

LEPF 02-14

**GENETIC DETECTION AND MONITORING OF AQUATIC NUISANCE GOBY
AND MUSSEL POPULATIONS IN LAKE ERIE**

**FINAL REPORT
2003-2005**

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round goby, tubenose goby, zebra mussel**

Objectives: To (1) identify invasion sources, corridors, and spread patterns for the nonindigenous goby and dreissenid mussel populations in Lake Erie using molecular genetic markers, (2) develop rapid discrimination methodology for distinguishing among species and genetic types, including those predicted to invade in the future, (3) analyze genetic changes in Lake Erie populations across the historical time courses of the invasions, and (4) apply environmental risk methodology and the results to model and predict genetic and population characters and invasion corridors for future monitoring and prevention. This proposal addressed the strategic action of the *Lake Erie Protection & Restoration Plan (B-1)* to fully implement Ohio's Aquatic Nuisance Species (ANS) Plan, specifically to develop and maintain monitoring programs in Lake Erie for early detection of new infestations and to identify transport pathways from shipping, spread from other areas in the Great Lakes, and changes to the invasive species' population structures in Lake Erie.

Methodology: This study used high-resolution molecular genetic markers as monitoring tools to discriminate among species, populations, and invasive sources/spread sites for exotic gobies (round *Neogobius melanostomus*, tubenose *Proterorhinus marmoratus*, and other species of *Neogobius* which are predicted to invade) and dreissenid mussels (zebra, quagga, and other species of *Dreissena*, such as *D. rostriformis*, which may invade). The population genetic structure of the exotic invasions of round gobies, tubenose gobies, and zebra and quagga mussels were analyzed from sites in Lake Erie and St. Clair in comparison with specimens from native Eurasian sites, as well as individuals sampled from an invasive population in the Gulf of Gdansk, Poland and samples from the other Great Lakes.

We also analyzed and developed genetic markers for the other species of Eurasian *Neogobius* that are predicted to invade the Great Lakes, enabling their rapid detection if/when they invade. Methodology included DNA sequencing and population genetic

statistical analysis of variation in the mtDNA cytochrome b gene, and some microsatellite DNA analyses of the round goby and the zebra mussel.

Results were analyzed using comparative population genetic statistics and maintained in a web-accessible database, as well as published in the National Institutes of Health (NIH) GenBank (www.ncbi.nlm.nih.gov). The data sets are projected to serve as a benchmark for future monitoring of nonindigenous populations, accessible and augmentable by state and federal agencies.

Rationale: The proposed project specifically meets the Strategic Objective of Priority Recommendations for Lake Erie to limit future introductions and spreading of exotic species in the Lake Erie basin, by analyzing the factors regulating their success. We worked with Ohio Division of Wildlife, the NOAA Great Lakes Aquatic Ecosystem Research Laboratory, and other state and federal agencies to obtain samples, evaluate types of invasive gobies and dreissenids, and developed a long-term monitoring data base. We also analyzed the data using environmental risk analysis methodology for modeling and predicting the role of genetic variability in interpreting invasion vectors, pathways, and relative success using these genetic data and other published studies.

Benefits: A genetic marker data set was developed and disseminated for present and future monitoring of Lake Erie invasions, facilitating the implementation of Ohio's Aquatic Nuisance Species (ANS) Plan. The project results specifically benefit scientists and fishery management agencies in assessing and quantifying the number of introduction events, the spread from one area to another, changes in invasive populations over time, and the identification of new invasions. This investigation will produce results directly usable by the Ohio Division of Wildlife and other Great Lakes Departments of Natural Resources, the Ontario Ministry of Natural Resources, the NOAA Great Lakes Aquatic Ecosystem Research Laboratory, and the U.S. Fish and Wildlife Service for interpreting and quantifying the ongoing problems of aquatic species introductions. This research produced data of the number and description of genetic types, their distributions, and their relative abundances in Lake Erie. The study benefited the public by providing data helpful to managing and preserving Lake Erie resources.

Students supported:

Joshua E. Brown, Ph.D. student, Department of Earth, Environmental, and Ecological Sciences, University of Toledo “Comparative population genetics of round gobies and dreissenid mussels”. (2004-5)

Matthew E. Neilson, Ph.D. student, Department of Earth, Environmental, and Ecological Sciences, University of Toledo “Phylogenetic and biogeographic relationships of neogobiin gobies” (2004-5)

Sergei Shirmam, undergraduate student, Department of Chemistry, Cleveland State University. “Population genetics of zebra and quagga mussels”. (2003-4)

Five Publications to date on the results: *=students supported through the grant

1. Stepien, C.A., C.D. Taylor, I.A. Grigorovich, S.V. Shirman*, R. Wei, A.V. Korniushev, and K.A. Dabrowska. 2003. DNA and systematic analysis of invasive and native dreissenid mussels: Is *Dreissena bugensis* really *D. rostriformis*? *Aquatic Invaders* 14(2):8-18.
2. Stepien, C. A., J. E. Brown*, M. E. Neilson*, and M. A. Tumeo. 2005. Genetic diversity of invasive species in the Great Lakes versus their Eurasian source populations: insights for risk analysis. *Journal of Risk Analysis* 25(4):1043-1060.
3. Stepien, C. A., and M. A. Tumeo. 2006. Invasion genetics of Ponto-Caspian gobies in the Great Lakes: a 'cryptic' species, absence of founder effects and comparative risk analysis. *Biological Invasions* 8:61-78.
4. Stepien, C.A., J.E. Brown*, and M.E. Neilson*. 2006. Genetic diversity and divergence patterns in exotic species introduced from the Ponto-Caspian to the North American Great Lakes. *Biology of Inland Waters* MAIK-Nauka, Russia. In Press.
5. Neilson, M.E.* and C.A. Stepien. 2006. Genetic identity, systematics, and biogeography of invasive neogobiids: Patterns in the Ponto-Caspian, the Great Lakes, and beyond. *Biology of Inland Waters* MAIK-Nauka, Russia. In Press.

Presentations at scientific and management conferences on the results:

*** =student supported by the grant**

1. **International Marine Bioinvasions Conference**, March 2003 held at Scripps Institution of Oceanography, La Jolla, CA. oral presentation by Carol A. Stepien. Genetic investigations of source areas, spread patterns, and changes over the time course of exotic invasions
2. **The International Conference on Aquatic Invasive Species**, June 2003, Windsor, Canada. Oral research presentation by Carol A. Stepien "Genetics and risk analysis of the round and tubenose goby invasions". Online at: http://www.aquatic-invasive-species-conference.org/powerpoint_pdf/Session%20B/Wednesday/carol_stepien
3. **American Fisheries Society Annual Meeting**, August 2003 invited oral research presentation by Carol A. Stepien in the symposium "Use of genetic markers for management and conservation". Quebec, Canada.
4. Departmental Seminar. **Bowling Green University**, October 2003. *Molecular genetics and systematic investigations of fishes, with comments on mentorship of women in science.*

5. **American Fisheries Society**, 2004, invited keynote presentation by Carol A. Stepien, "Genetics of exotic mussels and gobies in the Great Lakes: What does DNA tell us about invasions?" New York. February 2004.
6. C.A. Stepien **University of Toledo**, 2004. Departmental Seminar. "*What does DNA tell us about invasions? Genetics of exotic mussels and gobies in Lake Erie*". **Grand Valley State University**, Michigan. 2004.
7. **American Society of Ichthyologists and Herpetologists** June 2004, Norman, Oklahoma. **Society for Study of Evolution**. Oral research presentations by Carol Stepien "Invasion Genetics of Ponto-Caspian gobies in the Great Lakes"
8. J. E. Brown* and C. A. Stepien. "Zebra mussels: invaders in space and time". April 2005. University of Toledo **Sigma Xi Symposium** 2005, Toledo, OH, USA.
9. J. E. Brown* and C. A. Stepien. "Temporal and spatial genetic comparisons of the zebra mussel across its North American invasion versus Eurasia". **International Association for Great Lakes Research** annual meeting, 24-27 May 2005, Ann Arbor MI, USA
10. M. E. Neilson* and C. A. Stepien, 2005. "New genetic identity of tubenose goby from Great Lakes and Eurasian waterways. **International Association for Great Lakes Research** annual meeting, 24-27 May 2005, Ann Arbor MI
11. M. E. Neilson* and C. A. Stepien, 2005. "Molecular systematics of the neogobiin gobies". **American Society of Ichthyologists and Herpetologists** Annual Meeting, 7-12 July 2005, Tampa FL.
12. J.E. Brown*, M.E. Neilson*, and C. A. Stepien, 2005. "The Eurasian round goby: structure of an invasion". **American Society of Ichthyologists and Herpetologists** Annual Meeting, 7-12 July 2005, Tampa FL.
13. Stepien, C.A., J.E. Brown*, and M.E. Neilson*, 2005. "Genetic diversity and divergence patterns in exotic species introduced from the Ponto-Caspian to the North American Great Lakes" **2nd International Symposium on Invasion of Alien Species in Holarctic Waters** (BOROK-II). Russian Academy of Sciences, I.D. Papanin Institute of Biology of Inland Waters, Borok, Yaroslavl Province, Russia, 27 September - 1 October 2005.
14. M. E. Neilson* and C. A. Stepien, 2005. "Genetic identity, systematics, and biogeography of invasive neogobiins: patterns in the Ponto-Caspian, the Great Lakes, and beyond". **2nd International Symposium on Invasion of Alien Species in Holarctic Waters** (BOROK-II). Russian Academy of Sciences, I.D. Papanin Institute of Biology of Inland Waters, Borok, Yaroslavl Province, Russia, 27 September - 1 October 2005.

15. C.A. Stepien and J. E. Brown*. “Genetics, ecology, and invasional success: examples from the Great Lakes”. October 2005. **Estuarine Research Foundation ERF 2005**, Norfolk, VA, USA

16. C.A. Stepien. “Invasion Genetics of Ponto-Caspian Gobies in the Great Lakes”. Departmental Seminars at **New Mexico State University** 10-05, **Bowling Green State University** 9-05, and the **Field Museum of Natural History**, Chicago. 2-15-06.

17. Posters based on results from this grant were presented at the Risk Analysis of Invasive Species in the Great Lakes Conference, Cleveland State University, October 2004, and at the February 2005 and 2006 annual conferences of the Ohio Fish and Wildlife Department of Natural Resources in Columbus, Ohio.

Summary of Grant Project Findings

Objective 1: To identify invasion sources, corridors, and spread patterns for the nonindigenous goby and dreissenid mussel populations in Lake Erie using molecular genetic markers.

-and-

Objective 2: To develop rapid discrimination methodology for distinguishing among species and genetic types, including those predicted to invade in the future.

A. Dreissenid Mussels – zebra mussel *Dreissena polymorpha* and quagga mussel *Dreissena bugensis*

We analyzed DNA samples using sequences from the mitochondrial cytochrome b gene from a total of 188 zebra mussels and 78 quagga mussels, as well as outgroup species (*Dreissena rostriformis* and *D. stankovici*), in order to develop rapid discrimination markers for species and populations, and to evaluate their relationships (see Fig. 1). Zebra mussels were tested from eighteen locations in Eurasia and fifteen locations in North America, representing much of their native and invasive ranges. Quagga mussels were sampled from nine Eurasian sites and eleven in North America. Overall, both species showed high levels of connectivity between North America and Eurasia, consistent with the hypothesis of several large inoculations from multiple donor sites. Dreissenid source identification using the mitochondrial cytochrome b gene was complicated by the life history of these organisms. They broadcast spawn and have planktonic veliger larvae, facilitating rapid long distance dispersal and population mixing.

Results of our study indicate that the dreissenid invasions were not isolated events, but were rather the product of multiple inoculation events drawing on a large range of donor populations, with subsequent mixing in the invasive range (Figure 1). We identified 19 total haplotypes for *Dreissena polymorpha*. Fourteen were recovered from the North American locations, and twelve were identified from Eurasia. Seven of these were shared between the two continents, indicating that additional sampling in Eurasia is needed in further study. The remaining seven North American haplotypes were likely drawn from regions of Eurasia that we have not yet sampled, illustrating the scope of this invasion. The two continents (when all population samples were grouped) were not significantly different from each other ($F_{ST} = 0.002$, $p = 0.455$), with most of the variation being located within individuals of any given population, once again indicating the scope of both the invasion, and the subsequent mixing that has occurred in the invasive range.

Fifteen mtDNA cytochrome b haplotypes of quagga mussels were identified from 78 individuals sampled, with 15 types in North America and 14 in Eurasia, and only 40% and 43% of these respectively were shared with the other continent. As in the case of the zebra mussel, additional individuals need to be analyzed in order to gain a more complete understanding of this wealth of genetic variation among native and invasive dreissenid populations. Haplotypic/gene diversity levels were very high in the quagga mussel - even greater than that found in the zebra mussel - according to both the mtDNA and the nuclear DNA data (Table 1). As in the zebra mussel, the distribution of genetic variation was not significant between the continents in an AMOVA analysis since so much variation was introduced during the invasion, but was significant among sites within continents (Table 1).

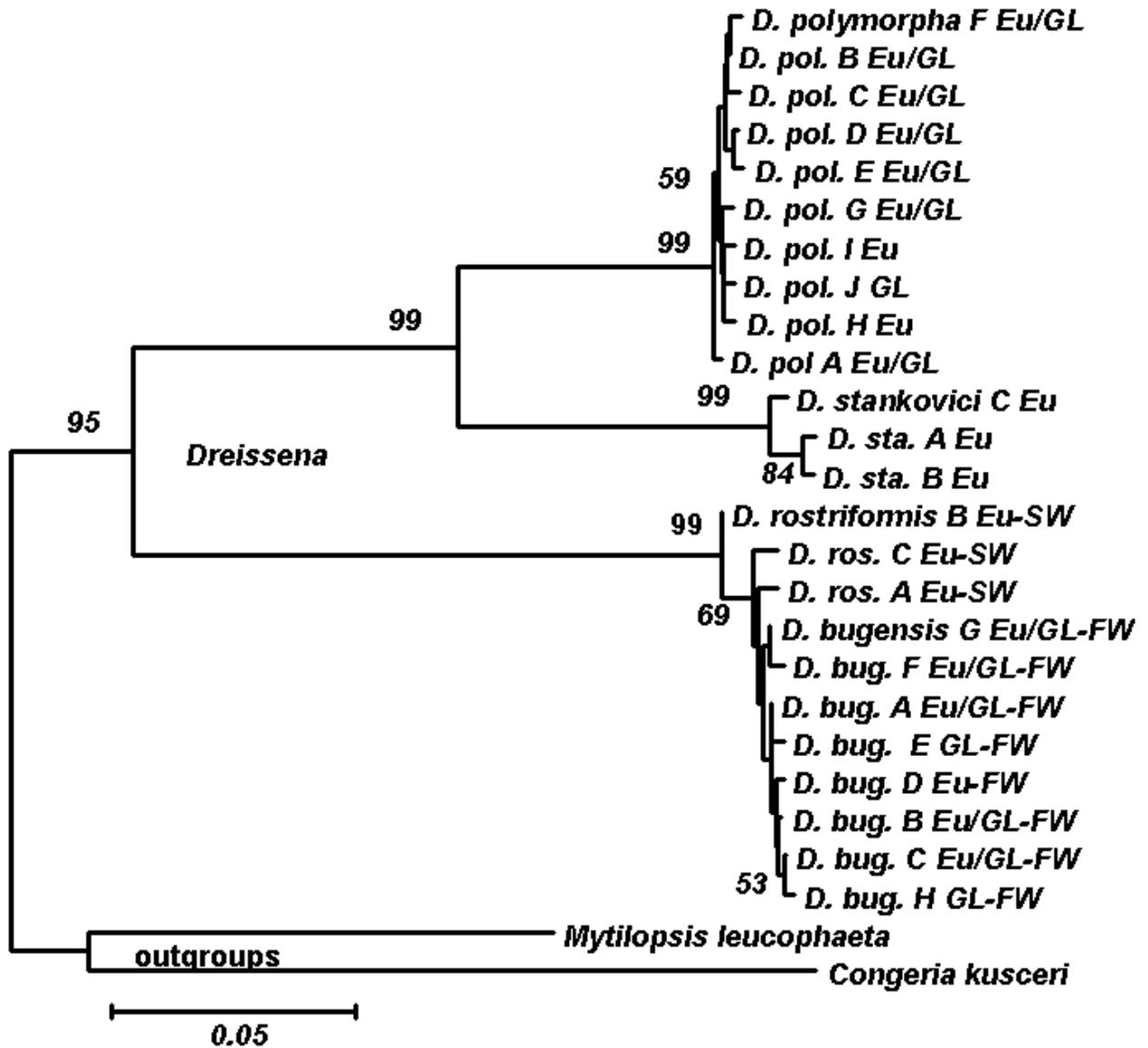


Fig. 1. Neighbor-joining tree depicting the evolutionary relationships among *Dreissena* mtDNA cytochrome *b* gene sequence haplotypes; including the primary types of zebra mussel *D. polymorpha* and quagga mussel *D. bugensis* (numbering 3 or more individuals; thus rare haplotypes are excluded here). We compare these species with variation among all four species in the genus *Dreissena* - including the saline variant *D. rostriformis* (which appears little diverged from *D. bugensis*) and *D. stankovici* (endemic to lakes in Macedonia and Albania) - as well as its sister genera *Congeria* and *Mytilopsis*. The tree was constructed using Kimura (1980) 2-parameter genetic distances in MEGA 2.1, with pairwise deletion and 1000 bootstrap replications (bootstrap support values greater or equal to 50% are indicated at nodes). Adapted from: Stepien, C. A., J. E. Brown, M. E. Neilson, and M. A. Tumeo. 2005. Genetic diversity of invasive species in the Great Lakes versus their Eurasian source populations: insights for risk analysis. *Journal of Risk Analysis* 25(4):1043-1060.

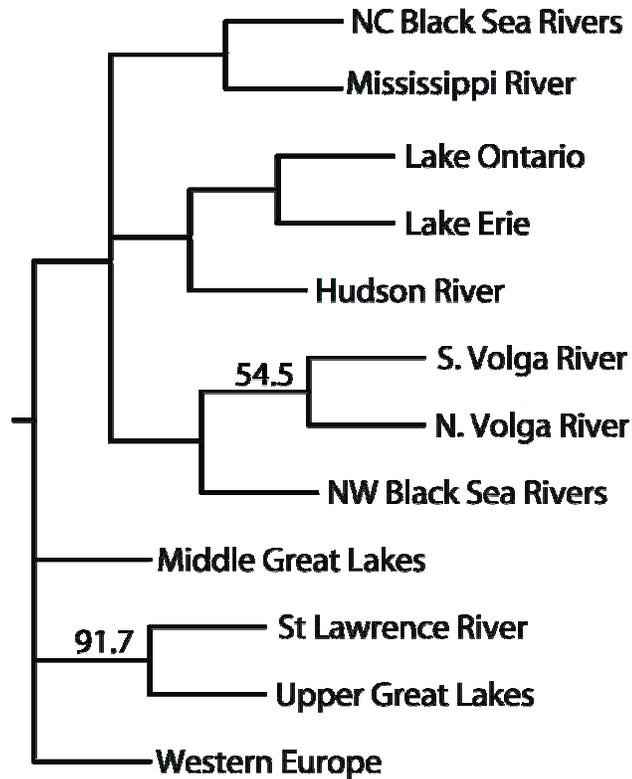


Fig. 2. Dollo-Parsimony tree illustrating the relationships among zebra mussel populations. (Unpublished, part of dissertation work by Ph.D. student Joshua E. Brown).

Table 1. Allelic variation and genetic variability comparisons within and among sites from North America and Eurasia. N.S. = not significant, p = statistical probability. Adapted from: Stepien, C.A., J.E. Brown, and M.E. Neilson. 2006. Genetic diversity and divergence patterns in exotic species introduced from the Ponto-Caspian to the North American Great Lakes. Biology of Inland Waters MAIK-Nauka, Russia. In Press. (note: additional data were collected for the present study that are not included in this paper, which was accepted fall 2005).

	Zebra mussel North America	Zebra mussel Eurasia	Quagga mussel North America	Quagga mussel Eurasia	Round goby North America	Round goby Eurasia
N individuals per continent	111	77	49	29	101	148
N sampling geographic regions per continent	7	5	3	2	4	7
N sampling locations per continent	15	18	10	8	7	9
N DNA alleles/haplotypes per continent and % of total	14 74%	12 63%	15 65%	14 61%	9 45%	13 65%
Mean N alleles/haplotypes	4.4	5.4	6.7	8	4.5	3.4

per region (and range) per continent	(3-8)	(3-7)	(5-10)	(6-10)	(3-7)	(3-6)
N haplotypes and % of total shared with the other continent	7 50%	7 58%	6 40%	6 43%	2 22%	2 15%
Haplotype/gene diversity (h) +/- sampling variance	0.672 +/- .041	0.691 +/- .050	0.887 +/- .028	0.884 +/- .021	0.748 +/- .023	0.566 +/- .041
Mean haplotype/gene diversity (h) per region within continent (and range)	0.579 (.080-.800)	0.693 (.451-.873)	0.861 (.800-.905)	0.884 (.867-.900)	0.558 (.257-.692)	0.458 (.083-.758)
Nucleotide diversity (π) on continent	0.0042 +/- .0027	0.0040 +/- .0027	0.0048 +/- .0031	0.0054 +/- .0034	0.0027 +/- 0.0020	0.0028 +/- 0.0020
Mean nucleotide diversity (π) within regions on continent	.0041	.0039	.0048	.0048	.0018	.0016
Mean Divergence between continents (F_{ST})	.002 N.S.	.002 N.S.	.039 p<.017	.039 p<.017	0.114 p<.0001	0.114 p<.0001
Mean Divergence among regions within continent (F_{ST})	.069	.066	.164	.182	0.283	0.428
Mean Migration between continents (N_m)	3.46	3.46	8.67	8.67	3.90	3.90
% Variation between continents (AMOVA)	0.11% N.S.	0.11% N.S.	0.57% N.S.	0.57% N.S.	5.17% p<.0001	15.25% N.S.
% Variation among regions within continent (AMOVA)	5.65% p<.023		10.52% p<.0001		47.2% p<.0001	
% Variation within sampling sites (AMOVA)	93.28% p<.0001		88.91% p<.0001		47.63% p<.0001	

B. Gobies – round goby *Apollonia (Neogobius) melanostomus* and tubenose goby *Proterorhinus (marmoratus) semilunaris*

Using mitochondrial cytochrome b, we tested 188 individuals sampled from populations from Lake Michigan, Lake Huron, Lake St. Clair and Lake Erie in North America, and from the Gulf of Gdansk in Poland, the Danube and Dnieper Rivers, and the Black and the Caspian Seas. We made comparisons with Eurasian relatives and developed molecular genetic markers for distinguishing among them, to facilitate rapid identification (see Fig. 3). There were considerably greater divergences among round goby populations when compared with the dreissenids (Tables 1 and 2). The cytochrome b sequence data for the round goby revealed significant overall F_{ST} divergences between pooled data for the continents of North America versus Europe (Table 2). F_{ST} values were higher and migration values lower than those characterizing dreissenid mussel populations (Table 1). Significant differences were discerned among locations within both continents, revealing appreciable geographic structure across their native and invasive ranges.

The round goby prefers brackish waters in its native habitats (Charlebois et al. 1997), although it is confined to freshwater in its present North American invasive range. The round goby thus appears likely to extend its range into North American salt marsh and estuarine habitats, where it may significantly impact benthic fish communities. It will encounter an abundant food supply in native blue mussels along North American coasts, to which they are pre-adapted (since they feed on *Mytilus* congeners in their Black and Caspian Sea marine/estuarine habitats).

The Caspian Sea did not contribute to the North American invasion. Most of the round gobies in the Great Lakes appear to have originated from the region near the mouth of the Dneiper River, and the north central Black Sea. The two different genetic markers employed showed slightly different patterns. Using mtDNA cytochrome b, the Lake Erie populations are most closely related to those in Lake St. Clair and Lake Huron but not to those in Lake Michigan (Table 2), whereas all Great Lakes populations are significantly different using nuclear microsatellites (Table 3). The Dneiper River also shows a population genetic connection to the Black Sea, which is not surprising since the Dneiper empties into the Black Sea (Table 2). Using four microsatellite loci, we found a similar connection between the North American Great Lakes and the lower Dnieper River system (Table 3).

Sample sizes for tubenose gobies are limited at present, since prior analyses showed that they comprise separate marine and freshwater species (Stepien and Tumeo 2006). The freshwater tubenose goby data show no significant F_{ST} divergence and high migration values between samples from the Great Lakes and rivers draining into the Black Sea. However, there is considerable genetic divergence among individual native river locations in Eurasia. The samples from the Great Lakes contained as many haplotypes or more than were found in the individual Eurasian river sites, indicating that a large number of propagules were introduced into the Great Lakes. Overall, haplotype and nucleotide diversity values were almost as large in the Great Lakes as for pooled Eurasian data. Those values for individual sites in the Great Lakes were equivalent and greater, respectively, than the mean values for Eurasian river sites. Results indicate that overall genetic diversity of tubenose goby in the Great Lakes is as high as or higher than in a typical native Eurasian location, and the variety of genotypes suggests multiple founding sources. As in dreissenids and round gobies, a large number of propagules founded the Great Lakes invasion by the tubenose goby and there is no appreciable founder effect.

The neighbor-joining tree (Fig. 4) shows marked separation between the freshwater tubenose goby *P. semilunaris* and the marine/estuarine *P. marmoratus*, along with relatively high divergences among haplotypes. Since congeners are often also successful in invasions, it appears likely that *P. marmoratus* may be successfully introduced to North American coastal estuaries.

Table 2. Round Goby Population Divergences among sites (F_{ST} divergences) using cytochrome b. Results with ** were significantly different after Bonferroni correction. Those with * were different before correction only. NS= not significant (indicates high gene flow). Results show the Dnieper River as a likely founding source for the Great Lakes.

	Lake Michigan	Lake Huron	Lake St. Clair	Lake Erie	Baltic Sea	Danube River	Dneiper River	Black Sea
Lake Huron	0.354 p < 0.0001 **							
Lake St. Clair	0.519 p < 0.0001 **	0.050 p = 0.136 NS						
Lake Erie	0.320 p < 0.0001 **	0.000 p = 0.989 NS	0.045 p = 0.121 NS					
Baltic Sea	0.284 p < 0.0001 **	0.070 p = 0.081 NS	0.144 p = 0.035 *	0.080 p = 0.034 *				
Danube River	0.829 p < 0.0001 **	0.362 p < 0.0001 **	0.158 p < 0.0001 **	0.275 p < 0.0001 **	0.460 p < 0.0001 **			
Dneiper River	0.387 p < 0.0001 **	0.062 p = 0.107 NS	0.095 p = 0.071 NS	0.071 p = 0.063 NS	0.000 p = 0.710 NS	0.404 p = 0.002 *		
Black Sea	0.467 p < 0.0001 **	0.127 p = 0.002 *	0.026 p = 0.154 NS	0.141 p < 0.0001 **	0.141 p = 0.008 *	0.402 p = 0.018 *	0.049 p = 0.074 NS	
Caspian Sea	0.875 p < 0.0001 **	0.792 p < 0.0001 **	0.803 p < 0.0001 **	0.756 p < 0.0001 **	0.756 p < 0.0001 **	0.932 p < 0.0001 **	0.846 p < 0.0001 **	0.777 p < 0.0001 **

Table 3. Round Goby Population Divergences among sites (F_{ST} divergences) using four nuclear microsatellite loci. Results with ** were significantly different after Bonferroni correction. Those with * were different before correction only. NS= not significant (indicates high gene flow). Results show the Dnieper River as a likely founding source for the Great Lakes.

	Lake St. Clair	Lake Huron	Lake Erie	Baltic Sea	W Black Sea	C Dnieper R.	S Bug R.	S Dnieper R.	C Black Sea
Lake Huron	0.031 P=.020 *								
Lake Erie	0.168 P<.0001 **	0.207 P<.0001 **							
Baltic Sea, Gulf of Gdansk	0.202 P<.0001 **	0.218 P<.0001 **	0.202 P<.0001						
W Black Sea Varna	0.142 P<.0001 **	0.164 P<.0001 **	0.128 P<.0001 **	0.067 P<.001 *					
C Dnieper R., Kiev	0.227 P<.0001 **	0.359 P<.0001 **	0.394 P<.0001 **	0.358 P<.0001 **	0.276 P<.0001 **				
S Bug R.	0.157 P<.0001 **	0.187 P<.0001 **	0.124 P<.0001 **	0.107 P<.0001 **	0.036 P<.0001 **	0.356 P<.0001 **			
S Dnieper R.	0.036 P=.020 *	0.124 P<.0001 **	0.216 P<.0001 **	0.236 P<.0001 **	0.173 P<.0001 **	0.160 P<.0001 **	0.177 P<.0001 **		
C Black Sea	0.169 P<.0001 **	0.190 P<.0001 **	0.152 P<.0001 **	0.088 P<.001 *	0.033 P<.001 *	0.305 P<.0001 **	0.064 P<.0001 **	0.207 P<.0001 **	
Caspian Sea, Nabran	0.191 P<.0001 **	0.220 P<.0001 **	0.208 P<.0001 **	0.086 P<.0001 **	0.065 P<.0001 **	0.361 P<.0001 **	0.111 P<.0001 **	0.247 P<.0001 **	0.052 P<.0001 **

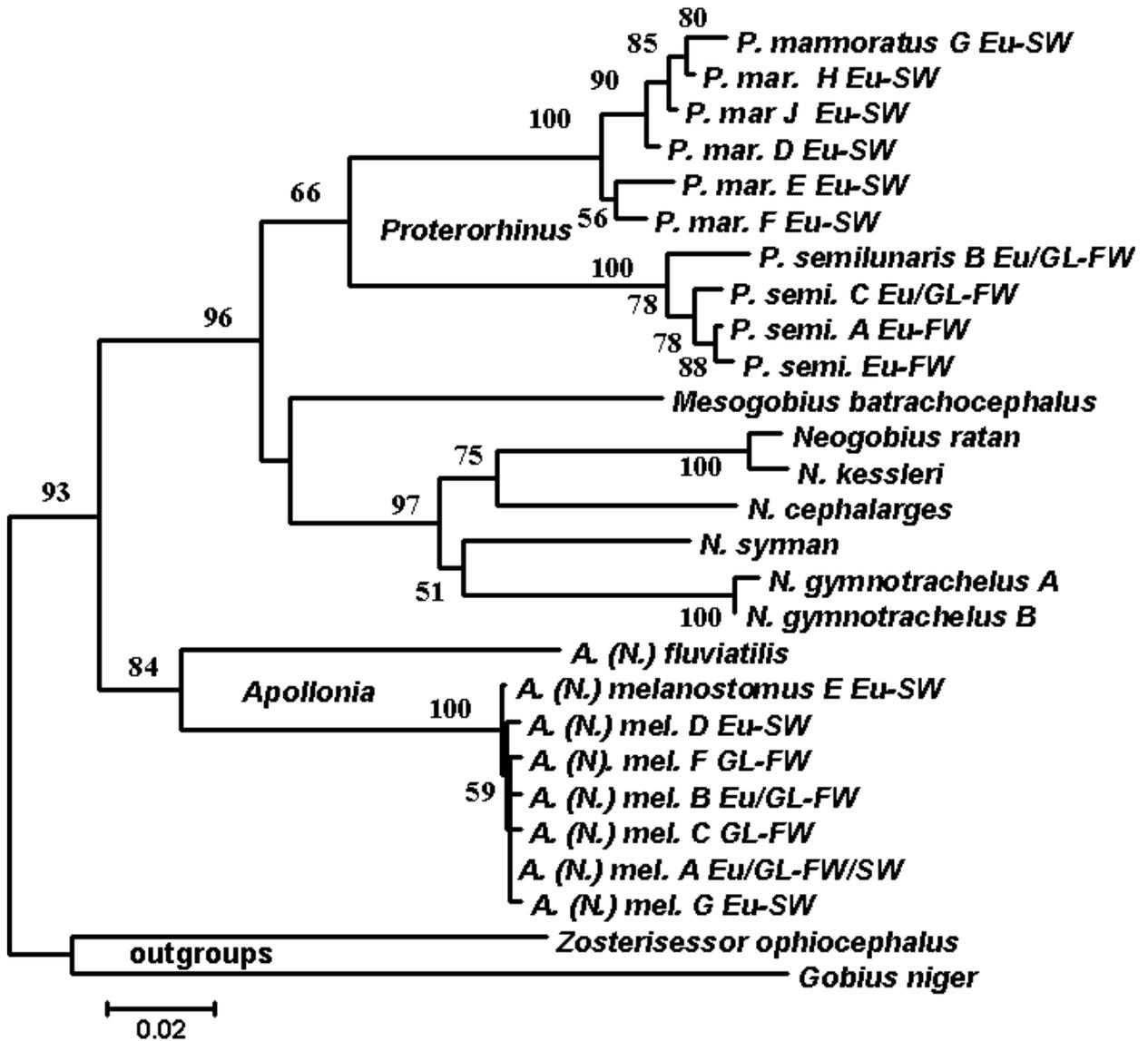


Fig. 3. Neighbor-joining tree depicting the evolutionary relationships among neogobiin mtDNA cytochrome *b* gene sequence haplotypes, including round goby *Apollonia* (*Neogobius*) *melanostomus*, freshwater tubenose goby *Proterorhinus semilunaris* (formerly part of *P. marmoratus*), and the marine tubenose goby *P. marmoratus* (including samples from the type locality at Sevastopol, Crimea, Ukraine). We compare these species with variation among other neogobiin species and the remaining neogobiin genera, including monkey goby *Apollonia* (*Neogobius*) *fluviatilis*, syрман goby *Neogobius syman*, racer goby *N. gymnotrachelus*, ratan goby *N. ratan*, ginger goby *N. cephalarges*, bighead goby *N. kessleri*, and knout goby *Mesogobius batrachocephalus*. Outgroups include two members of the subfamily Gobiinae, grass goby *Zosterisessor ophiocephalus* and black goby *Gobius niger*. The tree was constructed using Kimura (1980) 2-parameter genetic distances in MEGA, with pairwise deletion and 1000 bootstrap replications (bootstrap support values greater or equal to 50% are indicated at nodes). (Adapted from Stepien et al. 2005).

C. DNA Markers for Rapid Identification.

Using the mitochondrial cytochrome b gene we have developed we can readily distinguish the invaders from their congeners, and we have been adding additional species from their native range to a reference library. For gobies, we have a library of 27 congeners and confamilials available for comparison, which allow us to identify unknown fish rapidly and accurately. For the mussels we have a library of two other dreissenid species and several outgroups, which provides a similar degree of speed and accuracy.

These reference sequences are available through N.I.H. GenBank (Table 4).

Table 4: GenBank Accession Numbers.

Species	Accession Number	Species	Accession Number
<i>Dreissena polymorpha</i>	DQ0721117-26	<i>Neogobius ratan</i>	AY884585
<i>Dreissena bugensis</i>	DQ072130-7	<i>Neogobius cephalarges</i>	AY884588
<i>Apollonia melanostomus</i>	AY884582-3, U53673-7	<i>Neogobius syrman</i>	AY884586
<i>Proterorhinus marmoratus</i>	AY88572-5	<i>Mesogobius batrachocephalus</i>	AY884590
<i>Apollonia fluviatilis</i>	AY884584	<i>Gobius niger</i>	AY884591
<i>Neogobius gymnotrachelus</i>	AY884589	<i>Zosterisessor ophiocephalus</i>	AY884592
<i>Neogobius kessleri</i>	AY884587		

Objective 3: Analyze genetic changes in Lake Erie populations across the historical time courses of the invasions.

Time series comparisons for both zebra mussels and round gobies are underway by Ph.D. student Joshua Brown, and preliminary patterns are presented here. For both species tested, locations were chosen that had collections taken early in the invasion, and then samples from approximately the same location taken later. For zebra mussels, we used samples from before 1996 for our early time period and those after 2000 for our late time interval. For round gobies, early was set at before 2000, while those after 2000 were considered late. These time intervals were determined in part by the dates available, and also by the timing and spread of the species in question.

Preliminary data for zebra mussels from Stone Lab show moderate but not statistically significant allelic shifts over time, whereas populations in the Hudson River show evidence of temporal change in allelic composition in the mussels in that system

(Table 5). Joshua Brown is currently completing this work and will update these preliminary findings.

Table 5. Zebra Mussel Temporal Comparisons

	Haplotype	Early %	Late %	Change
Gibraltar Island w. Lake Erie	A	28	23	No (p = 0.202)
	B	36	69	
	C	36	0	
	E	0	8	
Hudson River	A	71	46	Yes (p = 0.029)
	B	12	0	
	C	0	38	
	D	6	0	
	E	6	0	
	N	6	0	
	O	0	8	
	P	0	8	

Round gobies from Stone Lab populations sampled in the 1990s and those collected in 2005 show no temporal differences, despite the changes from a zebra mussel dominated community to a quagga mussel community. However, samples from Saginaw Bay showed a significant shift in genetic composition, with the inclusion of two previously undetected haplotypes (Table 6). The Saginaw Bay site also showed a doubling in haplotype diversity over the same time period, indicating possible additions from overseas locations or spread from other locations.

Table 6. Round Goby Temporal Shifts

	Haplotype	Early %	Late %	Change
Gibraltar Island w. Lake Erie	A	50	46	No (p = 0.506)
	B	41	31	
	C	4	15	
	F	4	0	
	H	0	8	
Saginaw Bay	A	70	20	Yes (p = 0.009)
	B	0	30	
	H	30	20	
	N	0	30	

The lack of a clear change through time for both zebra mussels and round gobies around Gibraltar Island, western Lake Erie suggests that those populations derive many of their recruits from local reproduction, rather than from external migration. However, further testing with larger sample sizes is warranted, and will be accomplished by Ph.D. student Joshua Brown.

Objective 4: Apply environmental risk methodology and the results to model and predict genetic and population characters and invasion corridors for future monitoring and prevention.

A significant advantage of the genetic data collected in this study is their application in determining the risk of multiple invasion events and invasions from related taxa. Table 7 lists the elements and provides an example of how the genetic data collected in this study could be used to produce a qualitative estimate of risk for each of the seven elements shown.

An important step in the risk analysis process involves examining what kind of risk management could be implemented to eliminate or reduce the impact of the hazard (in this case, the establishment of an invasive species). The best solution is to prevent the introduction altogether. International cooperative efforts for stemming ballast water introductions have moved strongly in this direction. Genetic data can be used in conjunction with mathematical models to predict likely source areas as well as areas with a high probability of being invaded. The type of genetic data presented in the present study can greatly assist in targeting efforts in this regard because it is likely that related species have similar risk for entry and establishment. For example, it appears highly likely that the round goby will spread to salt marsh and estuarine habitats in North America, where they will encounter abundant native prey in *Mytilus* mussels. Their genetic diversity and divergence patterns in the Great Lakes suggest that genotypes already here will likely be successful in more saline habitats. It is also likely that *Dreissena rostriformis* and the marine tubenose goby *Proterorhinus marmoratus* will be introduced and become established in North American salt marsh and estuarine habitats.

In conclusion, qualitative risk assessment using genetic characters of invasive populations indicates that high genetic variability, large number of founders, and introduction of several invasion founding source populations leads to high probability of establishment and persistence. The combination of DNA variation data and risk assessment procedures offers an important diagnostic and monitoring tool for evaluating the relative success of exotic species invasions.

Risk assessment was treated in two papers from the study:

Stepien, C. A., J. E. Brown, M. E. Neilson, and M. A. Tumeo. 2005. Genetic diversity of invasive species in the Great Lakes versus their Eurasian source populations: insights for risk analysis. *Journal of Risk Analysis* 25(4):1043-1060.

Stepien, C. A., and M. A. Tumeo. 2006. Invasion genetics of Ponto-Caspian gobies in the Great Lakes: a 'cryptic' species, absence of founder effects and comparative risk analysis. *Biological Invasions* 8:61-78.

Table 7. Risk Assessment and Relation to Genetic Studies of Exotic Species. Adapted From: Stepien, C. A., J. E. Brown, M. E. Neilson, and M. A. Tumeo. 2005. *Genetic diversity of invasive species in the Great Lakes versus their Eurasian source populations: insights for risk analysis. Journal of Risk Analysis* 25(4):1043-1060.

SECTION 1: PROBABILITY OF ORGANISM ESTABLISHMENT

Element (per ANSTF Guidance)	Important Considerations in Assessing Element	Application of Data Collected in this study
Estimate probability of the organism being on, with, or in the pathway (ballast water in this case)	Organism's temporal and spatial association with the pathway.	Genetic data show that highly successful Ponto-Caspian invasions in the Great Lakes are characterized by (1) a large number of introduced genotypes, with genetic diversities of invasive populations comparable to that of native population sites, (2) equivalent effective population sizes of native and introduced populations, indicating that the introductions did not undergo "genetic bottlenecks", and (3) considerable genetic differentiation and large number of haplotypes. Results indicate that multiple founding source populations were involved for zebra and quagga mussels and the round goby. Hence, related populations and species in the areas of origin likely have a high probability to be in ballast water and to survive transport. In addition, the invasive population areas for zebra mussels, quagga mussels and round gobies, including those in different Great Lakes, show genetic divergence and match different source populations, suggesting that different founding sources contributed differentially to the new populations. It thus is likely that several nearly simultaneous invasions occurred from several different sources, in these cases.
Estimate probability of the organism surviving in transit.	Organism's hitchhiking ability in commerce, ability to survive during transit, stage of life cycle during transit, number of individuals expected to be associated with the pathway; or whether it is deliberately introduced (e.g. biocontrol agent or fish stocking).	
Estimate probability of the organism colonizing and maintaining a population.	Organism's ability to come into contact with an adequate food resource, probability of encountering appreciable abiotic and biotic environmental resistance, and the ability to reproduce in the new environment. Introduction of co-evolved predator and prey species, such as the round goby and zebra mussel may result in ecological facilitation and enhanced invasive success.	
Estimate probability of the organism spreading beyond the colonized area.	Ability for natural dispersal, ability to use human activity for dispersal, ability to readily develop races or strains, and the estimated range of probable spread.	

SECTION II: CONSEQUENCE OF ESTABLISHMENT

Element (per ANSTF Guidance)	Important Considerations in Assessing Element	Application of Data Collected in this study
Estimate economic impact if established.	Economic importance of hosts, damage to crop or natural resources, effects to subsidiary industries, exports, and control costs.	The risk consequence indicated by genetic data – i.e., a large number of genotypes, high genetic diversity, and significant divergence among colonizing population sites appears to be extremely high in terms of our unfortunate inability to "control" an invasion once it has become established, as is the case with dreissenid mussels as well as the round goby.
Estimate environmental impact if established.	Ecosystem destabilization, reduction in biodiversity, reduction or elimination of keystone species, reduction or elimination of endangered/threatened species, and effects of control measures, impacts on the human environment (e.g. human parasites or pathogens would also be captured under this element.)	
Estimate impact from social and/or political influences.	None provided	Genetic data not applicable.

Future Directions

We are continuing to expand our sampling, both in terms of numbers of individuals and numbers of sites. Time series are being looked at for additional sites in both North America and Eurasia, and those that we have started are being brought up to larger sample sizes, which should allow for finer resolution. In addition, we are employing nuclear microsatellite DNA in tandem with the mitochondrial data already under consideration in order to improve spatial and temporal resolution.

Publication Abstracts * = students supported by this grant

Stepien, C. A., J. E. Brown*, M. E. Neilson*, and M. A. Tumeo. 2005. Genetic diversity of invasive species in the Great Lakes versus their Eurasian source populations: insights for risk analysis. *Journal of Risk Analysis* 25(4):1043-1060.

Combining DNA variation data and risk assessment procedures offers important diagnostic and monitoring tools for evaluating the relative success of exotic species invasions. Risk assessment may allow us to understand how the numbers of founding individuals, genetic variants, population sources, and introduction events affect successful establishment and spread. This is particularly important in habitats that are “hotbeds” for invasive species—such as the North American Great Lakes. This study compares genetic variability and its application to risk assessment within and among three Eurasian groups and five species that successfully invaded the Great Lakes during the mid 1980s through early 1990s; including zebra and quagga mussels, round and tubenose gobies, and the ruffe. DNA sequences are compared from exotic and native populations in order to evaluate the role of genetic diversity in invasions. Close relatives are also examined, since they often invade in concert and several are saline tolerant and are likely to spread to North American estuaries. Results show that very high genetic diversity characterizes the invasions of all five species, indicating that they were founded by very large numbers of propagules and underwent no founder effects. Genetic evidence points to multiple invasion sources for both dreissenid and goby species, which appears related to especially rapid spread and widespread colonization success in a variety of habitats. In contrast, results show that the ruffe population in the Great Lakes originated from a single founding population source from the Elbe River drainage. Both the Great Lakes and the Elbe River populations of ruffe have similar genetic diversity levels—showing no founder effect, as in the other invasive species. In conclusion, high genetic variability, large numbers of founders, and multiple founding sources likely significantly contribute to the risk of an exotic species introduction’s success and persistence.

Stepien, C. A., and M. A. Tumeo. 2006. Invasion genetics of Ponto-Caspian gobies in the Great Lakes: a ‘cryptic’ species, absence of founder effects and comparative risk analysis. *Biological Invasions* 8:61-78.

Genetic variability and structure of nonindigenous versus native populations are compared for the Eurasian round goby *Neogobius melanostomus* and the tubenose goby *Proterorhinus marmoratus*, which both invaded Lake St. Clair of the North American Great Lakes about 1990. The round goby spread rapidly to all of the Great Lakes and the

tubenose goby largely has been restricted to Lake St. Clair, with some recent range extension into western Lake Erie. Risk analyses may indicate whether genetic variability of colonizers is predictive of their relative invasive and establishment successes. The present investigation examined DNA sequence variation across the left domain of the mitochondrial DNA cytochrome b gene in round and tubenose gobies from Eurasian and Great Lakes locations. We also sequenced six additional *Neogobius* species (including the monkey *N. fluviatilis*, racer *N. gymnotrachelus*, and bighead *N. kessleri* gobies that have been ‘on the move’ in Europe) and the knout goby *Mesogobius batrachocephalus* from the Black Sea in order to develop diagnostic genetic characters to identify them in case of future and/or undetected invasions and to delineate their phylogenetic relationships. Results show that a diverse number of haplotypes characterize round and tubenose goby populations from both North America and Eurasian sites, fitting a risk analysis prediction of high genetic variability in their successful introductions. Phylogenetic results indicate that the current genus *Neogobius* is paraphyletic and that the subgenus *Apollonia* thus should be elevated to the level of genus, containing *Apollonia* (*N.*) *melanostomus* (the round goby) and *A. (N.) fluviatilis* (the monkey goby). In addition, there appear to be two separate species of *Proterorhinus marmoratus*, a marine *P. marmoratus* Pallas 1814 in the Black Sea (matching the original type locality), and a ‘cryptic’ freshwater species in the Danube and Dnieper Rivers and probably other Eurasian freshwater habitats, as well as invasive in the Great Lakes. We suggest resurrecting the name *P. semilunaris* Heckel 1837 for the freshwater species (a taxon that was originally described from rivers draining into the Aegean Sea and the Danube River, but was later placed in synonymy with *P. marmoratus*).

Stepien, C.A., C.D. Taylor*, I.A. Grigorovich, S.V. Shirman,* R. Wei, A.V. Korniuschin, and K.A. Dabrowska*. 2003. DNA and systematic analysis of invasive and native dreissenid mussels: Is *Dreissena bugensis* really *D. rostriformis*? Aquatic Invaders 14(2):8-18.

Prior studies by our Great Lakes Environmental Genetics Laboratory have shown the utility of DNA characters in resolving the systematic relationships and identifications of the bivalve family Dreissenidae. The present study analyzes the phylogenetic relationships among four extant species of *Dreissena* — including the zebra mussel *D. polymorpha*, the quagga mussel *D. bugensis*, the Lake Okhrid native *D. stankovici*, and the Caspian Sea native *D. rostriformis*. We compare results from sequences of the mitochondrial 16S ribosomal DNA region and the cytochrome b gene. This is the first published DNA sequence analysis of the species *D. stankovici* and *D. rostriformis*. We also examine intraspecific divergence levels among invasive and native ranges, and discuss the phylogeographic history of the genus and component taxa. Results show that the genus diverged into two clades in the region of the Paratethys Sea — the ancestral *D. polymorpha/stankovici* and the *D. rostriformis/bugensis* lineages about 9 to 11 million years ago. The former clade has been restricted to freshwater habitats, while the latter inhabited fresh, brackish, and saltwater areas. *Dreissena stankovici* diverged from *D. polymorpha* about 3 to 4 million years ago, and *D. bugensis* (restricted to the Dnieper-Bug estuarine region before 1940) and *D. rostriformis* (endemic to mesohaline waters of the Caspian Sea area, 10-12.7 ppt) differentiated about 300,000 years ago during the mid-Pleistocene Epoch. We also recognize a divergent form of *D. polymorpha* in the Volga

River delta dating to about 430,000 years ago, and dispute taxonomic validity of the putative subspecies of *D. rostriformis* in the Caspian Sea. Genetic evidence shows that the invasions of *D. polymorpha* and *D. bugensis* in the Great Lakes each were founded by large numbers of genotypes from multiple sources.

Stepien, C.A., J.E. Brown*, and M.E. Neilson*. 2006. Genetic diversity and divergence patterns in exotic species introduced from the Ponto-Caspian to the North American Great Lakes. Biology of Inland Waters MAIK-Nauka, Russia. In Press.

Genetic variability and population structure of nonindigenous versus native populations are compared for dreissenid mussels (zebra mussel *Dreissena polymorpha* and quagga mussel *D. bugensis*) and neogobiin gobies (round goby *Apollonia* (formerly *Neogobius) melanostomus* and freshwater tubenose goby *Proterorhinus semilunaris*; formerly *P. marmoratus*), which invaded the North American Great Lakes from the Ponto-Caspian region of Eurasia via ballast water during the mid 1980s through early 1990s. DNA sequence variation in the mitochondrial DNA cytochrome *b* gene is analyzed from invasive populations in the Great Lakes and Eurasia and native Ponto-Caspian populations, in order to elucidate possible founding sources and evaluate whether genetic variability influences invasive success. Congeners and close relatives are analyzed to examine evolutionary patterns as well as to develop diagnostic genetic characters for identifying “cryptic” species and relatives in case of undetected and/or new invasions. Results show that a diverse number of haplotypes characterize these invasive populations in both North American and Eurasian sites and - surprisingly - indicate little or no founder effects. Data also show that there were multiple founding sources for the mussel and goby invasions of the Great Lakes and their genetic diversity levels are similar to those across their native Ponto-Caspian ranges. In summary, these results show that the number of founding invasion propagules were large in all three cases, and that multiple founding sources were most likely involved. A large number of founding individuals representative of their native genetic diversity and multiple founding sources likely increase the success of invasions, aiding rapid spread and adaptation to new habitats.

Neilson, M.E*. and C.A. Stepien. 2006. Genetic identity, systematics, and biogeography of invasive neogobiins: Patterns in the Ponto-Caspian, the Great Lakes, and beyond. Biology of Inland Waters MAIK-Nauka, Russia. In Press.

Systematic identity is an important consideration in understanding the biology of a species, and is of particular importance with invasive species, where taxonomic confusion can influence ecological impact assessments and management efforts. We investigated the systematics of the neogobiin gobies: a small species flock endemic to the Ponto-Caspian region (Black/Caspian Seas and associated drainages) that are invasive in the North American Great Lakes and central/eastern Europe, and whose taxonomic position and systematic relationships are poorly understood. We analyzed DNA sequence data from the mitochondrial cytochrome *c* oxidase I gene and previously published cytochrome *b* data to infer relationships among members of this subfamily. Results indicate that the genus *Neogobius* appears to be paraphyletic, with marked divergence between the former subgenus *Apollonia* (comprising the round goby *A.*

(formerly *N.*) *melanostomus* and monkey goby *A. (N.) fluviatilis*) and all other neogobiin taxa. Significant divergence is also seen between marine and freshwater types of tubenose goby *Proterorhinus marmoratus*, indicating species level separation between the two forms. The freshwater type of *Proterorhinus* was originally described as *P. semilunaris*, but was later synonymized with *P. marmoratus*. These data support the resurrection of *P. semilunaris* for the freshwater tubenose goby, and the elevation of *Apollonia* to generic status, rendering four neogobiin genera.