

# Dispersal strategies, secondary range expansion and invasion genetics of the nonindigenous round goby, *Neogobius melanostomus*, in Great Lakes tributaries

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## Abstract

Dispersal strategies are important mechanisms underlying the spatial distribution and colonizing ability of all mobile species. In the current study, we use highly polymorphic microsatellite markers to evaluate local dispersal and colonization dynamics of the round goby (*Neogobius melanostomus*), an aquatic invader expanding its range from lake to river environments in its introduced North American range. Genetic structure, genotype assignment and genetic diversity were compared among 1262 round gobies from 20 river and four lake sites in three Great Lakes tributaries. Our results indicate that a combination of short-distance diffusion and long-distance dispersal, collectively referred to as 'stratified dispersal', is facilitating river colonization. Colonization proceeded upstream yearly (approximately 500 m/year; 2005–2009) in one of two temporal replicates while genetic structure was temporally stable. Contiguous dispersal from the lake was observed in all three rivers with a substantial portion of river fish (7.3%) identified as migrants. Genotype assignment indicated a separate introduction occurred upstream of the invasion front in one river. Genetic diversity was similar and relatively high among lake and recently colonized river populations, indicating that founder effects are mitigated through a dual-dispersal strategy. The remarkable success of round goby as an aquatic invader stresses the need for better diffusion models of secondary range expansion for presumably sessile invasive species.

**Keywords:** colonization, conservation genetics, invasive species, secondary range expansion, stratified dispersal

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## Introduction

Successful colonization of habitat beyond a species current range requires the appropriate dispersal strategy to facilitate the initial introduction. Dispersal into new environments can occur by regional diffusion—where the population expands into adjacent habitats as a function of population growth rate and a diffusion coefficient (Shigesada *et al.* 1995; Thibault *et al.* 2009)—or by long-distance dispersal where dispersal is not limited to nearby patches, but instead can lead to disjunct populations located far from the species range core (Kareiva

*et al.* 1990). It is now recognized that multiple dispersal mechanisms such as short-distance diffusion combined with long-distance jump events, collectively termed stratified dispersal, contribute to the range expansion of many contemporary colonizing species (Muirhead *et al.* 2006; Worthington Wilmer *et al.* 2008; Darling & Folino-Rorem 2009; Huebner 2010). Stratified dispersal can lead to complex species distribution patterns and increasing rates of expansion when a species is colonizing a new area (Shigesada *et al.* 1995; Henne *et al.* 2007).

Species expanding their range into new environments are faced with selective pressures, which will challenge their dispersal strategy and potentially limit their expansion. Selective forces include abiotic factors such

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as different climate, different chemical composition and physical barriers to dispersal (Moyle & Light 1996; Ricciardi & MacIsaac 2000; Kestrup & Ricciardi 2009) and biotic factors such as new predators, parasites and community dynamics (Moyle & Light 1996; Sakai *et al.* 2001; Chun *et al.* 2010). Dispersal is expected to be favoured in peripheral and expanding populations. Critical factors that affect a species' dispersal strategy include dispersal ability (Hochkirch & Damerau 2009), the location of the disperser within the species range (Gros *et al.* 2006; Dytham 2008) and its ability to respond to environmental heterogeneity (Kestrup & Ricciardi 2009). For example, an adaptation for increased dispersal capacity at the invasion front of the prolific cane toad in Australia has led to an accelerating rate of expansion into territories that were not expected to be colonized by cane toads based on models derived from their native region (Phillips *et al.* 2007). Population genetic theory states that adaptive potential depends on genetic diversity, that is, populations with higher genetic diversity will have a greater opportunity to resist detrimental founder effects and adapt to different environments (Lee 2002). At the same time, genetic drift and selective forces associated with colonizing new environments will act to deplete a population's genetic diversity (Ciosi *et al.* 2008; Peacock *et al.* 2009).

Recent research shows that genetic diversity may be maintained in colonization events and ongoing invasions, despite theoretical expectations for a loss of genetic diversity through founder effects and genetic bottlenecks (Colautti *et al.* 2005; Stepien *et al.* 2005; Stepien & Tumeo 2006; Hochkirch & Damerau 2009). Suggested mechanisms that may account for this anomaly include high propagule pressure (Lockwood *et al.* 2005) and gene flow among independently colonized populations (Kolbe *et al.* 2004; Stepien *et al.* 2005). Human-mediated introductions are known to have high propagule numbers and multiple invasions from genetically distinct source populations (Kolbe *et al.* 2004; Theriault *et al.* 2005; Simon-Bouhet *et al.* 2006; Stepien & Tumeo 2006; Roman & Darling 2007; Zalewski *et al.* 2010). Human-mediated dispersal within new environments will also promote gene flow among newly established populations, therefore increasing genetic diversity and the potential to produce novel genotypes within these new populations (Lombaert *et al.* 2010). Colonization patterns associated with human-mediated dispersal are different from those created by natural dispersal, and thus, if short-distance diffusion and long-distance dispersal events are both contributing to range expansion (i.e., 'stratified dispersal'), the rate of expansion is expected to increase (Shigesada *et al.* 1995). Stratified dispersal is increasingly being accepted as a mechanism that can also maintain genetic diversity at

the invasion front of an expanding population (Simon-Bouhet *et al.* 2006; Tobin & Blackburn 2008). Furthermore, it has been suggested that high levels of genetic diversity at the invasion front will increase the probability of an introduction becoming a successful invasion by providing genetic variation for phenotypic traits associated with colonizing ability on which selection can act (Roman 2006; Parisod & Bonvin 2008).

The round goby, *Neogobius melanostomus*, is a highly successful invader originating from the Ponto-Caspian region. Round gobies were first detected in the Laurentian Great Lakes in 1990 in the St. Clair River (Jude *et al.* 1992), and by 1995, they were reported in all five Great Lakes. Presently, round gobies continue to be detected in new areas throughout the Great Lakes watershed (Jude *et al.* 1995; Phillips *et al.* 2003; Schaeffer *et al.* 2005; Pennuto *et al.* 2010; Poos *et al.* 2010). The rapid spread of round goby populations throughout the Great Lakes is mainly attributed to ballast water transfer among Great Lakes ports postintroduction (Hensler & Jude 2007; Brown & Stepien 2009). Successful establishment of round goby populations has been also facilitated by their high environmental tolerance, rapid population growth rate and superior competitive ability (Dubs & Corkum 1996; Charlebois *et al.* 2001). Despite their rapid spread, round goby populations were expected to remain limited to the Great Lakes basin within preferred rocky habitat (Clapp *et al.* 2001; Ray & Corkum 2001). However, round gobies have recently expanded their range upstream into river systems in their introduced ranges in North America and Europe (Phillips *et al.* 2003; Sapota & Skora 2005; Wiesner 2005; Pennuto *et al.* 2010; Poos *et al.* 2010). River expansion is occurring in areas void of ballast water vectors and therefore suggests that alternate dispersal mechanisms are facilitating upstream spread. Paradoxically, round gobies are expected to have poor natural dispersal ability, especially upstream, because of their benthic morphology and small home range (Wolfe & Marsden 1998; Ray & Corkum 2001; but see Hensler & Jude 2007).

The ability of round goby to disperse and colonize river systems creates new dispersal corridors for their continued expansion into connected waterways and inland lakes, which would increase their ecological and economic impact (Ricciardi & MacIsaac 2000). It is critical to identify factors governing secondary spread into tributary watersheds if we are to accurately predict the ultimate distribution and potential impacts that may result from the round goby invasion. Here, we examine the invasion genetics of the round goby river invasion front in three selected Great Lakes tributaries. Specifically, we test two hypotheses: (i) river colonization occurs by contiguous diffusion or long-distance dispersal and (ii) river dispersal results in a change in

genetic diversity within a colonizing population relative to the nonexpanding lake population. Genetic characterization of the colonization dynamics and dispersal mechanisms associated with successful invasions will provide useful results for accurate risk assessment and effective management strategies. Additionally, the current study will contribute to the growing knowledge of how species expand their range into new environments.

## Methods

### Sample collection

A total of 1262 round gobies were collected from lake and river sites at three Great Lakes tributaries from May to October in 2005 to 2009. Round gobies were collected from lake and river sites at the Maitland River, Goderich, Ontario, Canada, in 2005 and 2006 as part of another study (Dufour 2007; Table 1). Samples collected from the Maitland River lake site in 2005 and 2006 were combined to attain a reasonable sample size ( $n = 31$ ) after testing for genetic similarity. Round gobies were also collected

at the Maitland River from 2007 to 2009 ( $n = 160, 281$  and  $254$ , respectively; Fig. 1, Table 1). Round gobies were collected from lake and river sites at the Grand River, from Port Maitland to Brantford, Ontario, Canada, in 2008 ( $n = 109$ ) and 2009 ( $n = 188$ ) and the Saugeen River that drains into Lake Huron at Southampton, Ontario, Canada, in 2009 ( $n = 102$ ; Fig. 1, Table 1). As part of another study examining round goby dispersal within the Great Lakes, round gobies were collected from 31 lakes sites in Lake Huron, Erie and Ontario ( $n = 1927$ ; Table S1, Supporting information; Dufour 2007). All fish were collected by a combination of techniques including a single pass with Smith-Root LR-24 backpack electrofisher (Smith-Root Inc., Vancouver, WA, USA), three to six 100-m seine hauls with a 30-m beach seine with 6.35-mm mesh, four or more minnow traps baited with luncheon meat and/or hook and line baited with worms. Round gobies were considered absent at a site when no specimens were captured with a minimum of 1200 electroshocking seconds, three 100-m seine hauls or four angling hours plus four baited minnow traps depending on suitability of each sampling technique at each site.

**Table 1** Watershed and location, site description, site code, GPS coordinates, distance to river mouth (km), sampling year and number of samples per site ( $n$ ). Number of first-generation migrants per site as identified by genotype exclusion in brackets. Dash (–) indicates no sampling was performed

| Watershed and location     | Site Description | Site Code | Latitude      | Longitude     | Distance to river mouth (km) | 2005    | 2006   | 2007   | 2008   | 2009   |
|----------------------------|------------------|-----------|---------------|---------------|------------------------------|---------|--------|--------|--------|--------|
| Lake Huron: Maitland River | Lake             | ML1       | 43°44'40.57"N | 81°43'17.61"W | 0.9                          | 31* (0) | 31*(0) | 44 (1) | 84 (3) | 64 (2) |
|                            | Mouth            | MR1       | 43°44'54.37"N | 81°43'26.69"W | 0.3                          | 17 (1)  | 31 (2) | 0      | 52 (8) | 9 (1)  |
|                            | River            | MR2       | 43°45'6.48"N  | 81°42'52.32"W | 1.1                          | 16 (0)  | 24 (2) | 16 (1) | 61 (4) | 48 (1) |
|                            | River            | MR3       | 43°45'11.34"N | 81°42'40.02"W | 1.5                          | 4 (0)   | 29 (1) | 44 (1) | 26 (3) | 35 (2) |
|                            | River            | MR4       | 43°45'15.78"N | 81°42'22.98"W | 1.9                          | 7 (0)   | 17 (0) | 41 (8) | 28 (1) | 15 (1) |
|                            | River            | MR5       | 43°45'12.00"N | 81°42'5.70"W  | 2.3                          | 0       | 12 (1) | 1 (0)  | 4 (0)  | 17 (2) |
|                            | River            | MR6       | 43°45'1.98"N  | 81°42'0.36"W  | 2.7                          | 0       | 0      | 2 (0)  | 14 (1) | 22 (2) |
|                            | River            | MR7       | 43°44'57.66"N | 81°41'56.40"W | 2.9                          | 0       | 0      | 12 (1) | 10 (1) | 35 (3) |
|                            | River            | MR8       | 43°44'43.86"N | 81°41'52.74"W | 3.4                          | –       | 0      | 0      | 2 (1)  | 6 (0)  |
| Lake Erie: Grand River     | River            | MR9       | 43°44'34.92"N | 81°41'39.06"W | 3.9                          | –       | –      | 0      | 0      | 3 (0)  |
|                            | Lake             | GL1       | 42° 51.183'N  | 79°34'94.8"W  | 0.7                          | –       | –      | –      | 13 (0) | 34 (1) |
|                            | Lake             | GL2       | 42°51.121'N   | 79°34'78.5"W  | 0.5                          | –       | –      | –      | 37 (0) | 40 (0) |
|                            | Mouth            | GR1       | 42°51'24.72"N | 79°34'40.53"W | 0.1                          | –       | –      | –      | 26 (3) | 41 (6) |
|                            | River            | GR2       | 42°54'2.09"N  | 79°37'7.45"W  | 7.9                          | –       | –      | –      | 10 (1) | 33 (1) |
|                            | River            | GR3       | 42°55'37.38"N | 79°42'41.40"W | 8.3                          | –       | –      | –      | 4 (0)  | 5 (0)  |
|                            | River            | GR4       | 42°56'56.28"N | 79°51'37.08"W | 32.7                         | –       | –      | –      | 3 (0)  | 2 (0)  |
|                            | River            | GR5       | 43°4'26.73"N  | 79°57'29.51"W | 51.1                         | –       | –      | –      | 3 (0)  | 11 (0) |
|                            | River            | GR6       | 43°4'31.98"N  | 79°57'52.32"W | 51.8                         | –       | –      | –      | 2 (0)  | 0      |
| Lake Huron: Saugeen River  | River            | GR7       | 43°4'41.70"N  | 79°58'16.86"W | 52.9                         | –       | –      | –      | 10 (0) | 13 (0) |
|                            | River            | GR8       | 43°5'38.15"N  | 80° 1'54.02"W | 58.9                         | –       | –      | –      | 1 (0)  | 4 (0)  |
|                            | Lake             | SL1       | 44°30.089'N   | 81°22'522"W   | 0.1                          | –       | –      | –      | –      | 19 (0) |
|                            | Mouth            | SR1       | 44°30'4.08"N  | 81°22'18.20"W | 0.2                          | –       | –      | –      | –      | 42 (4) |
|                            | River            | SR2       | 44°30'17.34"N | 81°20'53.64"W | 2.4                          | –       | –      | –      | –      | 13 (0) |
|                            | River            | SR3       | 44°30'15.39"N | 81°19'58.69"W | 4.3                          | –       | –      | –      | –      | 13 (1) |

\*Genotype data from samples collected in 2005 and 2006 at site ML1 were combined in a previous study to increase sample size after confirming no difference in allele frequency distribution. Total sample size is 31.

Samples were collected at the river mouth, in the lake close to the river mouth, and at upstream intervals until the invasion fronts were identified (Fig. 1). The invasion fronts in each river were determined as the farthest upstream site where round gobies were captured when round gobies were absent at a minimum of two consecutive upstream sites. Additional random sites upstream from the invasion fronts (Maitland: 11.4 river kilometres, Grand: 21.2 river kilometres, Saugeen: 4.8, 6.6 and 7.8 river kilometres upstream of the invasion front) were sampled to check for possible disjunct round goby populations (Fig. 1). All Universal Transverse Mercator (UTM) coordinates were recorded with a portable GPS unit (Table 1). All round gobies were humanely euthanized with an overdose of clove oil (25 ppm), and fin clip samples were fixed in 95% ethanol and stored at room temperature until DNA extraction.

#### DNA extraction

A 0.5–10.0 mg clip of caudal fin tissue was pressed and air-dried to remove all ethanol from the tissue sample prior to proteinase K digestion. We extracted DNA following an automated silica plate-based extraction protocol (Elphinstone *et al.* 2003) and resuspended DNA in 100 µL Tris–EDTA buffer (pH = 8.0). Samples were genotyped at eight polymorphic nuclear microsatellite loci; Nme2, Nme3, Nme4, Nme5, Nme6, Nme7, Nme9 and Nme10 (Dufour *et al.* 2007). Polymerase chain reactions (PCR) contained approximately 50 ng DNA, 1.0× PCR reaction buffer (Applied Biosystems), primer-specific concentrations of MgCl<sub>2</sub> (Nme 2 = 2.1 mM; Nme4, Nme9 and Nme10 = 2.4 mM; Nme 1, Nme3 and Nme5 = 2.7 mM; Nme6 and Nme7 = 2.8 mM), 0.19 mM of each dNTP (Promega), 0.057 µM reverse primer (Sigma Genosys), 0.038 µM IR700 or 800 dye-labelled forward primer (MWG Biotech AG) and 0.05 U Taq polymerase (5 U/µL; Applied Biosystems) with ddH<sub>2</sub>O to a final reaction volume of 11 µL. PCR followed the protocol of 95 °C initial denaturation for 120 s; 35 cycles of 95 °C for 15 s, optimized primer-specific annealing temperature (Nme3, Nme5, Nme7 and Nme10 = 48 °C; Nme2 and Nme4 = 55 °C; Nme9 = 58 °C; Nme6 = 59 °C) for 15 s, 72 °C extension for 30 s; followed by 72 °C final extension for 120 s and a 4 °C holding temperature. The PCR products were run on a LiCor DNA analyzer for visualization. Amplicons were manually analysed and scored to size with one base pair resolution using GeneImagIR 4.05 software (Scanalytics, Inc.).

#### Genetic analyses

Microsatellite markers were tested for Hardy–Weinberg equilibrium and linkage disequilibrium within each

sampling site using an exact probability test in Genepop 4.0.10 (Raymond & Rousset 1995). Significance was adjusted for multiple comparisons using sequential Bonferroni correction.

#### Genetic structure

Regional level genetic structure has been previously reported in the Great Lakes round goby distribution (Stepien *et al.* 2005; Stepien & Tumeo 2006; Dufour 2007), and therefore, we focus our analyses on a local scale (i.e. each river independently). Population genetic structure within each river was inferred for each sampling year using the Bayesian cluster method in Structure 2.2 (Pritchard *et al.* 2000). Genotype clustering uses all individuals as units simultaneously with no prior knowledge of the spatial distribution of sampling sites (Pritchard *et al.* 2000). Results were generated under the admixture model using 10<sup>5</sup> burnins and 10<sup>6</sup> iterations of a Markov chain Monte Carlo simulation. The number of genetic clusters (*K*) was determined by the highest negative ln probability (–ln *P*) of data of all possible *K* values, when *K* ≤ the number of sampling sites within each river. Genetic structure results were used to cluster river sites for subsequent analyses.

We also estimated genetic differentiation between river sites, and between river and lake sites within each sampling year, by calculating pairwise *F*<sub>ST</sub> in Arlequin 3.11 (10 000 permutations; Excoffier *et al.* 2005). Significance was corrected for multiple comparisons following the sequential Bonferroni method.

#### Upstream dispersal

Individual- and population-level genotype assignment and exclusion were performed in GeneClass 2.0 (Piry *et al.* 2004) to infer population connectivity and gene flow patterns. More specifically, we wanted to identify (i) individual fish whose genotype is excluded from the lake or river population in which they were captured, with the river populations as previously determined by cluster analyses; (ii) the most likely source population/region of each migrant and (iii) the most likely source population for each river population (with migrants excluded) using group genotype assignment.

Genotype assignment has been shown to be a more powerful approach to detect spatial patterns of dispersal compared to population-level approaches that are based on the relationship between genetic differentiation and geographic distance (i.e. isolation by distance; Castric & Bernatchez 2004).

First, we used individual genotype exclusion (Rannala & Mountain 1997) with Monte Carlo resampling of 10<sup>4</sup> simulations (Paetkau *et al.* 2004) to identify migrant

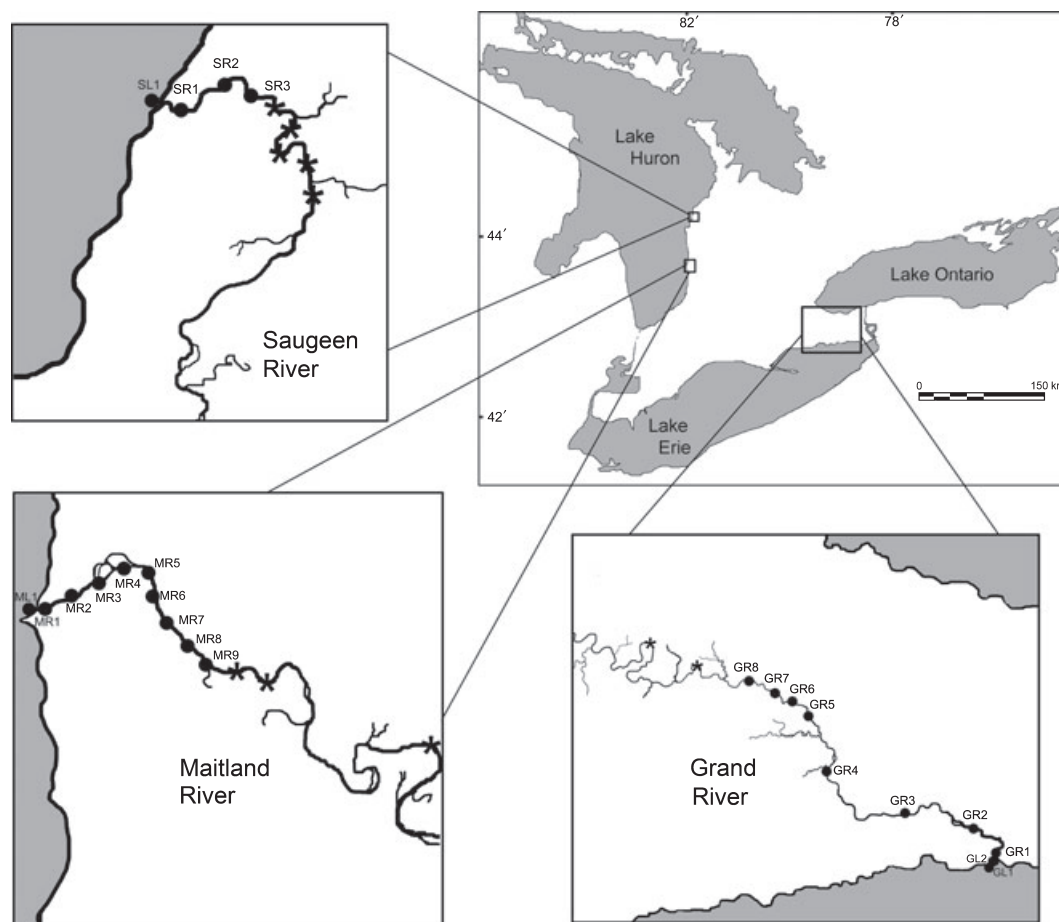


Fig. 1 Map of the round goby, *Neogobius melanostomus*, sampling sites in three watersheds of southwestern Ontario. Site abbreviations are as shown in Table 1. Sites where no round gobies were captured are indicated by an asterisk (\*).

fish whose genotype is excluded from the lake or river population in which they were captured and thus are likely first-generation immigrants to that population. We identified migrants as fish that were excluded from the population based on a 5%, 10% and 20% type I error threshold. The evaluation of migrant fish at the three threshold levels allows us to assess the sensitivity of our analyses to the choice of threshold value. We subsequently use the 5% threshold value as a conservative level and report those results. The assignment exclusion method by Rannala & Mountain (1997) is suitable to identify recent immigrants to a population when source and sink populations have relatively low genetic divergence among them, and this approach is also robust to population genetic disequilibrium (Rannala & Mountain 1997). We performed a cross-tabulation analysis in Systat 7.0 to test for differences in number of migrants in the lake vs. the river.

Next, we used genotype data from 31 baseline round goby lake populations ( $n = 1927$ ) from the lower Great Lakes region that were sampled and genotyped in a

previous study (Dufour 2007) and the nine lake populations associated with our sampled rivers as reference populations to identify the potential source of each migrant. We performed genotype exclusion to identify migrant fish whose genotype is excluded from all potential source lake populations. Bayesian rank-based genotype assignment (Rannala & Mountain 1997) was performed for the remaining migrants (migrants not excluded from all potential source populations) to identify a putative source population for these migrants. The rank-based method calculates the likelihood of each reference population as the putative source for each migrant fish. Successful assignment was achieved if the score for the most likely source population was a minimum of four times greater than the score for the second-ranked population (Beneteau *et al.* 2009). Low genetic differentiation among sites can decrease assignment success. In such cases, we grouped the top-ranked source populations into regional groups for assignment if the criteria of geographic proximity ( $< 70$  km) and nonsignificant genetic differentiation ( $F_{ST}$ ) were met.

Lastly, we performed Bayesian rank-based group assignment (Rannala & Mountain 1997) to identify the most likely source lake population for each river population (with migrants removed). Criteria for successful assignment to a source population were as in step 2; the score for the most likely source population must be a minimum of four times greater than the score for the second-ranked population (Beneteau *et al.* 2009). We identified three possible outcomes: (i) river populations that were assigned to their associated lake population are colonization events via contiguous diffusion dispersal; (ii) river populations that were not assigned to the associated lake population although were successfully assigned to a lake population located within 150 km from the site of capture (as measured by shortest water distance in Google Earth) are short-distance colonization, or 'regional diffusion'; and (iii) river populations assigned to a lake population located more than 150 km away are long-distance colonizations. The 150-km threshold used to distinguish among contiguous and long-distance dispersal events was determined by examining the peaks in the frequency distribution of dispersal distances travelled by migrant fish (as identified in step 2; Fig. 2). The first peak consisted of dispersal distances up to 150 km which most likely represents natural dispersal, and thus, we used 150 km as a threshold distance to distinguish between contiguous and long-distance dispersal events (potentially human mediated). River populations were defined by genetic structure analyses described earlier.

### Genetic diversity

To evaluate genetic diversity differences among lake and river populations (river populations as defined by genetic structure analysis), we calculated allelic richness (A) as the mean of all loci, with values corrected for unequal samples sizes by rarefaction to 12 individuals, in FSTAT v.2.9.3 (Goudet 2001) and expected heterozygosity ( $H_E$ ) in Arlequin 3.11 (Excoffier *et al.* 2005). We calculated mean A and  $H_E$  and 95% confidence intervals for all lake populations combined ( $n = 40$ ) including 31 baseline populations from a previous study (Table S1, Supporting information; Dufour 2007). We tested for differences in genetic diversity (A and  $H_E$  independently) between the river populations and the combined lake populations by identifying river population values that fell outside of the 95% confidence limits generated by the lake values. Genetic diversity (A and  $H_E$ ) was estimated in river populations with and without long-distance migrants included to determine the effect that long-distance dispersal has on genetic diversity during colonization. Low genetic diversity can

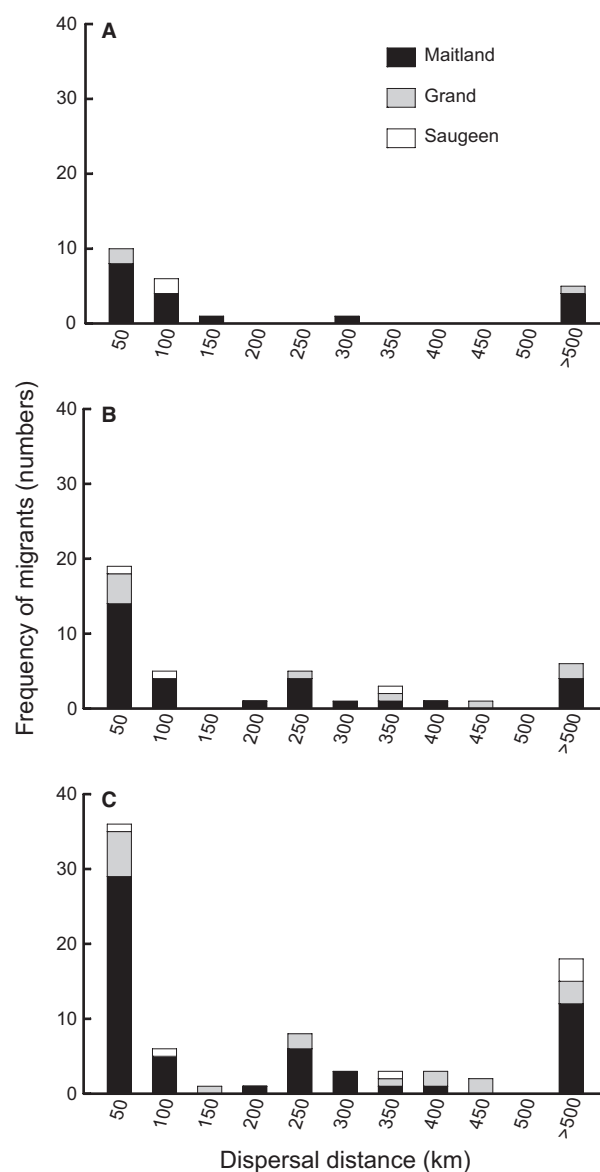


**Fig. 2** Dispersal distance frequency of round goby, *Neogobius melanostomus*, river migrants from the Maitland River (2005–2009), Grand River (2008–2009) and Saugeen River (2009) that successfully assigned to a source population/region by genotype assignment (Rannala & Mountain 1997). Migrants were identified by genotype exclusion using (A) 5%, (B) 10% and (C) 20% exclusion threshold values.

result from founder and population bottleneck events and is expected to be the result of recent colonization.

Relatedness was estimated within lake and river populations (with migrants excluded) to determine whether there is a family effect associated with upstream dispersal (e.g. dispersal genotype). Pairwise relatedness (Queller & Goodnight 1989) between individuals was estimated within each lake ( $n = 40$ ) and river population ( $n = 10$ ) in Genalex 6.1 (99 permutations, 99 bootstraps;

Peakall & Smouse 2006). Within-population mean relatedness ( $r$ ) was calculated from pairwise relatedness between individuals. We tested for differences in relatedness between lake and river populations (with migrants excluded) by examining whether point estimates of  $r$  within river populations are within the 95% confidence interval (CI) derived from the lake values. River estimates outside of the 95% CI would be considered significantly different based on a 5% error threshold.

### Temporal stability

Because the round goby river invasion process is both recent and highly dynamic, we expected the river populations to show temporal instability in spatial distribution and genetic structure in the two rivers for which we had temporal replicates (Maitland and Grand Rivers). To examine temporal stability in spatial distribution, GPS coordinates were recorded at each sampling site within each river. The shortest water distance separating the river mouth and the uppermost invasion front site was measured in Google Earth and compared among years in the Maitland River (2005–2009) and Grand River (2008–2009). Temporal stability in allele frequency distribution was tested by performing exact tests within river populations (Maitland 2005–2009, Grand River 2008–2009) across adjacent years in TFPGA (Miller 1997). Significance was Bonferroni corrected to account for multiple comparisons.

### Results

A total of 1262 round gobies were genotyped (2005: 44; 2006: 144; 2007: 160, 2008: 390; 2009: 524; Table 1). All eight microsatellite markers were polymorphic with four to 18 alleles per locus. Departures from Hardy–Weinberg equilibrium were found in 21 of the 464 loci by sampling site comparisons. The majority of departures were found within Maitland River sites (19/21: 90%), and the remaining two departures were found in 2009-GR1 and 2009-SL1 (Site abbreviations are as shown in Table 1). The majority of Hardy–Weinberg deviations were because of heterozygote deficiency (12/21: 57%). We ran Microchecker 2.2.3 (van Oosterhout *et al.* 2004) and identified the possibility of null alleles. Other potential causes of Hardy–Weinberg disequilibrium include small sample size in some of the river sites as well as population substructure in the Maitland lake site. Significant linkage disequilibrium was observed between two locus pairs with overall significance driven by one significant population comparison each (Nme3–Nme10: 2007-MR3; Nme2–Nme9: 2009-ML1). We recovered 76 alleles from all river sites and 65

alleles from lake sites (lake sites associated with the sampled rivers) with an overall mean of 10 alleles per locus. Lake and river sites share 63 alleles with two of 65 (3%) lake alleles only found in lake sites and 13 of 76 (17%) river alleles only found within river sites. The number of private alleles identified ranged from zero to one in the lake sites and zero to two in river sites.

### Genetic structure

The highest negative  $\ln$  probability values ( $-\ln P$ ) for individual genotype clustering within each river in each year inferred one cluster in the Maitland River in each sampling year (2005–2009) and one cluster in the Saugeen River (2009). We thus combined all of the Maitland and Saugeen river sites into a single ‘river population’ (MR and SR, respectively) within each year for subsequent analyses. Two clusters were identified in the Grand River in each sampling year (2008 and 2009). The spatial distribution of Grand River clusters was similar across years and corresponds to an upper and lower river distribution. River sites GR1 and GR2 were combined into a lower Grand River population (GR<sub>LOW</sub>) in each year. River sites GR4 to GR8 were combined to make the upper Grand River population (GR<sub>UP</sub>) in each year. Fish from river site GR3 had a mixed membership among GR<sub>LOW</sub> and GR<sub>UP</sub> in each year and were assigned accordingly. In 2008, two fish from GR3 clustered with GR<sub>LOW</sub> and two fish clustered with GR<sub>UP</sub>. In 2009, three fish from GR3 clustered with GR<sub>LOW</sub> and two fish clustered with GR<sub>UP</sub>.

Pairwise  $F_{ST}$  values for genetic divergence among sampling sites within a sampling year ranged from 0 to 0.25 (Table S2, Supporting information). All pairwise  $F_{ST}$  estimates among sites in the Maitland and Saugeen River system were nonsignificant and sometimes negative (negative values were nonsignificant and reported as zero). For the Grand River sites, 23 of 81 (28.4%) pairwise comparisons were significant after Bonferroni correction (Table S2, Supporting information). Significant  $F_{ST}$  comparisons generally correspond to differentiation among upper and lower Grand River sites in 2009, mirroring our genotype clustering results.

### Upstream dispersal

The results of the individual genotype exclusion identified seven of 366 (1.9%) lake fish and 65 of 896 (7.3%) river fish that were excluded from the lake or river population (river populations defined by genetic structure analyses) in which they were captured and therefore were likely first-generation migrants to that population (Table 1). Six migrants were identified in the Maitland lake population in 2007–2009 (ML1: 2007  $n = 1$ , 2008

$n = 3$ , 2009  $n = 2$ ). One migrant was identified in one Grand River lake population (GL1: 2009). No migrants were identified in the Saugeen lake population (SL1). River migrants were identified at all sampling sites in the Maitland River population (MR) with the exception of the uppermost upstream site (MR9-2009; Fig. 3). The frequency of migrants in the Maitland River within each year ranged from 2.3% (2005:  $n = 1$ ) to 9.6% (2008:  $n = 19$ ; Table 1). Migrants were identified at two of three sites (SR1:  $n = 4$ , SR3:  $n = 1$ ) in the Saugeen River population (SR) and made up 7.4% of all samples collected from the Saugeen River (Table 1; Fig. 3). The lower Grand River population (GR<sub>LOW</sub>) was composed of 6.8% ( $n = 4$ ) migrants in 2008 and 5.6% ( $n = 7$ ) migrants in 2009, collected from sites GR1 and GR2 in each year (Table 1; Fig. 3). Interestingly, no migrants were detected in the upper Grand River population (GR<sub>UP</sub>) in either sampling year (2008 and 2009; Fig. 3). We present the number of river migrants from 2008 and 2009 only as these represent years that intensive sampling was conducted (Fig. 3). The cross-tabulation analyses revealed a significant difference between migrant loads in lake and river populations in the Maitland River ( $P = 0.010$ ,  $\chi^2 = 1.02$ , d.f. = 1) and the Grand River ( $P = 0.002$ ,  $\chi^2 = 9.87$ , d.f. = 1). In both rivers, the migrant load was higher than in the corresponding lake population (Maitland: 49/612 (8.0%) river migrants and 6/221 (2.7%) lake migrants; Grand: 11/104 (10.6%) lower river (GR<sub>LOW</sub>) migrants and 1/126 (0.8%) lake migrants). No significant difference in migrant load was detected between the lake and river population in the

Saugeen River, although our sample size was small ( $n = 87$ ; lake:  $n = 19$ , river:  $n = 68$ ).

Assignment to a specific population or region was achieved for three of seven (42.9%) lake migrants and 23 of 65 (35.4%) river migrants, based on our assignment criteria (the score for the first-ranked population is four times greater than the score for the second-ranked population). Genotype assignment indicated that 18 migrants (lake:  $n = 1$ , river:  $n = 17$ ) assigned to source populations located within 150 km of their site of capture, three migrants (lake:  $n = 2$ , river:  $n = 1$ ) assigned to a source approximately 205–270 km away and five river migrants assigned to source populations located over 500 km away by shortest water distance (Fig. 2). Migrants from the Maitland populations (lake and river) that successfully assigned to a source lake population ( $n = 21$ : lake  $n = 3$ , river  $n = 18$ ) were assigned to populations in the western basin of Lake Erie ( $n = 4$ : lake  $n = 2$ , river  $n = 2$ ), eastern Lake Erie (river  $n = 3$ ), eastern Lake Ontario (river  $n = 1$ ) and Lake Huron ( $n = 13$ : lake  $n = 1$ , river  $n = 12$ ). Successful assignment of the Grand River migrants to a lake population ( $n = 3$ ) assigned to populations in eastern Lake Erie ( $n = 2$ ) and Lake Huron ( $n = 1$ ). Migrants to the Saugeen River that successfully assigned to a source population ( $n = 2$ ) assigned to a population in Lake Huron close to the mouth of the Maitland River ( $n = 2$ ). Assignment results should be interpreted with caution as four of seven (57.1%) lake migrants and 19 of 65 (29.3%) river migrants failed to assign to any potential source population based on our assignment criteria.

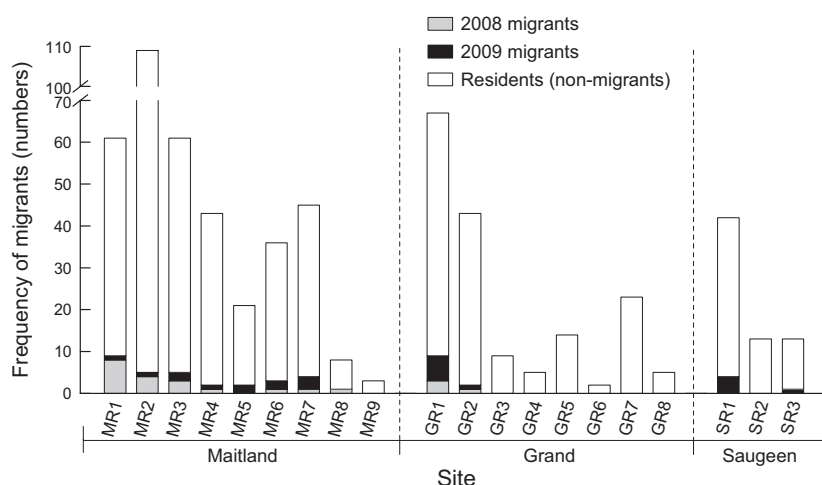


**Fig. 3** Number of round goby, *Neogobius melanostomus*, migrants identified by genotype exclusion (Rannala & Mountain 1997) in the Maitland, Grand and Saugeen river populations (river populations as determined by cluster analysis) in 2008 (grey bars) and 2009 (black bars; Saugeen: 2009 only). Residents (nonmigrants: white bars) represent fish whose genotype was not excluded from the river population based on a 5% exclusion threshold. Downstream to upstream sites within each river population are positioned from left to right. Refer to Table 1 for site abbreviations.

Failed assignment most likely occurred because the source population was not sampled as 23/46 (50%) unassigned migrants were excluded from all lake populations ( $n = 40$ ) based on a 5% error threshold. Round goby lake populations have significant genetic structure in the Great Lakes which may occur at a smaller spatial scale than our 40 baseline source populations were sampled (Dufour 2007; Brown & Stepien 2009). Additionally, the 40 putative source populations primarily represented the mid- to lower Canadian regions of the Great Lakes (Lake Huron, Lake Erie and Lake Ontario; Dufour 2007).

Seven of 10 river populations (with migrants excluded) successfully assigned to their associated lake population and therefore were most likely established by contiguous dispersal from the lake. River populations that assigned to their nearby lake population included all Maitland River populations (2005–2009) and the two lower Grand River populations (GR<sub>LOW</sub>: 2008 and 2009). Group assignment scores were high and ranged from 97.2% (GR<sub>LOW</sub> 2009) to 99.9% (MR 2005, 2006, 2008). Of the remaining three river populations, one was assigned to a lake population within 150 km (SR 2009: assignment score 99.9%), one was assigned to a lake population located over 150 km (shortest water distance) from the river mouth (GR<sub>UP</sub> 2008: assignment score 99.99%) and one river population failed to assign to any of the available source populations (GR<sub>UP</sub> 2009). The Saugeen River population (SR 2009) successfully assigned to a Lake Huron lake population located near the mouth of the Maitland River (89 km shortest water distance) and therefore was considered established by regional diffusion. The 2008 upper Grand River population (GR<sub>UP</sub>) also assigned to the Lake Huron population near the mouth of the Maitland River. The shortest water distance separating these two populations (GR<sub>UP</sub> and its putative source population in Lake Huron) is approximately 561 km suggesting that the upper Grand River population (2008 only) was established by a long-distance dispersal event. The most likely source population for the 2009 upper Grand River population (GR<sub>UP</sub>) was also the Lake Huron population near the mouth of the Maitland River, although the assignment score was relatively low (59.6%) and was not four times higher than the second-ranked source population (40.4%; located in the western basin of Lake Erie near Colchester, Ontario).

### Genetic diversity

Mean allelic richness (A), calculated as a mean of all loci after rarefaction to 12 individuals, within lake and river populations (river populations as defined by genetic structure analysis) ranged from 2.84 to 5.08 with

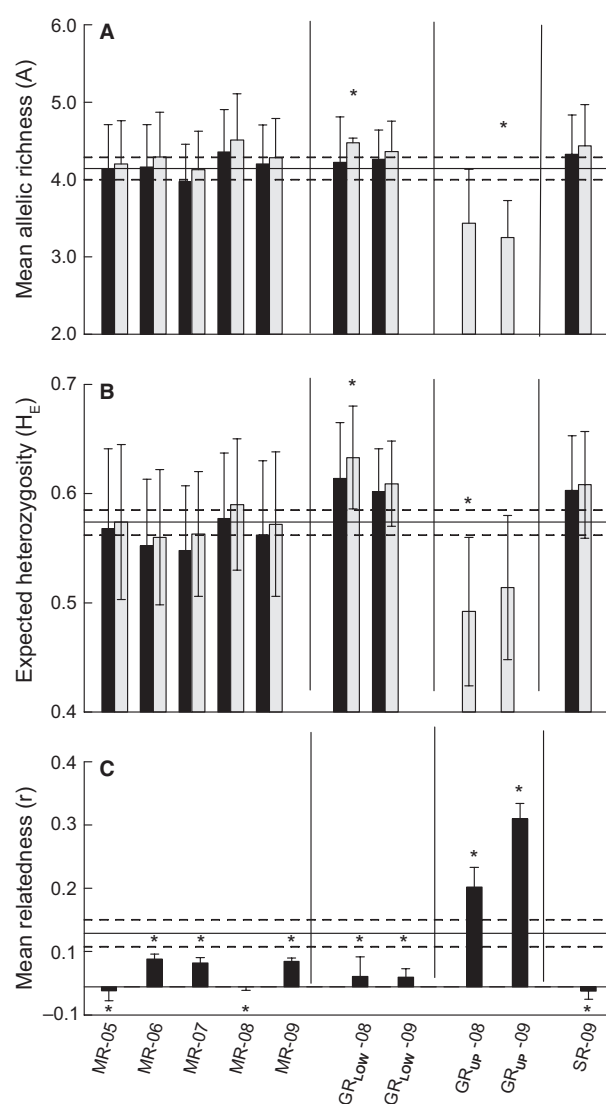


**Fig. 4** Genetic diversity, as measured by (A) mean allelic richness, after rarefaction to 12 individuals to account for sample size differences, (B) expected heterozygosity ( $H_E$ ) and (C) mean relatedness ( $r$ ), in round goby, *Neogobius melanostomus*, populations in the Maitland, Grand and Saugeen Rivers. River population values represent 'with' (grey bars; A and  $H_E$  only) and 'without' (black bars) migrants. Mean (solid line) and 95% confidence interval (dashed lines) represent expected values as calculated from 40 baseline lake populations. Asterisk (\*) indicates a significant difference between the river and generalized lake population. Site abbreviations are as indicated in Table 1.

a global mean A of 4.14. Expected values as determined by the mean A (4.14) of the lake populations ( $n = 40$ ) bounded by the 95% confidence interval ranged from 4.00 to 4.29. Mean allelic richness (A) within river populations (with migrants removed) was 4.03 and ranged from 3.25 (GR<sub>UP</sub> 2009) to 4.36 (MR 2008: Fig. 4A). When

migrants were included, mean  $A$  within river populations was 4.14 and ranged from 3.25 (GR<sub>UP</sub> 2009) to 4.51 (MR 2008). One of 10 river populations (GR<sub>LOW</sub> 2008) had significantly higher  $A$  than the lake values when migrants were included, and one river population (GR<sub>UP</sub> 2009) had significantly lower  $A$  than the lake values (Fig. 4A).

Mean  $H_E$  in the lake and river populations ranged from 0.49 to 0.64 (global mean: 0.57). Mean  $H_E$  for the lake populations ( $n = 40$ ) was 0.57 and 95% confidence interval ranged from 0.56 to 0.59. Mean  $H_E$  in river populations with migrants removed was 0.56 and ranged from 0.49 (GR<sub>UP</sub> 2008) to 0.61 (GR<sub>LOW</sub> 2008; Fig. 4B). When long-distance migrants were included in river populations, mean  $H_E$  was 0.57 and ranged from 0.49 (GR<sub>UP</sub> 2008) to 0.63 (GR<sub>LOW</sub> 2008). One of 10 river populations (GR<sub>LOW</sub> 2008) had significantly higher  $H_E$  and one river population (GR<sub>UP</sub> 2008) had a significantly lower  $H_E$  than the lake populations when migrants were included (Fig. 4B).

Genetic diversity ( $A$  and  $H_E$ ) was elevated in all river populations (with the exception of GR<sub>UP</sub> that had no long-distance migrants) when migrants were included compared to when migrants were removed from the analyses, although the difference was not statistically significant.

### Relatedness

Mean within-population relatedness ( $r$ ) values ranged from  $-0.047$  to  $0.325$  in the lake and  $-0.008$  to  $0.298$  in the river populations (with migrants removed). All river population estimates were outside the 95% confidence interval for the lake populations (Fig. 4C). Mean relatedness was significantly higher when compared to the generalized lake population in two of 10 river populations (GR<sub>UP</sub> 2008–2009). The remaining eight of 10 river populations (MR 2005–2009, GR<sub>LOW</sub> 2008–2009, SR 2009) had significantly lower within-population mean relatedness compared to lake values.

### Temporal stability

The temporal stability in spatial distribution of round goby river populations that were colonized from the lake (i.e. contiguous dispersal) was examined across five years (2005–2009) in the Maitland River and two years (2008–2009) in the Grand River. In 2005, the uppermost river site (MR4) in the Maitland River colonized by round gobies from the lake (i.e. contiguous dispersal) was located 2.0 km from the river mouth at Goderich, ON (Table 1; Fig. 1). By 2009, river colonization by contiguous dispersal had advanced upstream to site MR9 located approximately 4.0 km from the

river mouth (Table 1; Fig. 1). Round goby colonization of the Maitland River expanded at a rate of approximately 500 m/year. No disjunct populations were identified in the Maitland River upstream from the natural invasion front in any sampling year. The uppermost river site in the Grand River that was colonized by contiguous dispersers from the lake was located approximately 8.3 km (GR3) from the river mouth at Port Maitland, ON in 2008 (Table 1; Fig. 1). Contiguous dispersers were not identified upstream from site GR3 in 2009 suggesting that the round goby population is relatively stable or moving at a rate undetectable by our sampling regime.

Exact tests of temporal change in allele frequency distribution within the Maitland River population across subsequent years returned one significant pairwise comparison (MR 2008–2009;  $P = 0.00$ ,  $\chi^2 = 56.77$ , d.f. = 16). Pairwise comparisons of allele frequency distribution in the Grand River populations (GR<sub>LOW</sub> and GR<sub>UP</sub>) between the two sample years (2008–2009) were nonsignificant.

### Discussion

Colonization events provide unique opportunities to examine invasion dynamics associated with expanding populations. An expanding population can therefore yield valuable insight into mechanisms associated with colonization success and hence range expansion. Genetic methods have allowed researchers to characterize mechanisms of dispersal during colonization which has led to an increase in studies reporting stratified dispersal as a mechanism facilitating secondary range expansion (Colautti *et al.* 2005; Parisod & Bonvin 2008; Darling & Folino-Remo 2009). Additionally, modelling approaches have emphasized the importance of stratified dispersal in secondary spread (e.g. Muirhead *et al.* 2006). The results of our study suggest that round goby river colonization proceeds by a stratified dispersal strategy; contiguous and long-distance dispersal both operating together.

Contiguous dispersal moves individuals into adjacent territory at a rate of spread relative to the population growth rate multiplied by a diffusion coefficient (Shigesada *et al.* 1995). Genetic panmixia and nonequilibrium processes are typical in the early stages of colonization by contiguous dispersal with a stepping stone pattern of genetic structure developing over time as population expansion proceeds. We identified contiguous dispersal from the lake as the primary mechanism of upstream expansion on a local scale. A temporally unstable spatial distribution led to a constant rate of spread across years. Despite spatial instability, the genetic structure of the colonizing populations was

temporally stable. Genetic stability during colonization was unexpected but may be explained by expansion dominated by fish from the lake population. Alternatively, downstream movement of fish from the river invasion front (e.g. larvae being swept downstream by the river flow) may have contributed to the lack of genetic structure observed among the sample sites in the rivers. Juvenile round goby are benthic and lacks a pelagic larval phase, although they do vertically migrate at night to feed (Hensler & Jude 2007). There may thus be passive downstream dispersal in rivers; however, higher allelic richness in rivers suggests that downstream displacement of river fish is not a dominant force distributing alleles uniformly between lake and river populations. It is more likely that river colonization and genetic panmixia are the results of recent and ongoing upstream colonization dominated by multiple genotypes from the lake population. These results suggest that there is a fundamental difference between round goby dispersal patterns reported for the Great Lakes (Brown & Stepien 2009) and local dispersal associated with river colonization. Substantial genetic structure and genetic differentiation among Great Lakes round goby populations was reported early in their invasion of the Great Lakes (Stepien & Tumeo 2006) suggesting human activity was responsible for their rapid spread and regional distribution (see Wolfe & Marsden 1998) followed by limited gene flow postestablishment. In the current study, population expansion by contiguous dispersal suggests round gobies are undergoing secondary range expansion naturally. Dispersal from the range margin is expected to be under strong selective forces (Dytham 2008) that can act to limit range expansion because of unsuitable habitat and physical barriers to dispersal. Rivers act as dispersal barriers for species limited to passive movement as downstream flow prevents upstream migration. The hydrodynamic flow regime of rivers can also act to limit dispersal among species employing an active dispersal strategy as sufficient swimming ability is required to hold their position and to move within high flow stochastic environments (Langerhans 2008). The recent colonization of river systems by round gobies through natural dispersal suggests that the costs associated with dispersing through such dispersal barriers have been reduced (Bonte *et al.* 2010), although the mechanism of that reduction is not clear.

A significantly higher migrant load was identified in river populations associated with contiguous dispersal compared to the associated lake population. Assignment of river migrants resulted in a wide range of dispersal distances, suggesting a combination of short- and long-distance dispersal vectors. Peaks in the frequency distribution of migrant dispersal distance likely repre-

sent a combination of natural dispersal (shorter dispersal distance) and human activity (longer dispersal distance); however, distinguishing between dispersal vectors remains speculative at this point. Migrant path analysis based on genotype assignment is limited by the level of genetic divergence among the putative source populations. However, the round goby display surprisingly high levels of genetic structure in the Great Lakes (Dufour 2007; Brown & Stepien 2009), which allows robust migrant dispersal identification, at least at the regional level where geographically close populations are not differentiated. Darling & Folino-Rorem (2009) proposed stratified dispersal as the mechanism responsible for the genetic structure patterns observed in the invasive hydrozoan, *Cordlophora*, in the Great Lakes. It was suggested that natural current-driven dispersal was responsible for local-scale colonization while long-distance dispersal was human mediated because of vessel movement through the Great Lakes. On the other hand, a combination of multiple natural dispersal mechanisms (i.e. pollen and seed dispersal) in the European wildflower, *Biscutella laevigata*, has resulted in short- and long-distance dispersal into marginal populations (Parisod & Bonvin 2008). Although the current study focuses on dispersal at a local-scale, river colonization by a dual-dispersal strategy suggests that founder effects may be mitigated by gene flow from distinct source populations (Parisod & Bonvin 2008).

A colonizing population is expected to have lower genetic diversity compared to its source population because of a limited number of founders (Ciosi *et al.* 2008; Darling & Folino-Rorem 2009; Peacock *et al.* 2009; Ramakrishnan *et al.* 2010). Genetic diversity is expected to recover over time as additional dispersers migrate into the new population and gene flow increases (Ibrahim *et al.* 1996, Brown & Stepien 2008; Parisod & Bonvin 2008; Darling & Folino-Rorem 2009). River habitats are located at the periphery of the round goby Great Lakes distribution and therefore were expected to have low genetic diversity as a result of limited gene flow because of population fragmentation and drift effects associated with small founding populations. Indeed, lower genetic diversity was observed in one river population (GR<sub>UP</sub>); however, this can be attributed to a long-distance founding event that established a relatively isolated upstream population. Population isolation of GR<sub>UP</sub> is also supported by significantly higher relatedness compared to the generalized lake population. In the remaining river populations (those associated with contiguous dispersal), there was no reduction in genetic diversity relative to expected values from the generalized lake population. In fact, one river population (GR<sub>LOW</sub> 2008) had significantly higher genetic diversity relative to the mean lake values when

long-distance migrants were included in the analyses. A previous study on round goby Great Lakes populations reported high genetic diversity, similar to that observed in the native Ponto-Caspian region, but also identified reduced genetic diversity in some peripheral expansion areas indicating secondary founder effects (Brown & Stepien 2009). In all cases, when long-distance migrants were included in our analyses, genetic diversity was elevated, as expected, which resulted in 17% of river alleles only recovered from river populations (i.e. private alleles). High genetic diversity in peripheral expanding populations has previously been attributed to stratified dispersal in which long-distance dispersal facilitates gene flow from genetically distinct populations (Roman & Darling 2007; Parisod & Bonvin 2008; Brown & Stepien 2009). In a study investigating local-scale colonization dynamics, a cline in genetic diversity was observed within a core region with decreasing diversity towards the edge, as expected from multiple founding events during postglacial recolonization (Parisod & Bonvin 2008). However, when marginal regions were compared to the core region, no difference in genetic diversity was observed. Genetic structure among recently established populations in peripheral regions, combined with evidence for short- and long-distance dispersal, suggested the maintenance of genetic diversity at the periphery was because of stratified dispersal and population admixture processes during expansion. Therefore, despite the potential diversity reducing effects of dispersal and colonization, stratified dispersal can replenish genetic diversity in peripheral populations through direct migration into the new population and indirectly through population coalescence following establishment. Lower relatedness in the river populations makes it unlikely that the observed variation in genetic diversity is because of family dispersal biases. Long-distance dispersers make up a substantial component of river populations and thus have probably been supplementing river genetic diversity for some time as migrants become naturalized into the population. Gene flow from long-distance dispersal may provide the genetic diversity necessary to overcome bottlenecks and facilitate rapid colonization of a presumably nonideal habitat (Kolbe *et al.* 2004; Stepien *et al.* 2005).

We identified two genetic clusters in the Grand River that coincided with the spatial distribution of fish in upper and lower portions of the river. Group assignment to a source population over 500 km away indicates that the upper population (GR<sub>UP</sub>) likely originated from a separate, long-distance introduction. Interestingly, migrants were not detected in GR<sub>UP</sub> in either year. The absence of recent migrants, high relatedness and low diversity suggests that GR<sub>UP</sub> is a founding

population that remains relatively isolated from the downstream river and lake populations. Despite round goby possession and transportation bans, the practice of using round gobies as bait may still occur, and therefore, 'bait bucket' transfer may explain the disjunct upstream population in the Grand River (GR<sub>UP</sub>). Alternatively, natural dispersal vectors such as avian piscivores may contribute to movement of round goby, although dispersal from only nearby populations would be expected. Natural long-distance dispersal by round gobies may also contribute to the establishment of disjunct populations, as a previous mark-recapture study identified a single fish that dispersed considerably farther than the majority of the population (Wolfe & Marsden 1998). In our study, the long-distance dispersal most likely reflects human activity, and therefore, management efforts promoting public awareness, stewardship and compliance to regulations should be pursued immediately to reduce unwanted introductions into new river areas and to mitigate gene flow among established populations. Geographic distance and physical barriers to dispersal (i.e. river weirs) may facilitate further isolation of GR<sub>UP</sub> but are not expected to prevent dispersal as fishways connect the lower, middle and upper reaches of our Grand River study region. Indeed, genetic clustering results revealed that site GR3 had a mixed membership from the upper and lower Grand River populations. The location of GR3 is immediately upstream from the first potential dispersal barrier, the Dunnville dam, a weir equipped with a Denil fishway. Admixture of fish from GR<sub>LOW</sub> and GR<sub>UP</sub> at site GR3 suggests that the Dunnville dam is a permeable barrier which may eventually lead to further population coalescence. Reid *et al.* (2008) reported low genetic divergence among populations of the black redhorse (*Moxostoma duquesnei*) separated by weirs in the Grand River. Unlike black redhorse, round goby dispersal capacity is expected to be low (Wolfe & Marsden 1998; Ray & Corkum 2001), yet is sufficient to allow population connectivity across this potential dispersal barrier. It should be noted that if round goby populations can penetrate rivers beyond physical barriers such as weirs, the Saugeen River population is poised at Denny's dam (SR3) for further upstream expansion.

Multiple introductions have been proposed as a mechanism to facilitate the successful establishment of introduced species (Colautti *et al.* 2005; Stepien *et al.* 2005; Stepien & Tumeo 2006; Darling & Folino-Rorem 2009; Bjorklund & Almqvist 2010). Similarly, multiple dispersal vectors into a recipient population will increase propagule pressure during colonization as well as supplement genetic diversity through gene flow from genetically distinct source populations. In the current study, we demonstrate that a multiple dispersal strategy

can facilitate colonization of new territories and can supplement genetic diversity in expanding populations. Contiguous diffusion dispersal acts to expand a species range on a local scale and can be an important mechanism of range expansion into new territory, while long-distance dispersal can increase, or at least maintain, genetic diversity in an expanding population through gene flow from distant source populations. We therefore predict that round goby populations will continue to spread upstream in river environments possibly at an increasing pace. Physical dispersal barriers within river corridors are expected to slow upstream range expansion yet long-distance introductions as well as gradual diffusion dispersal across such barriers can occur. We have established baseline information on the round goby river invasion front in three Great Lakes tributaries and identified contiguous dispersal as the predominant mechanism of upstream expansion. River colonization is recent and dominated by fish from the nearby lake population. Although complete eradication of round goby populations is not possible, a rapid response strategy incorporating population reduction near the river mouth and physical barriers to upstream dispersal may be suitable to slow the spread in select tributaries (Dimond *et al.* 2010).

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This work resulted from the graduate thesis research of J.E.B. and B.A.D. J.E.B. continues to work on invasive aquatic species with the Department of Fisheries and Oceans, Canada, while B.A.D. is an environmental consultant. D.M.H. studies sensory physiology in a variety of fish taxa, and his research program includes a broad physiological examination of the invasive round goby. D.D.H. has interests in the evolutionary and ecological implications of dispersal in fishes, and uses genetic approaches to address conservation and management issues.

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## Supporting information

Additional supporting information may be found in the online version of this article.

**Table S1** Site, location, GPS coordinates and sample size (n) for 31 baseline round goby, *Neogobius melanostomus*, lake populations from Lake Erie, Ontario and Huron used as reference populations for genotype assignment of river migrants (Dufour 2007).

**Table S2** Pairwise comparisons of Weir & Cockerham's (1984)  $F_{ST}$  among round goby, *Neogobius melanostomus*, sampling sites at the Maitland (2005–2009), Grand (2008–2009) and Saugeen (2009) Rivers.

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