contained the true (N_e) are 427.1-1151.5 (parametric) and 240.5 to infinity (jackknife on loci). Per population, at a P_{crit}=0.02, the harmonic mean sample size of Misery Bay is 31.2, with 1647 independent comparisons, and an estimated (N_e) of -379.2, with 95% CI for (N_e) at 206.8-infinity (parametric) and 196.4-infinity (jackknife on loci). Twenty Mile Creek Embayment harmonic mean sample size is 6.8, with 862 independent comparisons, and an estimated (N_e) of -49.3, with 95% CI for (N_e) at 16.7-infinity (parametric) and 38.9-infinity (jackknife on loci). Twenty Mile Creek Seine harmonic mean sample size is 28.4, with 1453 independent comparisons, and an estimated (N_e) of 93.2, with 95% CI for (N_e) at 45.6-957.3 (parametric) and 35.1-infinity (jackknife on loci). Elk Creek Seine (2013) harmonic mean sample size is 36.2, with 1831 independent comparisons, and an estimated (N_e) of -369.8, with 95% CI for (N_e) at 306.4-infinity (parametric) and 248.1-infinity (jackknife on loci). Elk Creek Seine (2014) harmonic mean sample size is 24.9, with 1490 independent comparisons, and an estimated (N_e) of 86.5, with 95% CI for (N_e) at 41.0-1823.9 (parametric) and 37.0-infinity (jackknife on

loci). Elk Creek Embayment (2013) harmonic mean sample size is 10, with 1021 independent comparisons, and an estimated (N_e) of -51.6, with 95% CI for (N_e) at 49.8-infinity (parametric) and 94.5-infinity (jackknife on loci). Elk Creek Embayment (2014) harmonic mean sample size is 29.1, with 1448 independent comparisons, and an estimated (N_e) of -487.1, with 95% CI for (N_e) at 134.6-infinity (parametric) and 91.3-infinity (jackknife on loci). Walnut Creek Embayment harmonic mean sample size is 15.6, with 1350 independent comparisons, and an estimated (N_e) of 383.1, with 95% CI for (N_e) at 41.5-infinity (parametric) and 49.8-infinity (jackknife on loci). Lake Erie Trawl harmonic mean sample size is 61.2, with1860 independent comparisons, and an estimated (N_e) of 368.9, with 95% CI for (N_e) at 155.2-infinity (parametric) and 107.3-infinity (jackknife on loci). Port of Erie Terminal harmonic mean sample size is 9.1, with 660 independent comparisons, and an estimated (N_e) of -130.9, with 95% CI for (N_e) at 18-infinity (parametric) and 24.2-infinity (jackknife on loci). Marina Lake (2013) harmonic mean sample size is 11.4, with 1051 independent comparisons, and an estimated (N_e) of 81.1, with 95% CI for (N_e) at 19.2-infinity (parametric) and 26.4-infinity (jackknife on loci). Marina Lake (2014) harmonic mean sample size is 28.0, with 1354 independent comparisons, and an estimated (N_e) of -312.0, with 95% CI for (N_e) at 144.3-infinity (parametric) and 130.0-infinity (jackknife on loci).

The methods considered in LDNe are based on a genetic index that has two components: one from genetic drift (the signal) and one due to sampling a finite number of individuals. Unbiased estimators are dependent on knowing the sample size, so that the expected magnitude of sampling error can be calculated, and subtracting that value from the index. The exact amount of sampling error can be greater than expected, which presents the possibility for the correction to result in a negative estimate of (N_e), e.g., Misery Bay, Twenty Mile Creek Embayment, Elk Creek Seine (2013), Elk Creek Embayment (2014), Port of Erie Terminal and Marina Lake (2014). The usual interpretation in this case is that the estimate of (N_e) is *infinity*, i.e., there is no evidence for

variation in the genetic characteristic caused by a finite number of parents, which can be explained by sampling error. Similarly, an equivalent phenomenon also can occur with unbiased estimators of genetic distance of F_{ST} values. Therefore, the value is reported as infinity because we do not have enough information to determine otherwise. The Evanno Method employed by STRUCTURE HARVESTER (Earl and vonHoldt 2012) found K to equal 1(Figure 4-2).

DISCUSSION

Invasive species are often characterized by genetic plasticity, thus allowing for rapid adaptation to novel environments (Kornis et al. 2013). Round Goby are relatively recent invaders to North America (less than 30-years) and as previously noted, less than 20-year (1996) residents of Lake Erie. The need for a population genetics assessment of the Round Goby existed despite the large lake-wide populations that have few barriers impeding their ability to interbreed. Ecological theory predicts that recent invasions are likely to be founded by only a few individuals containing a fraction of the source population's genetic diversity, which may limit adaptive potential and success (Frankham 2005; Poulin et al. 2005, Brown and Stepien 2009). Some exotic introductions, however, experience little or no reduction in genetic diversity, due to large numbers of founding propagules and multiple founding sources (e.g., multiple ballast water purges from trans-Atlantic shipping vessels), which increases species adaptive potential (Brown and Stepien 2009). Brown and Stepien's (2009) invasion genetics research of Round Goby included samples from all five Great Lakes as well as native ranges in the Black and Caspian seas and the Dnieper River in the Ukraine.

Low genetic diversity in an invasive species typically results from founder and population bottleneck events and is considered to be the result of recent colonization (Bronnenhuber et al. 2011). Results from the present study and previous research (Brown and Stepien 2009) suggests that multiple introductions of the species from ballast water purging introduced multiple founders. It should be noted that while Brown and Stepien's range-wide

assessment showed high levels of genetic structure in Round Goby (2009) across the Great Lakes, regional levels that are geographically close to one another are less differentiated (Bronnenhuber et al. 2011). Collections of Round Goby for my study were made within a 30-mile centralized region. Brown and Stepien (2009) noted that in some peripheral expansion zones, reduced genetic diversity was an indication of secondary founder effects. Bronnenhuber et al. (2011) found that when peripheral or marginal zones were compared to the core region (i.e., locations near the invasion's origin in the St. Clair River), there were no observable differences in genetic diversity. They concluded that genetic structure among newly established populations in peripheral zones, when combined with evidence for short and long-distance dispersal, suggested the maintenance of genetic diversity due to dispersal stratification and population admixture during expansion (Bronnenhuber et al. 2011).

The Round Gobies collected and genotyped for this study appear to have reached a genetic drift mutation equilibrium displaying no current signs of a population bottleneck and there does not appear to be a strong signal of a founding event. Frequency data and Delta plots generated in STRUCTURE version 2.3.4 visually indicate panmixia for the Round Goby collections used for this study (Figure 4-4). Round Goby collected from the relatively close geographical ranges for this study appear to have little detectable genetic differentiation among populations (Table 4-10, 4-11; Figure 1-4, 2-4 and 3-4). The unknown but presumably large number of introductions via ballast water and the large number of founding individuals appear to be randomly mating with no barriers to reproduction within the study area.

Figure 4-2. Frequency distribution and plot of relatively even admixture proportions at the population level of, from the top down, *K*=2, *K*=3, *K*=4, and *K*=5. These plots were generated using STRUCTURE and further demonstrate the suggested inference that Round Goby populations along stream, shoreline, bay and lake habitats are a large, randomly mating panmictic population.

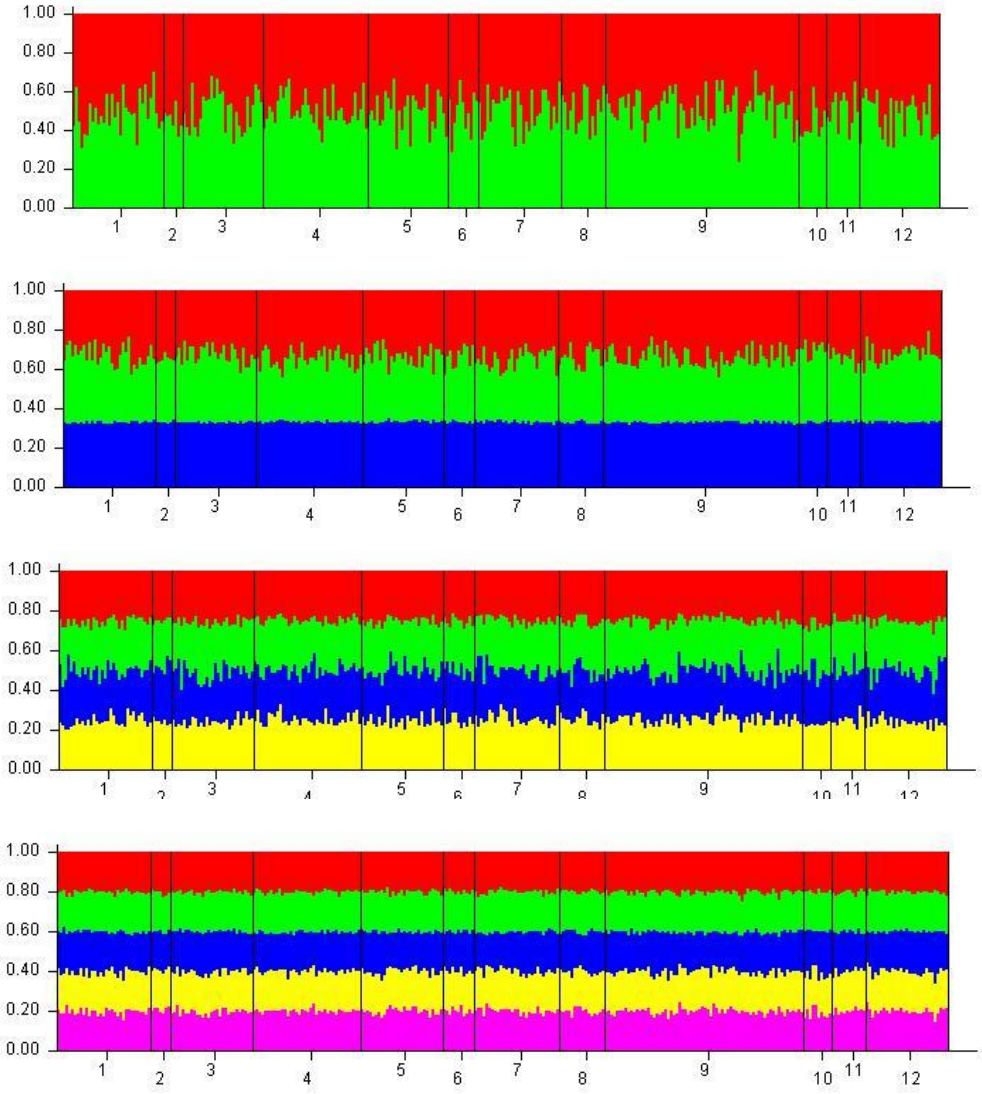


Table 4-4. GenAlEx output displaying Allele Frequencies for 12 loci (Nme_2125, Nme_1514, Nme_2703, Nme_2097, Nme_12681, Nme_10641, Nme_2565, Nme_00109, Nme_2649, Nme_1571, Nme_12042, Nme_3048) by Population for Codominant Data for 12 populations of *Neogobius melanostomus*. Population location names and acronyms are listed here: (Misery, Misery Bay); (20Mile_E, Twenty Mile Creek Embayment); (20Mile_S, Twenty Mile Creek Seine); (ElkCr_S, Elk Creek Seine May, 2013); (ElkCr_S2, Elk Creek Seine July, 2013); (ElkCr_E, Elk Creek Embayment 2013); (ElkCr_E14, Elk Creek Embayment 2014); (Walnut_E, Walnut Creek Embayment); (LE_T, Lake Erie Trawl); (POET, Port of Erie Terminal); (MarinaL_S13, Marina Lake Seine 2013) and (MarinaL_S14, Marina Lake Seine 2014).

Locus	Allele/ n	Misery	20Mile _E	20Mile_ S	ElkCr_ S	ElkCr_ S2	ElkCr_ E	ElkCr_ E14	Walnut _E	LE_T	POET	Marina L_S13	Marina L_S14
NmeQ3	**N**	35	7	29	40	29	11	30	15	78	10	14	30
	149	0.186	0.357	0.224	0.125	0.207	0.273	0.133	0.100	0.141	0.100	0.286	0.183
	152	0.114	0.000	0.086	0.138	0.172	0.045	0.117	0.200	0.109	0.000	0.071	0.067
	155	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.013	0.000	0.000	0.000
	158	0.229	0.286	0.172	0.150	0.086	0.318	0.150	0.233	0.212	0.200	0.107	0.217
	167	0.071	0.143	0.086	0.163	0.138	0.136	0.117	0.033	0.154	0.050	0.107	0.017
	170	0.343	0.214	0.328	0.388	0.379	0.227	0.450	0.433	0.353	0.650	0.321	0.433
	173	0.057	0.000	0.103	0.038	0.017	0.000	0.017	0.000	0.019	0.000	0.107	0.083
	uHe	0.786	0.468	0.715	0.786	0.884	0.759	0.677	0.765	0.675	0.740	0.578	0.351
NmeQ17	**N**	30	6	29	37	31	11	30	15	73	11	14	30
	198	0.000	0.000	0.000	0.027	0.048	0.045	0.033	0.000	0.014	0.000	0.036	0.050
	201	0.100	0.083	0.086	0.149	0.081	0.182	0.150	0.067	0.144	0.136	0.107	0.200
	204	0.717	0.750	0.724	0.676	0.677	0.636	0.700	0.533	0.740	0.682	0.750	0.533
	207	0.050	0.000	0.000	0.041	0.000	0.000	0.067	0.067	0.014	0.000	0.036	0.000
	210	0.117	0.167	0.172	0.095	0.177	0.136	0.050	0.300	0.089	0.136	0.071	0.183
	213	0.017	0.000	0.017	0.014	0.016	0.000	0.000	0.033	0.000	0.045	0.000	0.033
NmeQ15	**N**	33	7	29	38	28	10	29	15	72	10	12	29
	243	0.121	0.214	0.172	0.105	0.071	0.200	0.155	0.200	0.153	0.000	0.125	0.138
	246	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.000	0.000	0.017
	249	0.106	0.286	0.052	0.066	0.071	0.100	0.155	0.067	0.097	0.150	0.083	0.086
	252	0.015	0.000	0.017	0.039	0.018	0.000	0.017	0.033	0.021	0.000	0.000	0.000
	255	0.485	0.214	0.397	0.395	0.464	0.400	0.328	0.333	0.375	0.550	0.417	0.466
	258	0.000	0.000	0.034	0.000	0.036	0.050	0.017	0.000	0.021	0.000	0.083	0.069
	261	0.152	0.214	0.190	0.289	0.232	0.100	0.172	0.067	0.174	0.100	0.292	0.121
	264	0.106	0.071	0.138	0.079	0.107	0.100	0.155	0.200	0.125	0.150	0.000	0.103
	267	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.007	0.050	0.000	0.000
	273	0.000	0.000	0.000	0.026	0.000	0.050	0.000	0.067	0.014	0.000	0.000	0.000
NmeQ16	**N**	35	7	29	38	31	11	29	15	78	10	14	30

Table 4-4. Extended

	208	0.043	0.000	0.000	0.053	0.016	0.045	0.034	0.067	0.026	0.000	0.071	0.033
	218	0.000	0.000	0.052	0.026	0.016	0.000	0.017	0.000	0.013	0.050	0.036	0.033
	223	0.186	0.000	0.052	0.053	0.081	0.045	0.086	0.033	0.090	0.050	0.107	0.067
	228	0.043	0.071	0.017	0.013	0.000	0.045	0.017	0.000	0.000	0.000	0.071	0.083
	233	0.200	0.214	0.328	0.224	0.242	0.273	0.241	0.300	0.276	0.200	0.143	0.183
	243	0.343	0.500	0.397	0.382	0.355	0.364	0.397	0.233	0.404	0.450	0.250	0.333
	253	0.171	0.214	0.155	0.250	0.290	0.227	0.207	0.367	0.192	0.250	0.321	0.267
	258	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NmeQ23	**N**	31	7	29	39	23	10	29	15	68	8	13	28
	197	0.065	0.000	0.017	0.051	0.000	0.000	0.086	0.033	0.037	0.000	0.000	0.018
	203	0.065	0.071	0.017	0.051	0.022	0.000	0.017	0.033	0.051	0.000	0.077	0.036
	206	0.048	0.000	0.052	0.051	0.043	0.100	0.069	0.067	0.044	0.125	0.115	0.125
	209	0.097	0.214	0.121	0.064	0.152	0.250	0.017	0.000	0.081	0.188	0.000	0.161
	212	0.032	0.000	0.000	0.051	0.022	0.050	0.017	0.000	0.015	0.000	0.077	0.036
	215	0.016	0.071	0.000	0.000	0.087	0.000	0.000	0.000	0.015	0.000	0.038	0.018
	221	0.226	0.143	0.155	0.141	0.196	0.150	0.155	0.233	0.147	0.563	0.115	0.143
	224	0.000	0.000	0.000	0.051	0.043	0.000	0.069	0.033	0.029	0.000	0.077	0.018
	227	0.065	0.071	0.138	0.077	0.022	0.000	0.069	0.033	0.147	0.000	0.038	0.036
	233	0.210	0.286	0.345	0.333	0.348	0.350	0.328	0.400	0.265	0.125	0.346	0.357
	236	0.048	0.143	0.034	0.013	0.043	0.000	0.086	0.033	0.059	0.000	0.000	0.000
	242	0.081	0.000	0.121	0.051	0.000	0.100	0.052	0.067	0.081	0.000	0.038	0.054
	248	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.000
	251	0.032	0.000	0.000	0.051	0.022	0.000	0.000	0.033	0.022	0.000	0.038	0.000
	257	0.016	0.000	0.000	0.013	0.000	0.000	0.034	0.033	0.000	0.000	0.038	0.000
NmeQ22	**N**	33	7	29	40	30	11	30	15	78	9	14	30
	195	0.015	0.071	0.017	0.013	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.033
	201	0.000	0.071	0.000	0.013	0.033	0.000	0.000	0.000	0.019	0.056	0.000	0.017
	207	0.030	0.071	0.017	0.013	0.067	0.000	0.033	0.033	0.013	0.111	0.000	0.000
	210	0.258	0.214	0.172	0.250	0.183	0.227	0.217	0.300	0.263	0.167	0.286	0.367
	213	0.242	0.143	0.379	0.413	0.300	0.500	0.267	0.300	0.250	0.167	0.464	0.233
	216	0.348	0.214	0.259	0.225	0.333	0.136	0.300	0.200	0.314	0.167	0.179	0.217
	219	0.061	0.000	0.034	0.038	0.017	0.091	0.067	0.000	0.083	0.167	0.000	0.067

Table 4-4. Extended

	225	0.015	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.026	0.167	0.000	0.000
	228	0.015	0.071	0.121	0.025	0.067	0.045	0.033	0.133	0.026	0.000	0.036	0.000
	231	0.015	0.143	0.000	0.013	0.000	0.000	0.017	0.033	0.006	0.000	0.036	0.067
NmeQ6	**N**	35	6	29	39	32	11	29	15	75	11	13	30
	135	0.343	0.250	0.293	0.256	0.297	0.318	0.310	0.233	0.320	0.273	0.154	0.267
	138	0.257	0.250	0.310	0.231	0.250	0.227	0.241	0.100	0.113	0.364	0.192	0.217
	144	0.386	0.417	0.397	0.500	0.453	0.455	0.448	0.667	0.553	0.364	0.654	0.517
	147	0.014	0.083	0.000	0.013	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000
NmeQ11	**N**	35	7	27	36	29	10	30	15	58	11	12	29
	150	0.014	0.000	0.000	0.014	0.052	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	156	0.014	0.000	0.019	0.028	0.000	0.000	0.017	0.000	0.009	0.000	0.000	0.000
	159	0.129	0.071	0.167	0.125	0.328	0.050	0.183	0.167	0.138	0.500	0.250	0.172
	162	0.200	0.071	0.130	0.250	0.224	0.200	0.283	0.233	0.276	0.091	0.167	0.241
	163	0.029	0.071	0.093	0.083	0.017	0.000	0.117	0.167	0.095	0.000	0.042	0.069
	165	0.157	0.071	0.130	0.181	0.103	0.250	0.067	0.133	0.095	0.364	0.125	0.172
	168	0.400	0.643	0.389	0.236	0.138	0.300	0.233	0.267	0.276	0.045	0.292	0.293
	171	0.057	0.071	0.074	0.083	0.138	0.200	0.100	0.033	0.112	0.000	0.125	0.052
NmeQ14	**N**	34	7	28	38	31	9	30	14	59	11	11	29
	220	0.176	0.214	0.179	0.132	0.113	0.056	0.183	0.143	0.178	0.227	0.364	0.138
	226	0.103	0.071	0.125	0.132	0.161	0.056	0.117	0.071	0.102	0.182	0.000	0.069
	229	0.162	0.214	0.143	0.132	0.226	0.278	0.167	0.321	0.186	0.091	0.000	0.172
	235	0.044	0.214	0.125	0.053	0.065	0.056	0.133	0.036	0.068	0.136	0.045	0.052
	238	0.515	0.286	0.429	0.553	0.435	0.556	0.400	0.429	0.458	0.364	0.545	0.569
	241	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.045	0.000
NmeQ24	**N**	36	7	29	40	32	11	29	15	76	11	13	29
	111	0.000	0.000	0.034	0.025	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.017
	126	0.431	0.357	0.448	0.325	0.297	0.273	0.310	0.567	0.395	0.364	0.308	0.379
	147	0.236	0.143	0.121	0.238	0.203	0.091	0.241	0.067	0.132	0.182	0.192	0.207
	153	0.000	0.000	0.000	0.000	0.000	0.045	0.000	0.000	0.000	0.000	0.000	0.000
	159	0.069	0.000	0.017	0.075	0.016	0.000	0.000	0.067	0.072	0.091	0.077	0.069
	162	0.139	0.214	0.103	0.188	0.172	0.273	0.155	0.033	0.197	0.136	0.231	0.121
	165	0.028	0.000	0.052	0.013	0.047	0.045	0.017	0.000	0.013	0.045	0.000	0.000

Table 4-4. Extended

	174	0.028	0.000	0.034	0.050	0.031	0.000	0.017	0.000	0.033	0.000	0.000	0.034
	189	0.000	0.000	0.069	0.000	0.000	0.000	0.017	0.000	0.000	0.091	0.000	0.000
	192	0.000	0.000	0.034	0.000	0.047	0.136	0.034	0.133	0.039	0.000	0.000	0.017
	201	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.017
	234	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.000
	237	0.014	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.017
	240	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	243	0.056	0.286	0.086	0.088	0.156	0.136	0.207	0.133	0.086	0.091	0.192	0.121
	249	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.000
NmeQ4	**N**	35	7	29	38	29	11	30	13	71	10	13	29
	220	0.314	0.643	0.397	0.355	0.241	0.409	0.433	0.462	0.437	0.400	0.346	0.259
	223	0.029	0.071	0.000	0.000	0.000	0.000	0.033	0.000	0.007	0.000	0.000	0.034
	225	0.000	0.071	0.000	0.039	0.000	0.000	0.017	0.038	0.000	0.000	0.038	0.000
	227	0.029	0.000	0.034	0.000	0.034	0.045	0.017	0.000	0.028	0.000	0.000	0.000
	230	0.057	0.000	0.172	0.066	0.103	0.091	0.067	0.077	0.085	0.050	0.077	0.155
	231	0.571	0.214	0.362	0.513	0.586	0.364	0.433	0.423	0.415	0.500	0.538	0.552
	232	0.000	0.000	0.034	0.026	0.034	0.091	0.000	0.000	0.028	0.050	0.000	0.000
NmeQ13	**N**	36	7	29	39	31	11	30	15	78	11	14	30
	258	0.222	0.429	0.397	0.410	0.339	0.409	0.300	0.267	0.506	0.364	0.250	0.100
	264	0.778	0.571	0.603	0.590	0.661	0.591	0.700	0.733	0.494	0.636	0.750	0.900

Table 4-5. Results of summary of Chi-Square tests for Hardy-Weinberg Equilibrium (HWE) in Round Goby sampled for this project in 12 populations at 12 loci for 314 individual specimens. Each specimen was tested at each of the 12 loci used for this study. Results were generated using the computer software GenAlEx 6.5 (Peakall and Smouse 2006).

Locus	Chi Square	DF	Probability	Significance
NmeQ3	19.715	21	0.539	ns
NmeQ17	29.833	15	0.013	***P<0.05***
NmeQ15	31.211	45	0.941	ns
NmeQ16	15.285	28	0.975	ns
NmeQ23	356.570	105	0.000	***P<0.001***
NmeQ22	73.077	45	0.005	***P<0.01***
NmeQ6	1.629	6	0.950	ns
NmeQ11	44.012	28	0.028	***P<0.05***
NmeQ14	14.518	15	0.487	ns
NmeQ24	263.111	120	0.000	***P<0.001***
NmeQ4	7.147	21	0.998	ns
NmeQ13	0.106	1	0.745	ns

Table 4-6. Heterozygosity, F-statistics and Polymorphism by Population for Codominant Data. The following table lists the Sample Size (N), Number of Alleles (N_a), Number of effective Alleles (N_e), Information Index (I), Observed Heterozygosity (H_o), Expected Heterozygosity (H_e), Unbiased Expected Heterozygosity (uH_e) and Fixation Index (F). These data represent 12 populations screened for 12-loci with an original count of 333 individual specimens.

Pop	Locus	*N*	N_a	N_e	*I*	H_o	H_e	uH_e	*F*
Misery	**NmeQ3**	35	6.000	4.430	1.617	0.914	0.774	0.786	-0.181
	NmeQ17	30	5.000	1.852	0.938	0.400	0.460	0.468	0.130
	NmeQ15	33	7.000	3.382	1.496	0.727	0.704	0.715	-0.033
	NmeQ16	35	7.000	4.438	1.635	0.686	0.775	0.786	0.115
	NmeQ23	31	13.000	7.657	2.271	0.710	0.869	0.884	0.184
	NmeQ22	33	9.000	3.967	1.590	0.848	0.748	0.759	-0.134
	NmeQ6	35	4.000	3.006	1.144	0.571	0.667	0.677	0.144
	NmeQ11	35	8.000	4.070	1.629	0.657	0.754	0.765	0.129
	NmeQ14	34	5.000	2.987	1.314	0.735	0.665	0.675	-0.105
	NmeQ24	36	8.000	3.703	1.582	0.750	0.730	0.740	-0.027
	NmeQ4	35	5.000	2.324	1.050	0.600	0.570	0.578	-0.053
	NmeQ13	36	2.000	1.528	0.530	0.444	0.346	0.351	-0.286
20Mile_E	**NmeQ3**	7	4.000	3.630	1.334	0.857	0.724	0.780	-0.183
	NmeQ17	6	3.000	1.674	0.721	0.167	0.403	0.439	0.586
	NmeQ15	7	5.000	4.455	1.537	0.714	0.776	0.835	0.079
	NmeQ16	7	4.000	2.882	1.195	0.714	0.653	0.703	-0.094
	NmeQ23	7	7.000	5.444	1.810	0.857	0.816	0.879	-0.050
	NmeQ22	7	8.000	6.533	1.970	0.857	0.847	0.912	-0.012
	NmeQ6	6	4.000	3.273	1.265	0.833	0.694	0.758	-0.200
	NmeQ11	7	6.000	2.279	1.227	0.571	0.561	0.604	-0.018
	NmeQ14	7	5.000	4.455	1.537	0.714	0.776	0.835	0.079
	NmeQ24	7	4.000	3.630	1.334	0.857	0.724	0.780	-0.183
	NmeQ4	7	4.000	2.130	0.991	0.714	0.531	0.571	-0.346
	NmeQ13	7	2.000	1.960	0.683	0.286	0.490	0.527	0.417
20Mile_S	**NmeQ3**	29	6.000	4.698	1.661	0.828	0.787	0.801	-0.051
	NmeQ17	29	4.000	1.780	0.818	0.414	0.438	0.446	0.056
	NmeQ15	29	7.000	4.063	1.598	0.828	0.754	0.767	-0.098
	NmeQ16	29	6.000	3.398	1.398	0.690	0.706	0.718	0.023
	NmeQ23	29	9.000	5.112	1.849	0.690	0.804	0.819	0.143

Table 4-6. Expanded

	NmeQ22	29	7.000	3.894	1.532	0.724	0.743	0.756	0.026
	NmeQ6	29	3.000	2.946	1.090	0.552	0.661	0.672	0.165
	NmeQ11	27	7.000	4.405	1.683	0.815	0.773	0.788	-0.054
	NmeQ14	28	5.000	3.742	1.469	0.857	0.733	0.746	-0.170
	NmeQ24	29	10.000	4.083	1.817	0.655	0.755	0.768	0.132
	NmeQ4	29	5.000	3.121	1.270	0.759	0.680	0.691	-0.116
	NmeQ13	29	2.000	1.918	0.672	0.517	0.479	0.487	-0.081
ElkCr_S	**NmeQ3**	40	6.000	4.255	1.603	0.800	0.765	0.775	-0.046
	NmeQ17	37	6.000	2.040	1.057	0.486	0.510	0.517	0.046
	NmeQ15	38	7.000	3.795	1.566	0.763	0.736	0.746	-0.036
	NmeQ16	38	7.000	3.780	1.512	0.684	0.735	0.745	0.070
	NmeQ23	39	13.000	6.259	2.194	0.795	***0.840***	0.851	0.054
	NmeQ22	40	9.000	3.497	1.482	0.725	0.714	0.723	-0.015
	NmeQ6	39	4.000	2.709	1.090	0.615	0.631	0.639	0.024
	NmeQ11	36	8.000	5.515	1.829	0.861	0.819	0.830	-0.052
	NmeQ14	38	5.000	2.777	1.283	0.632	0.640	0.648	0.013
	NmeQ24	40	8.000	4.678	1.725	0.700	0.786	0.796	0.110
	NmeQ4	38	5.000	2.524	1.112	0.658	0.604	0.612	-0.089
	NmeQ13	39	2.000	1.938	0.677	0.359	0.484	0.490	0.258
ElkCr_S2	**NmeQ3**	29	6.000	4.112	1.551	0.690	0.757	0.770	0.089
	NmeQ17	31	5.000	2.002	0.987	0.452	0.501	0.509	0.098
	NmeQ15	28	7.000	3.416	1.502	0.750	0.707	0.720	-0.060
	NmeQ16	31	6.000	3.626	1.406	0.839	0.724	0.736	-0.158
	NmeQ23	23	11.000	5.062	1.927	0.435	0.802	0.820	0.458
	NmeQ22	30	7.000	4.082	1.581	0.800	0.755	0.768	-0.060
	NmeQ6	32	3.000	2.809	1.066	0.781	0.644	0.654	-0.213
	NmeQ11	29	7.000	4.778	1.705	0.690	0.791	0.805	0.128
	NmeQ14	31	5.000	3.527	1.415	0.774	0.716	0.728	-0.081
	NmeQ24	32	10.000	5.278	1.867	0.781	0.811	0.823	0.036
	NmeQ4	29	5.000	2.410	1.123	0.655	0.585	0.595	-0.120
	NmeQ13	31	2.000	1.811	0.640	0.613	0.448	0.455	-0.368
ElkCr_E	**NmeQ3**	11	5.000	4.033	1.468	0.545	0.752	0.788	0.275

Table 4-6. Expanded

	NmeQ17	11	4.000	2.180	1.010	0.545	0.541	0.567	-0.008
	NmeQ15	10	7.000	4.255	1.679	0.900	0.765	0.805	-0.176
	NmeQ16	11	6.000	3.781	1.480	0.818	0.736	0.771	-0.112
	NmeQ23	10	6.000	4.348	1.609	0.600	0.770	0.811	0.221
	NmeQ22	11	5.000	3.025	1.313	0.455	0.669	0.701	0.321
	NmeQ6	11	3.000	2.782	1.059	0.636	0.640	0.671	0.006
	NmeQ11	10	5.000	4.255	1.501	0.500	0.765	0.805	0.346
	NmeQ14	9	5.000	2.531	1.164	0.889	0.605	0.641	-0.469
	NmeQ24	11	7.000	5.042	1.751	0.636	0.802	0.840	0.206
	NmeQ4	11	5.000	3.143	1.310	0.909	0.682	0.714	-0.333
	NmeQ13	11	2.000	1.936	0.677	0.273	0.483	0.506	0.436
ElkCr_E14	**NmeQ3**	30	7.000	3.696	1.550	0.733	0.729	0.742	-0.005
	NmeQ17	30	5.000	1.921	0.978	0.500	0.479	0.488	-0.043
	NmeQ15	29	7.000	4.765	1.676	0.655	0.790	0.804	0.171
	NmeQ16	29	7.000	3.738	1.503	0.759	0.732	0.745	-0.036
	NmeQ23	29	12.000	6.050	2.110	0.483	***0.835***	0.849	0.422
	NmeQ22	30	9.000	4.592	1.739	0.833	0.782	0.795	-0.065
	NmeQ6	29	3.000	2.813	1.066	0.724	0.644	0.656	-0.124
	NmeQ11	30	7.000	5.085	1.738	0.733	0.803	0.817	0.087
	NmeQ14	30	5.000	3.956	1.495	0.667	0.747	0.760	0.108
	NmeQ24	29	8.000	4.473	1.647	0.586	0.776	0.790	0.245
	NmeQ4	30	6.000	2.620	1.155	0.633	0.618	0.629	-0.024
	NmeQ13	30	2.000	1.724	0.611	0.533	0.420	0.427	-0.270
Walnut_E	**NmeQ3**	15	5.000	3.409	1.367	0.800	0.707	0.731	-0.132
	NmeQ17	15	5.000	2.601	1.171	0.600	0.616	0.637	0.025
	NmeQ15	15	8.000	4.839	1.778	0.733	0.793	0.821	0.076
	NmeQ16	15	5.000	3.516	1.363	0.733	0.716	0.740	-0.025
	NmeQ23	15	11.000	4.327	1.861	0.600	0.769	0.795	0.220
	NmeQ22	15	6.000	4.167	1.540	0.800	0.760	0.786	-0.053
	NmeQ6	15	3.000	1.965	0.840	0.533	0.491	0.508	-0.086
	NmeQ11	15	6.000	5.000	1.671	0.933	0.800	0.828	-0.167
	NmeQ14	14	5.000	3.187	1.313	0.857	0.686	0.712	-0.249

Table 4-6. Expanded

	NmeQ24	15	6.000	2.727	1.334	0.667	0.633	0.655	-0.053
	NmeQ4	13	4.000	2.504	1.043	0.615	0.601	0.625	-0.025
	NmeQ13	15	2.000	1.642	0.580	0.533	0.391	0.405	-0.364
LE_T	**NmeQ3**	78	7.000	4.444	1.634	0.756	0.775	0.780	0.024
	NmeQ17	73	5.000	1.736	0.835	0.397	0.424	0.427	0.063
	NmeQ15	72	10.000	4.535	1.760	0.847	0.780	0.785	-0.087
	NmeQ16	78	6.000	3.510	1.405	0.718	0.715	0.720	-0.004
	NmeQ23	68	14.000	7.265	2.249	0.765	0.862	0.869	0.113
	NmeQ22	78	9.000	4.183	1.621	0.692	0.761	0.766	0.090
	NmeQ6	75	4.000	2.372	0.996	0.653	0.578	0.582	-0.130
	NmeQ11	58	7.000	4.954	1.717	0.828	0.798	0.805	-0.037
	NmeQ14	59	6.000	3.438	1.433	0.763	0.709	0.715	-0.076
	NmeQ24	76	12.000	4.391	1.807	0.711	0.772	0.777	0.080
	NmeQ4	71	6.000	2.688	1.172	0.648	0.628	0.632	-0.032
	NmeQ13	78	2.000	2.000	0.693	0.577	0.500	0.503	-0.154
POET	**NmeQ3**	10	4.000	2.105	0.982	0.600	0.525	0.553	-0.143
	NmeQ17	11	4.000	1.984	0.945	0.455	0.496	0.519	0.083
	NmeQ15	10	5.000	2.778	1.278	0.800	0.640	0.674	-0.250
	NmeQ16	10	5.000	3.226	1.327	0.800	0.690	0.726	-0.159
	NmeQ23	8	4.000	2.612	1.157	0.250	0.617	0.658	0.595
	NmeQ22	9	7.000	6.480	1.898	0.889	0.846	0.895	-0.051
	NmeQ6	11	3.000	2.951	1.090	0.818	0.661	0.693	-0.238
	NmeQ11	11	4.000	2.547	1.073	0.364	0.607	0.636	0.401
	NmeQ14	11	5.000	4.102	1.504	0.818	0.756	0.792	-0.082
	NmeQ24	11	7.000	4.745	1.744	0.818	0.789	0.827	-0.037
	NmeQ4	10	4.000	2.410	1.013	0.800	0.585	0.616	-0.368
	NmeQ13	11	2.000	1.862	0.655	0.545	0.463	0.485	-0.179
MarinaL_S13	**NmeQ3**	14	6.000	4.455	1.629	0.714	0.776	0.804	0.079
	NmeQ17	14	5.000	1.719	0.882	0.429	0.418	0.434	-0.024
	NmeQ15	12	5.000	3.470	1.398	0.667	0.712	0.743	0.063
	NmeQ16	14	7.000	4.780	1.725	0.714	0.791	0.820	0.097
	NmeQ23	13	11.000	5.828	2.084	0.615	0.828	0.862	0.257

Table 4-6. Expanded

	NmeQ22	14	5.000	3.015	1.260	0.857	0.668	0.693	-0.282
	NmeQ6	13	3.000	2.048	0.883	0.538	0.512	0.532	-0.052
	NmeQ11	12	6.000	4.800	1.657	0.833	0.792	0.826	-0.053
	NmeQ14	11	4.000	2.305	0.979	0.636	0.566	0.593	-0.124
	NmeQ24	13	5.000	4.390	1.532	0.769	0.772	0.803	0.004
	NmeQ4	13	4.000	2.397	1.023	0.615	0.583	0.606	-0.056
	NmeQ13	14	2.000	1.600	0.562	0.357	0.375	0.389	0.048
MarinaL_S14	**NmeQ3**	30	6.000	3.571	1.461	0.700	0.720	0.732	0.028
	NmeQ17	30	5.000	2.765	1.231	0.567	0.638	0.649	0.112
	NmeQ15	29	7.000	3.657	1.585	0.690	0.727	0.739	0.051
	NmeQ16	30	7.000	4.358	1.644	0.633	0.771	0.784	0.178
	NmeQ23	28	11.000	5.074	1.929	0.714	0.803	0.818	0.110
	NmeQ22	30	7.000	4.063	1.581	0.800	0.754	0.767	-0.061
	NmeQ6	30	3.000	2.597	1.025	0.567	0.615	0.625	0.079
	NmeQ11	29	6.000	4.738	1.647	0.759	0.789	0.803	0.038
	NmeQ14	29	5.000	2.632	1.235	0.724	0.620	0.631	-0.168
	NmeQ24	29	10.000	4.485	1.785	0.655	0.777	0.791	0.157
	NmeQ4	29	4.000	2.522	1.083	0.448	0.603	0.614	0.257
	NmeQ13	30	2.000	1.220	0.325	0.200	0.180	0.183	-0.111

Table 4-7. Mean and SE over Loci for each Population. The following table lists Number of Different Alleles (N_a), number of Effective Alleles (N_e), Shannon's Information Index (I), Observed Heterozygosity (H_o), Expected Heterozygosity (H_e), Unbiased Exptected Heterozygosity (uH_e) and Fixation Index (F).

Pop		N	N_a	N_e	I	H_o	H_e	uH_e	F
Misery	**Mean**	34.000	6.583	3.612	1.400	0.670	0.672	0.682	-0.010
	SE	0.550	0.811	0.460	0.128	0.043	0.043	0.043	0.043
20Mile_E	**Mean**	6.833	4.667	3.529	1.300	0.679	0.666	0.719	0.006
	SE	0.112	0.482	0.429	0.112	0.067	0.041	0.043	0.076
20Mile_S	**Mean**	28.750	5.917	3.597	1.405	0.694	0.693	0.705	-0.002
	SE	0.179	0.668	0.296	0.109	0.040	0.034	0.035	0.032
ElkCr_S	**Mean**	38.500	6.667	3.647	1.427	0.673	0.689	0.698	0.028
	SE	0.359	0.801	0.391	0.118	0.041	0.033	0.034	0.027
ElkCr_S2	**Mean**	29.667	6.167	3.576	1.398	0.688	0.687	0.699	-0.021
	SE	0.711	0.737	0.333	0.110	0.038	0.034	0.035	0.060
ElkCr_E	**Mean**	10.583	5.000	3.443	1.335	0.642	0.684	0.718	0.059
	SE	0.193	0.426	0.283	0.090	0.058	0.029	0.030	0.083
ElkCr_E14	**Mean**	29.583	6.500	3.786	1.439	0.653	0.697	0.709	0.039
	SE	0.149	0.764	0.379	0.119	0.032	0.038	0.039	0.053
Walnut_E	**Mean**	14.750	5.500	3.324	1.322	0.700	0.664	0.687	-0.069
	SE	0.179	0.669	0.315	0.109	0.037	0.036	0.037	0.044
LE_T	**Mean**	72.000	7.333	3.793	1.443	0.696	0.692	0.697	-0.012
	SE	2.045	0.980	0.442	0.130	0.035	0.038	0.039	0.026
POET	**Mean**	10.250	4.500	3.150	1.222	0.663	0.640	0.673	-0.035
	SE	0.279	0.417	0.390	0.102	0.062	0.034	0.036	0.080

Table 4-7. Expanded

MarinaL_S13	**Mean**	13.083	5.250	3.401	1.301	0.646	0.649	0.675	-0.004
	SE	0.288	0.653	0.409	0.128	0.043	0.045	0.047	0.038
MarinaL_S14	**Mean**	29.417	6.083	3.474	1.378	0.621	0.666	0.678	0.056
	SE	0.193	0.753	0.329	0.125	0.047	0.049	0.050	0.035

Grand Mean and SE over Loci and Pops

		N	***Na***	***Ne***	***I***	***Ho***	***He***	***uHe***	***F***
Total	**Mean**	26.451	5.847	3.528	1.364	0.669	0.675	0.695	0.003
	SE	1.446	0.206	0.105	0.032	0.013	0.011	0.011	0.015

Table 4-8. F-statistics and Estimates of N_m over all Populations for each Locus. The following functions are listed: F_{IS} = The inbreeding coefficient within individuals relative to the subpopulation. It measures the reduction in heterozygosity of an individual due to non-random mating within its subpopulation (Peakall and Smouse 2012).

$$F_{IS} = \frac{\overline{H}_e - \overline{H}_o}{\overline{H_e}}$$

F_{IT} = The inbreeding coefficient within individual relative to the total. This statistic takes into account the effects of both non=random mating within subpopulations and genetic differentiation among the subpopulations (Peakall and Smouse 2012).

$$F_{IT} = \frac{H_T - \overline{H}_O}{H_T}$$

F_{ST} =The inbreeding coefficient within subpopulations relative to the total. This statistic measures the genetic differentiation between subpopulations, i.e., the proportion of the total genetic diversity distributed among the subpopulations. F_{ST} is almost always ≥ 0 (Peakall and Smouse 2012).

$$F_{ST} = \frac{H_T - \overline{H}_e}{H_T}$$

F-Statistics and Estimates of Nm over All Pops for each Locus

All Pops.	**Locus**	F_{is}	F_{it}	F_{st}	N_m
	NmeQ3	-0.017	0.022	0.038	6.244
	NmeQ17	0.087	0.109	0.024	10.113
	NmeQ15	-0.021	0.009	0.030	8.087
	NmeQ16	-0.005	0.015	0.020	12.213
	NmeQ23	0.219	0.252	0.043	5.618
	NmeQ22	-0.026	0.012	0.037	6.556
	NmeQ6	-0.052	-0.024	0.026	9.296
	NmeQ11	0.056	0.114	0.061	3.822
	NmeQ14	-0.103	-0.066	0.034	7.168
	NmeQ24	0.059	0.086	0.029	8.498
	NmeQ4	-0.108	-0.064	0.040	6.066
	NmeQ13	-0.036	0.017	0.051	4.687
	Mean	0.004	0.040	0.036	7.364
	SE	0.026	0.026	0.003	0.691

Figure 4-3. Mean Allelic Patterns across 12 Populations of *Neogobius melanostomus* in Erie County, PA, and the offshore Pennsylvania waters of Lake Erie (Provided by Dr. Tim L. King, USGS Leetown Science Center)**.**

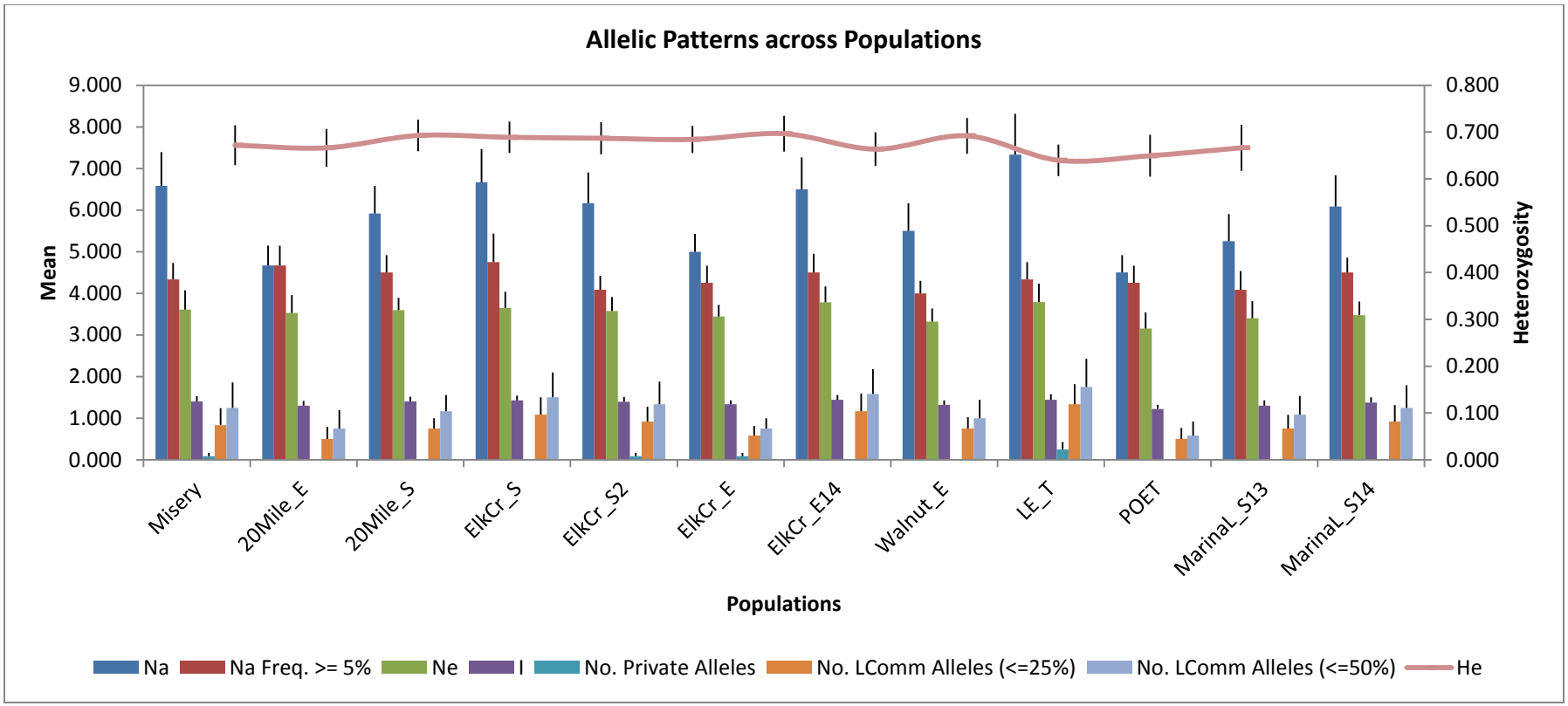
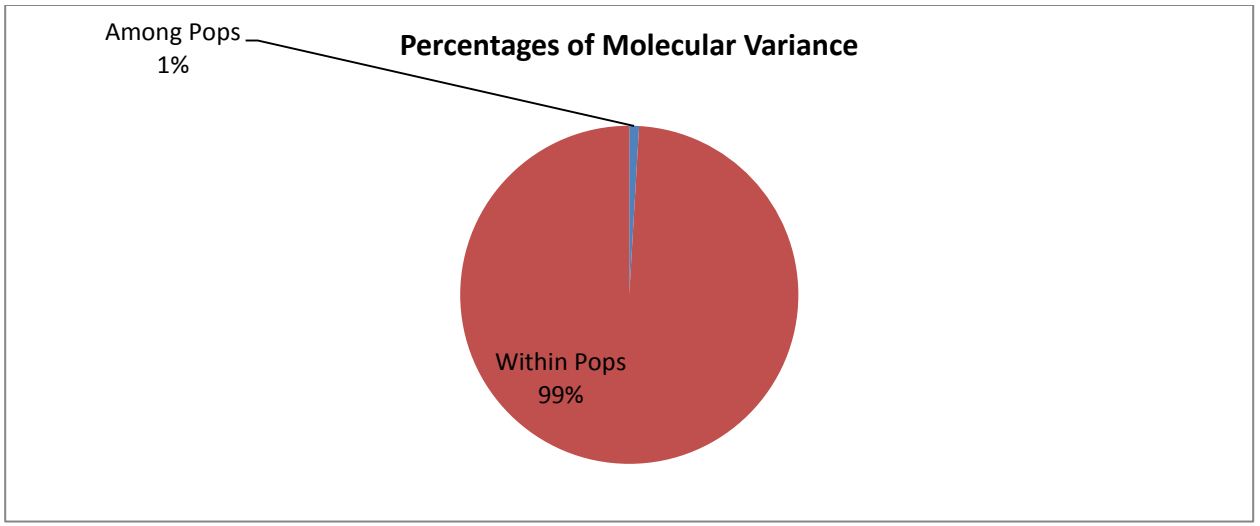
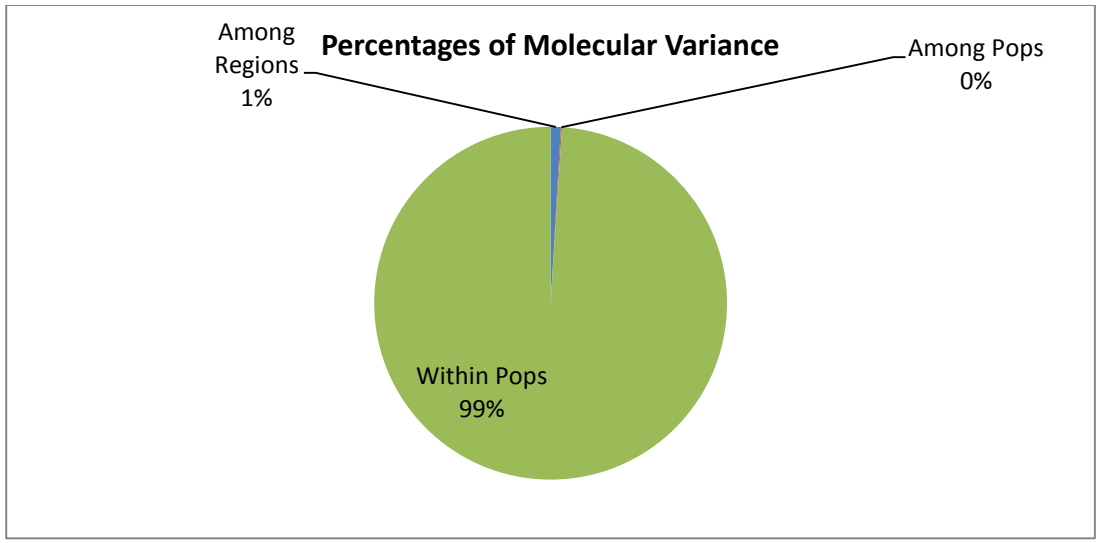


Figure 4-4. Results of AMOVA as generated by GenAlEx. Input as codominant allelic distance matrix for calculation of F_{ST} (within individual analysis suppressed) and corresponding Summary AMOVA Table. The chart and table display only 1-percent variation among populations suggesting panmixia.



Summary AMOVA Table					
Source	**df**	**SS**	**MS**	**Est. Var.**	**%**
Among Pops	11	68.770	6.252	0.040	1%
Within Pops	616	2616.279	4.247	4.247	99%
Total	627	2685.049		4.287	100%

Figure 4-5. Results of AMOVA as generated by GenAlEx. Input as codominant allelic distance matrix for calculation of F_{ST} (within individual analysis suppressed) and corresponding Summary AMOVA Table. The chart and table display only 1-percent variation among regions and zero-percent among populations, thus suggesting panmixia.



Summary AMOVA Table					
Source	**df**	**SS**	**MS**	**Est. Var.**	**%**
Among Regions	6	46.653	7.775	0.040	1%
Among Pops	5	22.118	4.424	0.004	0%
Within Pops	616	2616.279	4.247	4.247	99%
Total	627	2685.049		4.291	100%

Table 4-9. Top table represents Results of Pairwise Population F_{ST} Analysis. Input as codominant allelic distance matrix for calculation of F_{ST} (within individual analysis suppressed. Results were obtained from 314 Round Goby specimens representing 12 populations after 999 permutations in GenAlEx.

Table 4-10. Bottom table represents results of Pairwise population F_{ST} values. The F_{ST} values are assumed to be zero, and the values above the diagonal are the probability that the F_{ST} is greater than zero.

Pairwise Population Fst Values													
	Misery	**20Mile_E**	**20Mile_S**	**ElkCr_S**	**ElkCr_S2**	**ElkCr_E**	**ElkCr_E14**	**Walnut_E**	**LE_T**	**POET**	**MarinaL_S13**	**MarinaL_S14**	
Misery	0.000	0.092	0.099	0.103	0.117	0.226	0.310	0.079	0.003	0.010	0.136	0.413	**Misery**
20Mile_E	0.014	0.000	0.437	0.061	0.023	0.441	0.334	0.064	0.103	0.007	0.050	0.009	**20Mile_E**
20Mile_S	0.005	0.000	0.000	0.231	0.038	0.444	0.312	0.165	0.034	0.005	0.066	0.006	**20Mile_S**
ElkCr_S	0.004	0.017	0.002	0.000	0.202	0.475	0.412	0.087	0.035	0.004	0.439	0.008	**ElkCr_S**
ElkCr_S2	0.005	0.028	0.009	0.003	0.000	0.331	0.247	0.043	0.007	0.094	0.143	0.034	**ElkCr_S2**
ElkCr_E	0.005	0.000	0.000	0.000	0.003	0.000	0.382	0.266	0.442	0.013	0.449	0.114	**ElkCr_E**
ElkCr_E14	0.001	0.004	0.002	0.000	0.002	0.002	0.000	0.237	0.063	0.028	0.261	0.062	**ElkCr_E14**
Walnut_E	0.009	0.023	0.006	0.008	0.014	0.005	0.004	0.000	0.028	0.016	0.128	0.194	**Walnut_E**
LE_T	0.013	0.013	0.006	0.006	0.012	0.000	0.005	0.011	0.000	0.001	0.025	0.001	**LE_T**
POET	0.025	0.051	0.029	0.027	0.013	0.031	0.020	0.033	0.034	0.000	0.004	0.007	**POET**
MarinaL_S13	0.008	0.027	0.012	0.000	0.007	0.000	0.004	0.011	0.017	0.043	0.000	0.283	**MarinaL_S13**
MarinaL_S14	0.000	0.033	0.016	0.011	0.009	0.009	0.007	0.005	0.028	0.029	0.003	0.000	**MarinaL_S14**
	Misery	**20Mile_E**	**20Mile_S**	**ElkCr_S**	**ElkCr_S2**	**ElkCr_E**	**ElkCr_E14**	**Walnut_E**	**LE_T**	**POET**	**MarinaL_S13**	**MarinaL_S14**	
Fst Values below diagonal. Probability, P(rand >= data) based on 999 permutations is shown above diagonal.													

Misery	20Mile_E	20Mile_S	ElkCr_S	ElkCr_S2	ElkCr_E	ElkCr_E14	Walnut_E	LE_T	POET	MarinaL_S	MarinaL_S14		
0.000												Misery	
0.048	0.000											20Mile_E	
0.019	-0.002	0.000										20Mile_S	
0.015	0.062	0.007	0.000									ElkCr_S	
0.017	0.102	0.032	0.010	0.000								ElkCr_S2	
0.017	-0.021	-0.025	-0.019	0.010	0.000							ElkCr_E	
0.003	0.014	0.006	-0.006	0.008	0.006	0.000						ElkCr_E14	
0.029	0.080	0.021	0.026	0.048	0.017	0.013	0.000					Walnut_E	
0.044	0.047	0.022	0.020	0.042	-0.007	0.018	0.037	0.000				LE_T	
0.081	0.176	0.096	0.091	0.045	0.107	0.068	0.107	0.116	0.000			POET	
0.026	0.091	0.038	-0.004	0.025	0.001	0.012	0.036	0.057	0.136	0.000		MarinaL_S13	
0.000	0.111	0.053	0.036	0.030	0.030	0.024	0.015	0.092	0.093	0.011	0.000	MarinaL_S14	
F'st Values below diagonal.													

Table 4-11. Mean allelic patterns for codominant data across populations.

Population	Misery	20Mile_S	ElkCr_S2	ElkCr_E14	Walnut_E	LE_T	POET	MarinaL_S13	MarinaL_S14
Na	6.583	6.417	7.167	6.750	5.583	7.250	4.250	5.083	6.083
Na Freq. >= 5%	4.167	4.250	4.167	4.500	4.167	4.250	4.250	3.917	4.500
Ne	3.644	3.673	3.730	3.840	3.381	3.767	3.040	3.300	3.499
I	1.402	1.431	1.453	1.459	1.335	1.444	1.178	1.283	1.381
No. Private Alleles	0.083	0.000	0.083	0.083	0.000	0.250	0.000	0.000	0.000
No. LComm Alleles (<=25%)	0.500	0.583	0.667	0.750	0.500	0.583	0.083	0.500	0.500
No. LComm Alleles (<=50%)	0.667	0.750	0.917	1.000	0.667	0.833	0.083	0.667	0.667
He	0.674	0.696	0.695	0.703	0.668	0.693	0.628	0.643	0.668
uHe	0.685	0.706	0.701	0.712	0.690	0.698	0.662	0.672	0.680
Standard Error (SE) values									
Population	Misery	20Mile_S	ElkCr_S2	ElkCr_E14	Walnut_E	LE_T	POET	MarinaL_S13	MarinaL_S14
Na	0.811	0.712	0.920	0.789	0.679	0.993	0.411	0.529	0.753
Na Freq. >= 5%	0.366	0.411	0.386	0.529	0.322	0.372	0.411	0.468	0.359
Ne	0.464	0.317	0.391	0.378	0.338	0.420	0.374	0.377	0.335
I	0.129	0.112	0.120	0.118	0.110	0.129	0.102	0.121	0.125
No. Private Alleles	0.083	0.000	0.083	0.083	0.000	0.179	0.000	0.000	0.000
No. LComm Alleles (<=25%)	0.230	0.229	0.225	0.250	0.195	0.193	0.083	0.195	0.230
No. LComm Alleles (<=50%)	0.284	0.279	0.336	0.348	0.333	0.322	0.083	0.225	0.284
He	0.042	0.035	0.034	0.036	0.035	0.037	0.035	0.044	0.049
uHe	0.043	0.035	0.034	0.036	0.036	0.037	0.037	0.046	0.050

Table 4-12. Summary of Private Alleles by Population

Pop	Locus	Allele	Freq
Misery	2097	258	0.014
ElkCr_S2	1571	240	0.016
ElkCr_E14	1571	153	0.045
LE_T	12681	248	0.007
LE_T	1571	234	0.007

Table 4-13. Results of Pairwise Population F_{ST} Analysis.F_{ST} values were calculated via Frequency Option in GenAlEx with 12-populations, 1 region, and 999 permutations. Below are the Pairwise F_{ST} Values.

Pairwise Population Fst Values													
	Misery	**20Mile_E**	**20Mile_S**	**ElkCr_S**	**ElkCr_S2**	**ElkCr_E**	**ElkCr_E14**	**Walnut_E**	**LE_T**	**POET**	**MarinaL_S13**	**MarinaL_S14**	
Misery	0.000												**Misery**
20Mile_E	0.030	0.000											**20Mile_E**
20Mile_S	0.011	0.019	0.000										**20Mile_S**
ElkCr_S	0.010	0.029	0.009	0.000									**ElkCr_S**
ElkCr_S2	0.011	0.035	0.011	0.007	0.000								**ElkCr_S2**
ElkCr_E	0.019	0.025	0.012	0.011	0.017	0.000							**ElkCr_E**
ElkCr_E14	0.009	0.023	0.009	0.007	0.009	0.016	0.000						**ElkCr_E14**
Walnut_E	0.020	0.037	0.019	0.018	0.020	0.023	0.016	0.000					**Walnut_E**
LE_T	0.014	0.022	0.008	0.006	0.012	0.013	0.008	0.019	0.000				**LE_T**
POET	0.026	0.051	0.028	0.027	0.023	0.037	0.024	0.039	0.030	0.000			**POET**
MarinaL_S13	0.016	0.037	0.019	0.012	0.016	0.023	0.015	0.025	0.020	0.039	0.000		**MarinaL_S13**
MarinaL_S14	0.010	0.044	0.022	0.018	0.016	0.024	0.016	0.018	0.026	0.034	0.018	0.000	**MarinaL_S14**
	Misery	**20Mile_E**	**20Mile_S**	**ElkCr_S**	**ElkCr_S2**	**ElkCr_E**	**ElkCr_E14**	**Walnut_E**	**LE_T**	**POET**	**MarinaL_S13**	**MarinaL_S14**	

Table 4-14. Outcomes of Tests for Hardy-Weinberg Equilibrium. Key: ns=not significant; *=P<0.05; **=P<0.01; ***=P<0.001. The four boxes below represent Round Goby sample populations from Misery Bay (Misery), Twenty Mile Creek Embayment (20Mile_E), Twenty Mile Creek Seine (20Mile_S), and Elk Creek Seine 2013 (May) (ElkCr_S).

Summary by locus for Misery

Locus	ChiSquare	DF	Prob	
NmeQ3	12.757	15	0.621	ns
NmeQ17	6.200	10	0.798	ns
NmeQ15	15.795	21	0.781	ns
NmeQ16	11.587	21	0.950	ns
NmeQ23	88.061	78	0.204	ns
NmeQ22	47.417	36	0.097	ns
NmeQ6	6.563	6	0.363	ns
NmeQ11	27.751	28	0.478	ns
NmeQ14	9.331	10	0.501	ns
NmeQ24	22.158	28	0.774	ns
NmeQ4	10.168	10	0.426	ns
NmeQ13	2.939	1	0.086	ns

Summary by locus for 20Mile_E

Locus	ChiSquare	DF	Prob	
NmeQ3	3.570	6	0.735	ns
NmeQ17	6.074	3	0.108	ns
NmeQ15	10.306	10	0.414	ns
NmeQ16	4.841	6	0.564	ns
NmeQ23	21.000	21	0.459	ns
NmeQ22	24.111	28	0.676	ns
NmeQ6	4.293	6	0.637	ns
NmeQ11	14.778	15	0.468	ns
NmeQ14	10.111	10	0.431	ns
NmeQ24	3.570	6	0.735	ns
NmeQ4	2.160	6	0.904	ns
NmeQ13	1.215	1	0.270	ns

Summary by locus for 20Mile_S

Locus	ChiSquare	DF	Prob	
NmeQ3	13.660	15	0.551	ns
NmeQ17	5.345	6	0.500	ns
NmeQ15	20.176	21	0.510	ns
NmeQ16	8.491	15	0.903	ns
NmeQ23	58.874	36	0.009	**
NmeQ22	17.402	21	0.686	ns
NmeQ6	1.816	3	0.611	ns
NmeQ11	26.949	21	0.173	ns
NmeQ14	11.248	10	0.339	ns
NmeQ24	57.425	45	0.101	ns
NmeQ4	6.870	10	0.738	ns
NmeQ13	0.189	1	0.664	ns

Summary by locus for ElkCr_S

Locus	ChiSquare	DF	Prob	
NmeQ3	15.741	15	0.399	ns
NmeQ17	18.078	15	0.259	ns
NmeQ15	21.043	21	0.456	ns
NmeQ16	21.531	21	0.427	ns
NmeQ23	85.713	78	0.257	ns
NmeQ22	13.316	36	1.000	ns
NmeQ6	3.918	6	0.688	ns
NmeQ11	28.168	28	0.456	ns
NmeQ14	10.997	10	0.358	ns
NmeQ24	39.553	28	0.072	ns
NmeQ4	4.875	10	0.899	ns
NmeQ13	2.599	1	0.107	ns

Table 4-14. Continued Outcomes of Tests for Hardy-Weinberg Equilibrium. Key: ns=not significant; *=P<0.05; **=P<0.01; ***=P<0.001. The four boxes below represent Round Goby sample populations from Elk Creek Seine 2013 (July) (ElkCr_S2), Elk Creek Embayment 2013 (ElkCr_E), Elk Creek Embayment 2014 (ElkCr_E14), and Walnut Creek Embayment (Walnut_E).

Summary by locus for ElkCr_S2

Locus	ChiSquare	DF	Prob	
NmeQ3	14.255	15	0.506	ns
NmeQ17	3.445	10	0.969	ns
NmeQ15	14.621	21	0.841	ns
NmeQ16	7.486	15	0.943	ns
NmeQ23	130.540	55	0.000	***
NmeQ22	17.411	21	0.686	ns
NmeQ6	6.473	3	0.091	ns
NmeQ11	17.072	21	0.707	ns
NmeQ14	11.409	10	0.327	ns
NmeQ24	46.369	45	0.416	ns
NmeQ4	6.324	10	0.787	ns
NmeQ13	4.202	1	0.040	*

Summary by locus for ElkCr_E

Locus	ChiSquare	DF	Prob	
NmeQ3	12.331	10	0.264	ns
NmeQ17	5.862	6	0.439	ns
NmeQ15	11.875	21	0.943	ns
NmeQ16	9.243	15	0.864	ns
NmeQ23	22.585	15	0.093	ns
NmeQ22	12.251	10	0.269	ns
NmeQ6	3.278	3	0.351	ns
NmeQ11	16.544	10	0.085	ns
NmeQ14	5.760	10	0.835	ns
NmeQ24	18.944	21	0.589	ns
NmeQ4	7.257	10	0.701	ns
NmeQ13	2.090	1	0.148	ns

Summary by locus for ElkCr_E14

Locus	ChiSquare	DF	Prob	
NmeQ3	16.241	21	0.756	ns
NmeQ17	4.076	10	0.944	ns
NmeQ15	23.839	21	0.301	ns
NmeQ16	9.804	21	0.981	ns
NmeQ23	107.378	66	0.001	***
NmeQ22	33.029	36	0.611	ns
NmeQ6	0.914	3	0.822	ns
NmeQ11	17.323	21	0.691	ns
NmeQ14	8.909	10	0.541	ns
NmeQ24	18.330	28	0.918	ns
NmeQ4	6.568	15	0.969	ns
NmeQ13	2.184	1	0.139	ns

Summary by locus for Walnut_E

Locus	ChiSquare	DF	Prob	
NmeQ3	7.938	10	0.635	ns
NmeQ17	16.609	10	0.083	ns
NmeQ15	26.567	28	0.542	ns
NmeQ16	5.877	10	0.825	ns
NmeQ23	68.112	55	0.110	ns
NmeQ22	10.046	15	0.817	ns
NmeQ6	8.067	3	0.045	*
NmeQ11	13.736	15	0.546	ns
NmeQ14	5.315	10	0.869	ns
NmeQ24	7.734	15	0.934	ns
NmeQ4	3.736	6	0.712	ns
NmeQ13	1.983	1	0.159	ns

Table 4-14. Continued Outcomes of Tests for Hardy-Weinberg Equilibrium. Key: ns=not significant; *=P<0.05; **=P<0.01; ***=P<0.001. The four boxes below represent Round Goby sample populations from Lake Erie Trawls (LE_T), Port of Erie Terminal (POET), Marina Lake Seine 2013 (MarinaL_S13), and Marina Lake Seine 2014 (MarinaL_S14).

Summary by locus for LE_T

Locus	ChiSquare	DF	Prob	
NmeQ3	16.523	21	0.740	ns
NmeQ17	10.073	10	0.434	ns
NmeQ15	67.433	45	0.017	*
NmeQ16	9.551	15	0.847	ns
NmeQ23	130.555	91	0.004	**
NmeQ22	41.295	36	0.250	ns
NmeQ6	2.959	6	0.814	ns
NmeQ11	11.332	21	0.956	ns
NmeQ14	14.120	15	0.516	ns
NmeQ24	110.852	66	0.000	***
NmeQ4	6.575	15	0.968	ns
NmeQ13	1.851	1	0.174	ns

Summary by locus for POET

Locus	ChiSquare	DF	Prob	
NmeQ3	10.710	6	0.098	ns
NmeQ17	8.262	6	0.220	ns
NmeQ15	5.262	10	0.873	ns
NmeQ16	8.316	10	0.598	ns
NmeQ23	13.432	6	0.037	*
NmeQ22	17.000	21	0.711	ns
NmeQ6	4.087	3	0.252	ns
NmeQ11	5.631	6	0.466	ns
NmeQ14	6.967	10	0.729	ns
NmeQ24	27.500	21	0.155	ns
NmeQ4	4.025	6	0.673	ns
NmeQ13	0.351	1	0.554	ns

Summary by locus for MarinaL_S13

Locus	ChiSquare	DF	Prob	
NmeQ3	14.853	15	0.462	ns
NmeQ17	3.905	10	0.952	ns
NmeQ15	14.557	10	0.149	ns
NmeQ16	16.689	21	0.730	ns
NmeQ23	65.642	55	0.154	ns
NmeQ22	6.387	10	0.782	ns
NmeQ6	3.925	3	0.270	ns
NmeQ11	9.361	15	0.858	ns
NmeQ14	2.750	6	0.840	ns
NmeQ24	5.485	10	0.856	ns
NmeQ4	2.308	6	0.889	ns
NmeQ13	0.032	1	0.859	ns

Summary by locus for MarinaL_S14

Locus	ChiSquare	DF	Prob	
NmeQ3	8.977	15	0.879	ns
NmeQ17	5.031	10	0.889	ns
NmeQ15	17.157	21	0.702	ns
NmeQ16	26.847	21	0.176	ns
NmeQ23	66.580	55	0.136	ns
NmeQ22	16.075	21	0.765	ns
NmeQ6	5.262	3	0.154	ns
NmeQ11	27.082	15	0.028	*
NmeQ14	8.579	10	0.573	ns
NmeQ24	36.568	45	0.811	ns
NmeQ4	7.035	6	0.318	ns
NmeQ13	0.370	1	0.543	ns

CHAPTER 5

Morphometric and Meristic Analyses of Round Goby in Lake, Bay and Tributary Habitats in Erie County, Pennsylvania

INTRODUCTION

I wanted to use morphometric and meristic analyses of Round Goby in this study to determine if these benthic invasive fish had significant ($p<0.05$) differences in morphology across the various collection sites sampled in Erie County, Pennsylvania. Comparing morphological data of Round Goby across bay, lake, tributary and embayment habitats may reveal phenotypic plasticity observed in body shape due to habitat occupied and possibly dietetic availability. While similar techniques have been performed to describe new species within the same genus (Ciccotto et al. 2011), the purpose of this portion of my research was to determine whether the morphometric and meristic data could be used to detect specific populations of Round Goby. These morphological data could then be compared to the population genetics assessment (microsatellite analyses) of the research area to determine whether significant differences ($p<0.05$) in body shape were indicative of unique metapopulation structuring or if both analyses exist irrespective of one another.

METHODS

Morphometric measurements and meristic counts were taken from (N=90) Round Goby specimens from collections made in 2013 and 2014. An effort was made to choose at least 10 of the largest specimens from each collection site. In the case of the Twenty Mile Creek (embayment) collection (N=7) and Walnut Creek (seine) collection (N=1) there were less than 10 specimens collected per site (Walnut Creek tributary data were excluded due to one specimen collected). Additionally, while (N=30) specimens from Twenty Mile Creek proper were seined, fin-clipped, preserved in formalin, and genotyped, none of those fish were pinned and/or measured for morphometric and meristic analysis.

I recorded morphometric measurements (N=35) and meristic counts (N=9) for each of the 90 specimens used for the phenotypic portion of the study (see **CHAPTER 2** Table 2-3). I entered meristic counts manually into Microsoft Excel after I visually obtained counts (e.g., fin rays) through a 10x21 power Wild Heerbrugg stereo light microscope in the laboratory. I designed a geometric truss structure for the Round Goby (see **CHAPTER 2** Table 2-3) and performed measurements using a set of FowlerTools Sylvac digital calipers model S235, which are capable of measuring to the accuracy of one-hundredth of a millimeter (0.01mm). I connected this digital caliper to a Lenovo X60s laptop computer using the *WinWedge® Pro* utility tool,

which imported the caliper measurement data into Microsoft Excel as raw data. Morphometric and meristic data were imported into SAS v.9.3 software for principle component analysis.

Morphometric data were analyzed with sheared principal component analyses (SPCA), with the co-variance matrix factored and meristic data analyzed with principal component analyses with the correlation matrix factored. The second sheared principle components of the morphometric characters were compared and plotted against the first principal components of the meristic data. Once plotted, minimal polygon clusters were generated for each population's characteristics. I selected the three (3) largest characters outputted from SAS v.9.3 for each morphometric and meristic sheared Principal Components Analysis (PCA) that contributed the largest variance. Using sheared principle component analysis, I compared all populations (groups 1-9) to determine which morphometric characters and meristic counts contributed the most variance across all collections.

GROUP	NAME	HABITAT TYPE
1	MARINA LAKE	BAY
2	PENBASE	OFFSHORE
3	FOUR MILE CREEK	OFFSHORE
4	PORT OF ERIE	BAY
5	MISERY BAY	BAY
6	TWENTYMILE CREEK EMBAYMENT	EMBAYMENT
7	ELK CREEK TRIBUTARY	TRIBUTARY
8	ELK CREEK EMBAYMENT	EMBAYMENT
9	WALNUT CREEK EMBAYMENT	EMBAYMENT

Table 5-1. Group listings used for morphometric and meristic analyses from collections of Round Goby from Lake, Bay,Tributary and Embayment habitats of Erie County, Pennsylvania.

The output scores I plotted had minimal polygon clusters constructed around each population to illustrate sample location/population morphology and meristic scores. Following Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT), I examined grouping and means. I considered populations to be significantly different if p-values did not exceed a critical alpha value (<0.05) in ANOVA. Duncan's Multiple Range Testing allowed for delineating populations significantly different from one another in morphology and meristic counts.

The three greatest SPCA2 morphometric characters contributing variance were used to determine what morphological and meristic differences, if any, are observed in Round Goby collected from tributary, embayment, offshore, and bay habitats. Such examinations are necessary to compare statistical results to life-history traits of Round Goby, therefore making it possible for me to draw inference into biological significance of observed statistical results.

RESULTS

Morphometric and meristic data for all populations are summarized in **Table 5-2**. The detailed measurement codes are summarized in **CHAPTER 2.** Analyses of these data for all populations and selected comparisons among specific populations are summarized below.

All Populations

Sheared PC1 (SPC1) was primarily influenced by size and accounted for 96% of the total variance, while sheared principle components two (SPC2) explained 16.5% of the remaining variance. For SPCA2, the variables with the three highest loadings were caudal peduncle length (-0.64), distance from the posterior portion of the first dorsal fin to the pelvic fin insertion (0.33), and length from the posterior second dorsal fin to the ventral section of the caudal fin (-0.23).

The PC1 for the meristic data explained 23% of the variance for Round Goby collections. Those factors with the highest loadings were head pore counts (0.54), lower gill rakers (0.50), and gill rakers found in the preopercular region (0.37).

When the first principal components of the meristic data were plotted against the second sheared principal components of the morphometric data there was a great amount of overlap among populations. Although there were significant differences ($p<0.05$) among the minimum polygons for the populations along the meristic axis, a Duncan's Multiple Range Test (DMRT) illustrated that no one group was significantly different ($p<0.05$) from the others. The DMRT for morphometric data, however, illustrated that populations from Peninsula Base (offshore), Port of Erie (Presque Isle Bay), Four Mile Creek (offshore), Twentymile Creek Embayment, and Elk Creek Embayment to be significantly different ($p<0.05$) from collections made in Marina Lake (Presque Isle Bay), Elk Creek tributary, Walnut Creek Embayment, and Misery Bay (Presque Isle Bay) (See **Table 5-1** for group name and habitat).

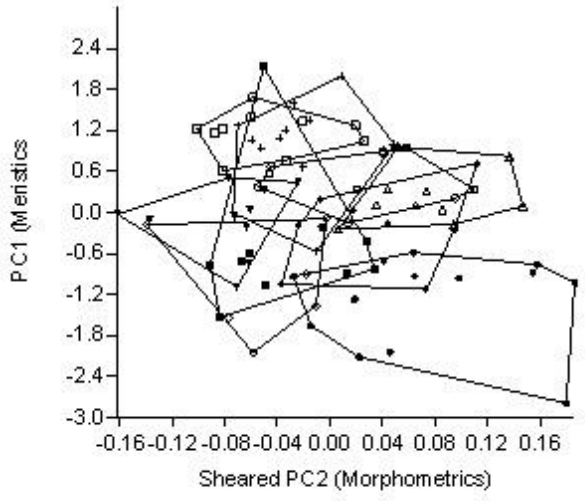


Figure 5-1. Sheared PCA Minimal Polygon Plots illustrating phenotypic grouping for all populations grouped together. The y-axis is meristics principle component one (PC1) and the x-axis is morphometrics sheared principle component two (SPC2). This illustrates the variance being analyzed in ANOVA.

Elk Creek Tributary vs. Elk Creek Embayment

Sheared PC1 (SPC1) was primarily influenced by size and accounted for 91% of the total variance, while sheared principle components two (SPC2) explained 20% of the remaining variance. For SPCA2, the variables with the three highest loadings were the posterior first dorsal fin to the pelvic fin insertion (0.37), the posterior second dorsal fin to the posterior anal fin (-0.29), and posterior second dorsal fin to the ventral insertion of the caudal fin (0.46).

The PC1 for the meristic data explained 29% of the variance for Round Goby collections. Those factors with the highest loadings were pelvic fin rays (0.42), head pore counts (.50), and gill rakers located in the preopercular region (.52).

When the first principal components of the meristic data were plotted against the second sheared principal components of the morphometric data there was very little overlap among populations. There were significant differences ($p<0.05$) among the minimum polygons for the populations along the morphometrics axis as well as the meristic axis for Elk Creek tributary and Elk Creek embayment collections of Round Goby as illustrated by Duncan's Multiple Range Test (See **Table 5-1** for group name and habitat).

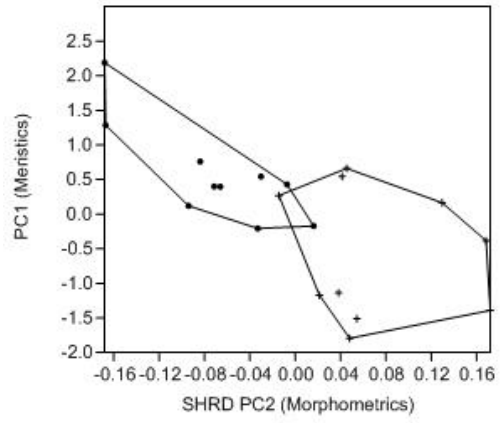


Figure 5-2. Sheared PCA Minimal Polygon Plots illustrating phenotypic grouping for Elk Creek tributary (●)vs. Elk Creek embayment (+)populations of Round Goby. The y-axis is meristics principle component one (PC1) and the x-axis is morphometrics sheared principle component two (SPC2). This illustrates the variance being analyzed in ANOVA.

Embayment, Presque Isle Bay, and Offshore Collections

Sheared PC1 (SPC1) was primarily influenced by size and accounted for 97% of the total variance, while sheared principle components two (SPC2) explained 19.7% of the remaining variance. For SPCA2, the variables with the three highest loadings were the body depth at (-0.26), caudal peduncle length (0.56), and the length from the posterior second dorsal fin to the posterior anal fin (-0.27).

The PC1 for the meristic data explained 24% of the variance for Round Goby collections. Those factors with the highest loadings were anal fin rays (0.28), pectoral fin rays (-0.35), and head pore counts (0.53).

When the first principal components of the meristic data were plotted against the second sheared principal components of the morphometric data there was overlap among populations. The Duncan's Multiple Range Test for morphometric data shows Presque Isle Bay collections to be significantly (<0.05) different from offshore and embayment collections. The DMRT used for meristic data analyses showed offshore collections of Round Goby to be significantly (<0.05) different from collections made in embayments and Presque Isle Bay.

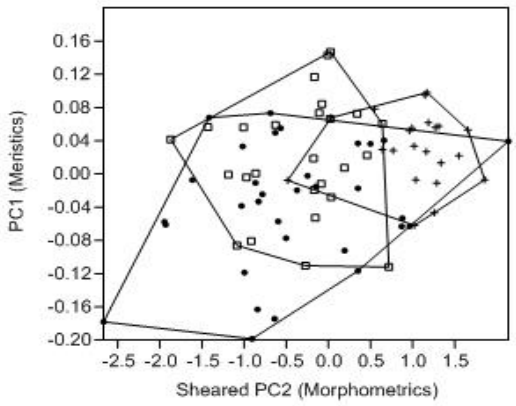


Figure 5-3. Sheared PCA Minimal Polygon Plots illustrating phenotypic grouping for embayment (■), bay (♦), and offshore (+) populations of Round Goby. The y-axis is meristics principle component one (PC1) and the x-axis is morphometrics sheared principle component two (SPC2). This illustrates the variance being analyzed in ANOVA.

Elk Creek Tributary vs. All Populations

For morphology of Round Goby collected in Elk Creek tributary vs. all other specimen collection sites (including Elk Creek Embayment) sheared (PC1) (SPC1) accounted for 96% of the data, while sheared principle components two (SPC2) explained the remaining 16.5% of the data. For sheared principle components analysis (PCA), the highest loadings were selected from three morphological measurements displaying the most variance recorded in greatest absolute value. Those measurements (PC2) were caudal peduncle length (-.65), posterior first dorsal fin to pelvic fin origin (.33), and posterior second dorsal fin to ventral caudal fin insertion (-.24).

Sheared (PC1) explained 23% of the variance in meristic counts for Round Goby collections. Those factors with the highest loadings were anal fin rays (0.27), pelvic fin rays (-0.36), and head pore counts at (0.54).

The Duncan's Multiple Range Test (DMRT) for morphometric measurement data showed that gobies from Elk Creek tributary to be significantly (<0.05) different from all other sample populations. Elk Creek tributary collections were not significantly (>0.05) different when compared to all other populations for meristic counts using DMRT.

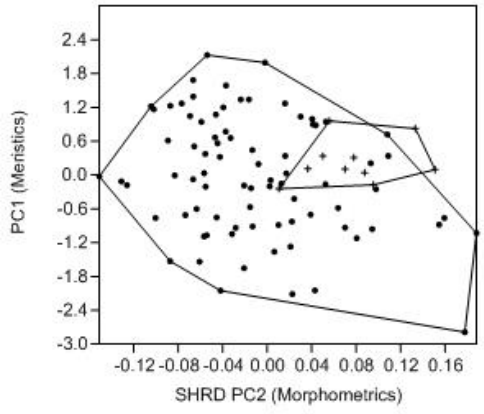


Figure 5-4. Sheared PCA Minimal Polygon Plots illustrating phenotypic grouping for tributary (+) vs. all other collection populations (●)of Round Goby. The y-axis shows principle component one (PC1) and the x-axis displays morphometrics sheared principle component two (SPC2). This illustrates the variance being analyzed in ANOVA.

Table 5-2. Morphometric and meristic values of *Neogobius melanostomus* (n=90) collected from Bay, Tributary, Offshore, and Embayment habitats in Erie County, Pennsylvania.

Neogobius melanostomus (as % of SL)	Mean	Bay range	Tributary range	Offshore range	Embayment Range
Standard length	61.1	49.8-94.6	44.2-68.4	46.1-110.4	26.7-69.9
Head length	17.8	13.5-30.6	13.0-19.7	14.8-33.0	7.7-19.5
Head width	9.7	7.8-16.8	6.4-9.4	7.5-19.3	4.1-11.6
Head diameter	11.4	9.5-20.5	7.5-12.7	8.8-22.5	4.2-13
Upper jaw length	5.3	3.7-10.1	3.8-5.6	4.3-11.5	1.9-6.3
Lower jaw length	6.4	5.3-11.8	4.5-6.3	4.7-13.2	2.8-7.3
Snout length	6.1	4.1-11.4	4.0-6.8	4.3-12.7	2.5-6.7
Post orbital head length	8.1	5.0-15.1	5.4-8.6	5.0-15.2	3.5-8.6
Horizontal eye diameter	4.4	3.7-6.3	4.0-5.4	4.5-6.5	2.5-5.0
Vertical eye diameter	4.2	4.2-6.4	4.0-5.5	3.3-7.0	2.2-5.1
Body depth	13.2	10.7-25.2	10.0-14.9	8.9-23.6	5.1-17.2
Caudal peduncle length	9.6	8.0-16.2	6.0-10.5	5.8-21.4	3.7-9.0
Least Caudal peduncle length	6.5	5.0-11.5	4.4-7.1	4.5-12.5	2.6-7.8
Snout to anterior first dorsal fin	21.5	17.5-36.2	15.6-24.4	15.8-38.6	9.7-24.0
Snout to posterior first dorsal fin	32.2	26.6-53.3	22.3-38.0	24.0-57.3	14.3-36.3
Snout to posterior seond dorsal fin	51.8	41.1-83.4	36.5-58.0	37.6-93.0	21.6-60.2
Snout to pelvic fin (ventral origin)	18.9	14.2-31.0	12.7-21.6	14.9-34.7	8.4-24.2
Anterior first dorsal fin to pelvic fin insertion	13.4	11.7-25.0	9.9-15.3	10.0-24.5	5.5-17.2
Posterior first dorsal fin to pelvic fin insertion	18.5	15.0-32.5	13.4-38.9	13.0-32.7	6.9-22.8
Posterior second dorsal fin to pelvic fin origin	34.6	28.3-58.0	25.4-38.8	24.6-63.2	12.6-42
Anterior first dorsal fin to anterior anal fin	19.2	16.1-33.4	13.7-22.2	14.4-35.2	8.0-21.9
Anterior first dorsal fin to posterior anal fin	32	26.4-52.0	21.8-36.0	25.5-58.4	13.4-37.8
Posterior second dorsal fin to posterior anal fin	12.8	10.2-23.7	8.7-14.2	9.4-23.7	5.3-15.0
Posterior first dorsal fin to anterior anal fin	21.7	17.8-36.1	14.9-24.3	16.8-38.9	8.3-23.8
Posterior first dorsal fin to posterior anal fin	19.2	15.2-31.6	12.3-20.3	13.9-36.0	7.3-23.1
Posterior second dorsal fin to anterior anal fin	7.8	6.0-14.5	4.8-7.8	5.5-14.7	3.0-9.1
Posterior first dorsal fin to ventral caudal fin	30.5	24.8-47.4	20.5-33.4	24.6-53.8	11.2-34.1
Posterior second dorsal fin to ventral caudal fin	11.6	9.8-17.7	8.1-12.8	8.5-20.3	5.5-12.2
Posterior anal fin to dorsal caudal fin insertion	13	10.2-19.2	9.8-13.8	11.0-24.3	6.6-13.7
Pelvic fin base length	4.5	3.6-7.7	31-5.0	3.4-8.3	1.9-5.6
Anal fin length	15.4	12.9-23.0	9.4-16.3	12.5-28.1	5.6-18.1
First dorsal fin length	11.2	8.8-19.0	6.3-14.5	7.5-20.0	4.8-12.9
Second dorsal fin length	20.1	16.8-31.8	13.6-21.3	16.1-36.6	7.0-23.9
Pectoral fin base length	7.1	5.5-12.5	4.7-7.3	5.4-13.9	3.0-8.7
First dorsal fin rays	6	6.0-6.0	6.0-7.0	5.0-6.0	6.0-6.0
Second dorsal fin rays	16	16.0-16.0	15-16	15-17	14-17
Anal fin rays	12.8	12.0-13.0	12.0-14	13-14	12.0-14
Pectoral fin rays	5.1	5.0-6.0	5.0-5.0	4.0-5.0	5.0-5.0
Pelvic fin rays	18	18-18	17-19	17.0-19	15-18
Head canal pores	8.5	8.0-10.0	8.0-10.0	9.0-11.0	7.0-10.0
Gill raker lower	6.9	8.0-8.0	6.0-7.0	6.0-9.0	5.0-7.0
Gill raker upper	2.6	2.0-3.0	2.0-3.0	2.0-3.0	2.0-3.0
Gill raker preopercular	9.9	10.0-10.0	10-12.0	9.0-13	7.0-12.0

DISCUSSION

Understanding the patterns of morphological variation among and across populations is a fundamental aspect of biodiversity research (Northrup et al. 2010). There have been reports of populations of the same species of fish in the same lake displaying different phenotypes (Northrup et al. 2010). Polacik et al. (2012) conducted a morphological and meristic comparison study of native (Bulgarian) and non-native (Slovak) populations of Round Goby. While my study contained 35 morphological characters and nine (n=9) meristic counts, Polacik et al. (2012) used 29 characters and five (n=5) meristic counts. In their study, Polacik et al. (2012) noted that post-orbital distance, head depth, and minimal body depth differentiated in populations only in females, while the overall trend was the same in both sexes. The range in fin ray numbers was similar between Bulgaria and Slovak populations (Polacik et al. 2012) and my observations were similar: Bulgaria and Slovak first dorsal ray mean=6.0 while range=6-6, Lake Erie mean=6.02 and range=5-7; Bulgaria and Slovak second dorsal ray mean=14.9 and range=14-17, Lake Erie mean=15.95 and range=14-17; Bulgaria and Slovak anal ray mean=12.1 and range=11-13, Lake Erie mean=12.82 and range=11-14; Bulgaria and Slovak pectoral ray mean=18.4 and range=17-20, Lake Erie mean=17.99 and range=15-19; Bulgaria and Slovak pelvic ray mean=12 and range=12-12, Lake Erie pelvic ray mean=5.13 and range=4-6 (Note: the Lake Erie specimens only had the left half of the pectoral ray measured. Had the whole fin been measured equally, the values would have likely doubled and been similar to Polacik et al. (2012) findings). Polacik et al. (2012) only collected Round Goby between 70-80 mm SL, whereas samples used in this study ranged from 25-110 mm SL. Their reasoning for that size range was to avoid problems linked to allometric growth, which they believed could potentially arise when comparing fish samples of different sizes (Polacik et al. 2012).

Lake (Erie, Port-of-Erie, Marina Lake, Misery Bay, Peninsula Base and Four Mile Creek) and embayment (Elk, Walnut, and Twenty Mile creeks) population collections were compared for morphometric and meristic variance. Morphologically, caudal peduncle length (cpl), posterior second dorsal to posterior anal (psdpa) and body depth (bd) were the greatest contributors to variance for embayment versus lake collections. For meristic counts, head count pores (hcp), gill rakers lower (grlow), and gill rakers preopercular (grpo) were the greatest contributors to variance. Morphology and meristics among populations of Round Goby were significantly different, $p=0.0003$ and $p=0.0741$, respectively. Duncan's Multiple Range Tests results indicated that while both populations were morphometrically different, they were not significantly different along the meristic axis when differing habitats were compared against one another.

Comparisons were made for embayment, Lake Erie, Presque Isle Bay, and the Port-of-Erie Terminal collections. Embayment collections remained the same, Lake Erie sample sites were Four Mile Creek and Peninsula Base, Presque Isle Bay samples were Marina Lake and Misery Bay, and the final sample site was Port-of-Erie Terminal habitat. The greatest morphological variances for the above listed sites were caudal peduncle length (cpl), posterior second dorsal fin to posterior anal fin (psdpa) and snout length (snl), respectively. The greatest variance between these populations meristically were head count pores (hcp), gill rakers lower (grlow), and gill rakers preopercular (grpo). Results from ANOVA implied that morphometrics and meristics among Round Goby populations in this comparison were significantly different ($p<0.05$). Duncan's Multiple Range Tests showed that at least one populations was morphometrically and meristically different when compared to all populations. Morphologically, specimens collected from sample sites in Presque Isle Bay were most differentiated from Embayment, Lake Erie, and Port-of-Erie Terminal specimens. For meristic counts, Lake Erie specimens were the most differentiated among all other population comparisons.

Generalist species such as percid and salmonid fishes have well documented ecological polymorphisms (Smith and Skulason 1996, Olsson and Eklov 2005, Bhagat et al. 2006, and Polacik et al. 2012). These fishes have excellent swimming abilities unlike the benthic Round Goby, which Hayden and Miner (2009) considered to be a molluscivore specialist. Polacik et al. (2012) believed it was reasonable to assume that different habitats within the same general bodies of water (in their case upper and lower sections of the same river) would be reflected in changes to Round Goby morphology. In the Polacik et al. (2012), significant ($p<0.0001$) differences between native and non-native populations were only observed between females and not the collections overall. The authors also believed that diet availability and type was a contributing factor to morphology and size. During the offshore PAFBC trawls, I noticed a large amount of Zebra and Quagga mussels each time the nets were retrieved. The mussels trawled were all at depths greater than sampled stream segments. This likely affects size of Round Goby in offshore and in bay habitats that have a greater abundance of mussel species available. External morphology differences in native and non-native Round Goby have been suggestively attributed to disparate environments and founder effects (Polacik et al. 2012) although for this study, no evidence of a genetic founder effect was found.

CHAPTER 6

Management Implications of Round Goby invaded waters and Summary of Study

MANAGEMENT IMPLICATIONS

Native ichthyofauna (e.g., Johnny Darter, Mottled Sculpin, Logperch) living in sympatry with Round Goby in Lake Erie, Presque Isle Bay, and surrounding tributaries may experience difficulty regaining their former competitive feeding abilities prior to Round Goby invasions (Laurer 2004). Karkowiak and Pennuto (2008) predicted that as Round Goby become more prominent in Lake Erie tributaries, their aggressive nature and diet plasticity would allow them to outcompete native fishes not only for food but also for nesting sites. Kipp and Ricciardi (2012) reported decreases in macroinvertebrate density and increases in algal blooms due to Round Goby preying on native algivores in the upper St. Lawrence River.

The likelihood that Round Goby will be eradicated from any of the Great Lakes is extremely low. Efforts can and must be made, however, to halt invasions into areas within close proximity to Round Goby infested waters. Fishery managers should continue to focus their efforts educating the public to not transfer invasive species between waterways. Round Goby are one of several species that anglers are prohibited from possessing as bait according to the Pennsylvania Fish and Boat Commission (Press Release, April 2010).

In August 2014, the PAFBC positively identified Round Goby presence in Lake LeBoeuf, a popular fishing area in Erie County's Waterford community (PAFBC Press Release, August 2014). The outflow of Lake LeBoeuf flows into French Creek, which according to the PAFBC contains several threatened and endangered fish and freshwater mussel species. In 2014, the PAFBC sought to petition state legislature to impose a $150 fine per Round Goby found in any bait shop aquaria (Birdsong, 2014). Nathan et al. (2014) reported that of a survey of 46 bait dealers in Pennsylvania, 43.8% of the species being sold were not on the state's approved bait list (LoVullo and Stauffer 1993), thus proving that Round Goby are being distributed whether

accidentally or intentionally through bait shops along the Great Lakes. A hallmark of the Nathan et al. (2014) research was the use of molecular markers to indicate non-native (both Round and Tubenose gobies) species presence. I believe that the marker developments made through my research in this thesis will aid future studies seeking the presence/absence detection of Round Goby in waters formerly uninhabited by this invasive Gobiid. Many Round Goby invasion protections methods thus far, unfortunately, have been reactive rather than proactively initiated.

Summary

The Round Goby has experienced widespread invasions throughout the Pennsylvania portion of Lake Erie and its tributaries. It has been shown that given no impassable physical barriers, Round Goby have the ability to invade a multitude of aquatic habitats and displace native fishes. At the time of this writing, Round Goby have established populations in lower French Creek, the most ichthyofaunaly diverse stream in Pennsylvania. While these fish were almost certainly introduced via bait bucket transfers, their impact on native fish and aquatic macroinvertebrates has yet to be determined.

This study began as an attempt to determine whether Round Goby were functioning as unique, detectable metapopulations in Lake Erie tributaries. The presence of young of year Round Goby in Elk Creek as late as August (Stauffer, pers. comm.) prompted the question if these fish were behaving as an independent population that were spawning without the influence of lake and bay residents gobies. To answer this question genetically, a suite of molecular markers were developed specifically from Round Goby collected at the study sites described in this manuscript. The development of a suite of species-specific markers added to a limited supply of existing Round Goby markers available to researchers. These markers will be useful for detecting Round Goby presence from water samples in locations were conventional fish collection methods (e.g., seining, electrofishing) may be difficult. Using microsatellite analysis, and making a genetic

comparison across the study region, it was determined that Round Goby in Erie County are essentially functioning as one large metapopulation that is experiencing true panmixia.

Round Goby were also compared for morphological and meristic differences across collection sites and various habitats. While there were significant differences ($p<0.05$) for some morphological characters and meristic counts, these differences were minimal and are likely the result of phenotypic plasticity due to site occupancy and possibly available food sources. Overall, these fish have similar traits and since they appear to be functioning as one metapopulation range-wide, they have yet to see any evolutionary change in morphology.

While it is unlikely that the Round Goby will be extirpated from the Great Lakes, there is a need to study its ability to adapt to novel environments. As noted, Round Goby not only have the ability to invade tributary systems at great lengths and displace native benthic fishes, but also to prey upon the eggs of economically important game fish that provide significant revenue to the State of Pennsylvania (Dufour et al. 2007, Chotkowski & Marsden 1999).

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