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ABSTRACT The round goby, *Neogobius melanostomus,* originally native to the Black and Caspian seas, was introduced into the Great Lakes via ballast water in the 1990's. Since then, the species has spread to all of the Great Lakes, thriving in the Lake Michigan region and spreading to surrounding bays and rivers. Invasive species are considered to have a high evolutionary potential. Differences in environmental conditions between native and introduced ranges stimulate adaptive evolution. Multiple introductions of an exotic species can result in separate instances of founder effects, further increasing the chance of evolutionary change. A total of 267 round goby specimens were collected using hook and line from lake, harbor, or river sites around the Chicagoland area in the Summers of 2012 and 2013. Along with basic measurements of length and weight, geometric morphometrics were performed on each specimen, allowing detailed comparisons of morphology. A discriminant function analysis was performed using body shape data to determine if a specimen's morphology was enough to correctly classify it by habitat. The results yielded 70.4% correct identification for lake vs. harbor specimens and 87.2% for lake vs. river specimens, indicating a significant difference in morphology of specimens by habitat.

INTRODUCTION

Populations of plants and animals employ a variety of techniques to move their genes from place to place, some of which involve using another organisms as transportation. The round goby, *Neogobius melanostomus* (Pallas, 1814) is one of the many invasive species that have become established in the Midwestern United States. While only a small fraction of introduced exotic species become established in their new habitat, the effects a new species could have on the local ecology can range from mild competition and predation to large-scale niche replacement, evolutionary change, and extinction (Mooney and Cleland, 2001).

Since fishes as a group tend to exhibit high levels of body shape variation compared to other taxa, and these changes are often linked to a difference in ecological niche (Reid and Peichel 2010), development of adaptations for inhabiting novel environments is possible. Body shape divergence, especially changes in the configuration of fins, the morphology of the

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mouth, and body depth, is common in adaptive radiation in fishes (Schluter, 2000). Body shape tends to reflect the hydrodynamic context of their habitat, whereas differences in composition and acquisition of diet can account for variation in mouth morphology (Matthews, 1998). Previously published works involving round goby have touched on their varied diet and widespread nature (Carman et al., 2006), but little information is known of the nature and degree of the adaptations present in round goby populations in newly founded habitats, and there is no previous data on body shape divergence for round goby in the Great Lakes region.

Determining the adaptability of the round goby to new environments could offer insight to the ecological implications of the spread of this species. If the species' tendency to be a voracious and opportunistic predator is paired with a significant potential to specialize to different aquatic habitats and niches, then the round goby could be capable of invading many of the native bodies of water in and around eastern Lake Michigan, while exploiting the natural resources these environments offer. It is important to assess the risk the round goby poses to the habitats they occupy in order to speculate on the impact they will have on the native inhabitants of the area and the quality of their habitats.

METHODS

A total of 267 round goby specimens were collected during the summers of 2012 and 2013, with the three sites sampled located on the north side, downtown, and south side of Chicago (Fig. 1). For each site, a lake, harbor, and river site was sampled, with the exception of a river downtown, where sampling of the river yielded no round goby specimens, and a harbor site on the south side, which was due to difficulty finding a suitable harbor site in the area.

Specimens were collected using fishing rods with wax worms as bait and euthanized immediately with MS-222. They were then fixed in 10% formalin for one week before being rinsed in water for 24 hours and transferred to 70% ethanol. Specimens collected were grouped into categories based on which of the three environments they were collected from, and had their length, sex and weight recorded. Using the data collected on their standard length and weight, the body condition (weight per unit length) of the specimens was scored and compared with those of fish from the other environments. In this way, the health and condition of the specimens from each habitat can be assessed (Ogle 2013).

Geometric morphometrics was used In order to accurately quantify differences in external morphology across the three different habitats and create models depicting body shape variation. Specimens were photographed, and using TpsDig 2.17 (Rohlf, 2013), 18 landmarks were digitized on the body. Using these universal markers on each specimen allowed the program to use the distances between landmarks to assess and compare the shape and orientation of each specimen, accounting for differences in size, and decipher any patterns in morphology (Aguirre et al., 2011). SPSS 11.0.0 was used to assess the body shape divergence in populations in different habitats, as well as determine the influence of allometry (change in shape due to body size) and sexual dimorphism on the outcome of the statistical tests. In this way, not only will morphological divergence across habitats be quantified, but the magnitude of divergence can be compared with differences in body shape related to sexual dimorphism and ontogenetic changes in morphology. The data for sex as well as size and stage in development were used to assess the results and determine if these factors account for any differences found in morphology by habitat.

A discriminant function analysis (DFA) was performed on the data collected, where the computer program uses the morphometric data and the habitat categories provided to assign each specimen to a habitat based on only its body shape. In this way, we can determine if it is likely that divergence in body shape would primarily be due to the specimen's habitat and measure the rate of successful classification. The results were cross-validated to ensure accuracy, where the program created the function to classify the specimens excluding the specimens to be classified one at a time. By using the invasion history of this species, harbor and river morphology could be compared directly to that of the ancestral lake population, creating a visual representation of how the body shape changes in populations that colonize new aquatic habitats.

RESULTS

Using the data collected on the weight and standard length of each specimen, the general body condition was assessed. Assuming a higher weight to length ratio indicates a better body condition, this information was plotted to look for differences in body condition by habitat (Figs 2-4). The lake population seemed to have a higher average body condition than the harbor specimens, and the other comparisons did not appear significant.

There was a considerably even distribution of sex between all the specimens caught, with 142 males and 125 females. Using the discriminant function analysis on SPSS, body shape morphology of the entire sample was used to test for sexual dimorphism, where 77.5% of male specimens were correctly classified as male and 75.2% of female specimens were correctly sexed (Table 1). Although there was significant evidence for sexual dimorphism in the sample, the impact of sexual dimorphism on habitat differences seemed to be negligible.

A general consensus for body shape was then generated for both sexes (Figs. 5 & 6). Using TPS, the data collected by geometric morphometrics was used to create an average model for the body shape morphology of each sex. Although there were size differences by sex, where the males generally tended to be larger than females, of the impact of size differences on sexual dimorphism appeared to be negligible. When the discriminant function analysis was performed to contrast harbor and lake specimens, the function was able to classify 69.1% of lake specimens and 73.8% of harbor specimens correctly (Table 2).

Comparing the body shape morphology of the lake and river specimens with the discriminant function analysis resulted in correct classification of 89.2% of lake fish and 68.2% of river fish. Using TPS, a general consensus for the body shape morphology of the lake specimens was created (Fig. 7). From this baseline morphology, models of the harbor and river morphs (Figs. 8 & 9) were generated as a function of the lake morphs, creating a visual depiction of how the body shape has changed from the ancestral morphology. When testing for differences in morphology of fish from the different habitats, allometry must be accounted for. The general consensus for the body shape of the smallest fish versus the largest was generated on TPS (Figs. 10 & 11), and this data suggested that the changes in body shape by habitat was not likely caused by allometry.

DISCUSSION

Considered to be the most diverse group of vertebrates, fishes not only include more described species than all other chordates combined, but also have a diversity of body shape that far exceeds that of the entire phylum (fishbase.org 2011). An understanding of the importance of the diversity of fish can lend insight into their life history, trace lineages back to speculate on evolutionary processes, and make predictions about how morphological changes coincide with the constant change of their environment.

The results of the discriminant function analysis were statistically significant, with 87.4% of original cases classified correctly, and the trial that was cross-validated to minimize error grouped 78.9% of the specimens in their correct habitat category. If there had been no correlation of body shape by habitat, the expected percentage of correctly grouped cases would be around 50%. These results indicate that the populations that have spread to different aquatic environments are physically distinguishable from the original round goby population in the lake.

First observed in 1990 in the St. Clair River, which connects the southern tip of Lake Huron with Lake Erie, (Misin.msu.edu) round goby had spread to all five great lakes by 1994. Considered to be a problem species due to their effect on fishing and native species, round goby are a hearty species that can live in poor quality environments (University of Wisconsin 2013).
If round goby exhibit phenotypic plasticity in their body shape based on environmental differences in their habitats, their body shape

could be defined by environmental alteration of gene expression without actually exhibiting genetic change. (Whitman 2009) Conversely, the patterns of morphology observed in the results could indicate the early stages of evolutionary change. Many instances of contemporary evolution involve species that have colonized novel environments, such as the changes in lateral plate armor and even the genome in populations of the marine threespine stickleback that have founded populations in freshwater lakes (Aguirre and Bell 2012). The definable pattern of morphology between fish in the habitats sampled could mean that round goby are in the process of diverging into different ecomorphs or developing habitat-specific traits. In order to speculate on the cause of patterns in morphology, a subsequent study could perform common garden experiments to assess the degree of heritability of the shape differences documented or even examine the genome of the specimens collected to look for direct evidence of genetic change. In order to enable the exploration of any connection between genetic patterns and the patterns in morphology, fin clips were taken from each specimen and frozen for future sequencing.

Although the round goby is considered to be an unfavorable invasive species, further study on their effects on the environment would indicate the scale of damage the species is causing to the native ecology. Using this information, the benefits of eradicating the species can be weighed against the potential damage to the environment by eradication efforts. Modifications of our human activity affecting wildlife could reduce the introduction of exotic species. Boats and their bilges need to be drained in the same environment where the water came from, to ensure that organisms from one environment do not enter another. Chicago and the surrounding areas are built around extensive wetlands, prairies, and other aquatic settings that are essential for nutrient cycling and other ecological processes. With 27 million people living in Chicago alone, a huge human population depends on the health of our natural habitats. Vigilant study needs to continue on the health of our aquatic environments, as population spikes increase the strain on natural resources. In this way, the information gathered on invasive species, population genetics, and general ecology of our nature reserves can maximize the potential to maintain a healthy wetland environment and a functioning city.

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	Classification Results			
	Original	Predicted M	Predicted F	Total
	Sex			
count	M	113	29	142
	F	24	101	125
%	M	79.6	20.4	100
	F	19.2	80.8	100
	Cross-validated			
count	M	110	32	142
	F	31	94	125
%	M	77.5	22.5	100
	F	24.8	75.2	100

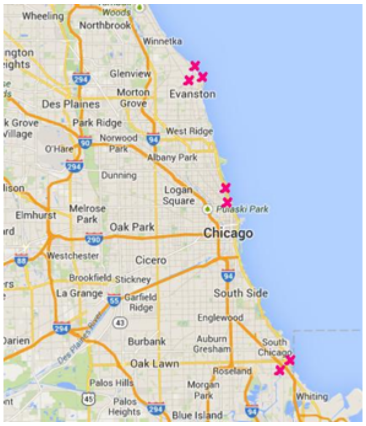
Table 1. With 80% of individuals classified into their correct sex and 76.4% of cross-validated grouped cases classified correctly, sexual dimorphism in body shape appears to be significant.

Classification Results				
Original		Predicted L	Predicted H	Total
	Habitat			
count	Lake	129	36	165
	Harbor	13	67	80
%	Lake	78.2	21.8	100
	Harbor	16.3	83.8	100
Cross-validated				
count	Lake	114	51	165
	Harbor	21	59	80
%	Lake	69.1	30.9	100
	Harbor	26.3	73.8	100

Table 2. *With 80% of the original grouped cases 70.6% of the cross-validated cases classified correctly, the DFA for lake vs. harbor specimens indicated a statistically significant difference in body shape between samples.*

Classification Results				
Original		Predicted L	Predicted R	Total
	Habitat			
count	Lake	158	7	165
	River	3	19	22
%	Lake	95.8	4.2	100
	River	13.6	86.4	100
	Cross-validated			
count	Lake	148	17	165
	River	7	15	22
%	Lake	89.7	10.3	100
	River	31.8	68.2	100

Table 3. With 94.7% of the original grouped cases and 87.2% of cross-validated cases correctly classified, the second DFA comparing body shape of lake and river specimens yielded a statistically significant result.



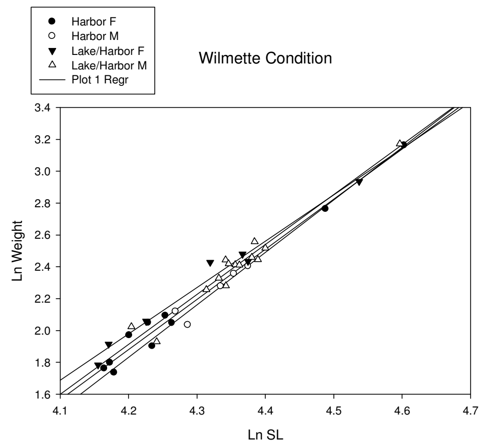
	Lake N	Harbor N	River N
Wilmette	45	19	5
Diversey	52	61	0
Calumet	65	0	17
Tot N	165	80	22

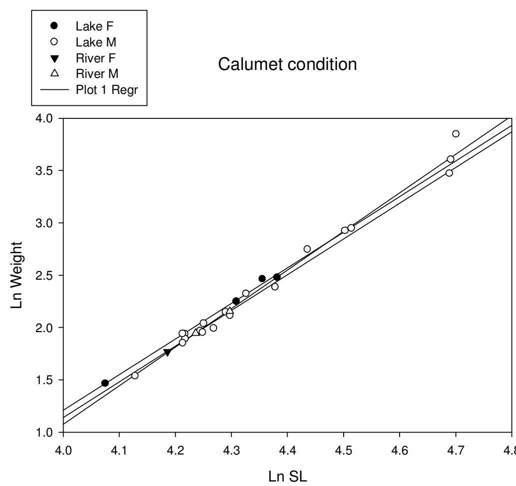
Fig. 1. Wilmette Lake (site A): 42 04' 38.3" N, 87 40' 52.5"W,

Wilmette Harbor (site B):41 42' 58.5, 87 31' 41.5", Wilmette River (site C):

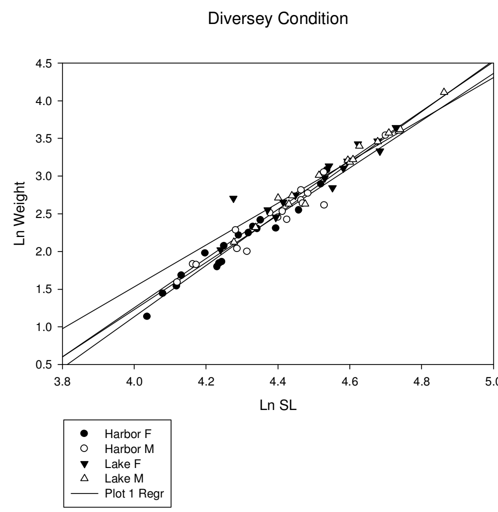
42 4' 21" N, 87 41' 29" W, Diversey Lake (Site D): 41 56' 7.5", 87 37' 53.5",

Diversey Harbor (site E): 41 55' 39.5", 87 38' 02" W, Calumet lake (site F): 41 43' 12.5", 87 31' 32" and Calumet river (side G):41 43'43", 87 32' 28".





Figs 2-4. Condition plots of the log of standard length plotted against the log of the weight. There appears to be correlation with lake specimens having a better body condition overall than harbor specimens.



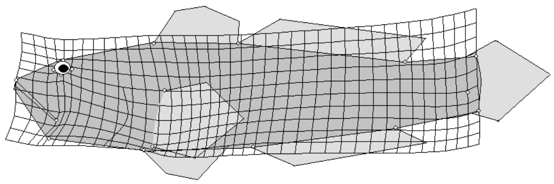


Fig 5. General body consensus for males. Features exaggerated 3 times to show detail

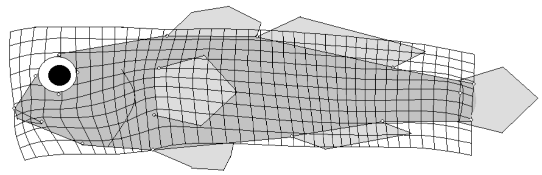


Fig 6. General body consensus for females. Features exaggerated 3 times to show detail

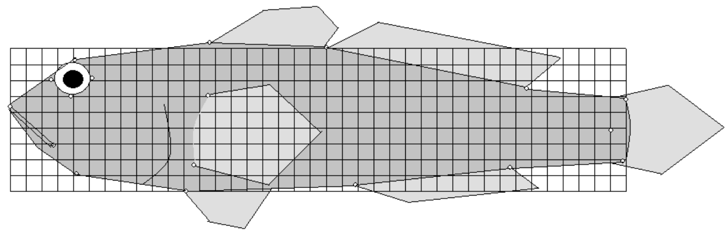


Fig 7. General consensus for lake morph

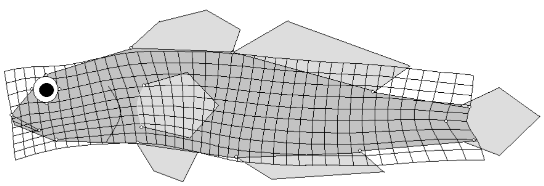


Fig 8. Harbor morph as a function of lake morph

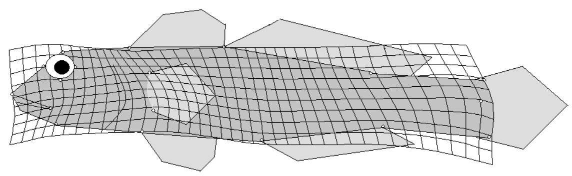


Fig 9. River morph as a function of lake morph

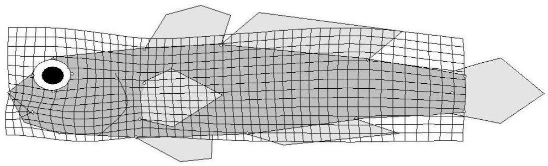


Fig 10. Body morph consensus for small gobies. Features exaggerated 3x to show detail

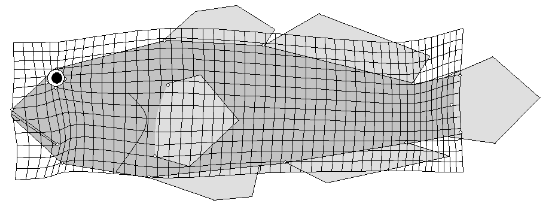


Fig 11. Body morph consensus for large gobies. Features exaggerated 3x to show detail