Effects of the dreissenid invasion on the genetic structure of *Lasmigona costata* (Bivalvia: Unionidae) in the Lake St. Clair delta and surrounding tributaries

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Introduction

In the Great Lakes region, there are over 40 native mussel species but invasive dreissenid mussels, *Dreissena polymorpha* and *D. rostriformis bugensis*, have devastated native mussel populations in the open waters of the Great Lakes; notably in Lake Erie and Lake St. Clair. *Lasmigona costata*, the Flutedshell, is one species that continues to persist in the region in several refuge areas along the coasts.

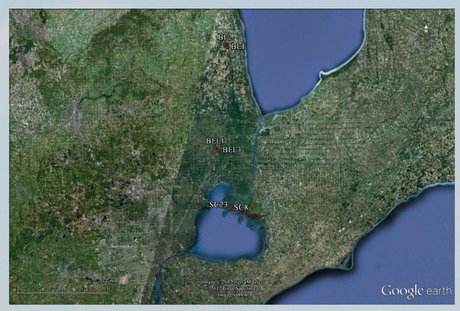

Figure 1. Collection sites for *Lasmigona costata* in Lake St. Clair and its tributaries (BL = Black River, BEL = Belle River, and SC = St. Clair delta).

After the introduction of *D. polymorpha*, the mean density of unionids was drastically reduced from 1.9 m^{-2} in 1986 to <0.1 m^{-2} in 1994 (Nalepa *et al.* 1996), a severe demographic bottleneck. A large and species diverse, but low density refuge for unionids has since been documented in the St. Clair delta (Zanatta *et al.* 2002).

Methods

The U.S. side of the St. Clair delta was surveyed for unionids including *L. costata* in July 2010 using the "circle-plot" method developed by Zanatta *et al.* (2002).

Table 1. Seventy-seven samples were collected in the summer of 2010 from Lake St. Clair delta and tributaries.

Site:	River:	Watershed:	Individuals:
BEL1	Belle River	St. Clair River	25
BEL3	Belle River	St. Clair River	6
BL1	Black River	St. Clair River	18
BL2	Black River	St. Clair River	4
SC8	Lake St. Clair Delta	St. Clair River	21
SC23	Lake St. Clair Delta	St. Clair River	3

All mussels collected had mantle tissue biopsies taken non-lethally and preserved in 95% ethanol solutions. DNA was extracted from tissue samples using an alcohol extraction method. Primers for seven different microsatellite loci (Galbraith *et al.* 2011) were optimized and amplified using PCR. Successfully amplified samples were sent to Trent University, ON, to be genotyped using an ABI 3730 automated sequencer.

Samples which amplified at >4 loci were scored using GENEMARKER v 1.80
Statistical analyses performed include:
Genotyping errors (Stuttering, large allele dropout, Null alleles):
MICROCHECKER v 2.2.3
Allelic richness: FSTAT v 2.9.3.2
Population Structure: STRUCTURE v 2.3.3, BAPS v 5.2
Genetic differentiation: F_{ST}, GENALEX v 6.41
Isolation By Distance (Mantel Test): GENEPOP v 4.0.10
Genetic Bottleneck: BOTTLENECK v 1.2.02

Objectives

•Is there regional genetic structure in the Flutedshell?

•Is gene flow occurring among St. Clair delta and tributaries?

•Could a genetic bottleneck have occurred following dreissenid invasion?

Table 2. Pairwise population F_{ST} value estimates (below diagonal) and *P*-values (above diagonal) for 7 microsatellite loci for *Lasmigona costata* from the St. Clair delta, the Belle and Black rivers, Michigan USA.

	Belle	Black	St. Clair
Belle	---	0.007	0.237
Black	0.011*	---	0.005
St. Clair	0.002	0.013*	---

* indicates significance after Bonferroni correction.

Table 3. Results of BOTTLENECK (Cornuet *et al.*, 1997) for *Lasmigona costata* from the St. Clair delta and surrounding tributaries, Michigan U.S.A. Models used for testing for genetic bottlenecks range from most to least sensitive test including the Infinite Alleles Model (I.A.M.), Two-Phase Model (T.P.M.), Stepwise Mutation Model (S.M.M., and Mode Shift test. *indicating significance (*P*<0.05).

Population	I.A.M.	T.P.M.	S.M.M.	Mode Shift
Belle	0.01953*	0.98047	0.99219	Normal L-Shaped
Black	0.05469	0.96094	0.98828	Normal L-Shaped
St. Clair	0.05469	0.98047	0.99219	Normal L-Shaped
All Sites	0.00781*	0.99609	1.00000	Normal L-Shaped

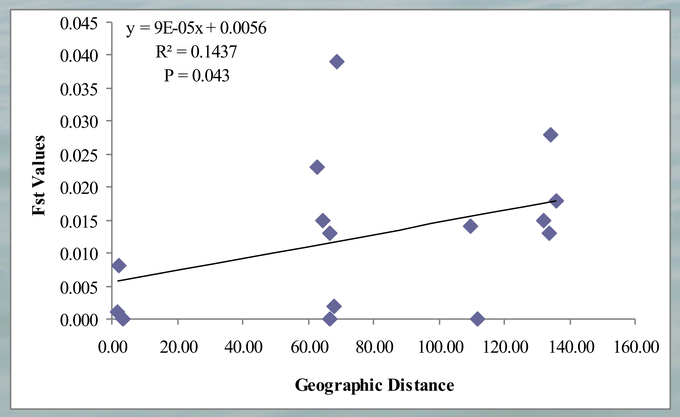

Figure 2. Regression of geographic (river) distance (km) versus genetic distance (F_{ST}) for microsatellite data from *Lasmigona costata* from the St. Clair delta and its tributaries, Michigan USA. *P*-value calculated using a Mantel test.

Results

•MICROCHECKER found that four of the seven loci showed an indication of null alleles using the Brookfield model (frequencies ranging from 0.00% to 22.6%). The mean frequency of null alleles was below 20%, which will not affect results of a population-level study.

•Loci were highly polymorphic, between 6 and 37 alleles per locus (mean = 20). The average allelic richness varied between 10.60 and 12.25 alleles (based on a rarified average number of individuals of 16). A Kruskall-Wallis test indicated that sampling locations had no significant differences in allelic richness (*P*=0.69).

•The results of AMOVA showed weak, but statistically significant genetic structure with 0.84% (*P*=0.020) of total genetic variation coming from among Flutedshell sampling locations, 21.70% among individuals, and 77.46% within individuals. Weak but significant genetic differentiation was found between the St. Clair delta and the Black River and between the Black River and the Belle River, however was no genetic differentiation between the St. Clair delta and Belle River (Table 2).

•BOTTLENECK showed little evidence for genetic bottlenecks, with only the Belle River and all sites giving a significant result using the very sensitive Infinite Alleles Model (Table 3).

•The results of the Mantel test showed a significant correlation between genetic differentiation and geographic (river) distances (R^2=0.1437, *P*=0.043; Figure 2). While the relationship is significant the correlation is not strong at the limited spatial scale investigated (maximum distance between sampling locations was only 135.84 km).

Conclusions

•Gene flow is occurring among the sites sampled and future conservation efforts should manage unionids in Lake St. Clair and its tributaries as a single genetic population.

•Flutedshell may be expanding its range and could be newly colonizing the delta, as it did not have a historically large population in Lake St. Clair.

•There was little support for the existence of a recent genetic bottleneck (Table 3). It may be too soon to detect this, as it has only been 25 years since the dreissenid invasion, with too few generations having passed in the Flutedshell population.

•Future studies could be compared to this baseline dataset and used as a foundation to observe ongoing changes and ultimately recovery of unionids in Lake St. Clair.

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