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Characterizing dispersal and colonization of the invasive round goby (Neogobius melanostomus) in the Great Lakes.

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CHARACTERIZING DISPERSAL AND COLONIZATION OF THE INVASIVE ROUND GOBY (*NEOGOBIUS MELANOSTUS*) IN THE GREAT LAKES

By

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ABSTRACT

As a Great Lakes invader, the round goby (*Neogobius melanostomus*) provides a valuable model system with which to study the evolution and dispersal dynamics of invasive species in novel environments. Their rapid expansion, aggressive behaviour and high population densities are of concern for conservation managers. Here, I describe the application of 10 novel polymorphic microsatellite markers to determine levels of genetic diversity and dispersal patterns in round goby populations in Ontario, Canada. Genetic analyses indicate that the established populations are highly genetically differentiated, and that specific geographical regions follow an isolation-by-distance pattern of differentiation. Recently established populations, as well as a novel invasion front, have retained high genetic diversity despite indications that founder events were small. Natural dispersal, ballast mediated jump dispersal events and multiple introductions from Eurasia likely led to the high diversity and unusual patterns of genetic differentiation among introduced round goby populations in the Great Lakes.

Dedication

"I dedicate this work to Danielle, her family and to my family for their unwavering faith, support and patience"

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1.0 GENERAL INTRODUCTION

1.1 GREAT LAKES INVASIVE SPECIES

An increase in global commerce has led to the widespread movement of once geographically restricted species beyond their natural range. This increase in human transport activity had been responsible for the introduction of many invasive plants and animals into the Laurentian Great Lakes (Mills *et al.* 1993, Ricciardi 2006). Many of these invaders have had profound impacts on the Great Lakes ecosystem, notable examples include: 1) the sea lamprey (*Petromyzon marinus*), 2) the zebra mussel (*Dreissena polymorpha*), 3) purple loosestrife (*Lythrum salicaria*), 4) Eurasian watermilfoil (*Myriophyllum spicatum*), and 5) the round goby (*Neogobius melanostomus*), all of which have had dramatic ecological and economic impacts. Between 1960 and 1990, there have been an average of 1.36 new invasive species discovered per year in the Great Lakes (Mills *et al.* 1993). Mills *et al.* (1993) identified 139 introduced species. In 2006, this list was updated and now includes 182 introduced species (Ricciardi 2006). In recent years there has been an increase in invasive species originating from Eurasia (Ricciardi & MacIsaac 2000). Since 1985, 70% of the invading species have originated from the Ponto-Caspian region (Ricciardi & MacIsaac 2000). Despite ballast water exchange guidelines initiated in 1993 (United States Coast Guard 1993), Ponto-Caspian invaders have continued to establish in the Great Lakes. Historically the Caspian Sea has undergone extended periods of reduced freshwater inputs (Dumont 1998). Currently in the Caspian Sea there exist several distinct salinity gradients, including exclusively fresh water near the outflow of the Volga River to near completely saline waters (Dumont 1998), resulting in many euryhaline taxa. The salinity tolerance of these species may have facilitated their survival during ballast water exchange practices prior to entering the Great Lakes. It was noted by Carlton *et al.* (1985) that several

freshwater taxa survived following ocean ballast exchange. Identifying dispersal vectors to the Great Lakes has helped researchers in recognizing new species that may invade the Great Lakes. Biological invasions into aquatic ecosystems have led to many unforseen ecological impacts, resulting in noticeable changes in biodiversity and ecosystem health (Kolar & Lodge 2001). The economic losses associated with invasive species can be equally high (see Pimentel *et al.* 2005). The Laurentian Great Lakes appear to be critically impacted by invasive species due to a recent influx of invasive species from the Ponto-Caspian region (Ricciardi & MacIsaac 2000).

1.2 DISPERSAL OF INVASIVE SPECIES

The spread of a newly colonized species can follow two basic models. Firstly, after initial colonization the species may simply spread naturally following logistic or exponential population growth. This model of spread has been termed reaction diffusion dispersal, where the extent of the spread is related to the populations' growth rate by a diffusion coefficient (Skellam 1951, in MacIsaac *et al.* 2001). This model predicts dispersal to be random, extending from a focal source. Extensions of this model can account for directional movement of individuals, such as species whose propagules are affected by wind or water currents (Shigesada *et al.* 1995). The second dispersal model by which invasive species can disperse is termed stratified diffusion, which is simply reaction diffusion that accounts for long distance dispersal, or "jump" dispersal, events. These long distance dispersal events have been documented in invaders such as the zebra mussel (Griffiths *et al.* 1991) and the Argentine ant (Saurez *et al.* 2001). The current primary mechanism of such long distance dispersal for aquatic species is through ballast water discharge between major shipping ports throughout the world. Ballast water is the leading mechanism for the initial introduction for many non-native species in the Great Lakes (see Mills *et al.* 1993).

Many of the Ponto-Caspian invaders of the Great Lakes have entered via ballast water transport, highlighting a strong invasion corridor (Ricciardi & MacIsaac 2000). MacIsaac *et al.* (2001) identified five major shipping corridors linking the Great Lakes with other global ports, although Colautti *et al.* (2002) noted that there was no direct link between the Ponto-Caspian region and the Great Lakes. The introduction of these species may have occurred through secondary hubs, where invasion spokes radiating from these hubs are facilitating secondary invasion events (i.e. to the Great Lakes) (Carlton 1996).

Most often it is not the initial introduction, but rather the secondary spread and in the introduced range that will determine the extent of the impact of the invasive species on the introduced region (Lodge *et al.* 1998). Having knowledge of this secondary spread aids researchers and managers in predicting the potential future spread as well as the direction and the rate of such spread (e.g. Ludwig & Leitch 1996, Schneider *et al.* 1998). Many fish species have extended their distribution beyond their native range via ballast water transport (Wonham *et al.* 2000). Human mediated dispersal may greatly exceed the rate of spread due solely to natural dispersal: human mediated dispersal was estimated to be 50,000 times greater than natural dispersal in an invasive crustaceous zooplankter (Hebert & Cristescu 2002). Identifying dispersal routes of invasive species is not only important for management reasons but will also increase our understanding of the importance of dispersal in colonizing species. These colonizing populations are generally characterized as genetically less diverse than source populations (e.g. Grapputo *et al.* 2005), and most often encounter a novel selective regime in the new environment to which they may not be adapted or have the required genetic variance to be able to adapt. The round goby represents an example of a successful Great Lakes invasive species. The rapid

spread of the round goby and high population growth provides a valuable model system in which to evaluate the genetic consequences of biological invasions.

1.3 ROUND GOBY BIOLOGY

The family Gobiidae is one of the largest families of fish in the world, comprising over 2000 species in 200 genera. Members of this family are found primarily in tropical and subtropical areas and many occur in marine or brackish shallow coastal waters around reefs. The family Gobiidae includes some of the smallest vertebrates in the world (<1cm, Genera: *Trimmaton* and *Pandaka*). Gobiids are generally differentiated from other fish by the presence of a fused pelvic fin, which forms a ventrally located adhesive suctoral disc. Gobiid fish also often adopt a reproductive strategy where males care for the eggs (Miller 1984).

Round Goby Life History

The round goby (*Neogobius melanostomus*) belongs to the subfamily Gobiinae, one of the five recognized subfamilies. They are native to the Sea of Azoz, Black Sea and Caspian basins, where they prefers shallow, brackish waters but also occurs in fresh water (Skora *et al.* 1999). During the spring in their native range, male round gobies are the first to appear on the spawning grounds where they set up territories after spending the winter in deeper waters (Kovtun 1980, Miller 1986). It is unknown whether individuals return to the same spawning areas the following year. In the Great Lakes, round gobies show high site fidelity during a single season, suggesting that they are philopatric and may return to the same spawning site the following year (Ray & Corkum 2001). Nest requirements for the round goby are stationary hard cavities in which there is only a single opening (Miller 1984). This can include crevices under logs and rocks as well as crayfish burrows (Charlebois *et al.* 1997). In Europe, male round

gobies mature at age 3 to 4 years, whereas females mature earlier, at age 2 to 3 years (Bil'ko 1971, Miller 1984). Typically, round gobies have a lifespan of 4-5 years within their native range (Charlebois *et al.* 1997). Introduced round gobies in the Detroit River have been found to mature earlier and at smaller sizes with a lifespan of 3 years (Corkum *et al.* 2004). North American female round gobies mature earlier (as young as age 1) than females in their native range (MacInnis & Corkum 2000a, b). Sexually mature males are dark black to slate grey in colour; they also have enlarged cheeks and are characterized by a larger size at maturity than females (Nilko'skii 1963, Miller 1984). Within a single nest there can be as many as 10,000 eggs deposited from four to six females, with fertilization success reaching as high as 95% (Charlebois *et al.* 1997). Round goby eggs are demersal and adhesive and are laid on the undersurface of the nest cavity (Miller 1984). A single round goby can produce 328 - 5,221 eggs and the eggs are among the largest of any gobiid species (3.2 mm; Kovtun 1978). Females can spawn several times in a single reproductive season and generally reproduce over multiple years. Male round gobies generally die after a single breeding season, as they do not feed while guarding the nest (Miller 1984). Sex-specific mortality can alter the sex ratio; Kovtun (1980) stated that the sex ratio was an important factor in predicting year class strength. In the Sea of Azov, juvenile survival is decreased if males were less numerous than females in the population (Kovtun 1980). In the Detroit River more females are found than males (1.27:1, MacInnis & Corkum 2000b). Within their native range, reproduction can start as early as April and extend into September (Miller 1986); however, the length of the spawning season is dependent on water temperature. Spawning can occur at temperatures ranging from 9 °C to 26 °C and at depths from 0.2 m to 1.5 m (Charlebois *et al.* 1997). In the Great Lakes, sexually mature round gobies have been observed on shipwrecks in Lake Erie at depths up to 11 m (Wickett and Corkum 1998).

Based on the capture of sexually mature round gobies and water temperatures of the Detroit River, it was estimated that the reproductive season may occur from mid-April to early November, where at least three spawning events could occur (MacInnis & Corkum 2000a).

Offspring are demersal and lack any true pelagic larval phase (Miller 1984). During their first year, male round gobies obtain sizes in the range of 100-130 mm standard length (SL); females are slightly smaller, 80-110 mm SL (Berg 1949 in MacInnis & Corkum 2000b). Round gobies in the Detroit River are considerably smaller than gobies in Eurasia at a given age: males and females had a mean SL of 58.4 mm and 62.8 mm respectively (MacInnis & Corkum 2000b).

Round Goby Feeding & Predation

Round gobies feed primarily on mollusks (e.g. zebra mussels). Their diet also includes zooplankton, crayfish, dragonflies, mayflies, fish eggs and larvae. Laboratory studies have confirmed that round gobies feed on the eggs of lake trout (*Salvelinus fontinalis*) (Chotkowski & Marsden 1998), and field studies have confirmed egg predation of lake sturgeon (*Acipenser fulvescens*) (in Corkum *et al.* 2004). Round gobies are a part of the diet of many important recreational piscivorous fish (in Corkum *et al.* 2004). The consumption of zebra mussels by round gobies has drawn many concerns. Contaminants in the benthos filtered and concentrated by zebra mussels have the potential to reach higher trophic levels through round goby predation by larger fish.

Potential Impacts

The elongated spawning season, high reproductive potential and aggressive nature of the round goby is a cause for concern as they have the potential to affect many native fish that occupy similar ecological niches. The round goby has been implicated in the decline of the mottled sculpin (*Cottus bairdi*) in both St. Clair River and southern Lake Michigan (Jude *et al.*

1995, Jude 1997). In the laboratory, round gobies also displace mottled sculpins from shelters (Dubs & Corkum 1996), potentially forcing mottled sculpins to sub-optimal habitats which may adversely affect their reproductive success. Round gobies have been implicated in similar population declines of the logperch (*Percina caprodes*) (Jude *et al.* 1995, Balshine *et al.* 2005). The round goby also has the potential to affect benthic species including other sculpins (e.g. slimy sculpin (*Cottus cognatus*)), darters (e.g. Johnny darter (*Etheostoma nigrum*)) and madtoms (*Noturus stigmosus*). Round goby densities in the central basin of Lake Erie are in the range of 1.8 – 17 /m^2. Higher densities (40/m^2) have been reported for Grand Calumet Harbour in southern Lake Michigan (Charlesbois *et al.* 1997). These high density aggregations have the potential to displace many native species from these areas. It was estimated that there were 9.9 billion round gobies in the western basin of Lake Erie in 2002 (Johnson *et al.* 2005).

Life History Traits Contributing to Invasion Success

The successful Great Lakes invasion by the round goby may be attributed to several factors. Round gobies are tolerant of various environmental conditions, such as low oxygen concentrations and changes in salinity, enabling them to have higher survival rates in poor water conditions found in ballast tanks. Tolerance to changes in salinity may be important in surviving ballast water exchange practices prior to entering the Great Lakes from continental sources. Round gobies also have a broad diet, allowing them to exploit different resources when food becomes limited. According to an invasional meltdown model suggested by Simberloff and Von Holle (1999), a previously established Ponto-Caspian invader, the zebra mussel, may have provided a plentiful food source that increased the likelihood of persistence for round gobies in the Great Lakes. Other possible contributing factors to the successful round goby colonization of the Great Lakes include their generally aggressive behaviour and their early maturation with the

ability to spawn numerous times throughout the spring, summer and fall. During initial colonization it is imperative that mates be able to find each other. The ability of the round goby to locate mates may have been aided by their pheromonal signaling and sensitive open lateral lines (Zielinski *et al.* 2003, Gammon *et al.* 2005). Finally, male parental care of eggs increases offspring survival and persistence in a novel environment.

1.4 CHAPTER 1 OBJECTIVES

The objective of Chapter 1 was to develop a molecular marker that can be used to assess round goby population structure. The genetic markers chosen were microsatellite DNA loci. Microsatellites were chosen because they are highly polymorphic and presumably neutral (not under selection). These markers were generated by developing a microsatellite DNA enriched genomic library, and screening it to identify 31 positive clones containing inserts of interest. Specific primer pairs were designed in the flanking region of the microsatellite repeat. Microsatellites were screened for variation and ease of amplification and 10 microsatellite loci were selected based on those criteria. All 10 markers were validated using 60 unrelated round gobies.

1.5 CHAPTER 2 OBJECTIVES

The objective of Chapter 2 was to use the microsatellite markers developed in Chapter 1 to assess population structure of 32 introduced round goby populations in the Great Lakes. Genotype assignment methods were used to assess recent patterns in migration. Combining the assignment analysis with measures of genetic diversity and divergence, I describe the dispersal

and colonization patterns of the round goby in the Great Lakes. The second objective was to evaluate temporal stability between five introduced populations that were sampled in 2005 and 2006.

1.6 CHAPTER 3 OBJECTIVES

The objective of Chapter 3 was to compare the genetic characteristics of recently colonized populations of the round goby that vary in time since establishment. I test for genetic bottlenecks and for effects on genetic diversity in a dynamic round goby invasion front (Maitland River). As a comparison, more established populations elsewhere in the Great Lakes were evaluated. As of 2006, round gobies had not been reported in the Maitland River. This active colonization event allowed me the chance to evaluate patterns of genetic differentiation. The invasion front in the Maitland River provides the opportunity to test population genetic theory predictions based on colonization events.

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2.0 TEN POLYMORPHIC MICROSATELLITE MARKERS IN THE INVASIVE ROUND GOBY (*NEOGOBIUS MELANOSTOMUS*) AND CROSS-SPECIES AMPLIFICATION

(Note: This Chapter has been accepted for publication in Molecular Ecology Notes)

2.1 INTRODUCTION

The round goby, *Neogobius melanostomus* is a small benthic fish native to the Ponto Caspian drainages in Eurasia. The invasiveness of the round goby can be attributed to its broad physiological tolerances to varying environmental extremes. Several life history characteristics, such as generalist diet, repeated annual spawning and aggressive behaviour also play a role in the successful establishment of round gobies in the Great Lakes, drawing concern that their spread may not only be limited to the Great Lakes region. The round goby was first reported in the Great Lakes basin in 1990 in the St. Clair River near Sarnia, Ontario (Jude et al. 1992), and they now inhabit all five Great Lakes (Charlesbois et al. 1997). The rapid proliferation and spread of the round goby may lead to many unforeseen ecological impacts on the natural biodiversity of the Great Lakes ecosystems. The round goby has the potential to impact native benthic fishes, such as the mottled sculpin, *Cottus bairdi*, through competitive displacement of preferred spawning sites (Dubs and Corkum 1996). It may also impact other benthic fish such as logperch, *Percina carprodes* and the already threatened darters. The round goby has been linked to a decrease in spawning success of lake trout, *Salvelinus fontinalis*, due to egg predation (Chotkowski and Marsden 1999). Round gobies are also egg predators of lake sturgeon, *Acipenser fulvescens* (Nichols et al. 2003), and smallmouth bass, *Micropterus dolomieu* (Steinhart et al. 2004). The round goby also has the potential to alter food web dynamics throughout the Great Lakes, as it is a voracious predator of zebra mussels (Ghedotti et al. 1995). Furthermore, simply its high abundance may replace native benthic fish as the preferred prey item for larger piscivores.

Previous genetic studies using mitochondrial sequence variation indicated high genetic diversity (Stepien & Tumeo 2006) among nonindigenous Great Lakes populations of round goby, yet little is known about dispersal mechanisms and gene flow among populations in North America. To evaluate dispersal and gene flow dynamics in the non-native round goby, I developed ten polymorphic microsatellite markers which will be useful in evaluating the current distribution and potential future range expansions of the round goby in the Great Lakes basin.

2.2 MATERIALS AND METHODS

Genomic DNA (gDNA) from a single round goby caudal fin was used to create a microsatellite-enriched gDNA library, following a protocol modified from Fisher and Bachman (1998). Genomic DNA was cut with one blunt-end cutting restriction enzyme (*RsaI*). Cut fragments were ligated to *Mlu*I adapter-primer complexes (21-mer: 5'-CTCTTGCTTACGCGTGGACTA -3' and a phosphorylated 25-mer: 5' – pTAGTCCACGCGTAAGCAAGAGCACA -3') with T4-DNA ligase (New England Biolabs, Ipswitch, USA). Ligated segments were hybridized with a $(GACA)_4$ biotinylated oligo probe and subsequently hybridized with streptavidin-coated magnetic beads (Roche, Indianapolis, USA). Beads were washed once with 2x SSC, 0.1 % SDS for 5 min at 25°C, once with 1x SSC for 5 min at 25°C and finally once with 1x SSC at 40°C. To elute enriched DNA, beads were resuspended in 300ul of ddH_2O and heated to 95°C for 2 min in a wet heating block. Enriched DNA was PCR amplified using 0.96 X PCR buffer (100 mM Tris HCl, 50mM KCl, Sigma-Aldrich, Oakville, Canada), 2.4 mM $MgCl_2$, 0.2mM of each dNTP, 0.048 U Taq polymerase (Sigma-Aldrich, Oakville, Canada), and 0.024μM 21-mer adaptor primer in a 13ul PCR reaction volume. The thermocycler profile was: 94°C for 1 min followed by 30 cycles of 94°C for 15 sec, 56°C for 30 sec, 72°C for 45 sec, with a final extension of 72°C for 2 min. The resulting

double-stranded microsatellite-enriched library was cloned into TOPO vectors and transformed into One Shot® competent *Escherichia coli* cells following the manufacturer's instructions (Invitrogen, Burlington, Canada). Positive colonies that contained the insert were white, these colonies were selected (N = 231) and clones were sequenced at the Genome Quebec Innovation Center (McGill University, QC). Thirty-one clones contained microsatellites, and primer pairs were designed flanking the microsatellite region using PRIMER3 (Rozen and Skaletsky 2000) and NETPRIMER (Premier Biosoft International) software. Ten of the 31 primer pairs were found to be easily amplified and polymorphic based on PCR amplification and visualization on an 1.8% agarose gel. Those 10 loci were characterized by genotyping 64 individuals using PCR conditions as follows: 50-100 ng of template DNA, locus specific concentrations of $MgCl_2$ (Table 1.1), 0.19 mM of each dNTP, 0.048 U *Taq* DNA polymerase (Applied Biosystems), 0.038 uM of forward dye-labeled primer (IR-700, IR-800 MWG Biotech), 0.057 uM reverse primer and 0.96X PCR buffer (100 mM Tris HCl, 50mM KCl) in a 13 uL reaction volume. The thermocycler profile used was: 94°C for 2 min, followed by 34 cycles of 94°C for 15 sec, annealing temperature (T_A, see Table 1.1) for 15 sec, 72°C for 30 sec, with a final extension of 72°C for 2 min. Dye-labeled PCR product was scored for fragment size on a LiCor 4300 DNA analyzer using Gene ImagIR 4.05 software (Scanalytics, Inc.) with three lanes containing manufacturers size standard (50bp – 350bp). Observed and expected heterozygosities as well as departure from Hardy-Weinburg equilibrium (HWE) were calculated using tools for populations genetic analyses (TFPGA) v1.3 (Miller 1997) and corrected for multiple simultaneous tests using the sequential Bonferroni method (significance at $P_i \leq \alpha/(1 + k - i)$; Rice 1989).

2.3 RESULTS

There was no evidence for departure from HWE at any of the 10 loci, although *Nme10* has an excess of homozygotes in this population. Observed and expected heterozygosities ranged from

0.3276 – 0.8571 and 0.3450 – 0.898 respectively (Table 2.1). Loci were tested for linkage disequilibrium using the program Arlequin ver 3.1(Excoffier and Schneider 2005) and *Nme6* and *Nme8* were in significant linkage disequilibrium after Bonferronni correction ($P < 0.0001$). The program MICRO-CHECKER (Oosterhout 2005) was used to check each locus for the presence of null alleles. One locus contained null alleles (*Nme10*).

I investigated the performance of our microsatellite DNA PCR primers in two or three animals from an additional five related taxa (Table 2.2). Successful amplification was observed for all but two of the marker loci (*Nme1* and *Nme3*), and there was considerable variation among species in the success of the primer pairs (Table 2.2). Curiously, the only congeneric species, *Neogobius gymnotrachelus*, was generally not very successfully amplified with my primers (Table 2.2).

2.4 CONCLUSION

Nonindegenous species not only pose threats to native biodiversity, they also represent valuable natural experiments in species colonization and range expansion. The microsatellite markers described here will enable researchers to better quantify and characterize population dynamics in introduced round gobies, as well as potentially predicting future population expansion. Therefore population genetic research involving the nonindigenous round goby will aid in developing management plans and aid in our understanding of the evolutionary responses and dispersal mechanisms of nonindegenous species after introduction into novel environments.

Table 2.1 Ten microsatellite loci for the round goby, *Neogobius melanostomus*, with number of individuals genotyped (N), accession number, repeat motif, primer sequence, annealing temperature (T_A), and $MgCl_2$ concentration. Allelic range, number of alleles (n) and observed (H_O) and expected heterozygosities (H_E) based on 64 fish from Lake Erie are shown. All markers were found to be in Hardy-Weinberg equilibrium.

Locus	Accession No.	Repeat Motif	Primer Sequence	T_A (°C)	[$MgCl_2$] (mM)	Allelic Range (bp)	*n*	H_O	H_E
Nme 1 N=64	DQ999976	$(GTCA)_8(GTCT)_{11}$ GC $(CTGT)_{10}$	F: CGAGCGCTAAAATAGAAGAAAA R: TCCAGTGGCTTGAGTGATGT	48	2.4	212-340	12	0.86	0.84
Nme 2 N=63	DQ999977	$(CA)_{13}$	F: TGTGTAATGACGTGGAATAGCC R: CAATAGGCCAGGATGAATGAG	55	2.1	230-242	4	0.44	0.52
Nme 3 N=53	DQ999978	$(AGAC)_{14}$	F: GCGGGAGTCAAGAATTGAAC R:TTGTTAGAATGTATTATGCCATAGCC	48	2.4	124-176	10	0.77	0.78
Nme 4 N=60	DQ999979	$(TCTG)_7$	F: TGTGCTTGGTTAAGGTGGTG R: CCGGACAGAAACAACTTAAAGC	55	2.4	89-117	4	0.33	0.35
Nme 5 N=64	DQ999980	$(CA)_4$ GC $(TCTG)_7$	F: GTCACACCGATCTTCGACTG R: GATTTACTTGATTCATCACT	48	2.4	127-141	7	0.37	0.38
Nme 6 N=64	DQ999981	$(TCTG)_9(TCTT)_{10}$ ACTTT $(CTTA)_6$	F: GCAACTCAACCTCGTAGCC R: CCGAAAAGCCAATTAAGCAC	59	1.9	227-307	10	0.69	0.52
Nme 7 N=60	DQ999982	$(AGAC)_5$	F: AATGGATGGGTCAATTGCAT R: AAGGTTGAGCTGCCACTGAG	48	2.4	158-170	3	0.41	0.52
Nme 8 N=64	DQ999983	$(TG)_8$	F: ATGGAGTTTCTGGGCAGTTG R: CTCCGTCGATTGTGTTCTGA	55	2.4	271-285	8	0.78	0.81
Nme 9 N=63	DQ999984	$(ATCC)_{12}$	F: GGGGTGCACTTGTTTAGCTC R: AACGGACAAGTGGAAGAAGG	59	2.4	161-213	7	0.57	0.57
Nme 10 N=61	DQ999985	$(AC)_{10}$	F: GCGATTATGAGGTTCGGAGA R: ATCAGCAACCCCTGAACAGA	48	2.4	267-287	8	0.71	0.73

Table 2.2 Cross-species amplification of 10 novel polymorphic microsatellite primer pairs developed for *Neogobius melanostomus*. Five related taxa were tested using these primer pairs (n is the number of individuals tested). Ø indicates non-amplification, MB indicates multiple bands, and where amplification was successful, then number of alleles observed is given (and alleles sizes are shown in parentheses).

Species	Locus Nme 1	Nme 2	Nme 3	Nme 4	Nme 5	Nme 6	Nme 7	Nme 8	Nme 9	Nme 10
Gobiosoma bosc (n = 3)	Ø	2 (140,200)	Ø	1 (105)	2 (133,179)	2 (143,307)	2 (182,206)	2 (240,346)	Ø	Ø
Ctenogobius boleosoma (n = 3)	Ø	1(136)	Ø	1 (281)	1 (141)	2 (251,299)	2 (178,324)	2 (240,346)	MB	Ø
Ctenogobius sagittula (n = 2)	Ø	2 (108,184)	Ø	1 (105)	Ø	2 (207,251)	2 (128,158)	1 (304)	3 (124-222)	1 (279)
Neogobius gymnotrachelus (n = 3)	Ø	Ø	Ø	Ø	1 (137)	Ø	1 (178)	1 (224)	Ø	Ø
Coryphopterus personatus (n = 2)	Ø	Ø	Ø	1 (105)	1 (127)	1 (251)	Ø	Ø	Ø	Ø

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3.0 DISPERSAL AND COLONIZATION OF THE INVASIVE ROUND GOBY (*NEOGOBIUS MELANOSTOMUS*) IN THE GREAT LAKES

3.1 INTRODUCTION

The colonization of novel habitats by invasive species is most often accompanied by dramatic shifts in selection pressures which can lead to rapid evolution (Mooney & Cleland 2001). These selection pressures likely vary over geographical scales and, combined with stochastic variation associated with founder events, may drive the differentiation of newly colonized reproductively isolated populations. Alternatively, gene flow among recently introduced populations may facilitate establishment of exotic species by increasing genetic variation and through the spread of rare introduced genotypes on which natural selection may act (Ellstrand & Schierenbeck 2000). Dispersal in the novel environment is also important for the establishment of an invasive species as their persistence and reproductive success is dependent on the availability of resources and the number of spawning sites in a given area. Dispersal is thus important to maximize resource use and to buffer against stochastic events that can increase local population extinction likelihoods. Alternatively, since dispersal is risky because resources and suitable spawning habitats may not be present outside their current range, successful introduced species may exhibit limited natural dispersal. The colonization of novel habitats by successful invasive species provides a means by which we can evaluate the importance of dispersal in their establishment. Evaluating dispersal of invasive species may provide a better understanding of the interactions between natural and human mediated dispersal and how such mechanisms can lead to drastic range expansions.

The round goby (*Neogobius melanostomus*) is a invasive benthic fish species. From its native range in the Ponto-Caspian region of Eastern Europe, the round goby has been extending

its home range. In recent decades the round goby has been dispersing in Europe and is now a part of the benthic community in many rivers draining into the Black sea, such as the Danube and Dneiper Rivers (Anhelt *et al.* 1998). The construction of navigation canals in Europe and an increase in commercial freighter traffic has allowed the round goby to increase its range at an unprecedented rate. The introduced round goby is now the dominant fish species in the Gulf of Gdansk in the Baltic Sea (Skora and Stolarski 1993, Sapota 2004). Over the same time period, the round goby has successfully invaded the Laurentian Great Lakes of North America, likely facilitated by ballast water transfer (Ricciardi & MacIsaac 2000). The adaptable life history and broad physiological tolerance of the round goby has enabled it to establish and spread throughout the Great Lakes and some tributaries. Specific traits that provide the round goby an invasive advantage include their tolerance to various environmental conditions, such as low oxygen and varying salinity concentrations. Other valuable invasive traits include high fecundity coupled with parental care, and general aggressive behaviour in acquiring food and spawning sites (Charlebois *et al.* 1997, Corkum *et al.* 1998, MacInnis and Corkum 2000). Now established, this species has become the dominant benthic fish in many areas of the Great Lakes and is of great concern as its effects on native fish species can be unpredictable (Jude 1997).

The ability of an invading species to persist and adapt to a novel habitat may depend on the genetic variation in the founding population(s) (Williamson 1996). However, many newly invaded populations are characterized by low genetic diversity, presumably due to small founding population size (e.g. Berg *et al.* 2002, Meimberg *et al.* 2006). If such populations remain at low abundance, they will also be affected by random genetic drift, which acts to further reduce genetic diversity and may result in the population being out of mutation-drift equilibrium. By nature, colonization events are stochastic, and are generally coupled with small founding

populations that must survive rapid transitions in the biological and chemical composition of the new habitat (Lee 1999). Thus, the genetic signature of a colonizing species is characterized by low heterozygosity and reduced frequency of rare alleles (Cornuet and Luikart 1996). Additionally, population differentiation should increase due to founder effects and genetic drift (Nei *et al.* 1975). Previous mitochondrial DNA sequence studies of the round goby in the Great Lakes have characterized them as having a large and diverse array of mtDNA haplotypes (Dillon and Stepien 2001). Given that the Great Lakes gobies also had levels of genetic diversity equivalent to that found in their native range, Stepien and Tumeo (2006) concluded that the Great Lakes round gobies were most likely founded by large introductions or multiple invasion events. Slight genetic differentiation was observed between two introduced populations (Stepien and Tumeo 2006) and previous sequence analyses of the faster evolving mtDNA control region identified higher genetic divergence between these two Great Lakes sites (Dillon & Stepien 2001). These results suggest that colonization was most likely from multiple Eurasian sources.

The potential for natural dispersal of the round goby may be limited; round gobies display high site fidelity and philopatry based on mark-recapture studies (Wolfe and Marsden 1998; Ray and Corkum 2001). In their native range, round gobies are found in littoral areas during the summer and migrate to deeper waters in the winter (Miller 1986), yet it is not known whether individuals return to the same area the following year. Given their apparent limited opportunity for natural dispersal, on-going ballast water transfer within the Great Lakes may be responsible for moving round gobies throughout much of the Great Lakes, seeding new areas and adding novel genotypes to established populations. Such a scenario is consistent with the sudden and substantial range expansions of the round goby throughout the Great Lakes (Wolfe and Marsden 1998). The once common practice of using round gobies as bait may also have played

a role in round goby dispersal, as round gobies have been found in areas where no ballast water is discharged (personal communication, Art Timmerman, OMNR, Guelph District). Alternatively, the natural dispersal potential of round gobies in the Great Lakes may be higher than anticipated based on our understanding of their life history in their native habitat. The dispersal and consequent range expansion of the round goby in the Great Lakes is likely a complex process characterized by multiple dispersal strategies including both natural and human mediated dispersal; however, the specific mechanisms by which they have so rapidly and effectively colonized the Great Lakes are not known.

The goal of this study is to estimate dispersal and characterize the genetic patterns that reflect the colonization history of the round goby among 32 sample sites in 3 of the Laurentian Great Lakes (Lakes Huron, Ontario and Erie) basins using 10 polymorphic microsatellite markers. The characterization of population genetic structure and dispersal patterns provides insight into the processes that led to the rapid range expansion of round gobies throughout the Great Lakes and may allow us to differentiate between natural versus human mediated dispersal. Understanding the patterns and history of these invasions will help in predicting the potential for future spread by identifying common dispersal pathways as well as source and sink populations. Identifying these source populations will also allow fisheries managers to spatially target management efforts in order to reduce further range expansion and establishment success. Invasive species also represent a unique opportunity to study adaptation and the effects of genetic drift in a colonization event and provide a valuable model in which we can study the rapid range expansion of a species in a novel environment.

3.2 MATERIALS AND METHODS

Sample collection and geographical information

Round gobies were caught in Lakes Huron, Erie and Ontario in the summer and fall of 2005 and 2006 at 32 sites (Fig. 3.1); five of those sites were sampled in both 2005 and 2006 to estimate temporal stability. The number of individuals taken per site ranged from 21-128, with an average of 61.2 individuals per site (total N = 1958). Fish were collected using three different capture techniques. Seine netting consisted of 2-3 separate passes of 10-20 m.At sites where shoreline access was possible, seine netting was used. At shoreline sites where access was limited, hook and line were used to capture round gobies. Offshore samples were collected by trawl nets. Sampling sites were selected based on known presence of round gobies, accessibility and ease of capturing a sufficient sample size. GPS coordinates were taken at all sites. A portion of the caudal fin was collected and stored in a 1.7 ml tube with 95% ethanol for future DNA extraction.

Microsatellite genotyping

DNA was extracted using a Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) or using a 96-well glass-fiber plate protocol described by Elphinstone *et al.* (2003). All round goby samples were genotyped at 10 polymorphic microsatellite loci (Chapter 2). PCRs were prepared in 7 µl total volumes, each reaction contained 50-100 ng of template DNA, 0.89X PCR buffer (Sigma-Aldrich, Oakville, Canada; 100 mM Tris-HCl, pH 8.3; 500 mM KCl), locus specific concentrations of $MgCl_2$ (see Chapter 2), 0.2 mM of each dNTP, 0.024 uM of reverse and fluorescence IRDye® infrared dye labeled forward primer (IR700, IR800, MWG Biotech, High Point, NC, USA) with 0.048 U of *Taq* DNA polymerase (Sigma-Aldrich,

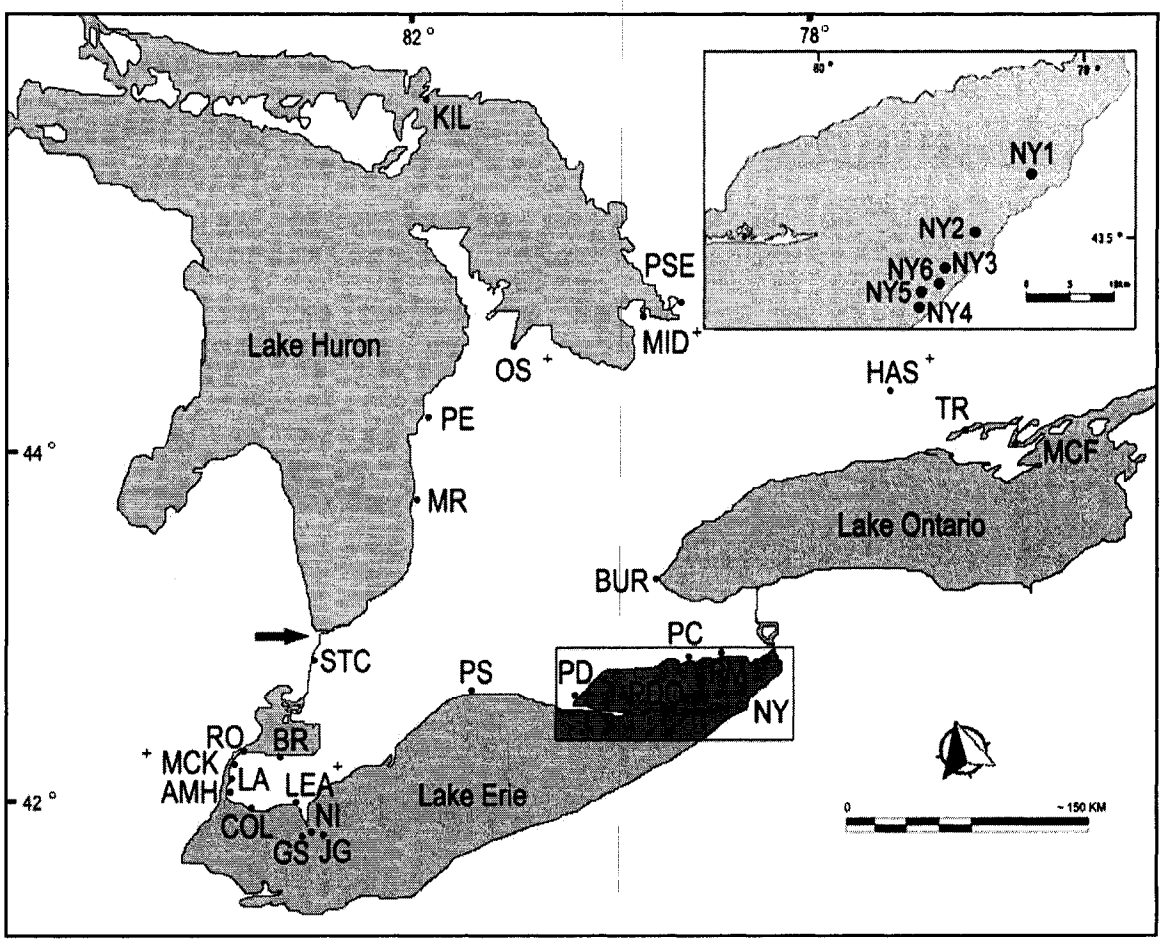

Figure 3.1 Map of 32 Great Lakes invasive populations of the round goby (*Neogobius melanostomus*) sampled during the summer and fall of 2005 and 2006. Site abbreviations are described in Table 1. Arrow indicates site of initial introduction. The shaded area is enlarged in the upper right corner and displays the locations of the samples from the eastern basin of Lake Erie. "+" indicates sites that were sampled in both years.

Oakville, Ontario, Canada). Thermocycler profiles consisted of a 2 min initial denaturation at 94 °C, followed by 35 cycles of 15 s at 94 °C, 15 s locus specific annealing temperatures (see Chapter 2), 30 s extension at 72 °C, with a final extension step at 72 °C for 2 min. Amplified PCR products were visualized on a LI-COR 4300 DNA Analysis System (Lincoln, Nebraska USA). Allele size scoring was accomplished using Gene ImagIR 4.05 (Scanalytics, Inc. Rockville, MD USA) using manufacturers' size standard (50bp-350bp). To avoid allele size scoring bias due to variation in gel consistency, multiple populations were run on all gels.

Genetic diversity

Mean observed (H_O) and expected heterozygosities (H_E) for all populations across all loci were calculated using ARLEQUIN VER. 3.1 (Excoffier *et al* 2005). Mean allelic richness (A) for all populations across all loci was estimated using FSTAT 2.9.3.2 (Goudet 2001). Deviations from Hardy-Weinberg equilibrium (HWE) for at loci in all populations were estimated using TFPGA 1.3 (Miller 1997) using an exact test employing a Markov chain algorithm with 10000 permutations; the test probabilities were corrected according to the sequential Bonferroni method (Rice 1989) for multiple simultaneous tests.

Population Structure

Population structure was assessed by estimating genetic divergence between sites using F-statistics calculated in ARLEQUIN VER. 3.1 (Excoffier *et al* 2005). Test probabilities were corrected for multiple simultaneous tests according to the sequential Bonferroni method (Rice 1989). A modified Fischer's exact test (Raymond and Rousseau 1995) in the program TFPGA 1.3 (Miller 1997) was used t test for differences in allelic frequency between sites, using the conventional Monte Carlo method, wit

10 batches and 1000 permutations per batch. All test statistics were corrected using the sequential Bonferroni method for multiple simultaneous tests (Rice 1989). The program POPULATIONS version 1.2.28 (Langella 2002) was used to construct a genetic distance matrix based of Cavalli-Sforza & Edwards' (1967) chord distances (D_C). To investigate migration among sites (gene flow) we used a Bayesian genotype assignment approach outlined by Rannala & Mountain (1997) in the computer program GENECLASS 2.0 (Piry *et al.* 2004). To identify successful assignment, the highest rank probability for assignment was divided by the second highest rank probability. If the number obtained was greater than four, meaning it was four times more likely to originate from the higher ranked population than the next highest ranked population, the individual was deemed successfully assigned. The number four was chosen arbitrarily, and did not differ significantly from other test numbers, only proportions changed. Based on this criterion, individuals were categorized into one of three categories: self assignment, migrant of known source (i.e. likelihood ratio > 4), or unidentified. To assess the exte to which a population is dispersive, a dispersal index was calculated as the number of migrants divided by the number of migrants plus the number of self-assigned. A highly dispersive population would ha a dispersal index of 1; a population where dispersal was low and self-assignment was high would have dispersal index of 0.

The two St. Clair River sites (Courtright (COUR) and Sombra (SP)) were found to be genetically indistinguishable and subsequently combined for the assignment analyses. This was based on low genetic divergence (F_{ST}= 0.01), non-significant exact test results (p>0.05), spatial proximity (~10.5 km) and an *a priori* expectation that these sample sites closely reflect the initial founding population in the Great Lakes based on geographical location of the initial site of introduction (Jude *et al.* 1992, Jude *et al.* 1995).

Isolation by distance

Isolation by distance (IBD) is a powerful null model of gene flow patterns among populations whereby we can test for anomalous dispersal patterns and hence infer the mechanisms behind the spread of the round goby in the Great Lakes. The IBD model assumes mutation-drift equilibrium which is likely inappropriate for rapidly expanding and recently established populations. We tested for IBD using a Mantel test in GENALEX V. 6 (Peakall and Smouse 2006), with geographical distance versus F_{ST} and D_C. IBD was first tested across all samples, then within Lakes Erie, Ontario, and Huron independently. IBD was also tested within three regions representing smaller spatial scales chosen based on the expectation that gene flow within those regions may be closer to equilibrium, given the historical spread of round gobies and hence where biological connectivity was judged to be most likely. The first region included 13 sites in the St. Clair River-Western Basin of Lake Erie corridor, including a site in the northern portion of the St. Clair River, presumably the initial colonists to the Great Lakes (Sites = STC, BR, RO, MCK, MCK2, LA, COL, LEA, LEA2, GS, NI and JG; see Fig. 3.1). The second region consisted of sites (N=12) in Eastern Lake Erie and one site from Lake Ontario. Included in the analysis were samples from Port Colborne, one of the earliest suddenly established populations (Sites = BUR, PC, PM, PST, PDO, PDO2, NY1, NY2, NY3, NY4, NY5 and NY6; see Fig. 3.1). The final region included sites (N=5) at both ends of the Trent Severn Waterway, including one site ~75 km upstream from the Bay of Quinte outlet (Sites = TR, MCF, HAS, HAS2, MID, and MID2; see Fig. 3.1).

Temporal Stability

Temporal stability was examined using data from five sites (LEA, MCK, OS, HAS and MID, see Fig. 3.1) that were sampled in 2005 and 2006. Sites were chosen based on sample availability in 2005. Temporal stability was assessed by testing for differences in allelic frequencies across years; this was done by computing exact tests of differentiation in the computer program TFPGA (Miller 1997). Genetic divergence (F_{ST}) was calculated between sites across both years in ARLEQUIN VER. 3.1 (Excoffier *et al* 2005) test probabilities were corrected according to Rice (1989). A temporal AMOVA was used to quantify the amount of variation that was due to among-site variation versus sample–year variation.

3.3 RESULTS

Genetic Diversity

Mean observed heterozygosities (H_E) across all sites ranged from 0.43 - 0.65, and expected heterozygosities ranged from 0.47 – 0.69 (Table 3.1). Measures of mean allelic richness (A) were found to be between 3.16 and 5.60 (Table 3.1). HAS2 and PSE had the lowest mean allelic richness values of 3.45 and 3.16 respectively, indicating lower diversity, whereas LA and RO had the highest mean A values of 5.60 and 5.54 respectively, indicating higher diversity. As expected, many loci were not in HWE: 43 locus x population comparisons out of 370 (11.6%) were out of HWE after Bonferroni correction (Table 3.1), likely due to their recent establishment, high population growth and insufficient time to establish equilibrium. In most cases the deviations from HWE could be attributed to an excess of homozygotes (Table 3.1).

Temporal Stability

Exact test of allelic differentiation between pairwise sample sites from 2005 and 2006 were all significant, indicating that allele frequencies at those sites have changed from 2005 to 2006 (P< 0.0001, after Bonferroni correction). The temporal AMOVA revealed significant variation among years within populations (4.06%; P <0.0001) and, as expected, a significant variation was found among populations (10.68%; P < 0001). F_{ST} values indicate that MCK and HAS were not significantly different between years, whereas LEA, MID and OS showed significant and stronger genetic divergence between years. Sample sites from 2005 and 2006 were thus not temporally stable and were treated as separate populations and were not combined for analyses.

Population Structure

Sites within the St. Clair River – Western Basin of Lake Erie corridor were the least genetically divergent from one another based on measures of *F-statistics*, with the exception of one site, JG, the furthest site east in the western basin of Lake Erie. Many sites in the St. Clair River – Western Basin of Lake Erie corridor were not significantly different from the NY sites in the eastern basin of Lake Erie (11/15 population comparisons, 73.3%) and to the sites in the Bay of Quinte (MCF and TR, 12/15 site comparisons, 80%) (Table 3.2). All New York sites (NY1-NY6) were genetically similar with the exception of NY2. Several sites were strongly divergent from all other sites. HAS had a mean pairwise F_{ST} value of 0.154 and was most genetically similar to HAS2 (mean F_{ST} = 0.164). Similarly, PSE, MID and MID2 had high mean pairwise F_{ST} values of 0.139, 0.087 and 0.083 respectively. Exact tests of allelic differentiation revealed that the allele frequencies of MCF were not significantly different than TR and also with sites in

Table 3.1 Characterization of variation at 10 polymorphic microsatellite loci used to determine population structure of the round goby (*Neogobius melanostomus*) in the Great Lakes from samples collected in 2005 and 2006 (ID refers to the abbreviated location name in Figure 3.1, and geographical coordinates are given). Expected heterozygosity (H_E), observed heterozygosity (H_O), number of individuals genotyped (n) and allelic richness (A) are presented. Loci out of HWE after Bonferonni correction are underlined and in bold-face type.

Site	Site Detail	ID		*Nme1*	*Nme2*	*Nme3*	*Nme4*	*Nme5*	*Nme6*	*Nme7*	*Nme8*	*Nme9*	*Nme10*	*Mean*
Trenton (2005)	River	TR	H_E	0.89	0.63	0.81	0.34	0.22	0.74	0.48	0.73	0.72	0.78	0.64
Lat. 44.1	Mouth		H_O	1	0.52	0.75	0.33	0.24	0.59	0.3	0.65	0.72	0.67	0.58
Long. 77.6			*A*	9.66	3.79	7	2.69	2.75	5.82	3.86	6.55	4.83	6.33	5.33
			n	25	27	24	27	21	17	27	26	25	24	24.3
Hastings (2006)	Lock	HAS	H_E	0.76	0.62	0.56	0.45	0.4	0.64	0.22	0.82	0.61	**0.68**	0.58
Lat. 44.3			H_O	0.86	0.72	0.51	0.5	0.47	0.47	0.25	0.66	0.71	0.28	0.54
Long. 78.0			*A*	4.33	3	4.27	2	2	3.64	2.3	6.13	3.34	4.75	3.58
			n	36	36	35	36	32	30	36	35	35	36	34.7
Hastings (2005)	Lock	HAS2	H_E	**0.76**	0.63	0.66	**0.44**	0.36	0.66	0.16	0.82	0.63	0.7	0.58
Lat. 44.3			H_O	0.74	0.63	0.6	0.37	0.33	0.44	0.13	0.87	0.6	0.43	0.52
Long. 78.0			*A*	5.03	3.2	4.13	2	2	3	2.08	6.01	3	4.06	3.45
			n	46	60	53	60	57	52	60	47	60	46	54.1
Burlington (2006)	Marina	BUR	H_E	0.71	0.47	0.62	0.32	0.12	0.67	0.56	0.73	0.68	0.77	0.56
Lat. 43.3			H_O	0.55	0.45	0.7	0.38	0.12	0.64	0.3	0.53	0.77	0.52	0.5
Long. 79.8			*A*	6.01	3.19	3.8	2.4	2.15	5.82	3.4	4.55	3.94	6.18	4.14
			n	53	53	53	53	50	47	53	51	53	50	51.6

Port Colborne (2006)	Canal	PC	H_E	0.79	0.54	0.7	0.48	0.3	0.63	0.52	0.8	0.54	0.79	0.61
Lat. 42.9			H_O	0.78	0.53	0.81	0.44	0.31	0.53	0.28	0.59	0.45	0.59	0.53
Long. 79.2			A	8.15	3.86	6.06	2	2.86	4.66	2.38	6.27	3.55	5.61	4.54
			n	32	30	32	32	32	32	32	32	31	32	31.7
Port Maitland (2006)	Pier	PM	H_E	0.74	**0.53**	0.8	0.56	0.52	0.6	0.51	0.8	0.56	0.57	0.62
Lat. 42.9			H_O	0.51	0.36	0.76	0.65	0.63	0.71	0.47	0.74	0.57	0.44	0.58
Long. 79.6			A	6.68	3.35	5.46	2.87	3.42	4.2	2.43	6.31	4.09	4.12	4.29
			n	49	44	45	49	48	41	49	43	42	45	45.5
Port Dover (2006)	Pier	PD	H_E	**0.76**	0.67	0.78	0.37	0.62	0.77	0.56	0.79	0.6	0.77	0.67
Lat. 42.6			H_O	0.63	0.48	0.79	0.4	0.48	0.49	0.46	0.74	0.71	0.56	0.57
Long. 80.4			A	5.87	3.23	6.35	2.23	4.54	6.48	3.07	6.21	4.07	6.65	4.87
			n	51	52	52	52	50	45	52	50	52	50	50.6
Port Stanley (2006)	Marina	PST	H_E	0.67	0.42	0.78	0.42	0.58	0.67	**0.51**	0.82	0.39	0.69	0.6
Lat. 42.7			H_O	0.28	0.41	0.82	0.47	0.42	0.7	0.49	0.76	0.4	0.52	0.53
Long. 81.2			A	5.84	2.98	5	2	3.62	5.45	2.27	7.06	2.73	5.94	4.29
			n	39	39	44	45	38	43	45	42	45	44	42.4
LaSalle (2006)	Marina	LA	H_E	0.85	0.69	0.77	0.44	0.58	0.67	0.6	0.85	0.62	**0.81**	0.69
Lat. 42.2			H_O	0.96	0.52	0.77	0.4	0.45	0.45	0.29	0.7	0.65	0.71	0.59
Long. 83.1			A	8.01	3.96	6.17	2.4	5.75	5.98	3.37	7.32	5.88	7.13	5.6
			n	28	31	30	30	22	29	31	30	31	31	29.3
Northern Indiana (2006)	Shipwreck	NI	H_E	**0.84**	0.64	0.78	0.41	0.33	0.62	0.52	0.79	0.57	**0.8**	0.63
Lat. 41.9			H_O	0.63	0.54	0.79	0.44	0.33	0.55	0.44	0.88	0.58	0.5	0.57
Long. 82.5			A	9.1	3.78	6.48	2.44	3.79	5.49	2.58	5.7	3.38	6.78	4.95
			n	46	46	48	48	48	47	48	48	48	48	47.5

Jay Gould (2006)	Shipwreck	JG	H_E	0.74	**0.54**	0.8	0.31	0.42	**0.58**	0.59	0.86	0.52	**0.78**	0.61
Lat. 41.9			H_O	0.4	0.51	0.6	0.38	0.4	0.64	0.7	0.7	0.51	0.49	0.53
Long. 82.4			*A*	6.7	2.97	6.13	2	3.62	4.99	3.46	7.4	3.41	6.63	4.73
			n	43	43	45	45	43	45	44	44	45	45	44.2
St.Clair River (2006)	Shore	STC	H_E	0.86	0.63	0.79	0.42	0.32	**0.74**	**0.51**	0.81	0.61	**0.78**	0.65
Lat. 42.8	Seine		H_O	0.85	0.37	0.75	0.41	0.34	0.48	0.41	0.68	0.63	0.53	0.55
Long. 82.5			*A*	8.76	3.69	6.54	2.31	3.81	6.99	2.61	6.73	5	6.44	5.29
			n	98	79	104	104	100	93	85	102	105	103	97.3
St.Rose Beach (2006)	Shore	RO	H_E	0.9	0.67	0.76	0.35	0.32	0.75	0.54	0.81	0.65	0.81	0.66
Lat. 42.3	Seine		H_O	0.81	0.5	0.78	0.32	0.27	0.42	0.32	0.73	0.57	0.5	0.52
Long. 83.0			*A*	10.23	3.89	5.82	2	3.72	7.29	3.41	6.35	5.57	7.14	5.54
			n	58	56	58	60	59	57	59	56	56	60	57.9
Amherstburg (2006)	Marina	AHM	H_E	0.85	0.67	0.75	0.43	0.51	0.6	0.55	0.85	0.6	0.78	0.66
Lat. 42.1			H_O	0.88	0.68	0.88	0.44	0.39	0.64	0.4	0.6	0.67	0.92	0.65
Long. 83.1			*A*	7.15	3.96	6.02	2	5.48	5.9	3.22	7.31	4.87	5.75	5.17
			n	24	25	24	25	23	22	25	25	24	24	24.1
Colchester (2006)	Pier	COL	H_E	0.84	0.56	0.8	0.48	0.41	0.66	**0.54**	0.85	0.59	0.66	0.64
Lat. 42.0			H_O	0.92	0.38	0.91	0.36	0.36	0.42	0.29	0.75	0.72	0.42	0.55
Long. 82.9			*A*	7.94	3.75	6.15	2.74	4.18	4.92	3.73	7.36	4.34	4.36	4.95
			n	25	24	23	25	25	24	24	20	25	24	23.9
Belle River (2006)	Beach	BR	H_E	0.87	0.68	0.75	0.34	0.31	0.75	0.56	0.84	0.62	0.74	0.65
Lat. 42.3	Seine		H_O	0.8	0.6	0.8	0.33	0.33	0.5	0.28	0.75	0.69	0.61	0.57
Long. 82.7			*A*	9.21	3.91	6.49	2.2	3.86	6.13	3.09	7.44	5.57	5.12	5.3
			n	59	58	59	60	60	56	60	59	58	56	58.5

George Stone (2006)	Shipwreck	GS	H_E	0.88	0.55	0.78	0.35	0.36	**0.68**	0.49	0.86	0.59	0.78	0.63
Lat. 41.9			H_O	0.78	0.45	0.79	0.38	0.39	0.56	0.47	0.72	0.5	0.58	0.56
Long. 82.6			A	9.31	3.5	5.6	2.34	4.02	5.72	2.78	7.38	3.88	6.98	5.15
			n	63	62	63	64	62	64	64	64	62	55	62.3
McFarland (2006)	Shore	MCF	H_E	0.85	0.65	0.79	0.32	0.13	**0.71**	**0.59**	0.83	0.7	**0.76**	0.63
Lat. 44.0	Seine		H_O	0.68	0.53	0.71	0.36	0.13	0.42	0.43	0.57	0.74	0.54	0.51
Long. 77.1			A	8.37	3.85	6.72	2.63	2.4	7.46	3.5	7.28	5.69	5.44	5.33
			n	34	40	38	42	31	36	40	37	42	35	37.5
McKee (2006)	Shore	MCK	H_E	0.85	0.71	0.79	0.28	**0.31**	**0.75**	0.59	0.75	0.7	**0.75**	0.65
Lat. 42.3	Seine		H_O	0.8	0.5	0.81	0.25	0.32	0.42	0.37	0.8	0.65	0.58	0.55
Long. 83.1			A	8.74	3.94	6.53	2.47	3.81	6.64	3.65	6.58	5.55	5.23	5.31
			n	59	60	59	60	59	57	59	60	55	57	58.5
McKee (2005)	Shore	MCK2	H_E	0.86	0.66	0.75	0.33	0.39	0.77	0.5	0.79	0.66	0.72	0.64
Lat. 42.3	Seine		H_O	0.89	0.55	0.75	0.31	0.39	0.53	0.29	0.62	0.6	0.61	0.55
Long. 83.1			A	8.87	3.86	6.15	2.56	3.75	6.24	2.37	6.03	5.75	6.38	5.2
			n	63	55	63	64	64	57	59	61	57	54	59.7
Leamington (2006)	Pier	LEA	H_E	**0.84**	0.54	0.77	0.4	0.62	0.76	0.57	0.78	0.61	**0.77**	0.66
Lat. 42.0			H_O	0.67	0.47	0.86	0.44	0	0.41	0.4	0.73	0.49	0.59	0.5
Long. 82.6			A	8.06	3.54	6.65	2.65	3.99	6.47	3.21	6.31	4.34	6.31	5.15
			n	63	64	64	64	62	64	63	64	63	63	63.4
Leamington (2005)	Pier	LEA2	H_E	0.85	0.52	0.79	0.34	0.4	0.7	0.53	**0.81**	0.57	0.74	0.62
Lat. 42.0			H_O	0.86	0.45	0.77	0.32	0.38	0.52	0.41	0.78	0.58	0.7	0.58
Long. 82.6			A	8.19	3.16	7.06	2.36	4.15	6.3	2.69	6.15	4.03	5.11	4.92
			n	64	62	53	59	64	63	59	64	62	60	61

Killarney (2005)	Shore	KIL	H_E	0.84	0.57	0.7	0.53	0.18	0.75	0.45	0.84	0.61	**0.77**	0.62
Lat. 46.0	Seine		H_O	0.78	0.43	0.67	0.7	0.19	0.63	0.36	0.81	0.63	0.63	0.58
Long. 81.6			*A*	8.45	3.64	4.7	2.57	2.56	5.71	2.19	6.91	4.78	6.45	4.8
			n	64	63	63	64	64	64	64	62	64	62	63.4
Midland (2006)	Marina	MID	H_E	0.8	0.66	0.58	0.22	0.34	0.71	0.55	**0.74**	0.44	**0.81**	0.59
Lat. 44.8			H_O	0.54	0.6	0.58	0.25	0.38	0.47	0.56	0.66	0.48	0.61	0.51
Long. 79.9			*A*	5.54	3.78	4.39	1.97	2.98	4.11	2.82	5.49	3.69	6.98	4.18
			n	59	63	60	64	64	64	64	62	61	59	62
Midland (2005)	Marina	MID2	H_E	0.81	0.64	0.58	0.13	0.47	0.63	0.55	0.75	0.53	0.74	0.58
Lat. 44.8			H_O	0.66	0.58	0.52	0.14	0.47	0.68	0.55	0.8	0.56	0.56	0.55
Long. 79.9			*A*	6.32	3.52	4.42	1.85	2.96	3.67	3.04	5.44	4.12	5.52	4.09
			n	59	55	56	58	59	60	60	56	41	57	56.1
Owen Sound (2006)	Shore	OS	H_E	**0.74**	0.52	0.59	0.34	0.13	**0.7**	0.57	0.7	0.68	**0.7**	0.57
Lat. 44.6	Seine		H_O	0.45	0.44	0.66	0.41	0.14	0.55	0.34	0.4	0.74	0.49	0.46
Long. 80.9			*A*	5.84	3.78	3.25	2.45	2.35	6.15	3.27	5.45	4.04	5.12	4.17
			n	42	43	47	46	37	42	44	45	43	45	43.4
Owen Sound (2005)	Shore	OS2	H_E	**0.71**	0.44	0.45	0.46	0.05	0.76	0.25	0.71	0.41	0.4	0.47
Lat. 44.6	Seine		H_O	0.5	0.33	0.47	0.53	0.05	0.83	0.27	0.67	0.39	0.38	0.44
Long. 80.9			*A*	4.67	2.95	3.76	2.84	1.56	4.89	1.99	5.35	2.6	4.09	3.47
			n	60	54	60	64	60	58	64	61	59	64	60.4
Port Elgin (2006)	Marina	PE	H_E	**0.74**	0.51	0.53	0.38	0.44	0.78	**0.32**	0.6	0.56	0.79	0.57
Lat. 44.4			H_O	0.66	0.43	0.52	0.38	0.54	0.63	0.34	0.5	0.63	0.47	0.51
Long. 81.4			*A*	7.87	3.77	3.45	2.61	3.44	5.97	2.82	4.73	4.81	6.64	4.61
			n	29	28	25	32	24	32	29	30	32	32	29.3

Maitland River (2006)	Electroshock	MR	H_E	**0.84**	0.49	0.6	0.41	0.39	0.81	0.55	0.76	0.65	0.80	0.63
Lat. 43.7			H_O	0.67	0.29	0.52	0.41	0.4	0.55	0.42	0.5	0.71	0.45	0.49
Long. 81.7			A	8.39	3.83	4.54	2	3.19	6.41	2.92	6.21	5.14	6.17	4.88
			n	30	31	31	29	30	31	31	30	31	31	30.5
Port Dover 2 (2005)	Offshore	PDO	H_E	**0.76**	0.46	0.78	0.47	0.52	0.7	0.52	0.71	0.58	0.78	0.63
Lat. 42.6	Trawl		H_O	0.52	0.61	0.76	0.41	0.52	0.71	0.44	0.75	0.51	0.63	0.59
Long. 80.3			A	5.69	2.47	7.03	2.57	3.89	6.17	2.47	5.57	3.22	6.46	4.55
			n	63	64	58	64	63	63	63	53	59	59	60.9
Port Severn (2005)	Shore	PSE	H_E	**0.61**	0.5	0.21	0.44	0.44	0.66	0.39	0.67	0.71	0.62	0.53
Lat. 44.8	Seine		H_O	0.33	0.33	0.24	0.38	0.47	0.33	0.39	0.54	0.77	0.49	0.43
Long. 79.7			A	3.77	2	1.97	2	2.94	3.81	2.2	4.71	3.92	4.27	3.16
			n	63	63	55	60	60	63	61	63	62	61	61.1
New York 1 (2005)	Offshore	NY1	H_E	**0.74**	0.49	0.78	0.39	0.43	**0.68**	0.5	0.76	0.44	0.69	0.59
Lat. 42.6	Trawl		H_O	0.24	0.4	0.8	0.42	0.43	0.46	0.39	0.77	0.38	0.56	0.49
Long. 79.3			A	6.13	3.46	5.41	2.23	3.36	4.7	2	6.22	2.61	4.82	4.09
			n	54	52	50	53	53	54	51	35	32	45	47.9
New York 2 (2005)	Offshore	NY2	H_E	0.74	0.34	0.79	0.43	0.41	0.73	0.54	0.79	0.56	0.81	0.62
Lat. 42.5	Trawl		H_O	0.25	0.28	0.83	0.38	0.38	0.68	0.36	0.65	0.55	0.64	0.5
Long. 79.4			A	6.21	2.5	5.85	2	3.3	5.03	2.88	6.65	4.47	6.54	4.54
			n	51	58	58	58	53	56	58	52	58	55	55.7
New York 3 (2005)	Offshore	NY3	H_E	**0.72**	0.45	0.78	0.4	0.3	0.65	0.57	0.82	0.5	0.81	0.6
Lat. 42.4	Trawl		H_O	0.28	0.39	0.64	0.32	0.35	0.59	0.46	0.75	0.37	0.62	0.48
Long. 79.6			A	5.53	2.94	5.74	2.19	2.55	4.45	3.15	8	3.25	6.62	4.44
			n	61	61	61	62	60	56	61	12	62	61	55.7

New York 4 (2005)	Offshore	NY4	H_E	0.8	0.47	0.81	0.28	0.05	**0.67**	0.49	0.79	0.61	0.75	0.57
Lat. 42.3	Trawl		H_O	0.47	0.44	0.88	0.33	0.05	0.52	0.39	0.9	0.68	0.62	0.53
Long. 79.7			A	7.05	2.701	5.929	1.995	1.6	4.136	2.203	6.336	4.138	5.75	4.18
			n	59	59	59	36	20	60	59	30	57	60	49.9
New York 5 (2005)	Offshore	NY5	H_E	0.88	0.42	0.79	0.4	0.42	0.57	0.57	0.82	0.55	0.76	0.62
Lat. 42.3	Trawl		H_O	0.36	0.44	0.85	0.44	0.38	0.55	0.56	0.8	0.44	0.62	0.54
Long. 79.7			A	9.31	2.59	5.67	2.21	3.8	4.35	3.2	6.93	4.08	6.89	4.9
			n	58	57	53	57	56	53	57	56	25	42	51.4
New York 6 (2005)	Offshore	NY6	H_E	0.81	0.41	0.78	0.4	0.3	0.77	0.5	0.83	0.57	**0.74**	0.61
Lat. 42.4	Trawl		H_O	0.4	0.41	0.82	0.38	0.27	0.5	0.55	0.54	0.71	0.59	0.52
Long. 79.6			A	7.58	2.66	5.81	2.18	3.18	5.24	2.33	6.81	4.34	5.52	4.57
			n	65	66	61	66	64	64	66	26	62	61	60.1

the lower Detroit River (LA and AMH) (Table 3.2). STC had allele frequencies that were not significantly different than AHM and COL in the Western Basin of Lake Erie. AHM was also not significantly different than sites in the St. Clair River – Western Basin of Lake Erie corridor (COL, GS, BR and MCK) as well MCF in the Bay of Quinte and PD2 in the eastern basin of Lake Erie. Three of 15 (20%) pairwise comparisons between all six NY sites were not significantly different based on allele frequency distributions. The frequency distribution of pairwise F_{ST} estimates shows a range of values (Fig. 3.2). A large portion of the F_{ST} estimates are found between 0 and 0.04 (46.7% of all values). A slight increase in frequency was observed for values in the range of 0.0141-0.016 (Figure 3.2).

Bayesian assignment analysis showed that self assignment among introduced sites ranged from 0 (NI, MCF and NY3) to 95 % (PSE). Identified migrants per population ranged from 0 (HAS, HAS2 and PSE) to 32% (AMH) off all fish. The water distance traveled by migrants ranged from 14 to 786 kilometers (Fig. 3.3). The frequency distribution distances traveled by migrants showed that 89 out of 224 (39.7%) identified migrants traveled a distance of less than 100 km (Fig. 3.3). Obvious peaks in distribution occurred in the range of 251-300 km and 601-650 km (Fig. 3.3), these distance ranges accounted for 38 of 224 migrants (17.0%). Dispersal index values revealed several distinctive patterns. Sites in Lake Huron were characterized as less dispersive (Fig. 3.4). Other sites that showed less dispersal included HAS, which was expected given its high genetic divergence and spatial restrictions along the Trent Severn Waterway. The eastern basin of Lake Erie also contained sites that were less dispersive, with the exception of the NY sites, where index values were variable. Dispersal index values were also variable for the St. Clair River – western basin of Lake Erie.

Table 3.2a Genetic relationship between 18 introduced round goby (*Neogobius melanostomus*) populations in three of the Laurentian Great Lakes. Pairwise F_{ST} values are presented in the table. Values that were significant after Bonferonni correction are in bold face type and underlined. Site abbreviations are described in Table 3.1.

	TR	HAS	BUR	PC	PM	PD	PS	LA	NI	JG	STC	RO	DU	COL	BR	GS	MCF	MCK
TR	---																	
HAS	**0.17**	---																
BUR	**0.06**	**0.22**	---															
PC	**0.07**	**0.17**	**0.08**	---														
PM	**0.07**	**0.15**	**0.07**	**0.08**	---													
PD	**0.07**	**0.15**	**0.08**	**0.07**	**0.03**	---												
PS	**0.10**	**0.14**	**0.10**	**0.08**	**0.04**	**0.05**	---											
LA	0.02	**0.14**	**0.08**	**0.05**	**0.07**	**0.05**	**0.09**	---										
NI	0.02	**0.14**	**0.07**	**0.04**	**0.04**	**0.03**	**0.05**	0.02	---									
JG	**0.05**	**0.15**	**0.07**	**0.06**	**0.04**	**0.03**	**0.02**	**0.05**	0.01	---								
STC	0.02	**0.15**	**0.06**	**0.03**	**0.06**	**0.04**	**0.07**	0.01	**0.01**	**0.04**	---							
RO	0.02	**0.16**	**0.05**	**0.04**	**0.07**	**0.05**	**0.08**	0.01	**0.02**	**0.05**	0.00	---						
DU	0.02	**0.15**	**0.06**	**0.04**	**0.06**	**0.05**	**0.07**	0.00	0.01	**0.04**	0.00	0.01	---					
COL	0.02	**0.18**	**0.04**	**0.04**	**0.05**	**0.04**	**0.07**	0.02	0.02	**0.04**	0.00	0.01	0.01	---				
BR	**0.04**	**0.16**	**0.07**	**0.04**	**0.08**	**0.06**	**0.09**	0.02	**0.04**	**0.06**	0.00	0.01	0.01	0.02	---			
GS	0.02	**0.15**	**0.04**	**0.05**	**0.04**	**0.04**	**0.04**	**0.02**	0.01	**0.02**	0.01	**0.02**	0.01	0.01	**0.03**	---		
MCF	0.01	**0.15**	**0.06**	**0.04**	**0.07**	**0.05**	**0.08**	0.00	0.01	**0.03**	0.00	0.00	0.00	0.01	**0.01**	0.00	---	
MCK	0.00	**0.15**	**0.05**	**0.06**	**0.07**	**0.06**	**0.08**	0.02	**0.03**	**0.04**	0.01	**0.02**	0.01	0.02	0.02	**0.02**	0.00	---

Table 3.2b Genetic relationship between 18 introduced round goby (*Neogobius melanostomus*) populations in three of the Laurentian Great Lakes. Pairwise F_{ST} values are presented in the table. Values that were significant after Bonferonni correction are in bold face type and underlined. Site abbreviations are described in Table 3.1.

	TR	HAS	BUR	PC	PM	PD	PS	LA	NI	JG	STC	RO	DU	COL	BR	GS	MCF	MCK
LEA	**0.03**	**0.15**	**0.09**	**0.07**	**0.07**	**0.06**	**0.07**	0.02	**0.04**	**0.06**	**0.04**	**0.05**	0.02	**0.03**	**0.06**	**0.04**	**0.03**	**0.05**
KIL	**0.03**	**0.13**	**0.06**	**0.04**	**0.06**	**0.07**	**0.08**	**0.04**	**0.03**	**0.06**	**0.02**	**0.03**	**0.02**	0.02	**0.04**	**0.03**	**0.03**	**0.04**
MID	**0.06**	**0.19**	**0.10**	**0.10**	**0.11**	**0.08**	**0.15**	**0.06**	**0.06**	**0.10**	**0.06**	**0.06**	**0.05**	**0.07**	**0.08**	**0.06**	**0.05**	**0.06**
OS	**0.05**	**0.21**	0.01	**0.07**	**0.06**	**0.07**	**0.09**	**0.07**	**0.06**	**0.07**	**0.04**	**0.04**	**0.04**	0.03	**0.06**	**0.03**	**0.05**	**0.03**
PE	0.03	**0.22**	**0.06**	**0.06**	**0.10**	**0.09**	**0.12**	**0.05**	**0.06**	**0.10**	**0.04**	**0.04**	**0.05**	**0.04**	**0.06**	**0.05**	**0.06**	**0.04**
MR	0.03	**0.16**	**0.05**	**0.04**	**0.07**	**0.07**	**0.08**	**0.05**	**0.05**	**0.07**	**0.02**	**0.03**	0.03	0.02	**0.03**	**0.04**	**0.03**	**0.03**
MID2	**0.04**	**0.19**	**0.11**	**0.11**	**0.11**	**0.07**	**0.13**	**0.04**	**0.05**	**0.08**	**0.05**	**0.05**	**0.04**	**0.07**	**0.07**	**0.06**	**0.03**	**0.04**
SL	**0.16**	**0.18**	**0.19**	**0.14**	**0.15**	**0.14**	**0.13**	**0.13**	**0.13**	**0.14**	**0.10**	**0.13**	**0.10**	**0.13**	**0.12**	**0.12**	**0.12**	**0.13**
NY1	**0.03**	**0.14**	**0.05**	**0.04**	0.02	0.01	0.01	**0.03**	0.00	0.00	0.01	**0.03**	0.02	0.02	**0.04**	0.00	0.02	**0.02**
NY2	**0.06**	**0.13**	**0.07**	**0.06**	**0.04**	**0.05**	**0.03**	**0.07**	**0.04**	**0.02**	**0.05**	**0.07**	**0.05**	**0.05**	**0.08**	**0.03**	**0.06**	**0.05**
NY3	0.01	**0.10**	**0.03**	0.00	0.01	0.01	0.00	0.02	0.00	0.00	0.00	**0.02**	0.00	0.02	**0.03**	0.00	0.01	0.01
NY4	**0.04**	**0.09**	**0.07**	0.02	0.00	0.00	0.00	0.02	0.00	0.00	0.00	**0.02**	0.01	0.01	**0.02**	0.00	**0.02**	0.01
NY5	**0.03**	**0.12**	**0.04**	**0.03**	**0.03**	**0.03**	0.01	0.02	0.01	0.00	**0.02**	**0.02**	0.00	0.02	**0.04**	0.00	0.01	**0.02**
NY6	**0.03**	**0.12**	**0.05**	**0.04**	**0.02**	**0.02**	0.00	**0.04**	0.00	0.00	**0.01**	**0.03**	0.02	0.03	**0.04**	0.00	**0.02**	**0.03**
LEA2	0.02	**0.15**	**0.06**	**0.06**	**0.07**	**0.05**	**0.06**	0.01	**0.02**	**0.03**	0.01	**0.02**	0.00	0.01	**0.02**	**0.01**	0.00	**0.02**
PD2	**0.05**	**0.14**	**0.06**	**0.05**	**0.04**	**0.03**	**0.04**	**0.03**	**0.03**	**0.02**	**0.04**	**0.04**	**0.03**	**0.03**	**0.05**	**0.02**	**0.03**	**0.05**
MCK2	0.01	**0.15**	**0.05**	**0.04**	**0.07**	**0.05**	**0.08**	0.01	**0.02**	**0.05**	0.01	0.01	0.01	0.02	**0.02**	**0.02**	0.00	0.00
OS2	**0.10**	**0.27**	**0.11**	**0.12**	**0.15**	**0.16**	**0.17**	**0.14**	**0.13**	**0.16**	**0.10**	**0.11**	**0.12**	**0.10**	**0.11**	**0.11**	**0.12**	**0.10**
HAS2	**0.18**	0.01	**0.23**	**0.17**	**0.17**	**0.15**	**0.16**	**0.15**	**0.14**	**0.16**	**0.14**	**0.16**	**0.15**	**0.19**	**0.16**	**0.15**	**0.16**	**0.15**

Table 3.2c Genetic relationship between 22 introduced round goby (*Neogobius melanostomus*) populations in three of the Laurentian Great Lakes. Pairwise F_{ST} values are presented in the table. Values that were significant after Bonferonni correction are in bold face type and underlined. Site abbreviations are described in Table 3.1.

	LEA	KIL	MID	OS	PE	MR	MID2	SL	NY1	NY2	NY3	NY4	NY5	NY6	LEA2	PD2	MCK2	OS2	H/
LEA	---																		
KIL	**0.06**	---																	
MID	**0.08**	**0.07**	---																
OS	**0.07**	**0.05**	**0.09**	---															
PE	**0.05**	**0.05**	**0.10**	**0.05**	---														
MR	**0.05**	**0.04**	**0.09**	0.02	0.01	---													
MID2	**0.07**	**0.08**	**0.03**	**0.09**	**0.1**	**0.1**	---												
SL	**0.12**	**0.12**	**0.15**	**0.18**	**0.21**	**0.16**	**0.15**	---											
NY1	**0.03**	**0.03**	**0.08**	**0.03**	**0.06**	**0.03**	**0.08**	**0.12**	---										
NY2	**0.06**	**0.06**	**0.11**	**0.07**	**0.09**	**0.06**	**0.11**	**0.13**	0.00	---									
NY3	**0.02**	0.01	**0.06**	0.02	**0.05**	0.02	**0.06**	**0.1**	0.00	0.00	---								
NY4	0.00	**0.03**	**0.07**	**0.06**	**0.09**	**0.03**	**0.05**	**0.09**	0.00	0.00	0.00	---							
NY5	**0.03**	**0.03**	**0.07**	**0.03**	**0.06**	**0.03**	**0.07**	**0.11**	0.01	0.00	0.00	0.00	---						
NY6	**0.04**	**0.02**	**0.08**	**0.04**	**0.07**	**0.03**	**0.08**	**0.11**	0.00	0.00	0.01	0.00	0.00	---					
LEA2	**0.03**	**0.03**	**0.07**	**0.05**	**0.06**	**0.04**	**0.06**	**0.12**	**0.02**	**0.04**	0.00	0.00	**0.02**	0.00	---				
PD2	**0.05**	**0.04**	**0.09**	**0.06**	**0.08**	**0.06**	**0.09**	**0.13**	**0.02**	**0.03**	0.00	0.00	0.01	0.01	**0.02**	---			
MCK2	**0.04**	**0.03**	**0.05**	**0.04**	**0.03**	0.02	**0.04**	**0.12**	**0.03**	**0.05**	0.01	0.01	**0.02**	**0.02**	**0.02**	**0.04**	---		
OS2	**0.13**	**0.09**	**0.16**	**0.1**	**0.06**	**0.05**	**0.18**	**0.25**	**0.12**	**0.15**	**0.12**	**0.14**	**0.14**	**0.11**	**0.12**	**0.14**	**0.08**	---	
HAS2	**0.16**	**0.15**	**0.19**	**0.22**	**0.24**	**0.17**	**0.2**	**0.21**	**0.16**	**0.13**	**0.13**	**0.1**	**0.14**	**0.15**	**0.15**	**0.16**	**0.16**	**0.29**	

Mantel tests revealed that patterns of genetic differentiation across all sites followed an IBD model (F_{ST}; r^2=0.19, p=0.010, D_C; r^2=0.28, p=0.010) (Fig. 3.5). Genetic differentiation on a lake scale followed an IBD model for only Lake Erie (F_{ST}, r^2=0.11, p=0.010, D_C; r^2=0.20, p=0.010) (Fig. 3.5). On a smaller spatial scale (lake basin), Mantel tests revealed that IBD was only significant at 1 of 3 regions. IBD was not apparent for the St. Clair River – western basin corridor as all sites were weakly divergent from one another irrespective of geographical distance (F_{ST}; r^2=0.01, p=0.270, D_C; r^2=0.01, p=0.180). Likewise, IBD was not evident along the Trent Severn Waterway (F_{ST}; r^2=0.01, p=0.380, D_C; r^2=0.03, p=0.110).

The significant and highly divergent HAS site likely led to the collapse of any IBD pattern of genetic differentiation. The regional scale analysis in the eastern basin of Lake Erie appeared to follow an IBD model of genetic differentiation (F_{ST}; r^2=0.35, p=0.020, D_C; r^2=0.36, p=0.03) (Fig. 3.5). This relationship was most likely driven by the NY sites, which were geographical close (greatest distance between any 2 sites ~29 km) and were also genetically indistinguishable based on measures of genetic distance and allele frequency distributions.

3.4 DISCUSSION

The genetic signature of an introduced population will depend on the effective population size of the invaders, as well as any preexisting genetic structure among source populations (Holland 2000). Many introduced populations are characterized by low genetic diversity, often the result of founder effects and population bottlenecks (e.g. Tsutsui *et al.* 2000, Colautti *et al.* 2005, Lindholm *et al.* 2005); however, multiple introductions and mixtures of introductions from

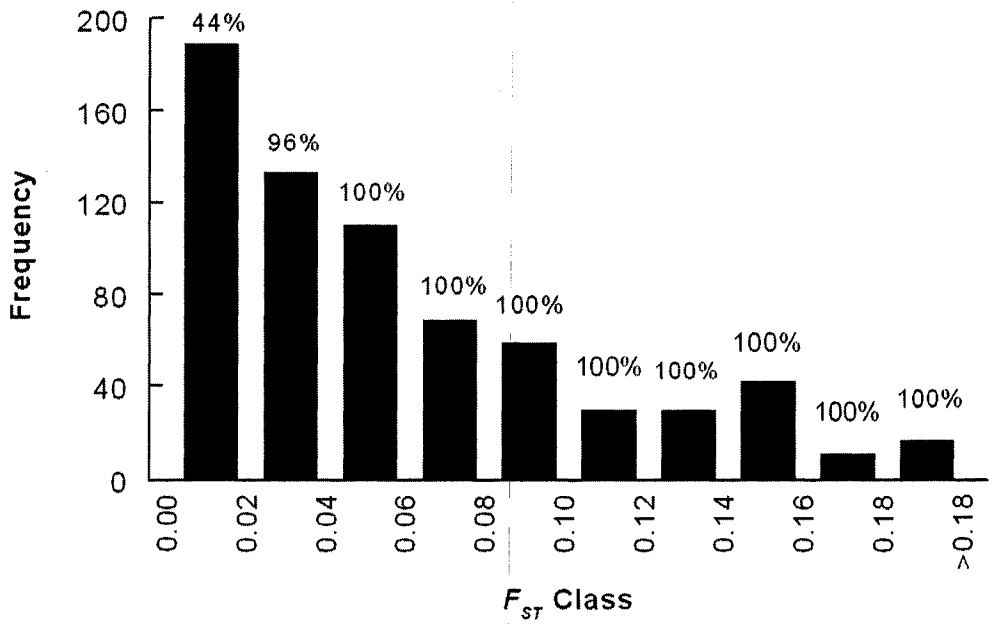

Figure 3.2 Frequency distribution of pairwise F_{ST} values among introduced round goby populations in the Great Lakes. The number above each bar indicates the percentage of pairwise F_{ST} values in that category that were significantly different from zero after Bonferroni correction. X-axis values indicate the upper values in the genetic divergence range.

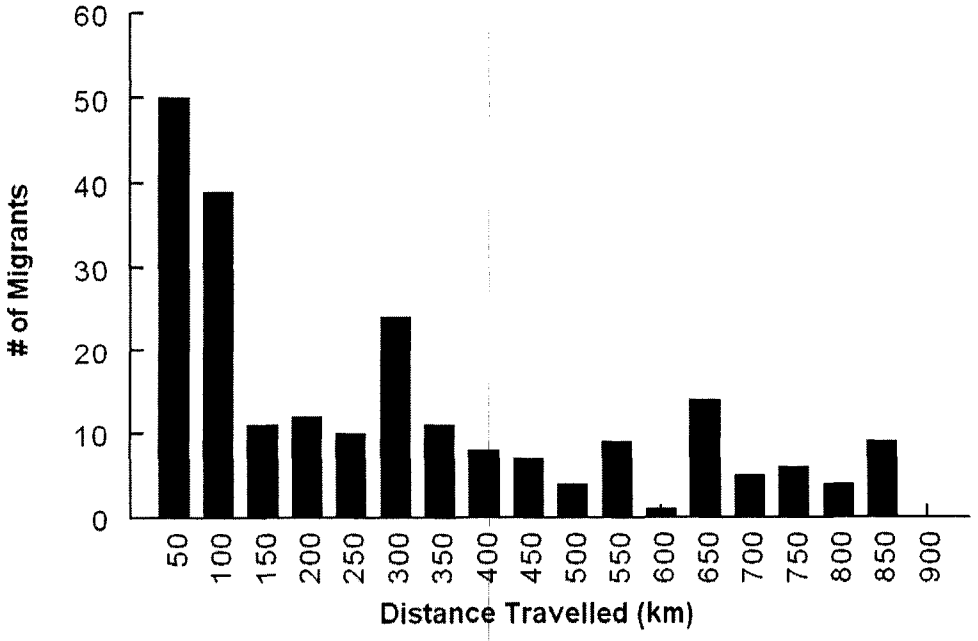

Figure 3.3 Distance traveled by individual round goby migrants in the Great Lakes. Migrants were identified having a likelihood ratio greater than four, when the highest rank probability was divided by the second highest rank probability. Distance is measured as the shortest in-water distance between sites.

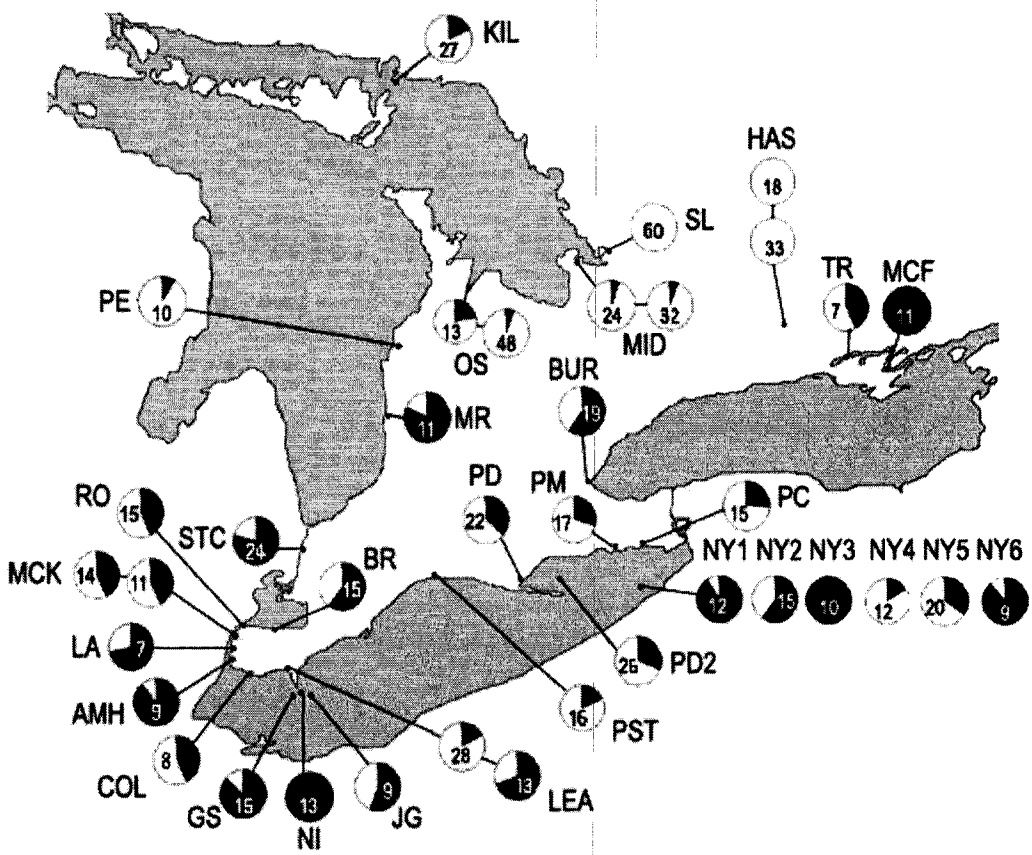

Figure 3.4 Great Lake round goby dispersal in 37 introduced populations inferred by genotype assignment analysis. Filled pie chart sections indicate dispersed individuals, while open pie sections indicate self-assigned, or resident, individuals. The number in the pie chart indicates the number of successfully assigned individuals for that population.

multiple sources can lead to elevated genetic diversity (e.g. Baker 1992, Kolbe *et al.* 2004). Furthermore, some researchers have postulated that high levels of genetic diversity in introduced species may facilitate invasion success, since elevated genetic diversity may provide increased opportunity for genetic adaptation (Lee 2002). Population bottlenecks associated with colonization events may act as barriers to establishment in that these populations are generally characterized by a reduction in genetic diversity (Mayr 1963).

The colonization of the Great Lakes by the round goby is characterized by not only high levels of genetic diversity in all sampled populations, but also very high levels of genetic differentiation among sites. Our results are consistent with previous genetic analyses of mtDNA haplotype diversity of Great Lakes round goby populations (Dillon & Stepien 2001, Stepien *et al.* 2005, Stepien & Tumeo 2006). Although high levels of genetic diversity can be explained by large propagule size or multiple introductions, our results are unexpected in that the genetic structure of recently introduced species, colonized over long distances is usually characterized by low or no genetic differentiation. This may be attributed to both high gene flow and panmixia processes associate with colonization (e.g. Duda 1994, Astanei *et al.* 2005) or due to a single inoculation event and insufficient time for significant divergence (e.g. Grapputo *et al.* 2006, Lindholm *et al.* 2005). Studies that report introduced populations exhibiting higher than expected genetic differentiation often attribute such structuring to physical or geographical barriers in the introduced range (e.g., Bousset *et al.* 2004, Demelo & Hebert 1994, Roman & Palumbi 2004, Herborg *et al.* 2006) or to multiple introductions (Durka *et al.* 2005).

In general, a number of possible mechanisms have been postulated to lead to high levels of genetic differentiation among introduced species populations. First, such a population genetic structure may be due to very small founding populations where subsequent drift effects and

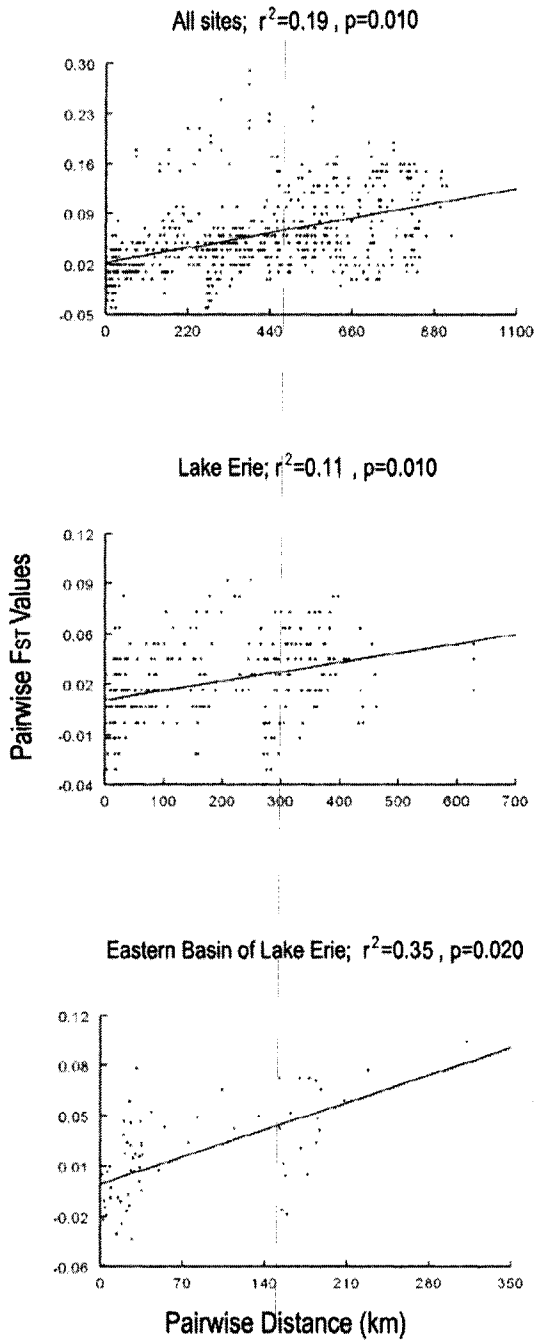

Figure 3.5 Plots of pairwise genetic distance (F_{ST}) versus geographical in-water distance across three different spatial scales of introduced round goby populations in the Great Lakes. The first scale encompasses all introduced sites (N=37), the second scale includes sites within Lake Erie (including the connecting corridor to the St. Clair River; N=24). The final scale include sites in the eastern basin of Lake Erie (N=12). Mantel tests were employed to test significance between the two distant matrices.

microhabitat selection led to population differentiation (Cabe & Alstad 1994). In my case, such a scenario would imply that only a few round gobies were able to colonize and establish in the Great Lakes, and that the subsequent range expansion was accomplished by ballast transport by Great Lakes freighters, also involving only a small effective population size. However, this scenario would lead to low levels of genetic diversity among introduced sites, no evidence of an IBD model of genetic differentiation and genetic signatures of population bottlenecks. Evidence for this is not apparent in this or previous studies (Dillon & Stepien 2001, Stepien *et al.* 2005, Stepien & Tumeo 2006), as round gobies in the Great Lakes are characterized by high levels of genetic diversity, and there is evidence of IBD across all sample sites and within two smaller spatial scales. I also found no evidence for current population bottlenecks, although, initial population establishment appears to have been reduced in size (Chapter 4). Thus it is unlikely that founder effects are the primary mechanism driving the genetic differentiation observed among the round goby populations in the Great Lakes.

An alternative explanation for high levels of genetic divergence among recently established invasive species populations may be multiple colonization events from divergent sources (e.g. Garnatje T *et al.* 2002, Shoji *et al.* 2007). In my study, the high degree and variation in pairwise genetic differentiation does suggest multiple introductions (Fig. 3.2). The frequency distribution of F_{ST} values suggests that pairwise populations with low genetic divergence are most likely the result of natural dispersal within the Great Lakes. The high F_{ST} values are not consistent with recent divergence, but rather suggest previously existing divergence within their native range. In this study several sites were extremely divergent from all other sites (HAS/HAS2, SL, OS2, MID/MID2, and BUR). Based on the grouping of these sites I estimate the minimum number of introductions to be at least five.

A third possible explanation of the pattern of round goby genetic differentiation is that the introduced populations are older than previously reported. However, this is unlikely since round gobies are generally easily detected as they are characterized by very fast population growth and very high population densities, making detection effortless. Thus, although the round goby colonization of the Great Lakes may predate the first report (Crossman *et al.* 1992, Jude *et al.* 1992), it is virtually certain they have not been present and widespread in the Great Lakes for sufficient time to account for the very high levels of genetic divergence documented here.

My results indicate that the colonization of the Great Lakes by the round goby resulted from a combination of small propagule introductions (driving founder effects and rapid genetic drift) and multiple introductions from genetically divergent sources within their native range. Secondary colonization events within the Great Lakes were also likely characterized by a small numbers of round gobies leading to additional founder effects. The pattern of genetic divergence observed among introduced round gobies suggests that management efforts may be difficult. Region or population specific plans must be developed in order to address the varying genetic composition of each population. The colonization of the round goby in the Great Lakes allows the evaluation of the consequences of a founding event involving a limited number of individuals. This study increases our understanding of the underlying evolutionary mechanism that allow invasive species to adapt and expand in the face of varied environmental and biological factors that ultimately act to force extinction. If persistence occurs, those same forces can ultimately drive the initial stages of speciation.

The spread of the round goby in the Great Lakes has been remarkably fast; since their initial appearance in 1990 the round goby colonized all five of the Great Lakes within five years (Charlebois *et al.* 1997). However, based on mark-recapture studies, round gobies were

characterized as highly philopatric with limited dispersal capabilities (Wolfe & Marsden 1998, Ray & Corkum 2001). It is thus unlikely that the observed spread of the round goby in the Great Lakes was due to natural dispersal alone. Several studies on the dispersal of invasive species have identified human transport processes as critical factors mediating dispersal and range expansion (e.g., Buchan & Padilla 1999, Suarez *et al.* 2001). In this study genotype assignment analyses characterized round goby dispersal in the Great Lakes as highly variable, with specific dispersal distances ranging as high as 835 km, clearly indicative of human mediated dispersal vectors. The dispersal distance histogram identified signs of natural dispersal, 39.7% of these migrants traveled between 0 and 100 km. These results are more consistent with natural dispersal, suggesting that this type of dispersal is greater than previously thought. Two peaks in the histogram likely reflect ballast water dispersal (Fig. 3.3). The peaks in the range of 601-650 km are identified migrants primarily moving between the lower Detroit River/western basin of Lake Erie corridor and the Bay of Quinte (MCF and TR). The second peak in the range of 251-300 km reflects migration between the east portion of the western basin of Lake Erie and the eastern basin of Lake Erie. These peaks in dispersal distance are consistent with F_{ST} values that reveal low genetic divergence between sites in the St. Clair River-western basin of Lake Erie corridor and the Bay of Quinte sites as well as between sites in the St. Clair River-western basin of Lake Erie corridor and the eastern basin of Lake Erie (see Table 3.2). Within-lake dispersal is a combination of natural/local dispersal with characteristic long-distance jump dispersal events, most likely facilitated by inter-basin ballast water transfer.

The spread of the round goby in the Great Lakes is most likely a combination of three dispersal strategies, natural dispersal, ballast water transfer within the Great Lakes, and multiple introductions to different areas within the Great Lakes from Eurasian sources. Partitioning

between these dispersal strategies is not possible, although speculation can be made through the identification of migrants and the distance traveled between sites of capture and origin. Through this study I estimate that there have been at least five introductions of round gobies to the Great Lakes. Within the Great Lakes dispersal is a combination of natural dispersal, which I estimate to account for approximately 40% of all dispersal (Fig. 3.3), and long distance jump dispersal, which I estimate to account for at least 16% of dispersal in the Great Lakes (Fig. 3.3).

The dynamic nature of a rapidly expanding invasion most often results in departures from population equilibrium. Based on temporal instability between five introduced sites and obvious departures from HWE across our 10 microsatellite loci, I conclude that round goby populations in the Great Lakes have not yet reached an equilibrium state. However, IBD was significant in some areas suggesting that these areas (i.e. Lake Erie and the eastern basin of Lake Erie) may be more stable than others. This may reflect the age of these populations, but rather most likely reflects the sampling regime and the non-significant genetic relationships between sites that were extremely close.

Here I demonstrate that a combination of multiple introduction of small number of individuals and subsequent genetic drift can drive the genetic differentiation of rapidly expanding populations. I also demonstrate that the rapid spread of an invasive species can be greatly accelerated by human-mediated vectors and that highly philopatric species can often disperse beyond their home range. The invasion of round gobies in the Great Lakes represents a unique genetic example of populations that are characterized by such unusual levels of divergence, high genetic diversity and that display rapid dispersal. These attributes pose many hurdles for conservation managers as there is a lack of understanding of the processes that led to the genetic patterns observed. This model system allows an analysis of how evolutionary

mechanisms such as drift and selection can act to drive the differentiation of populations suffering from founder effects during their colonization history.

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4.0 FOUNDER EFFECTS IN ROUND GOBY (*NEOGOBIUS MELANOSTOMUS*) INVASION FRONTS IN THE GREAT LAKES

4.1 INTRODUCTION

The successful establishment of an invasive species is dependent on the composition of the individuals making up the introduced population. Many invasion events are characterized by a small number of founding individuals, which consequently decreases the chance of persistence in the novel environment. The likelihood of persistence is decreased due to several reasons. Firstly, extinction of these small populations can occur simply by chance alone. Secondly, high mortality in the novel environment may rapidly select for specific genotypes, which will further reduce the founding population size. Population persistence and survival can reach close to zero if the population remains small. Finally, the likelihood of persistence can decrease because of inbreeding depression, caused by mating with close kin. This results in the expression of deleterious alleles, whereby fitness of the founding population is reduced. Founder effects associated with a colonization event can be defined as the effects resulting when a small number of individuals establish a population (Mayr 1963). Genetic diversity of this new population may be limited compared to the native population due to the population bottleneck associated with the colonization event (e.g. Tsutsui *et al.* 2000). The new population may subsequently differ genetically and phenotypically from the native population (e.g. Lee *et al.* 2003, Grosholz & Ruiz 2003, Philips & Shine 2003). Studying the genetic processes involved in colonization can provide the opportunity to evaluate rapid evolution (eg. Ellstrand & Schierenbeck 2000) and the responses to selective pressures generated in the novel environment during initial establishment and ensuing range expansion. Furthermore, small populations are strongly affected by the forces of random genetic drift. Genetic drift results from chance differences in survival and reproduction success over successive generations and it will drive alleles towards either loss or

fixation at a given locus. When small populations are involved, this drift alone may drastically alter the genetic composition of the introduced population compared to its source population. Genetic drift is one consequence of founder effects which, on an evolutionary time-scale, can lead to the beginning of species divergence (Mayr 1963).

The successful establishment of an invasive species is greatly influenced by the size of the introduced population and the amount of genetic diversity they possess. Most introduced species are founded by small populations that suffer founder effects (e.g., Cristescu *et al.* 2001, Berg *et al.* 2002, Grapputo *et al.* 2006). This scenario is highly likely, given the rarity of these events as well as a low probability of survival and establishment throughout each stage of the invasion event. Although many invasive populations have lower genetic diversity than their source or wild populations (Xu *et al.* 2003, Lasota *et al.* 2004, Lindholm *et al.* 2005), others are characterized by equal or greater genetic diversity relative to their source (Barbaresi *et al.* 2003, Kolbe 2004, Martel *et al.* 2004, Therriault *et al.* 2005, Chen *et al.* 2006). In the cases where invasive populations show high genetic diversity despite the expectations for population bottlenecks, several mechanisms are often postulated or demonstrated. A large number of founders may provide the genetic diversity required to adapt in the novel environment as well as to avoid inbreeding depression. Alternatively, repeated small invasions from multiple diverse sources may also inflate genetic diversity by mixing genotypes that may have been isolated in the native range (e.g., Ehrlich *et al.* 1989). Tolerance to founder effects and bottlenecks of some species may enable them to become established despite the reduction in population size. Most obligate sexual species will likely not successfully invade if the founder effects are too severe. In fact, the growth of small founder populations, following the initial survival stage may be limited by inbreeding depression because it reduces that population's ability to adapt and survive

(Nieminen *et al.* 2001). This inability to adapt increases the probability of extinction for these founding populations (Saccheri *et al.* 1998). Ultimately, a reduction in genetic diversity will limit that population's evolutionary potential when faced with a new selective regime (Frankham *et al.* 1999). Gene flow among invasive populations subsequent to initial establishment is another potentially powerful mechanism that substantially increases the likelihood of successful invasion since it could spread "invasive" or generally adaptive genotypes, and would increase genetic variation in populations still suffering from founder effects. Measuring gene flow among populations during a biological invasion provides valuable information for predicting the persistence and future spread of invasive populations.

Along with a loss in genetic diversity, the introduced population is also expected to exhibit reduced genetic equilibrium (e.g., DeWalt & Hamrick 2004, Colautti *et al.* 2005, Herborg *et al.* 2006). The reduction in population size associated with all introduction events will push the population towards a non-equilibrium state. Although the disequilibrium may last for as little as a single generation, drift, selection, and gene flow can act to extend the disequilibrium for many generations. Thus, recently established, small populations with very rapid population growth rates are expected to exhibit genetic instability at neutral loci.

The round goby (*Neogobius melanostomus*) is a successful invader, increasing its range in Europe as well as successfully establishing in the Great Lakes. Their aggressive nature, broad tolerance to varied environmental conditions, high reproductive rate, and high offspring survival likely helped the round goby become established in the Great Lakes and elsewhere. The recent establishment of many other Ponto-Caspian species in the Great Lakes has been facilitated by a common vector pathway: transoceanic transfer of ballast water (Mills *et al.* 1993). By its nature, ballast water uptake of fish is likely to result in the transfer of a limited number of individuals

(Wonham *et al.* 2000). The harsh conditions inside ballast tanks and ballast water exchange practices likely reduces this number even further, resulting in the transfer of only a handful of individuals (Wonham *et al.* 2005). Such a small number of colonizers will inevitably experience the negative effects associated with population bottlenecks. The rapid population growth exhibited by the round goby in the Great Lakes despite founder effects was likely the result of the addition of genetic diversity through gene flow. Round gobies in the Great Lakes have been shown to have high genetic diversity and high genetic differentiation among sites (Chapter 3, Stepien *et al.* 2005). There is also evidence that ballast water transfer within the Great Lakes as well as multiple introductions is partially responsible for their rapid spread.

Currently, round gobies have now colonized all five of the Great Lakes (Charlesbois *et al.* 1997), and have recently been observed inland along the Maitland River in Lake Huron. In Ontario, round gobies have also been found moving inland along the Trent-Severn Waterway, the Thames River, Pefferlaw Brook, and recently one specimen was found in Bellewood Lake (Guleph). The Maitland River provides a valuable one-dimensional invasion front that allows the opportunity to evaluate the genetic signature of an on-going invasion. This will allow the genetic characterization of secondary range expansion of the round goby in the Great Lakes and possibly the specific characteristics of dynamically invading round gobies.

The purpose of this project was to compare genetic characteristics of recently colonized populations of the round goby that have different times since establishment (15 years, nine years and actively colonizing). Specifically, I test for genetic bottlenecks and for effects on genetic diversity in a dynamic round goby invasion front in the Maitland River, Goderich, Ontario, Canada. I compare those populations to other, more established, populations elsewhere in the Great Lakes. Round gobies were first reported in Goderich in 1994, but until 2006 had not been

reported in the Maitland River, giving me the opportunity to evaluate patterns of genetic differentiation during a colonization event. Although the round goby does not generally appear to follow the predictions of colonization effects in population genetic theory, the invasion front in the Maitland River provides the chance to critically test some of those predictions.

4.2 MATERIALS AND METHODS

Sample collection and geographical information

Round gobies in the Maitland River were collect during the summer of 2006, where samples were caught at 5 different sites (Fig. 4.1). The first site was approximately 600 meters from the mouth of the Maitland River, while the four additional sites covered a distance 2.5 kilometers upstream from the first site of capture (Fig. 4.1). No round gobies were collected at any further upstream sites (~5 km and ~10km) despite exhaustive effort. Sample collections at these sites were equal in duration and intensity of the five lower sites (avg. ~2000 electroshocking seconds). Therefore the samples collected at the furthest upstream site in this study likely represent round gobies at the edge of their invasion front.

I also sampled five more sites spread over much of southern Ontario (Fig. 4.1) to collect round gobies from sites that have been established for approximately nine years. By selecting sites that are geographically widely distributed, I represent the expansion wave of round gobies in the lower Great Lakes. Samples from two of these sites were collected in multiple years (MCF and HAS) (Fig. 4.1). All of these sites were part of another study designed to measure dispersal and gene flow among Great Lake round gobies (Chapter 3).

Round gobies were also collected from the St. Clair River (Fig. 4.1), near the site of the first report of Great Lakes round gobies. These samples represent the longest-established

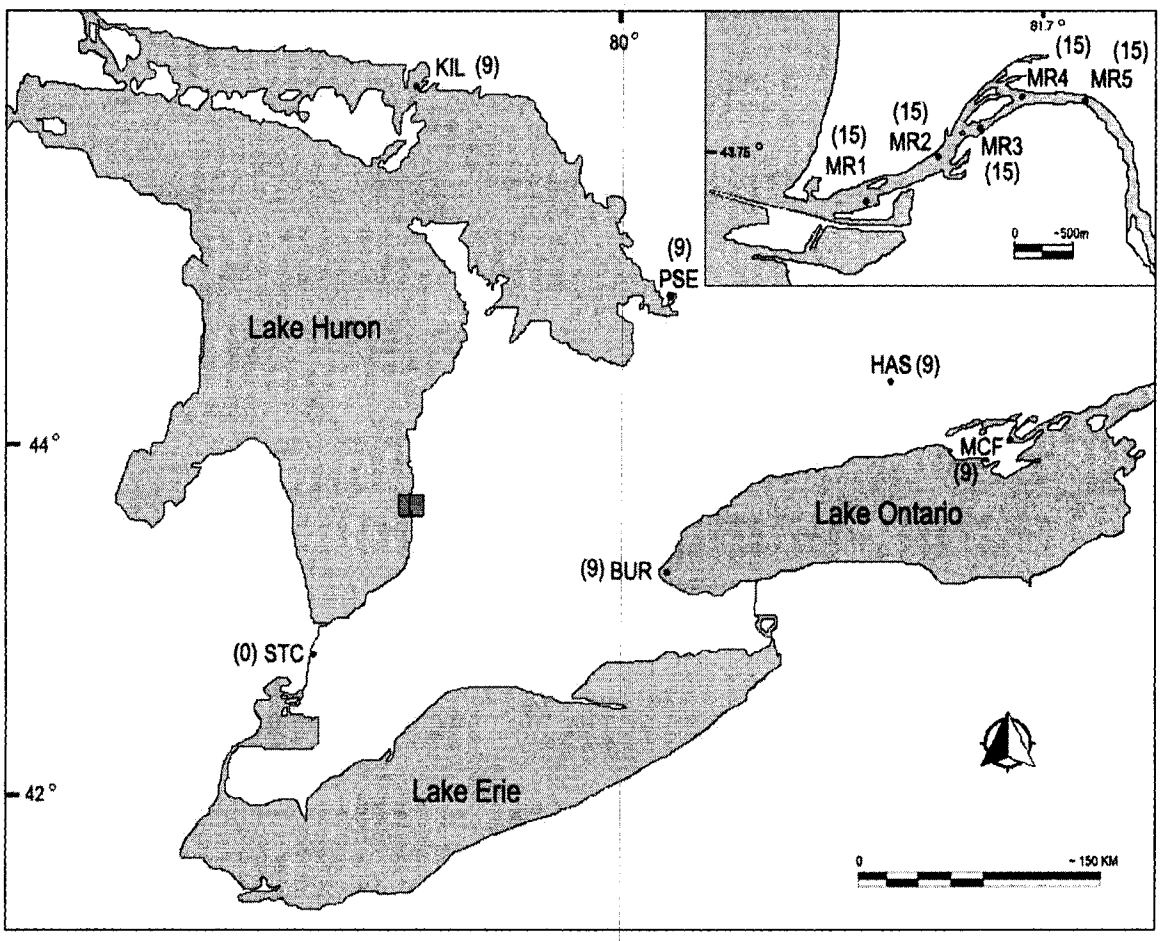

Figure 4.1 Locations of the sites where round gobies were caught during 2005 and 2006. Site abbreviations correspond to descriptions in Table 1. The shaded box indicates the Maitland River; map of the river appears in the upper right corner. The bracketed number indicates the approximate number of years since the initial invasion of the Great Lakes.

population of round gobies based on their proximity to the original site of identification, approximately 15 years ago (1992). This site was also part of another study designed to measure dispersal and gene flow among Great Lake round gobies (Chapter 3).

A total of 630 fish were caught through a combination of seine netting, hook and line and backpack electroshocking (Maitland samples only). A section of the caudal fin of each fish was removed and placed in a 1.7ml tube containing 95% ethanol for later DNA extraction.

Microsatellite genotyping

Refer to Chapter 3 for DNA extraction and PCR amplification protocols. Also refer to Chapter 2 for individual genotyping procedures.

Genetic diversity

Genetic diversity of recently established round goby populations was assessed by estimating observed (H_O) and expected heterozygosities (H_E) using the computer program ARLEQUIN VER. 3.1 (Excoffier *et al.* 2001). Allelic richness (A) per locus across all populations was estimated using FSTAT 2.9.3.2 (Goudet 2001), private alleles in each population were also identified.

Population stability was evaluated by testing for conformance to Hardy-Weinburg equilibrium using exact tests in the program TFPGA (Miller 1997). A Monte Carlo method to estimate the p-value, with 10 batches and 1000 permutations per batch was used. P-values were corrected according to the sequential Bonferroni method for multiple simultaneous tests across individual populations (Rice 1989). Linkage disequilibrium was evaluated among loci in the program ARLEQUIN VER. 3.1 (Excoffier *et al.* 2001). It is expected that reduced population sizes will show greater changes in the frequency of specific allelic combinations than would be expected under a scenario of non-random mating of much larger populations.

Population structure

To evaluate population structure and gene flow in an ongoing invasion event (Maitland River) we calculated conventional *F*-statistics using ARLEQUIN VER. 3.1 for the Maitland River samples only (Excoffier *et al.* 2001). Genetic distance was also estimated by calculating measures of chord distance (Cavalli-Sforza & Edwards 1967). To test for differences in allelic frequency distribution between locations, Fischer's exact test of allelic differentiation was calculated in the program TFPGA 1.3 (Miller 1997). Isolation by distance as a model of genetic differentiation was tested across the Maitland River sites: significance of the correlation between genetic (D_C (Cavalli-Sforza & Edwards 1967)) and a geographic distance matrix was assessed by Mantel tests in GENALEX V. 6 (Peakall and Smouse 2006). Genotype assignment analysis on the Maitland River samples was performed using the database of 32 populations described in Chapter 3. Briefly, individual genotypes from Maitland River were assigned to one of the 32 populations that was its most likely origin based on the distribution of allele frequencies in that source population. An assignment was deemed successful when the likelihood ratio between the probabilities of the two highest ranked populations exceeded four. The individual was then assigned to the highest ranked population, or unassigned if the ratio was < 4.

Population Bottleneck

We used three approaches to detect bottlenecks and recent population expansion in our three invasive population age categories since colonization (i.e., 15 years (N=1), 9 years (N=7), and active colonizers (N=5)). First, we used mean M ratio to detect a reduction in population size (Garza & Williamson 2001). M is calculated as the ratio of the total number of alleles found at a locus divided by the range in allele sizes measured in repeat number (or the total number of

possible alleles given a specific size range). The value of M will decrease when there is a significant bottleneck or founder effect. The severity and duration of the bottleneck is reflected by the magnitude of decrease in M. This approach has the ability to detect a population that has undergone a reduction in size due to a founder event, but is relatively insensitive to very recent or on-going bottlenecks (Garza & Williamson 2001). The second method is particularly sensitive to on-going population bottlenecks and is based on the detection of a distortion in allele frequency distributions, as described in Luikart *et al.* (1998). The distribution of allele frequencies at neutral loci displays a characteristic mode-shift immediately following a population bottleneck. Specifically, rare alleles are preferentially lost during bottlenecks, relative to alleles at intermediate frequencies. Although this approach is not quantitative, it provides a graphical representation of allele frequency distribution stability, and the mode shift is easily detected graphically. This method can only detect very recent or ongoing bottlenecks. Finally, the computer program BOTTLENECK v.1.2 (Cornuet & Luikart 1996) was used to detect expected changes in heterozygosity resulting from population bottlenecks. This program exploits the assumption that a reduction in population size corresponds to a reduction in allele numbers and heterozygosity at microsatellite loci. Allele diversity is reduced faster than heterozygosity in a population out of mutation-drift equilibrium. Thus it is expected that observed heterozygosity will be higher than the expected heterozygosity. This is only true when the expected heterozygosity is estimated based on observed number of alleles from loci in equilibrium. A Wilcoxon sign rank test was used to test for heterozygote excess under two mutation models (Infinite Allele Model (IAM) and the Stepwise Mutation Model (SMM)) and under the Two-Phase Model (TPM). This TPM model accounts for single step changes in the

microsatellite repeat motif as well as infrequent large changes in repeat number and most closely reflects the actual mutational process of microsatellite loci.

4.3 RESULTS

Genetic Diversity

Mean expected (H_E) and observed (H_O) heterozygosities ranged from 0.53 to 0.65 and 0.43 to 0.62 respectively, indicating high heterozygosity amongst all sites. Mean allelic richness (A) ranged from 3.16 (PSE) to 5.33 (MCF2) (Table 4.1), suggesting that a difference in allelic richness does exist between sites. The number of private alleles observed ranged from 0 (M1, M3 and M5) to 5 (STC) (Fig. 4.2).

Genetic Stability

Loci that were not in HWE accounted for 13% of all locus-by-population comparisons (17/130). Deviations from HWE were observed in all categories across the different colonization times. Five of the 17 non–equilibrium loci were found in the Maitland River sites (Table 4.1). After Bonferroni correction, significant linkage was observed in three populations, M1 had linkage between 1 pair of loci. PSE and HAS each had significant linkage disequilibrium pairs of loci.

Population Bottleneck

Mean M ratios across all populations ranged from 0.60 (HAS2 and MCF) to 0.68 (STC), the longest established site in this study (Fig. 4.3). All mean M ratios are consistent with populations that are either reduced or founders as described by Garza & Williamson (2001). Evaluating distortions in allele frequency distributions revealed that all populations had rare

alleles at the highest frequency, which is consistent with populations that are not experiencing severe population bottlenecks (Luikart *et al.* 1998). However, three populations, HAS/HAS2 and PSE, had allele frequency distributions that were shifted slightly to the right indicating a reduction in rare alleles compared to other populations (Fig. 4.3), consistent with on-going or recent population bottlenecks (Luikart *et al.* 1998). Based on the Wilcoxon test, eight of the populations showed significant heterozygote excess under the IAM, whereas, under the SMM no populations showed significant bottlenecks (Table 4.2). Under the TPM, three populations showed significant bottlenecks (HAS/HAS2 and PSE; Table 4.2). These three populations displayed significant heterozygote excess under both the IAM and TPM. This suggests that HAS, HAS2 and PSE are likely experiencing bottlenecks. These results are consistent with the observed shift in allele frequency distributions of these populations.

Population Structure

Exact tests of allelic differentiation (among Maitland River populations only) revealed that only M3 and M5 (1 out of ten comparisons) had allele frequencies that were significantly different from one another after Bonferonni correction ($p<0.001$). All Maitland River site *F*-statistics were not significantly different from zero indicating no population structure. Patterns of genetic differentiation weakly followed an isolation by distance pattern among the Maitland River samples ($r^2=0.063$, $p=0.04$). Genotype assignment analysis of the Maitland populations revealed that colonization of these sites was characterized by natural dispersal from nearby sites (PE) and from jump dispersal events from three distant regions. Identified migrants from the Maitland River populations successfully assigned to along the Detroit River, to sites throughout Lake Erie and to sites in the Bay of Quinte (MCF and TR) (Table 4.3).

Table 4.1 Measures of genetic diversity at 10 polymorphic microsatellite markers used in the analysis of population bottlenecks in five sites undergoing invasion (M1-5), five sites thought to be colonized in 1999, and one site representing the oldest known invaded site (STC). ID refers to the abbreviated population name, geographical coordinates are provided. Expected heterozygosity (H_E), observed heterozygosity (H_O), number of individuals (n) and allelic richness (A) are presented. Loci out of HWE after Bonferonni correction are in bold-face type and underlined. Mean values for all measures are given.

Site	ID		*Nme 1*	*Nme 2*	*Nme 3*	*Nme 4*	*Nme 5*	*Nme 6*	*Nme 7*	*Nme 8*	*Nme 9*	*Nme 10*	*Mean*
Maitland 1	M1	H_E	0.83	0.75	0.76	0.39	0.26	0.84	0.36	0.73	0.59	0.74	0.63
Lat. 43.7		H_O	0.79	0.72	0.73	0.38	0.24	0.81	0.34	0.70	0.57	0.71	0.60
Long. 81.7		*A*	7.00	4.00	5.65	2.00	2.91	5.99	2.83	5.98	5.81	7.63	4.98
		M	0.73	1.00	0.43	0.75	0.75	1.00	0.57	0.60	0.25	0.35	0.64
		n	10	10	12	12	11	11	12	11	11	11	11.1
Maitland 2	M2	H_E	0.85	0.57	0.61	0.39	0.38	0.80	0.54	**0.74**	0.59	**0.79**	0.63
Lat. 43.7		H_O	0.84	0.56	0.61	0.39	0.37	0.79	0.53	0.73	0.58	0.78	0.62
Long. 81.7		*A*	8.23	3.75	4.17	2.00	3.13	5.97	2.76	5.91	4.56	5.96	4.64
		M	0.64	1.00	0.43	0.75	0.63	1.00	0.57	0.55	0.43	0.50	0.65
		n	46	48	48	46	42	47	48	47	48	48	46.8
Maitland 3	M3	H_E	0.81	0.59	0.63	0.42	0.26	0.81	0.44	**0.75**	0.53	0.75	0.60
Lat. 43.7		H_O	0.80	0.58	0.62	0.41	0.25	0.80	0.43	0.74	0.53	0.74	0.59
Long. 81.7		*A*	6.97	3.87	5.14	2.27	2.52	6.04	2.44	6.44	3.89	6.08	4.57
		M	0.73	0.60	0.43	0.75	0.50	1.00	0.57	0.73	0.33	0.47	0.61
		n	39	39	39	37	35	39	40	40	39	40	38.7
Maitland 4	M4	H_E	**0.83**	0.52	0.76	0.36	0.21	0.80	0.54	0.77	0.48	**0.75**	0.60
Lat. 43.7		H_O	0.82	0.51	0.75	0.35	0.21	0.79	0.53	0.76	0.47	0.74	0.59
Long. 81.7		*A*	7.20	3.54	5.71	2.52	2.41	5.51	2.77	6.29	4.78	5.84	4.66
		M	0.73	0.60	0.64	0.75	0.75	1.13	0.57	0.73	0.30	0.43	0.66
		n	33	32	33	32	31	32	33	33	33	33	32.5
Maitland 5	M5	H_E	0.82	0.45	0.65	0.36	0.32	0.81	0.56	0.80	0.51	0.81	0.61
Lat. 43.7		H_O	0.80	0.44	0.63	0.35	0.32	0.79	0.55	0.78	0.50	0.80	0.60
Long. 81.7		*A*	7.34	3.68	4.07	2.00	2.47	6.75	2.90	6.23	3.75	6.88	4.60
		M	0.73	1.00	0.42	0.75	0.75	1.00	0.57	0.50	0.33	0.43	0.65
		n	34	40	38	42	31	36	40	37	42	35	37.5
McFarland 2004	MCF	H_E	0.81	0.69	0.76	0.32	0.22	0.78	0.66	**0.73**	0.66	0.76	0.64
Lat. 44.0		H_O	0.80	0.60	0.80	0.38	0.24	0.54	0.58	0.54	0.73	0.64	0.59
Long. 77.1		*A*	4.93	2.47	5.05	3.81	2.59	5.66	3.90	5.87	7.73	6.65	4.86
		M	0.64	0.60	0.50	0.57	0.67	1.00	0.57	0.71	0.36	0.40	0.60
		n	50	48	49	50	49	48	50	50	48	47	48.90

McFarland 2006	MCF2	H_E	0.85	0.65	0.79	0.32	0.13	0.71	0.59	0.83	0.70	**0.76**	0.63
Lat. 44.0		H_O	0.68	0.53	0.71	0.36	0.13	0.42	0.43	0.57	0.74	0.54	0.51
Long. 77.1		*A*	8.37	3.85	6.72	2.63	2.40	7.46	3.50	7.28	5.69	5.44	5.33
		M	0.55	0.60	0.50	0.67	0.67	1.00	0.57	0.69	0.36	0.48	0.61
		n	34	40	38	42	31	36	40	37	42	35	37.50
Hastings 2005	HAS	H_E	0.76	0.63	0.66	0.44	0.36	0.66	**0.16**	0.82	0.63	**0.70**	0.58
Lat. 44.3		H_O	0.74	0.63	0.60	0.37	0.33	0.44	0.13	0.87	0.60	0.43	0.52
Long. 78.0		*A*	5.03	3.20	4.13	2.00	2.00	3.00	2.08	6.01	3.00	4.06	3.45
		M	0.71	1.00	0.25	0.75	0.50	1.00	1.00	0.50	0.60	0.23	0.65
		n	46	60	53	60	57	52	60	47	60	46	54.10
Hastings 2006	HAS2	H_E	0.76	0.62	0.56	0.45	0.40	**0.64**	0.22	0.82	0.61	0.68	0.58
Lat. 44.3		H_O	0.86	0.72	0.51	0.50	0.47	0.47	0.25	0.66	0.71	0.28	0.54
Long. 78.0		*A*	4.33	3.00	4.27	2.00	2.00	3.64	2.30	6.13	3.34	4.75	3.58
		M	0.71	1.00	0.29	0.75	0.50	1.00	0.43	0.50	0.50	0.29	0.60
		n	36	36	35	36	32	30	36	35	35	36	34.7
Burlington	BUR	H_E	0.71	0.47	0.62	0.32	0.12	0.67	0.56	0.73	0.68	0.77	0.56
Lat. 43.3		H_O	0.55	0.45	0.70	0.38	0.12	0.64	0.30	0.53	0.77	0.52	0.50
Long. 79.8		*A*	6.01	3.19	3.80	2.40	2.15	5.82	3.40	4.55	3.94	6.18	4.14
		M	0.64	0.60	0.83	0.67	0.50	1.00	0.57	0.50	0.37	0.53	0.62
		n	53	53	53	53	50	47	53	51	53	50	51.60
Killarney	KIL	H_E	**0.84**	0.57	0.70	0.53	0.18	**0.75**	0.45	0.84	0.61	**0.77**	0.62
Lat. 46.0		H_O	0.78	0.43	0.67	0.70	0.19	0.63	0.36	0.81	0.63	0.63	0.58
Long. 81.6		*A*	8.45	3.64	4.70	2.57	2.56	5.71	2.19	6.91	4.78	6.45	4.80
		M	0.82	0.60	0.53	0.75	0.50	1.00	0.57	0.62	0.39	0.47	0.62
		n	64	63	63	64	64	64	64	62	64	62	63.40
Port Severn	PSE	H_E	0.61	0.50	0.21	0.44	0.44	0.66	0.39	0.67	0.71	**0.62**	0.53
Lat. 44.8		H_O	0.33	0.33	0.24	0.38	0.47	0.33	0.39	0.54	0.77	0.49	0.43
Long. 79.7		*A*	3.77	2.00	1.97	2.00	2.94	3.81	2.20	4.71	3.92	4.27	3.16
		M	0.50	1.00	0.80	0.75	0.50	0.83	1.00	0.50	0.15	0.36	0.64
		n	63	63	55	60	60	63	61	63	62	61	61.1
St. Clair River	STC	H_E	0.86	**0.63**	0.79	0.42	0.32	**0.74**	0.51	0.81	0.61	**0.78**	0.65
Lat. 42.8		H_O	0.85	0.37	0.75	0.41	0.34	0.48	0.41	0.68	0.63	0.53	0.55
Long. 82.5		*A*	8.76	3.69	6.54	2.31	3.81	6.99	2.61	6.73	5.00	6.44	5.29
		M	0.75	0.60	0.50	0.75	0.75	1.00	0.57	0.79	0.42	0.62	0.68
		n	98	79	104	104	100	93	85	102	105	103	97.3

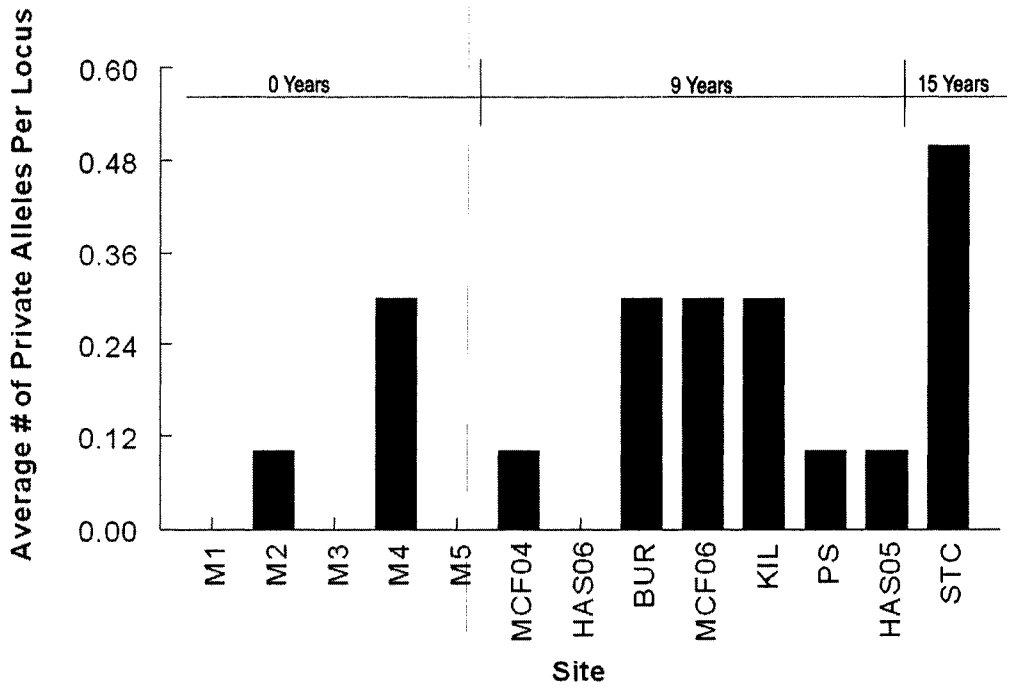

Figure 4.2 The number of private alleles average over all loci observed among three categories of invasion time since initial 1992 introduction to the Great Lakes (i.e., 0 years, 9 years and 15 years).

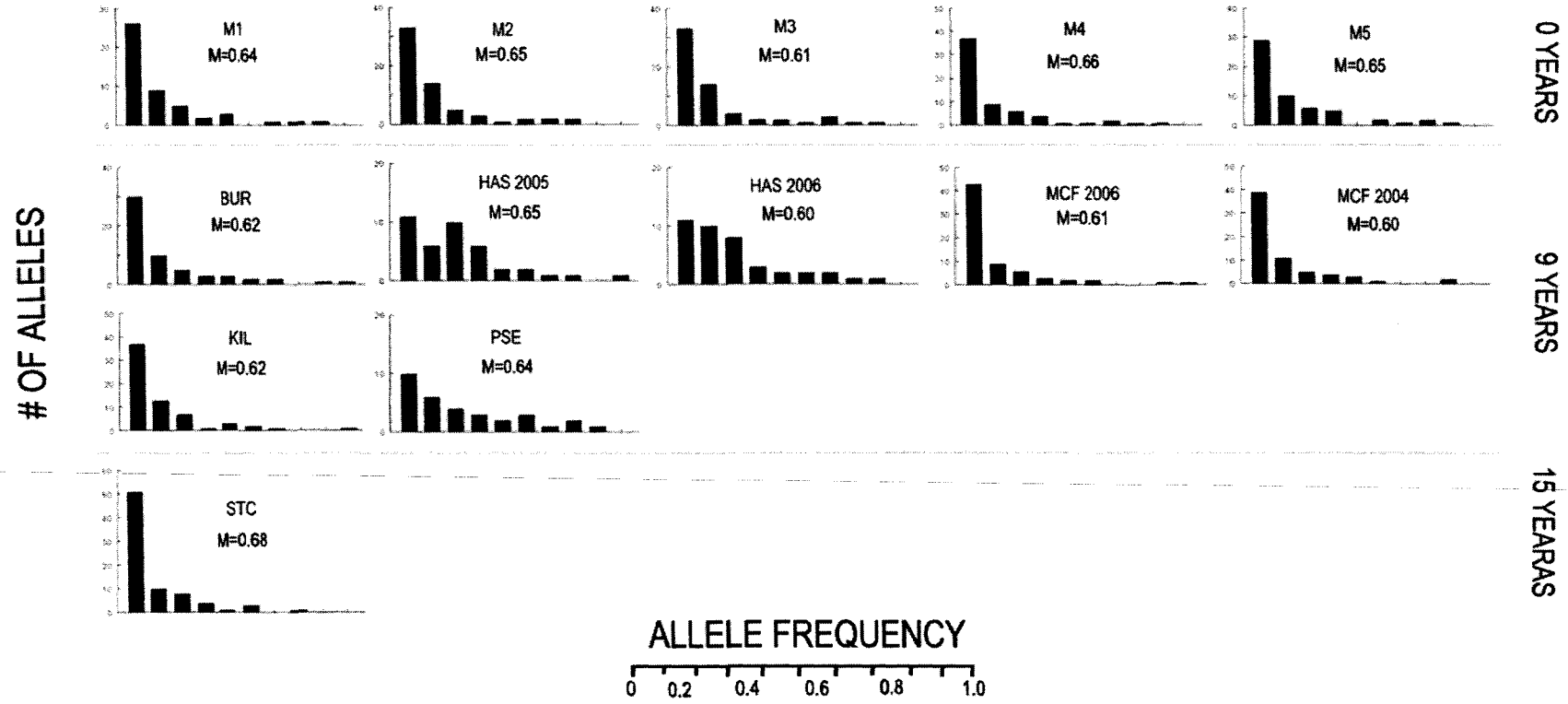

Figure 4.3 Allele frequency distribution across all round goby populations. Methods are based on detection of a mode-shift in the distribution following a population bottleneck (Luikart 1998). The scale of X-axis is the range of allele frequencies, low frequency alleles are on the left, while more frequent alleles are on the right. The left Y-axis indicates the number of alleles with across all loci for that fall within a defined frequency range. The mean M ratio (Garza & Williamson 2001) for each population is presented below the site name. The numbers of years after detection in the Great Lakes are presented along the right Y-axis.

Table 4.2 Detection of population bottlenecks in Great Lakes round goby populations. Heterozygote excess was assessed under three mutation models, Infinite Allele Model (IAM), Stepwise Mutation Model (SMM) and Two-phase Model (TPM). Significance was assessed using BOTTLENECK v. 1.2 (Cornuet & Luikart 1996). "X" denotes a significant heterozygote excess.

Site	IAM	SMM	TPM
M1			
M2	X		
M3	X		
M4			
M5	X		
MCF04	X		
HAS06	X		X
BUR			
MCF06			
KIL	X		
PSE	X		X
HAS05	X		X
STC			

Table 4.3 Genotype assignment test of the Maitland River samples using a reference database of round goby populations from Chapter 3. Individuals were successfully assigned if the likelihood ratio between the highest and second highest assignment probabilities exceeded four. Values indicate the number of migrants. Site abbreviations are described in Chapter 3 (Table 1, pg. 48).

	M1	M2	M3	M4	M5
Lake Ontario					
TR		3	1		1
HAS					1
BUR					
MCF					
Lake Erie					
PC		2	1		1
PM					
PD					
PS					
LA	1	2	4		
NI					
JG					
STC		1	1		
RO		1	1		
DU	1	3	1	1	2
COL	1	1		1	3
BR					
GS					
MCK					
LEA			1		
NY1			1		
NY2					
NY3					
NY4		1			
NY5					
NY6					
LEA2					
PD2					
MCK2					
Lake Huron					
KIL				2	1
MID					
OS					
PE		4	1	2	3
MID2					
SL		1	2	2	
OS2				2	
HAS2					

4.4 DISCUSSION

This study demonstrates that the examined round goby populations, despite the differences in colonization times, do not generally show signs of on-going population bottlenecks, although significant heterozygote excess was observed in three populations (HAS/HAS2 and PSE) indicating current bottlenecks. However, all populations appear to have undergone a reduction in size in the past. Population bottlenecks have been observed in many introduced species (Tsutsui *et al.* 2000, Colautti *et al.* 2005, Lindholm *et al.* 2005) where genetic diversity in introduced relative to native regions is reduced (Cristescu *et al.* 2001; Berg *et al.* 2002, Meimberg *et al.* 2006). This is not the case for round gobies in the Great Lakes, neither my, nor others data show any reduction in genetic diversity relative to native round goby populations (Chapter 3; Marsden *et al.* 1995, Stepien *et al.* 2006). Interestingly, an increase (or at least no reduction) in genetic diversity during colonization has been reported in a various taxa (Kolbe *et al.* 2004, Chen *et al.* 2006). The lack of any reduction in diversity in those studies indicates that either the populations did not undergo bottlenecks, or they rapidly recovered. It is generally accepted that accidental introduction events across long distances involve few individuals, so how can invasive species be genetically diverse? Two commonly cited scenarios include: 1) the introduction of a genetically diverse group of invaders from one or more sources in a single introduction event, or 2) multiple invasion events from diverse sources, followed by mixing and inter-breeding (e.g. Novak & Mack 1995; Durka *et al.* 2005). Finally, the assumption of small numbers of invading propagules may be incorrect, since a large number of genetically diverse individuals would provide an explanation of my results as well (Holland 2000).

The invasion front in the Mailtand River highlights the fact that during a novel colonization, round gobies are able to maintain high levels of genetic diversity. These

populations show comparable genetic diversity and display the same founder effects as longer established populations. During an extremely recent invasion, round gobies are still able to maintain unusual high genetic diversity, given the insufficient for time for multiple jump dispersal introductions; it is more likely that individuals along this invasion front are simply reflected by an already highly genetically differentiated Great Lakes sources, that I did not sample.

The high genetic diversity in the Maitland and other Great Lakes round goby populations was most likely not the result of a single introduction of a large number of individuals. It is more likely that colonization of the round goby is characterized by the invasion by a few individuals; multiple times (also see Chapter 3). Microsatellite data from other studies analyzed using population bottleneck detection methods described in Garza & Williamson (2001), showed populations that were historically described as reduced or founders had M ratios similar to those calculated in this study (e.g. northern wombat, mean M=0.618, Taylor *et al.* 1994; black bear, mean M=0.641, Paetkau *et al.* 1997). This indicates that recently established populations of round gobies in the Great Lakes have undergone a reduction in population size during their colonization history. The inability to detect on-going bottlenecks or lasting effects of severe founder effect may be due to multiple introduction events, by gene flow among introduced Great Lakes populations or a combination of the two. Although not tested in this study, the augmentation of invading genetic diversity from source populations has been documented in other invasive species (e.g. Baker 1992, Kolbe *et al.* 2004, Colautti *et al.* 2005) allowing newly founded populations to successfully pass through bottlenecks. Genetic analysis of 32 introduced round goby populations indicates that gene flow among Great Lakes sites may have enabled these populations to overcome bottlenecks (Chapter 3). Migration in the Great Lakes was

characterized by multiple dispersal strategies, allowing both short and long distance introduction of genetic diversity among sites (Chapter 3).

Panmixia during colonization has been documented in the invasion of several freshwater amphipods of the genus *Dikerogammarus* (Müller *et al.* 2002) and in the brown mussel, *Perna perna* (Holland 2001). The on-going invasion of the round goby along a 2 km stretch in the Maitland River is characterized by very weak or no genetic structure among 5 sampled sites. This panmixia is most likely the result of high levels of gene flow among the spatially close sites. Previous genotype assignment tests indicate that round gobies may be dispersing more readily than previously thought (Chapter 3). The lack of strong recent population bottleneck effects and high genetic diversity suggests that the successful invading front is composed of individuals that originated from genetically distinct sources within the Great Lakes. The absence of any defined structure along the invading front contradicts what was previously found among introduced populations. These studies reported high levels of genetic divergence among Great Lakes sites (Chapter 3, Stepien *et al.* 2005, and Stepien & Tumeo 2006). Currently expanding round gobies are characterized as genetically indistinguishable relative to older more established populations. Given their ability to maintain high genetic diversity while expanding its range, the round goby will likely be able to successfully establish a reproducing population with the introduction of only a limited number of founders. This causes great concern, as the threat of establishing in inland lakes and rivers can have dramatic consequences on native fish populations.

Studying the genetic changes that occur during a colonization event will enable scientists to evaluate the effects of drift and selection on invading populations. Identifying levels of genetic diversity as well as the population dynamics along an invasion front will allow managers to predict the risk for future spread. The most recently established round goby populations are

currently not experiencing population bottlenecks, yet mean M ratios suggest a historical bottleneck for all populations evaluated. The lack of a current bottleneck and signs of a historical reduction in population size suggest that initial colonization of these sites was by a limited number of individuals and migration of individuals through natural dispersal and long distance human mediated vectors enable the round goby to overcome the deleterious effects of population bottlenecks. Along the invasion front in the Maitland River, I found that these populations were no different from more established populations throughout the Great Lakes. This indicates that initial colonization of round gobies into novel ranges do not experience the expected genetic problems associated with colonization and thus their range expansion will continue without restrictions to any normal limitations of range expansion.

The Great Lakes round goby is defying the conventional invasion genetic expectations for range-limiting genetic founder effects. Thus there is an urgent need to further monitor invasion fronts as genetic changes through time will allow us to determine if the Maitland River invasion is an anomaly, or a new rapid invasion genetic model. Either way, such an evaluation will increase the accuracy of predicting future range expansion and persistence in upland systems around the Great Lakes. The ability to genetically quantify other currently invading systems will allow us to confirm these unique results and allow researchers to understand how small founding populations can retain such diversity in the presence of evolutionary forces that act to decrease diversity in these small populations.

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5.0 GENERAL DISCUSSION

Global transport through accidental ballast water discharge has been responsible for the introduction of many invasive species not only in the Great Lakes (Mills *et al.* 1993) but also worldwide (Wonham *et al.* 2000). These introductions can dramatically alter the recipient ecosystem through several means, which can include disruption of foodwebs (Vanderploeg *et al.* 2002, Mills *et al.* 2003) or through impacts on native biodiversity (Ricciardi *et al.* 1998, Yan *et al.* 2002). The detection of fish in ballast water has been rare (Carlton & Gellar 1993), although the importance of this vector should not be underestimated in mediating the invasion of fish species (Carlton 1985, Hensley 1993). Based on probability alone we can safely assume that populations introduced via ballast water were initially small. The genetic consequences associated with small population sizes can lead to the dramatic differentiation among introduced populations (Ellstrand & Elam 1993). The degree of these genetic changes is not only dependant on the composition of the founding population but also on ecological factors in the novel environment (Lambrinos 2004).

To detect the genetic signature of invasive populations, I developed highly polymorphic markers that could be used to detect recent changes in population dynamics. Microsatellite markers were chosen to help resolve the genetics of invasive species.

Using these highly mutable molecular markers I was able to observe that genetic diversity across all populations was not reduced (Chapter 3). This could have arisen by several means. Firstly, founding populations could have been large, this is not likely the case here, as fish abundance in ballast tanks are relatively low (Wonham *et* al. 2000). Secondly, round gobies could have been introduced multiple times. The high genetic divergence among introduced sites is more consistent with this hypothesis. The use of rapidly mutated markers is extremely

sensitive estimator of genetic diversity and allowed us to accurately characterize population variation.

Through this study I also found that genetic divergence among invasive sites was unusually high given their recent establishment in the Great Lakes, which is consistent with previous genetic analyses (Dillon & Stepien 2001, Stepien *et al.* 2005, Stepien & Tumeo 2006). A reduction in population size during colonization and multiple introductions from Eurasia likely led to the high genetic differentiation. Dispersal and range expansion in the Great Lakes can be attributed to a combination of three factors. Firstly, we observed patterns consistent with natural dispersal, which we estimate to be nearly 40%. Secondly, ballast water jump dispersal events were also characterized in the Great Lakes, we estimate that this vector of dispersal accounts for 16% of the range expansion. Lastly, multiple introductions from Eurasian sources likely played a role in the rapid spread of round gobies throughout the Great Lakes.

The observed high diversity along a 2 km current invasion front suggests that the diversity is not likely gained through the gradual increase of migrants but rather that initial colonization is characterized by high diversity from the start. From this we can conclude that the invasion process of round gobies is driven by invading groups with high genetic diversity that most likely resulted from the mixing of multiple invasions. This type of colonization pattern is previously unknown in vertebrates, which represents an unfavorable scenario that will lead to further successful rapid expansion.

Through this study I am able to make several suggestions in regard to conservation and management plans. Given that this study is the first to extensively evaluate dispersal and colonization of the round goby in the Great Lakes, it is important to note that additional work

needs to be done. Several key management recommendations can be made by evaluating the major points from this study:

- High genetic diversity – the introduction of even a limited number of individuals has the potential to generate genetically diverse invasive genotypes that can colonize additional inland lakes and river systems;
- High genetic divergence – over time isolated populations may become locally adapted, universal management plans may be ineffective ;
- Multiple invasions – continued invasions may further increase genetic diversity and facilitate further range expansion. and;
- High dispersal – within Great Lakes ballast water control measures should be implemented to reduce genetic exchange across long distances and to slow range expansion.

Future work should include:

- Identify source populations, which may be able to confirm multiple introductions and allow us to characterized natural genetic diversity. This can be accomplished through either genotype assignment tests, measures of genetic divergence between populations or by constructing neighbour joining trees based on estimates of genetic distance;
- Couple microsatellite population structure with historical mtDNA lineages. This can be accomplished by identifying haplotype diversity between introduced populations. Unique haplotypes may also be identified in the native range and could indicate source populations, and;

- Monitor, on an on-going basis the genetic signature of the invasion front. This can be done by sampling individuals along a continuous stretch until the invasion front is reach, presumably the where no round gobies are found.

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