THE GENETIC STRUCTURE OF REMNANT FATMUCKET MUSSEL (*LAMPSILIS SILIQUOIDEA*) POPULATIONS IN THE ST. CLAIR RIVER DELTA AND SURROUNDING TRIBUTARIES FOLLOWING THE INVASION OF DREISSENID MUSSELS

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I dedicate this to my father,
for teaching me
to not only look at life and wonder,
but to look at life *with* wonder

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ABSTRACT

THE GENETIC STRUCTURE OF REMNANT FATMUCKET MUSSEL (*LAMPSILIS SILIQUOIDEA*) POPULATIONS IN THE ST. CLAIR RIVER DELTA AND SURROUNDING TRIBUTARIES FOLLOWING THE INVASION OF DREISSENID MUSSELS

by Matthew T. Rowe

The St. Clair River Delta is a refuge habitat for native unionid mussels and has retained unionid populations while they have largely disappeared from the open waters of Lake St. Clair as a result of the invasion of dreissenid mussels. Eight variable microsatellite DNA markers were used to assess the genetic population structure of the Fatmucket mussel (*Lampsilis siliquoidea*) across 18 sites (n=326 individuals) within the delta and several of its tributaries with particular interest in determining how *Dreissena*-induced unionid declines may have impacted genetic structure. Four specific questions were addressed: 1) How much genetic diversity is present in the remnant populations of Fatmucket in the St. Clair Delta? 2) What is the level of gene flow occurring between sampling locations in the St. Clair Delta and surrounding watersheds? 3) Is genetic differentiation related to geographic isolation within the St. Clair Delta and its tributaries? And 4) is there any evidence of a recent genetic bottleneck in the Fatmucket populations of the St. Clair Delta? Results indicate that Fatmuckets within the various bays of the St. Clair River Delta and tributaries show limited genetic differentiation by geographic distance but still represent a single population with ongoing gene flow, relatively high allelic richness and locus polymorphism at all sites, and little evidence to support a recent genetic bottleneck. The Fatmucket is the most common species found in the delta and it can be assumed that the genetic health of this species represents a best-case scenario for mussel populations there. No evidence of a serious loss of genetic diversity or genetic structure in this species offers some hope that less

common and imperiled species found in the St. Clair Delta may have also retained their genetic diversity in the face of major demographic declines.

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CHAPTER I

INTRODUCTION

Unionid mussels (Bivalvia: Unionidae) are a varied group of freshwater bivalved mollusks with a worldwide distribution. The greatest diversity of unionids occurs in North America with two families containing almost 300 native species (Bogan 1993). North American species represent nearly a third of the world's total unionid diversity (Strayer *et al*. 2004, Graf and Cummings 2007). At least 40 of the 300 species are known to inhabit the lower Great Lakes region and of these 40 species, 32 have been identified in Lake St. Clair (Metcalfe-Smith *et al*. 1998) with 22 species having been reported within the last decade (Zanatta *et al.* 2002, McGoldrick *et al*. 2009). Unionids occupy vital roles in the ecology of many riverine and lacustrine habitats including: filtration of the water column, biodeposition of nutrients, nutrient cycling, resource limitation, bioturbation of sediments, and a source of habitat for other organisms(Vaughn and Hakenkamp 2001). Despite these critical roles, unionid mussels are considered to be the most imperiled group of organisms in North America with over 70% of native species being either assumed extinct (37 species) or vulnerable to extinction (165 species) (Strayer *et al.* 2004). As an important part of a healthy aquatic ecosystem, these unionid mussels are now in urgent need of effective management to prevent total extirpation of the fauna from the waters of the Great Lakes largely as a result of invasive dreissenid mussels.

Before the mid 1980's, unionid mussels dominated the biomass of benthic communities in the lower Great Lakes region. In 1983, mean unionid densities in Lake St. Clair reached 7 m^{-2} (University of Windsor 1984). Unionid biomass in this area (4.4 $g.m^{-2}$ dry weight) totaled four times the biomass of all other macroinvertebrates combined (Hudson 1986, Nalepa and Gauvin 1988). Despite these prodigious numbers, unionid mussel populations in the Great Lakes had

likely been in decline over the last century due to a variety anthropogenic effects (Nalepa *et al.* 1991, Nalepa *et al.* 1996). These effects included industrial, municipal and agricultural pollution; habitat destruction and changes in host fish communities (Bogan 1993). Studies of unionid populations in Lake St. Clair showed that densities had declined to approximately 1.9 m^{-2} by 1988 (Nalepa and Gauvin 1988).

A significant blow was dealt to unionids in North America when the European Zebra Mussel (*Dreissena polymorpha*) was introduced to Lake St. Clair in approximately 1986 (Hebert *et al*. 1989). In the eight years following this introduction, unionid mussel communities in the open waters of the lower Great Lakes were virtually eliminated (Nalepa *et al.* 1996, Schloesser *et al.* 1999). The decline of unionids since the mid-1980s has been well documented in several areas of the lower Great Lakes with multiple studies observing unionids in the Detroit River (Schloesser *et al.* 2006), Lake Erie (Schloesser and Nalepa 1994), and Lake St. Clair (Nalepa *et al.* 1996). These steep declines are thought to be largely due to fouling by dreissenid mussels (Mackie 1991, Gillis and Mackie 1994, Ricciardi *et al*. 1995, Schloesser *et al*. 1998, Nichols and Amberg 1999, Schloesser *et al*. 2006). In Lake St. Clair, unionids collected at 29 sites throughout the lake, dropped from 281 individuals and an average density of 1.9 m^{-2} in 1986 to 6 individuals with a density of 0.04 m^{-2} in 1994(Nalepa *et al*. 1996). Subsequent surveys conducted between 1997 and 2001 failed to locate any live unionids in the open waters of Lake St. Clair (Nalepa *et al.* 2001, Zanatta *et al.* 2002, Hunter and Simons 2004).

Despite this near extirpation, a shallow-water "refuge" was found in the St. Clair River Delta (Figure 1). This refuge was found to contain a relatively large remnant unionid community despite the presence of dreissenids (Zanatta *et al*. 2002, McGoldrick *et al*. 2009). The St. Clair River Delta is an area at the mouth of the St. Clair River covering a large portion of the

northeastern shore of Lake St. Clair (~100 km^2) (Figure 1). It contains numerous channels, islands, and bays averaging between one and three meters in depth. The combination of complex shoreline, soft sediment, and shallow water habitat has allowed for unionid mussels to persist at low densities while limiting dreissenid colonization (McGoldrick *et al.* 2009).

In order to properly manage unionids of conservation concern, it is important to assess the genetic health and structure of their populations (Berg *et al.* 2007, Kelly and Rhymer 2005, Strayer *et al*. 2004). Over time, small isolated populations can suffer from inbreeding depression and genetic drift, which can reduce genetic fitness (Freeland 2005). If supplemental stocking or translocation becomes necessary, it is also important to identify similar source populations and exclude those with divergent genotypes to avoid genetic homogenization (Hoftyzer *et al*. 2008, Jones *et al*. 2006, Cope and Waller 1995).

Because the conservation of remaining unionid populations in Lake St. Clair is so important to the continued existence of several unionid species in the lower Great Lakes, it is vital to understand the genetic structure of these populations. The Fatmucket mussel is a relatively common species within the study area (63% relative abundance; McGoldrick *et al.* 2009) and is a host generalist (Ohio State University Museum of Biological Diversity 2011). Because of these factors, the genetic health of this species can be used as a surrogate and best case scenario when assessing less common, host specialist, or endangered species which may not be present in sufficient numbers for sampling or may be too sensitive to stresses caused by handling and tissue biopsy.

In an effort to asses the genetic health of unionids in the St. Clair Delta, four factors were investigated: 1) how much genetic diversity is present in the remnant populations of Fatmucket in the St. Clair Delta 2) What is the level of gene flow occurring between sampling locations in

the St. Clair Delta and surrounding watersheds? 3) Is genetic differentiation related to geographic isolation within the St. Clair Delta and its tributaries? And 4) is there any evidence of a recent genetic bottleneck in the Fatmucket populations of the St. Clair Delta?

CHAPTER II

METHODS

Sample Collection

Fatmucket mussels were collected from several sites in five bays of the U.S. and Canadian portions of the St. Clair River Delta. Mussels were also collected from the major tributaries of the St. Clair River known to contain Fatmucket including: the Belle River, Pine River, Black River, and Clinton River (Figure 1). Delta sample sites were selected by visiting productive sites previously identified by Zanatta *et al.* (2002) and McGoldrick *et al.* (2009). Sampling sites in tributaries were selected by scouting accessible locations and investigating sites obtained from museum records (Ohio State University Museum of Biological Diversity. 2011. Freshwater mussel/host database. http://www.biosci.ohio-state.edu/~molluscs/OSUM2/. Accessed 18 May 2011). Unionid mussels are known to burrow beneath the surface in winter months to avoid extreme cold (Watters *et al.* 2001) and sampling was started in July to ensure most mussels had emerged above the substrate surface.

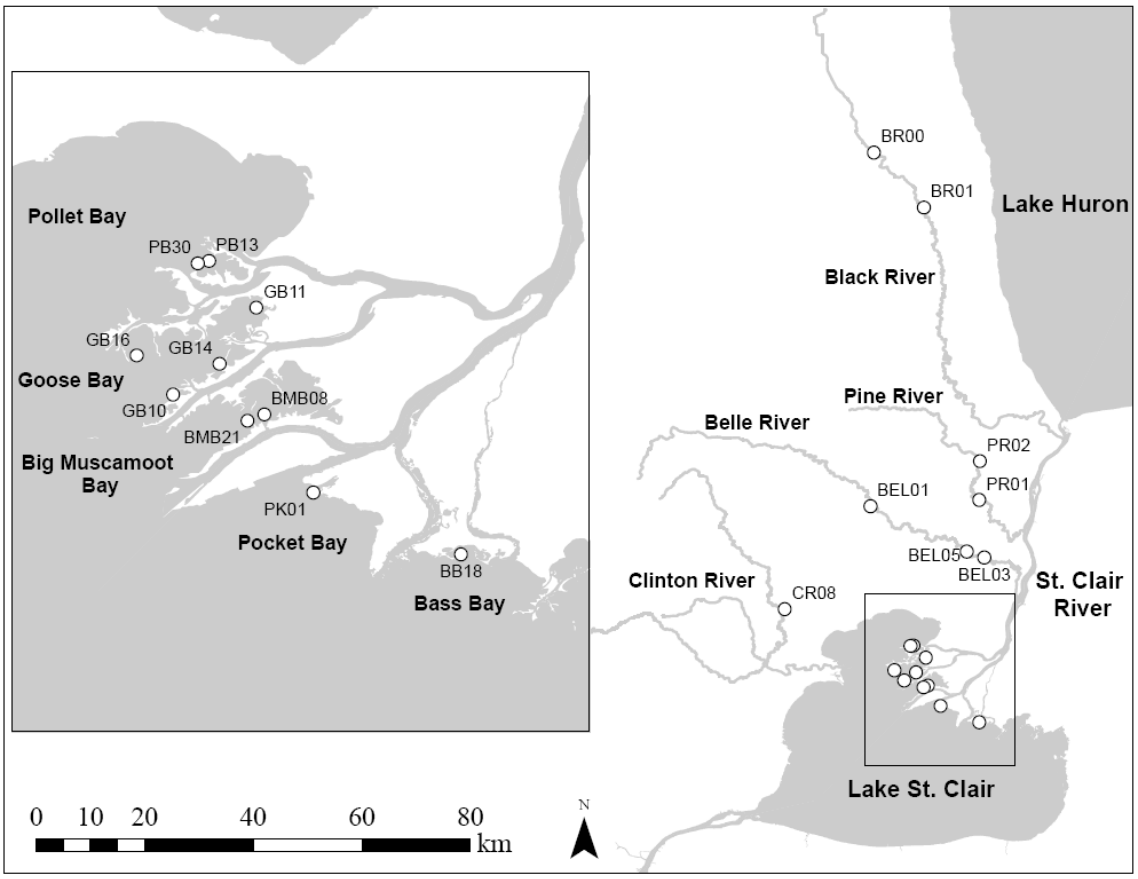

Figure 1. St. Clair River Delta and tributary sampling sites and sampling locations for *Lampsilis siliquoidea*.

Between 17 and 78 (mean = 38) individuals were collected from five of the major bays and four tributaries of the St. Clair River Delta (hereafter referred to as sampling locations) to acquire an adequate sampling of local genotypes (Table 1). A desired minimum number of 30 samples was deemed sufficient to provide adequate statistical power and avoid potentially erroneous allelic frequency results due to inadequately sampled populations (Freeland 2005; Piry *et al.* 1999). At only two of the sampling locations, Pocket Bay (n = 17) and Bass Bay (n = 29), were we unable to reach this threshold number of 30. Non-lethal tissue clips were collected from the mantle of *L. siliquoidea* following the procedure described by Berg *et al.* (1995) and preserved in ethanol for storage.

Table 1. St. Clair River Delta and tributary sampling sites and number of *Lampsilis siliquoidea* samples collected. Sites are grouped by sampling location.

Sampling Location	Site Code	Habitat	No. of samples	Totals by Sampling Location
Bass Bay	BB18	St. Clair Delta (CA)	29	29
Pocket Bay	PK01	St. Clair Delta (CA)	17	17
Big Muscamoot Bay	BMB08	St. Clair Delta (US)	30	59
Big Muscamoot Bay	BMB21	St. Clair Delta (US)	29	
Goose Bay	GB10	St. Clair Delta (US)	7	78
Goose Bay	GB11	St. Clair Delta (US)	27	
Goose Bay	GB14	St. Clair Delta (US)	14	
Goose Bay	GB16	St. Clair Delta (US)	30	
Pollet Bay	PB13	St. Clair Delta (US)	18	30
Pollet Bay	PB30	St. Clair Delta (US)	12	
Belle River	BEL01	St. Clair Tributary	7	36
Belle River	BEL03	St. Clair Tributary	23	
Belle River	BEL05	St. Clair Tributary	6	

Black River	BR01	St. Clair Tributary	13	30
Black River	BR00	St. Clair Tributary	17	
Clinton River	CR08	St. Clair Tributary	32	32
Pine River	PR01	St. Clair Tributary	20	30
Pine River	PR02	St. Clair Tributary	10	

DNA Extraction and Microsatellite Locus Amplification

DNA was extracted from a 0.10 - 0.25 cm^2 clip of tissue from each sample for genetic analysis using a modified alcohol extraction method, following Sambrook *et al.* (1989). A suite of nine primers developed and characterized for the congener *Lampsilis abrupta* by Eackles and King (2002) were used for PCR amplification of microsatellite loci (Table 2). Polymerase Chain Reaction (PCR) followed a modified method described by Eackles and King (2002). The PCR cocktail consisted of: 1.0 µl extracted genomic DNA working solution (10:100 genomic DNA:water), 1.0 µl 10X Taq buffer (Qiagen), 1.0 mM bovine serum albumin (BSA), 0.3 mM deoxyribonucleotide triphosphate (dNTP), 0.3 µl of 6-FAM or HEX fluorescent labeled forward primer, 0.3 µl reverse primer, 3.0 mM $MgCl_2$, and 0.05 U Taq (Qiagen) for a total reaction volume of 10 µl. Reactions were performed using Eppendorf thermocyclers (Eppendorf Mastercycler® epGradient). The amplification conditions were as follows: initial heating to 94°C for 2 min; then 45 cycles of 94°C for 40 sec., annealing at 58°C for 40 sec., and a 1 min extension time at 72°C followed by a final extension of 30 min at 72°C. Amplified PCR products were stained with SYBR green infused loading dye and confirmed using 1.5% agarose gel

electrophoresis at 92-98 volts for 90 min. All confirmed samples were genotyped on an ABI 3730 automatic gene sequencer. Genotypic data was scored using GENEMARKER ver. 1.80 (SoftGenetics LLC®).

Table 2. Microsatellite markers used for amplification of *Lampsilis siliquoidea* DNA. Modified from Eackles and King (2002).

Locus	Repeat Sequence	Size Range	No. of Alleles	Primer Sequence
LabC2	$(ATC)_2$ ... $(ATC)_3$ ... $(TCA)_8(GCA)_7$	145–167	8	F: ATGGACACCAGAAAGAAAAGG R: GAAGTCACAAGGTCAGGATCTC
LabC23	$(AGT)_6A(GCA)_8$	200–216	6	F: CAGTTGTTCCACTGTCGTAAAG R: TGGGACTAACATGGTGGTTAAG
LabC24	$(TGT)_5(TGC)_8$	138–237	23	F: TGGACCTATTCTTGCTTGTTG R: GTTCTTTCGCCTCCATGTATAG
LabC67	$(TAT)_9(TAG)_{12}(CAG)_7(TAG)_4$	158–213	19	F: AGTCTCTGGCTCAACCAACTC R: CAAATCAATTACTGCCTTTTC
LabD10	$(TATC)_{14}$	151–297	43	F: TTGTATAAACGGTCATGGAAAAC R: CCGTGACCACTTCTTCTAAAAC
LabD29	$(TATC)_{14}TACC(TATC)_2(TGTC)_7(TATC)_2$	181–263	24	F: TGTTCTTAGTTTATATTTATGGTTTGC R: GCAGAAAATCTCCAGTTTATGG
LabD111	$(ATCT)_{12}$ ... $(TGTC)_4$	200–262	20	F: TGCATCAACTCTATTCACAACC R: CAATGATAATGTAAATGTAAGCCTATC
LabD206	$(ATCT)_9$	189–259	20	F: AAGTGTAGAGGCAGAGAACTGAC R: TCACTGATACAGCATACATAATATAC
LabD213	$(ATCT)_{17}$	118–214	29	F: ATACACAGGGTGCTCTAAATGC R: TTGCCAAAACAACATAGTTCC

Data Analyses

The integrity of the collected molecular data was investigated by looking for sample amplification failures, linkage disequilibrium, genotyping errors and Hardy-Weinberg deviations that could potentially alter the results of the analysis. Samples that failed to amplify at more than four of the nine loci were removed from the analysis. Linkage disequilibrium was assessed with a log likelihood ratio statistic using the Markov chain method as implemented in GENEPOP v. 4.0.10. (Raymond & Rousset 1995). The presence of potential genotyping errors due to stuttering, large allele dropout, and null alleles were assessed using MICROCHECKER v. 2.2.3 (Oosterhout *et al.* 2004). Any loci with very high frequencies of null alleles (estimated frequencies >0.30) were removed from the analysis. Expected heterozygosities (H_e) were obtained using GENALEX v 6.41 (Peakall & Smouse 2006). These values were compared to observed heterozygosities (H_o) to identify locus-location combinations that deviated from Hardy-Weinberg equilibrium (HWE).

Population structure within the study area was assessed from genotype data using STRUCTURE v. 2.3.3 (Pritchard *et al.* 2000) and BAPS v. 5.2 (Corander & Marttinen 2006 and Corander *et al.* 1997). Analysis with STRUCTURE was conducted with 300,000 iterations and a burn-in of 200,000 iterations assuming admixture. Number of potential populations (K) was set at each value between one and ten with ten replicates for each potential value of K. Analysis with BAPS was conducted using clustering or groups of individuals with a default upper bound of 20 possible populations.

The level of genetic differentiation among sampling locations was assessed by computing pairwise F_{ST} values which were calculated using the AMOVA method (9999 permutations) in GENALEX (Peakall & Smouse 2006). Pairwise D_{est} (estimator of actual differentiation) values

(Jost 2008) were also calculated using SMOGD v. 1.2.5 (Crawford 2010). D_{est} values are considered to be a better estimate of genetic differentiation in highly polymorphic loci like those used in this study (Jost 2008). An analysis of molecular variance (AMOVA) was conducted to identify the amount and significance of variance among and within sampling locations and to calculate a global F_{ST} value.

An analysis of genetic isolation by geographic distance between all 18 sampling sites was conducted using a Mantel test (Mantel 1967) with 10 X 10^5 permutations in the program ISOLDE as implemented within GENEPOP v. 4.0.10. Geographic distance between sites was measured using ArcMap 10 (Environmental Systems Research Institute 2010) and was considered to be the shortest possible water distance between sites (river km in tributaries and straight lines across open water, avoiding land masses, within the delta). Genetic distance was calculated using Nei's standard genetic distance (Nei 1972). Mantel tests were also conducted in the same manner using F_{ST} and D_{est} values.

Allelic richness at each locus-location combination was estimated using FSTAT v. 2.9.3.2 (Goudet 1995). Sample sizes were standardized between sites through a process of rarefaction to ensure consistency. A Kruskall-Wallis test was conducted in Minitab V 16.1.0.0 (Minitab 2010) to identify any statistical differences in among site allelic richness for the assayed microsatellite loci.

In order to identify any recent (within $2N_e$-$4N_e$ generations) genetic bottlenecks in the study area, four tests with varying degrees of sensitivity were conducted. Wilcoxon sign rank tests were carried out using three models of evolution: the Infinite Allele Model (I.A.M.), Two Phase Model (T.P.M.), and the Stepwise Mutation Model (S.M.M.). Variance and S.M.M proportions for the T.P.M model were set at 12 and 95% respectively as recommended by Piry *et*

al. (1999). A mode shift test was conducted to identify significant changes in allelic frequency caused by a genetic bottleneck. All bottleneck tests were conducted using BOTTLENECK v. 1.2.02 (Piry *et al.* 1999).

CHAPTER III

RESULTS

Eight of the nine tested loci were successfully amplified for *L. siliquoidea*. Fifteen individuals that failed to amplify at four or more loci were removed from the original 341 collected samples leaving a total of 326 samples for genetic analysis. No significant linkage was detected between locus pairs. An analysis of the microsatellite data found no evidence of genotyping errors due to stuttering or large allele dropout however significant null alleles were detected at 6 of 9 loci with serious null alleles (frequency > 0.20) present at three out of nine loci. After gauging the effects of these null alleles on the results of the tests to be conducted, the locus with the most serious frequency of null alleles, LabC67, was removed to reduce the effect of this genotyping error on the results of the analysis. Significant deviations from HWE were detected at 34 of 72 locus-location combinations when locations were considered separately. When all sampling locations were combined into one population, seven of eight loci were found to be significantly out of HWE (Table 3). Deviations were likely caused by the relatively high estimated frequencies of null alleles in the dataset.

The remaining eight loci were found to be highly polymorphic with between 6 and 43 alleles per locus and a mean of 22 (Table 3). Allelic polymorphism was comparable, and in many cases, higher than those found in other unionid microsatellite studies including the same loci used in this study (Kelly & Rhymer 2005; Zanatta & Murphy 2007; Zanatta *et al.* 2007; and Zanatta & Murphy 2008). Genetic diversity for each sampling location was measured as the average allelic richness across all loci for each sampling location. Overall average allelic richness was found to be 4.0 alleles per locus with a range of 3.8 - 4.3 in a sample of three individuals obtained through process of rarefaction (Table 3). Using a Kruskall-Wallis test,

allelic richness was not determined to be significantly different among locations ($p = 0.919$). The rarefaction number was small due to a low number of successful PCR amplifications at one locus-location combination (LabC2/Pocket Bay). When Pocket Bay was removed from the allelic richness analysis, overall average allelic richness was found to be 10.0 alleles per locus with a range of 8.7 - 11.4 in a sample of 16 individuals. No significant differences were detected between sampling locations ($p = 0.918$)

Table 3. Number of alleles, observed heterozygosity (H_o), and expected heterozygosity (H_E) for *Lampsilis siliquoidea* by locus and sampling location. "All Sampling Locations" column was run separately by combining all sampling locations into a single population. (*) indicates locus-sampling location combinations which deviated significantly from Hardy-Weinberg Equilibrium. Allelic Richness values calculated from a rarefacted sample of three individuals.

	Clinton River	Pollet Bay	Goose Bay	Big Muscamoot Bay	Pocket Bay	Bass Bay	Belle River	Pine River	Black River	All Sampling Locations
# Collected	32	30	77	50	17	29	36	30	25	326
LabC2										
# alleles	5	4	5	5	2	4	4	3	4	8
# genotyped	26	28	68	44	3	26	31	25	23	274
H_o	0.19*	0.71	0.62	0.57*	0.00	0.42*	0.68*	0.32	0.30*	0.500*
H_E	0.54	0.58	0.57	0.52	0.44	0.59	0.49	0.49	0.61	0.562
LabC23										
# alleles	4	4	4	5	3	4	4	4	5	6
# genotyped	30	28	69	45	15	29	34	27	24	301
H_o	0.33	0.29	0.28	0.49*	0.27	0.34	0.48	0.41	0.29	0.355*
H_E	0.29	0.26	0.31	0.49	0.24	0.30	0.40	0.41	0.26	0.349
LabC24										
# alleles	12	10	11	10	8	10	12	10	9	23
# genotyped	32	23	44	16	17	28	36	16	25	237
H_o	0.66*	0.65	0.52*	0.75	0.65	0.54	0.56*	0.69	0.40*	0.595*
H_E	0.84	0.85	0.79	0.85	0.80	0.84	0.86	0.82	0.74	0.852
LabD10										
# alleles	23	19	28	28	17	16	13	17	13	43
# genotyped	30	26	62	43	16	22	18	21	18	256
H_o	0.67*	0.62*	0.61*	0.60*	0.50	0.41*	0.33*	0.61*	0.72*	0.578*
H_E	0.92	0.91	0.94	0.94	0.92	0.91	0.91	0.87	0.87	0.955

Table 3. Continued

	Clinton River	Pollet Bay	Goose Bay	Big Muscamoot Bay	Pocket Bay	Bass Bay	Belle River	Pine River	Black River	All Sampling Locations
LabD29										
# alleles	18	15	19	20	11	16	16	13	12	24
# genotyped	31	29	69	43	15	24	33	23	21	288
H_o	0.45*	0.59*	0.28*	0.47*	0.40*	0.42*	0.39	0.52*	0.67	0.438*
H_E	0.92	0.90	0.92	0.93	0.87	0.91	0.87	0.86	0.89	0.934
LabD111										
# alleles	10	14	17	15	10	11	13	14	13	20
# genotyped	31	28	70	38	17	28	28	28	24	292
H_o	0.87	0.86	0.91	0.47	0.76	0.89	0.82	0.93	0.83	0.873
H_E	0.84	0.86	0.85	0.93	0.82	0.86	0.84	0.89	0.83	0.872
LabD206										
# alleles	12	14	15	17	8	11	9	15	10	20
# genotyped	29	27	65	44	14	22	27	25	23	276
H_o	0.28*	0.48*	0.34*	0.32*	0.36	0.23*	0.40*	0.30*	0.43*	0.344*
H_E	0.77	0.85	0.85	0.86	0.72	0.87	0.88	0.77	0.78	0.868
LabD213										
# alleles	17	14	22	23	15	21	16	16	11	29
# genotyped	31	16	58	33	16	29	33	29	24	269
H_o	0.90	0.69	0.90	0.91	1.00	0.90	0.83	0.97	0.96	0.900*
H_E	0.91	0.91	0.92	0.93	0.90	0.92	0.92	0.90	0.86	0.927
Mean Allelic Richness	4.03	4.08	4.03	4.26	3.84	4.15	4.10	3.85	3.77	

Analysis of population structure using BAPS predicted the most likely number of populations within the study area to be one with a probability of 0.997. Analysis with STRUCTURE corroborated this finding and predicted one interbreeding population. An analysis of molecular variance (AMOVA) using 9999 permutations showed that among sampling location variance was responsible for only three percent of the total variance within these data with a global F_{ST} of 0.0356 ($p = 0.0001$). Pairwise F_{ST} values were determined to be significantly different from zero at 32 out of 36 pairwise location combinations after Bonferroni correction (corrected $\alpha = 0.0014$) (Table 4). F_{ST} values, though largely significant, showed limited differentiation between sampling locations. Pairwise D_{est} values (Table 4) were higher than F_{ST} values in general but still showed little genetic differentiation between sampling locations. Similar trends were observed in both the F_{ST} and D_{est} values with the highest values present in comparisons including Black River and Pocket Bay samples.

Table 4. Pairwise F_{ST} (below diagonal) and D_{est} (above diagonal) values for sampling locations in the St. Clair River Delta and tributaries. (*) indicates F_{ST} values are significantly different from zero after Bonferroni correction for multiple comparisons ($\alpha = 0.0014$).

	CR	**PB**	**GB**	**BMB**	**PK**	**BB**	**PR**	**BEL**	**BR**
CR	---	0.065	0.041	0.053	0.065	0.031	0.085	0.056	0.156
PB	0.034*	---	0.017	0.002	0.016	0.038	0.032	0.103	0.124
GB	0.028*	0.004	---	0.003	0.010	0.025	0.029	0.074	0.110
BMB	0.060*	0.022*	0.007	---	0.003	0.018	0.019	0.077	0.142
PK	0.054*	0.074*	0.069*	0.099*	---	0.032	0.045	0.093	0.155
BB	0.011	0.028*	0.021*	0.052*	0.057*	---	0.039	0.107	0.116
PR	0.030*	0.041*	0.033	0.062*	0.085*	0.024*	---	0.095	0.149
BEL	0.036*	0.024*	0.003*	0.015*	0.071*	0.020*	0.037*	---	0.220
BR	0.046*	0.044*	0.043*	0.080*	0.090*	0.022*	0.050*	0.045*	---

Genetic differentiation as represented by pairwise Nei's standard genetic distance values between all 18 sample sites were shown to be positively correlated with geographic water distance indicating that more geographically distant site combinations produced higher levels of genetic differentiation ($p = 0.0276$). While a significant correlation was detected, the analysis produced a low $R^2 = 0.15$. F_{ST} and D_{est} values also showed significant correlations with geographic distance (Figure 2).

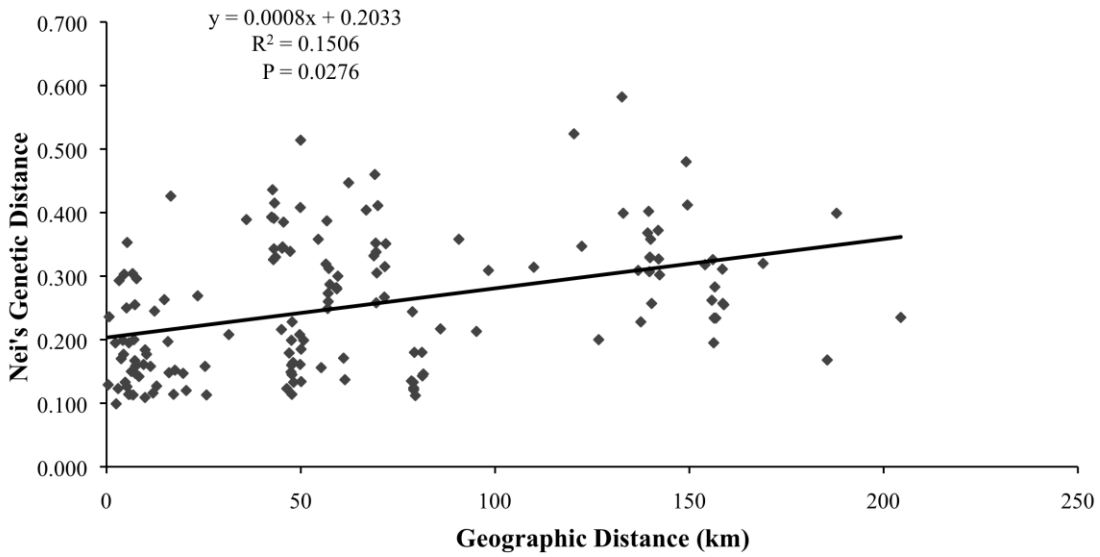

Figure 2. Nei's Genetic Distance versus geographic distance by water (km) for *Lampsilis siliquoidea* sampling sites in the St. Clair River Delta and tributaries. *p*-value calculated using a Mantel test.

Wilcoxon tests showed limited evidence for a recent genetic bottleneck at five of nine locations using the IAM model. TPM, SMM, and mode-shift tests did not indicate the presence of a recent bottleneck in any of the nine locations tested. Further bottleneck analysis when all sampling locations were combined into a single population (as suggested by population structure analyses) produced a similar result, with a significant indication of a recent bottleneck occurring only assuming the IAM of evolution (Table 5).

Table 5. Results of bottleneck analysis of *Lampsislis siliquoidea* from the St. Clair Delta and surrounding tributaries using Wilcoxon tests with three different models of evolution and a mode shift test. Wilcoxon tests were conducted using three models of evolution: Infinite Alleles Model (I.A.M), Two Phase Model (T.P.M), and Stepwise Mutation Model (S.M.M.). (*) Indicates significant evidence of a recent genetic bottleneck ($p < 0.05$).

Sampling Location	I.A.M	T.P.M	S.M.M	Mode Shift
Big Muscamoot Bay	0.006*	0.986	0.990	Normal L-shaped
Goose Bay	0.014*	0.986	0.996	Normal L-shaped
Pollet Bay	0.098	0.875	0.963	Normal L-shaped
Bass Bay	0.010*	0.320	0.527	Normal L-shaped
Pocket Bay	0.191	0.809	0.844	Normal L-shaped
Belle River	0.010*	0.996	0.998	Normal L-shaped
Black River	0.320	0.973	0.986	Normal L-shaped
Clinton River	0.125	0.980	0.980	Normal L-shaped
Pine River	0.006*	0.809	0.902	Normal L-shaped
All Sampling Locations	0.010*	0.994	0.996	Normal L-shaped

CHAPTER IV

DISCUSSION

Analyses of microsatellite DNA showed that while some slight genetic differentiation was present within the study area, *Lampsilis siliquoidea* sampling locations within the St. Clair Delta and its tributaries were all part of a single interbreeding population. Genetic diversity in dreissenid-impacted areas appeared to be high and was not significantly different from the tributary locations that were not impacted by dreissenids.

Despite population structure analyses detecting only a single genetic population, the majority of F_{ST} values were significantly different from zero indicating that some level of genetic differentiation and spatial structure was present in the study area. This small level of differentiation is potentially due to individuals having a higher probability of breeding with individuals in close proximity to one another. Unionid mussels have been shown to display patterns of spatial aggregation in the St. Clair Delta (Zanatta *et al.* 2002) and the surrounding watershed and this may be at least partially responsible for the differentiation detected. Further evidence of this can be seen in the statistically significant genetic differentiation by geographic distance. Significant F_{ST} values may indicate the beginning of population fragmentation, though this is not likely to have been caused by dreissenid-induced population declines due to such a short period of time having elapsed. It could indicate changes due to reduction of unionid populations previous to the most recent declines and recent effects of isolation and fragmentation of a once more continuously distributed population.

The significant deviations from Hardy-Weinberg equilibrium detected during the analysis were all skewed towards a heterozygote deficit. Heterozygote deficiencies in microsatellite data can be caused by a Wahlund effect, inbreeding, and the presence of null alleles (Dakin and Avise

2004). Assignment tests indicated only one interbreeding population was present in the study area supporting the conclusion that null alleles were the primary cause of HWE deviations. Wahlund effects and inbreeding are also less likely culprits because both of these factors usually show heterozygosity deficiencies across all loci (Dakin and Avise 2004) and three loci investigated in this study showed no significant deviation. Null alleles are a frequent problem encountered when dealing with microsatellite data and are common in mollusks (Li *et al.* 2003 and Astanei *et al.* 2005). In addition, the primers for the microsatellite loci utilized in this study were not originally designed for use with *L. siliquoidea* (Eackles & King 2002) and non-species specific primers can increase incidence of null alleles (Pemberton *et al.* 1995). It is possible that the presence of these null alleles may have also resulted in an increase in the degree of genetic differentiation detected among sampling locations, as null alleles have been shown to artificially inflate F_{ST} values; though this effect was more pronounced in populations with low levels of gene flow (Chapuis & Estoup 2007).

Null alleles could potentially impact the results of population structure analyses at the frequencies detected in the dataset (>0.20; Dakin and Avise 2004). In order to address this potential problem, iterative tests of population structure were conducted, beginning with the three loci that were free of null alleles and sequentially adding the additional six loci to the analysis in the order of null allele frequency severity. Results of assignment tests were consistent using both STRUCTURE and BAPS using between four and eight loci. Results differed when the locus with the highest null allele frequency was included in the dataset and this locus was removed. Because this method was used, it is not believed that null alleles have affected the results of the assignment tests.

Despite the decline of unionid populations is the St. Clair Delta by at least two orders of magnitude (from >1.00 m^{-2} to <0.05 m^{-2}) in the preceding 24 years (Nalepa *et al.* 1996, McGoldrick *et al.* 2009), little evidence supporting a genetic bottleneck was detected. There are numerous reasons for this. Bottlenecks can be avoided in populations showing rapid population size reduction by (1) high levels of gene flow, (2) rapid population recovery, (3) long species lifespan (or generation time), and (4) a failure of the population to reach a sufficiently low level to cause a bottleneck (Chapman *et al.* 2011).

Evidence of populations that have suffered demographic declines but do not exhibit strong evidence of recent genetic bottlenecks or declines in allelic diversity are not uncommon. Recent studies observing other long-lived species which have suffered population collapses include: the Wavy-Rayed Lampmussel (Zanatta *et al.* 2007), the European Spiny Lobster (Palero *et al.* 2011), the Black Redhorse (Reid *et al.* 2008), the Arctic Grayling (Swatdipong *et al.* 2010), the Smalltooth Sawfish (Chapman *et al.* 2011), the Wood Turtle (Spradling *et al.* 2010), and the Northern Map Turtle (Bennett *et al.* 2010), among others, have resulted in similar findings. It should be noted that these studies deal with populations which have been impacted by overfishing or habitat destruction and little research has yet been conducted on the genetic impacts on populations suffering from declines due directly to the introduction of an invasive species (Gasc *et al.* 2010) as has been observed in Great Lakes unionids.

Lampsilis siliquoidea in the St. Clair Delta may not show strong evidence of a genetic bottleneck despite a severe demographic bottleneck because the population in question meets three of the four criteria described above for avoiding a genetic bottleneck. (1) Population structure analysis, and genetic differentiation tests point to high levels of gene flow present within the study area. Gene flow appears to be counteracting the effects of population

fragmentation, inbreeding, and genetic drift on unionid populations in the region. Tributary-dwelling *L. siliquoidea* are quickly restoring any alleles lost from the sampling locations in Lake St. Clair due to the dreissenid invasion. (2) There is little evidence of any large recovery of unionids in Lake St. Clair to date as their densities remain at least two orders of magnitude lower than pre-dreissenid levels (McGoldrick *et al.* 2009; Lucy *et al.* in press). (3) The biology of unionids may make them resistant to genetic bottlenecks by virtue of their long lifespan and relatively long generation times. Some unionid mussels are among the longest-lived invertebrates and, while maximum age is difficult to determine, *L. siliquoidea* is known to live for greater than 19 years with some estimates being much longer (Anthony *et al.* 2001). This long lifespan could potentially confound the effects of genetic drift by retaining individuals in the population that were present before the demographic decline. If the bottleneck had reduced *L. siliquoidea* populations far below the threshold level for a genetic bottleneck, a more pronounced bottleneck signature in the dataset would be expected. It is also possible that the recent nature of the demographic decline may be masking the beginning of a genetic bottleneck or an ongoing genetic bottleneck. *Lampsilis siliquoidea* is known to become sexually mature at roughly five years of age (Nichols *et al.* 2001), therefore the maximum number of generations which could have passed since dreissenids were introduced to Lake St. Clair is four with only three generations since unionids were extirpated from the open waters of the lake. Even this number is likely an over-estimate due to the previously stated longevity of the species. Though most individuals selected for this study appeared to be roughly five to six years old based on cursory examination of external shell annuli (Haag and Commens-Carson 2008), the parents of these individuals could have potentially been present pre-dreissenid invasion (thus pre-bottleneck) making the generation interval somewhere between one and three. (4) Finally, it is possible that

the demographic bottleneck of unionids caused by the invasion of dreissenid mussels in the early 1990s was simply not severe enough to trigger a detectable genetic bottleneck or loss of allelic diversity.

Despite these factors, some evidence supporting a genetic bottleneck was detected using the Wilcoxon test under the infinite alleles model (IAM). The IAM is the most sensitive of the tests utilized and is what one would expect to find in population suffering from a weak, or recently begun, genetic bottleneck (Piry *et al.* 1999). Due to the sensitivity of this test, however, it is not possible to discount type I error. Without support from the less sensitive tests, it is difficult to conclusively determine that a genetic bottleneck has occurred.

It appears that in the St. Clair Delta and its surrounding tributaries, Fatmucket populations have retained relatively high levels of genetic diversity and display little evidence to support the existence of a genetic bottleneck. This result may be due to the high level of genetic diversity present in the pre-bottleneck population, high levels of gene flow occurring within the study area counter-acting the loss of the unionid in the open waters of Lake St. Clair, long species lifespan, the population failing to reach a sufficiently low population size to cause serious diversity loss, and the very recent nature of the demographic bottleneck.

The Fatmucket mussel is a widely distributed species, with a diverse host fish assemblage, and is still present in relatively high numbers in the St. Clair Delta (tens to hundreds of thousands of animals, McGoldrick *et al.* 2009; Lucy *et al.* in press). While the results of this study are encouraging, they only provide what can be considered a best-case scenario for unionids in the St. Clair Delta as *L. siliquoidea* is the most common unionid remaining. Unionid species vary considerably in biology, reproductive requirements, and abundance; even within the St. Clair Delta, and it would be unwise to assume that all unionids display similar levels of

genetic diversity and patterns of gene flow. Additional research investigating the genetic diversity and population structure of some less abundant species should be conducted to gain a more complete picture of overall health of the unionid community and regional population connectivity. The St. Clair Delta is the largest known remnant unionid community in the open waters of the Great Lakes and effectively managing this area is vital to preserving unionid mussel populations in the presence of invasive dreissenid mussel species. Continuing to monitor the genetic diversity and structure of unionids in this, and other, refuge habitats will be critical in understanding and managing unionid diversity and connectivity; providing managers with the information needed to preserve these imperiled specie

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