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Population structure of the invasive round goby (Neogobius melanostomus) along the eastern shore of Lake Michigan

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Abstract

Round gobies are an invasive species that have proliferated throughout the Great Lakes since 1990. Today, anthropogenic forces are causing an increasing number of invasions causing great economic and ecological damage. The round goby invasion, because of its high level of success and recent occurrence, may represent a model system to study the evolutionary dynamics and formation of genetic population structure in novel habitats. Our study is a fine scale examination of the round goby genetic population structure along the eastern shore of Lake Michigan.

Introduction

Round gobies were first brought to the Great Lakes via ship ballast water and had established populations in the St. Clair River by 1990. Within 5 years, they had proliferated throughout all five Great Lakes. Since their establishment, they have caused significant ecological effects including; extirpation of native benthic fish species, nest predation (Steinhart et. al 2004, Fitzsimons et. al 2006), transfer of toxins (Kwon et. al 2006), facilitation of avian botulism outbreaks (Yule et. al 2006), and becoming a food source for other predators of the Great Lakes (Truemper et. al 2006).

Previous genetic studies of round gobies have focused on patterns of diversity, differentiation and divergence at large temporal and geographical scales (Brown & Stepien 2008, 2009, Stepien & Tumeo 2006, Stepien et. al 2005). Studies have examined native Eurasian genetic population structure, and found two major lineages, the Black-Azov Sea basin and the Caspian Sea basin (Brown and Stepien 2008). Other studies have focused on genetic

differentiation in invaded Eurasia, as well as North America (Stepien et. al 2005), and have even identified the Black-Azov Sea basin and Dneiper River as the source populations of the Great Lakes invasion (Brown and Stepien 2009).

Our study compliments previous studies focusing on a smaller geographical scale, along the Eastern shore of Lake Michigan. Our study examines 8 pierheads within 375 km. to examine whether pierheads represent discrete populations within the Great Lakes, and quantifies the current levels of differentiation between them. We designated pierheads as possible discrete units of sub-populations because of their high rocky substrate, of which round gobies have a high affinity (Ray and Corkrum 2001), and the sandy shores located along eastern Lake Michigan, that may act as a barrier to gene flow between them.

Methods

Sampling

We collected round gobies using baited minnow traps, from eight pier head locations along the Eastern coast of Lake Michigan from May– July, 2008. Sites included (from north to south); Charlevoix (CV) Ludington (LD), Pentwater (PW), Whitehall (WH), Muskegon (MK), Grand Haven (GH), Holland (HL), and Saint Joseph (SJ) (Figure 1). Samples numbered between 25-52 fish per site, totaling 291 fish (Table 1). Caudal fin clippings were collected and stored individually in 95% ethanol.

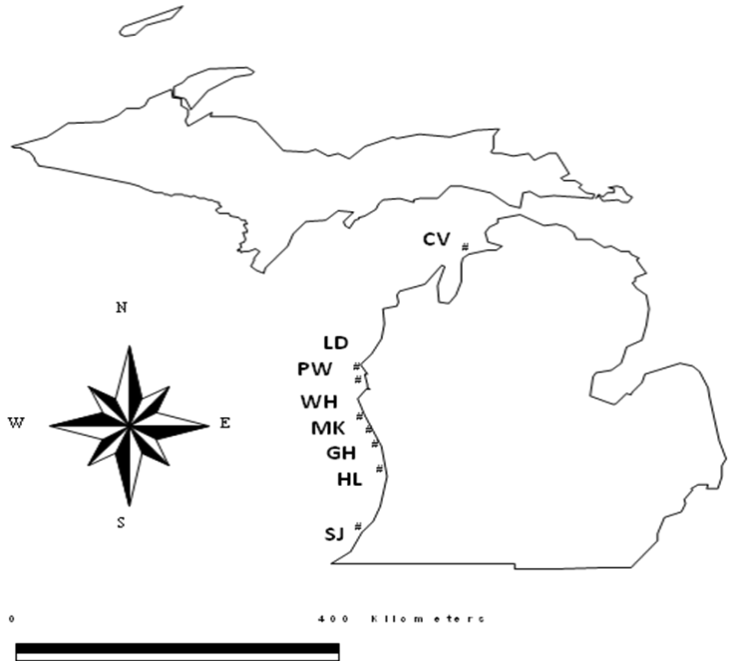

Fig. 1 Round Goby collection sites; Charlevoix (CV, n=28) Ludington (LD, n=40), Pentwater (PW, n=36), Whitehall (WH, n=30), Muskegon (MK, n=50), Grand Haven (GH, n=25), Holland (HL, n=30) and Saint Joseph (SJ, n=52)

Genetics

We extracted genomic DNA from the caudal fin clippings of all 291 fish (Table 1), using Qiagen DNeasy 96 kits. Six polymorphic microsatellite markers (Dufour 2007) were amplified using 0.2μM fluorescent primers from Dufour et. al (2007), 0.2mM dNTPs, 2.5mM $MgCl_2$, 1.0 U taq polymerase (PROMEGA), and 5X (PROMEGA) taq buffer. PCR parameters included; 2 min denaturation at 94°C, followed by 10 cycles of denaturation at 94°C,15 sec annealing at 55°C , and a 15 sec extension at 72°C, followed by 30 cycles of 94°C denaturation to 48°C annealing. Microsatellites were then multiplexed in two separate plates according to fluorescent label and size range (Table 2). Plate 1 included Nme1, Nme2, and Nme5, and plate 2 included Nme3, Nme8, and Nme9. We then sent the dilution plates with a 1:20, to the University of Illinois for fragment analysis.

Table 2 Six polymorphic microsatellites designed by Dufour et. al (2007) for the round goby with number of alleles (N_A), size range (bp), observed heterozygosity (H_O), expected heterozygosity (H_E), significance value and inbreeding coefficient (F_{IS})

Microsatellite	N_A	Size range (bp)	H_O	H_E	P-value	F_{IS}
Nme1	18	240-368	0.82692	0.84597	0.16503	0.04221
Nme2	4	238-250	0.11538	0.11128	1.00000	-0.01089
Nme3	11	135-187	0.59615	0.67401	0.11777	-0.00332
Nme5	6	134-148	0.09615	0.12864	0.18808	0.09206
Nme8	8	281-292	0.76471	0.80392	0.63515	-0.00606
Nme9	7	168-220	0.59615	0.63069	0.66535	-0.02250

Genetic analysis

We scored raw data in GENEMAPPER, after alleles were automatically scored, we used visual inspection in order to ensure alleles were scored properly (Pompanon et. al 2005). Originally, 10 microsatellite markers (Dufour et al. 2007), were examined. Allele calling is commonly the greatest source of error (Pompanon et. al 2005), hence, Nme4, Nme6 and Nme10 were excluded from further analysis due to scoring abnormalities. The presence of null alleles was checked for by MICROCHECKER version 2.23 (http://www.microchecker.hull.ac.uk, van Oosterhout et al. 2004 2006). MICROCHECKER flagged one microsatellite, Nme7, and after further investigation was concluded to contain null alleles due to the locus significantly deviating from HWE, thus excluding it from analysis. ARLEQUIN 3.1 (Excoffier et al., 1992) was then utilized for calculating linkage disequilibrium to examine whether proportions of associated alleles between two loci were non-random. No loci showed evidence for LDE across all sites and thus, all 6 remaining loci were used in analysis.

Our total population of eastern Lake Michigan round gobies was comprised of 8 pierhead sub-populations. F-statistics were used to measure population differentiation. We used ARLQUIN 3.1 to test for pairwise F_{ST} and for significance after Bonferroni corrections. Fischer exact tests were also performed to test for differentiation significance in ARLQUIN 3.1. In order

to examine whether population differentiation was related to geographic distance, known as isolation by distance, we performed a Mantel test using ARLQUIN 3.1.

Results

Genetic diversity

All loci at all sites were polymorphic. The average number of alleles per site ranges between 4.50 and 6.83 (Table 1). Observed heterozygosity vs. expected heterozygosity at each site shows that subpopulations do not significantly deviate from Hardy-Weinberg equilibrium (Table 1).

Table 2. Location site, sample size (N), average number of alleles (N_A), observed geterzygosity (H_O), expected heterozygosity (H_E), inbreeding coefficient (F_{IS})

	Location	**N**	**Ave. N_A**	H_O	H_E	Average F_{IS}
CV	Charlevoix	28	6.17	0.47619	0.48117	0.01054
LT	Ludington	40	5.50	0.57735	0.57000	-0.03601
PW	Pentwater	36	6.17	0.56944	0.55086	-0.03423
WH	Whitehall	30	5.83	0.53697	0.53720	-0.00633
MK	Muskegon	50	6.50	0.57571	0.55887	-0.03519
GH	Grand Haven	25	5.83	0.53361	0.52642	-0.05570
HL	Holland	30	4.50	0.47778	0.51629	0.07578
SJ	St. Joseph	52	6.83	0.49925	0.53242	0.05837

Genetic differentiation

Pairwise F_{ST} values indicate varying amounts of differentiation between pierhead populations ranging from 0.00 to 0.071 (Table 3). The highest degree of differentiation occurred between Holland and Charlevoix. Fischer exact tests showed an identical signature of significance levels of differentiation between pierhead populations (Table 3).

Table 3 Below the diagonal are pairwise F_{st} values including all six microsatellite markers between pairs of pierhead populations, above diagonal are Fischer exact test values with significance P-value

	SJ	HL	GH	MK	WH	PW	LD	CV
St. Joseph	-------- --	**inf (<.0001)**	**inf (<.0001)**	**66.79 (<.0001)**	**inf (<.0001)**	**inf (<.0001)**	**inf (<.0001)**	**34.145 (.0006)**
Holland	**0.033**		8.959 (.7064)	9.58 (.6537)	15.669 (.3969)	18.902 (.0909)	**74.528 (<.0001)**	**inf (<.0001)**

			(.7064)	(.6527)	(.2069)	(.0909)	**(<.0001)**	
Grand Haven	**0.028**	0	----------	9.293 (.6777)	7.723 (.8064)	12.945 (.3730)	**52.042 (<.0001)**	**inf (<.0001)**
Muskegon	**0.019**	0	0	----------	10.018 (.6144)	12.554 (.4023)	**inf (<.0001)**	**inf (<.0001)**
Whitehall	**0.002**	0	0	0	----------	22.621 (.03113)	**inf (<.0001)**	**inf (<.0001)**
Pentwater	**0.028**	0.006	0.003	0.001	0.005	----------	**65.927 (<.0001)**	**inf (<.0001)**
Ludington	**0.055**	**0.045**	**0.040**	**0.039**	**0.055**	**0.038**	----------	**inf (<.0001)**
Charlevoix	**0.013**	**0.071**	**0.064**	**0.050**	**0.053**	**0.047**	**0.056**	----------

Significant differentiation values after Bonferroni corrections (P < = 0.05) shown in bold

Using a Mantel test, we found a significant relation (P=0.012) between pairwise differentiation and geographic distance (Figure 2), which had a correlation value of 0.61. There was one significant outlier from our isolation by distance model. This point was our furthest sites apart, which had a relatively low F_{ST} of 0.013 (P=0.018), and geographic distance of 375km.

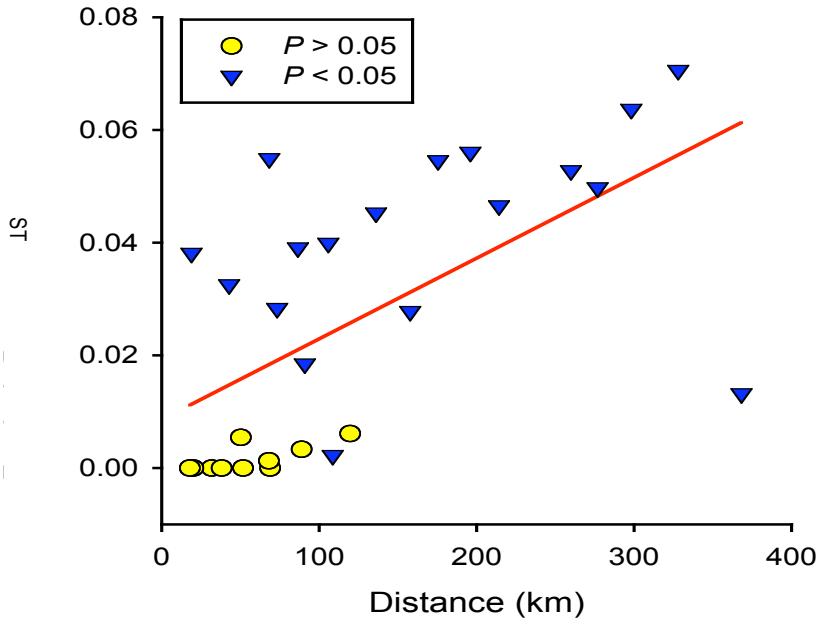

Figure 2. Mantel test = 0.61, P=0.012. Pairwise F_{ST} vs. geographic distance

Discussion

Population structure

Previous studies of round goby genetics have focused on both levels of genetic diversity, differentiation and divergence. Our study compliments previous studies with a fine-scale examination of population structure along the eastern shores of Lake Michigan. Examining 8 pierheads within 375 km. we found evidence for significant population structure occurring. This may not be surprising, as multiple causes for current levels of differentiation may exist, such as multiple founding populations and recentness of invasion. However, we have found that the degree of differentiation is positively correlated to geographic distance. This suggests that gene flow may be limited by dispersal capabilities. The round goby population structure in eastern Lake Michigan is an example of the evolutionary model known as isolation by distance, which suggests that gene flow between populations decrease with geographic distance. Our study clearly illustrates this model (Figure 2) as the current type of population structure occurring along eastern Lake Michigan.

Population Structure Dynamics

However, what our study cannot determine, is whether the population structure is at equilibrium or changing. Due to the recentness of the invasion, it is unlikely the population structure has reached equilibrium, and may be more likely to be increasing or dissolving. There are a multitude of evolutionary forces, creating the dynamics that have resulted in the current population structure. Currently, it is uncertain what level of isolation or gene flow is occurring between the pierhead populations. However, future temporal studies, examining the dynamics of the round goby genetic population structure would be insightful to quantifying the levels of

isolation/gene-flow occurring between pierhead populations. The round goby invasion may represent a model system to examine these types of dynamics that develop population structure in novel habitat.

References

Brown JE & Stepien CA (2008) Ancient divisions, recent expansions: phylogeography and population genetics of the round goby *Apollonia melanostoma*. *Molecular Ecology* **17**, 2598-2615.

Brown JE & Stepiend CA (2009) Invasion genetics of the Eurasian round goby in North America: Tracing sources and spread patterns. *Molecular Ecology* **18,** 64-79.

Dufour BA, TM Hogan, & Heath (2007) Ten polymorphic microsatellite markers in the invasice round goby (Neogobius melanostomus) and cross-species amplification. *Molecular Ecology Notes* **7**,1205-1207.

Excoffier L, Laval G, Schneider S (2005) ARLEQUIN ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online,* **1,** 47-50. http://cmpg.unibe.ch/software/arlequin3/

Fitzsimons J, B Williston, G Williston, G Barvener, JL Jonas, RM Claramunt, JE Marsden, & BJ Ellrot (2006) Laboratory estimates of salmonine egg predation by round gobies, sculpins, and crayfish. *Journal of Great Lakes Research* 32(2)227-241.

Van Oosterhout C, WF Hutchinson, DPM Willis, P Shipley (2006) http://www.microchecker.hull.ac.uk

Kwon AM, IK Barker, JW Austin, & RD Moccia (2006) Toxicity of *Clostridium botulinum* type E neurotoxin to Great Lakes fish: Implications for avian botulism. *Journal of Wildlife Diseases* 42(3) 479-493.

Kwon TD, SW Fisher, GW Kim, H Hwang, & JE Kim (2006) Trophic transfer and biotranformation of polychlorinated biphenyls in zebra mussel, round goby, and smallmouth bass in Lake Erie, USA. *Chemistry* 25(4)1068-1079.

Pompanon F, A Bonin, & P Taberlet (2005) Genotyping errors: causes, consequences and solutions. *Nature* **6**,847-859.

Ray WJ & LD Corkum (2001) Habitat and site affinity of the round goby. *Journal of Great Lakes Research* 27(3) 329-334.

Steinhart GB, EA Marshall, & RA Stein (2004) Round goby predation on smallmouth bass offspring in nests during simulated catch and release angling. *Transactions of the American Fisheries Society* 133(1) 121-131.

Stepien CA, Brown JE, Neilson ME, & Tumeo MA (2005) Genetic diversity of invasive species in the Great Lakes versus their Eurasian source populations: insights for risk analysis. *Risk Analysis* 25(4), 1043-1060.

Stepien CA & MA 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

Truemper HA, TE Lauer, TS McComish, & RA Edgell (2006) Response of yellow perch diet to a changing forage base in southern Lake Michign. *Journal of Great Lakes Research* 32(4)806-816.