

Source–sink dynamics sustain central stonerollers (*Campostoma anomalum*) in a heavily urbanized catchment

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SUMMARY

1. The influence of spatial structure on population dynamics within river–stream networks is poorly understood. Utilizing spatially explicit analyses of temporal genetic variance, we tested whether persistence of central stonerollers (*Campostoma anomalum*) reflects differences in habitat quality and location within a highly modified urban catchment in southwestern Ohio, U.S.A.
2. Estimates of genetic diversity did not vary with habitat quality. Nevertheless, evidence of weak but temporally stable genetic structure, location-dependent effective population sizes and rates of immigration among sites, together suggest that persistence of central stonerollers within the catchment may be attributable to source–sink dynamics driven by habitat heterogeneity.
3. Under this scenario, migrant-pool colonization from areas of relatively high habitat quality in the upper catchment sustains the presence of central stonerollers at degraded sites in the main stem and dampens population subdivision within the catchment. However, because intact habitat is restricted to the upper portion of the catchment, it is not possible to preclude net downstream dispersal as a mechanism contributing to source–sink dynamics. The slight genetic structure that persists appears to reflect weak isolation by distance diminished by high rates of immigration.
4. This study suggests that without a systems perspective of the conditions that sustain populations in degraded waterways, environmental assessments may underestimate levels of impairment. Conservation and management of stream fishes could be improved by maintaining habitat in areas that are net exporters of migrants or by remediation of impaired habitat.

Keywords: aquatic biological assessment, conservation, effective population size, migration, population genetics

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Introduction

Relating local demographic processes to spatial structure (e.g. habitat heterogeneity) is essential for understanding population and species persistence (Hanski & Gilpin, 1997; Fagan, 2002). Yet few studies have tested general hypotheses about the importance of

spatial patterns in determining population dynamics within river–stream networks (Lowe, Likens & Power, 2006). Metapopulation studies of stream biota such as freshwater fishes often relate site occupancy to the hierarchical structure of river–stream networks (Dunham & Rieman, 1999; Taylor & Warren, 2001), but rarely consider how colonization varies according to connectivity, dispersal geometry and spatial scale (Pannell & Charlesworth, 1999; Fagan, 2002; Lowe *et al.*, 2006). Similarly, studies focusing on movement of stream fishes often identify habitat and conditions that favour or constrain dispersal (Power, 1984; Skalski & Gilliam, 2000), but few effectively link movement patterns to the spatial distribution of source and sink habitats (Lowe *et al.*, 2006).

Urban catchments are natural laboratories for examining population persistence and dispersal of stream fishes. Runoff, physical habitat and flow regime modifications can lead to highly altered stream fish assemblages reflecting heterogeneous loss of diversity and persistence of pollution-tolerant species (Roy *et al.*, 2005). Variation in assemblage structure can be examined to relate site occupancy to habitat quality and spatial location (Power, 1984; Taylor, 1997; Gotelli & Taylor, 1999). Even in the most degraded and biologically depauperate ecosystems, it is possible to learn more about persistence and colonization from patterns of genetic variation among populations of remaining pollution-tolerant fishes. Urbanization can influence genetic variation of pollution-tolerant species by altering levels of genetic drift, migration, and natural selection (Bickham *et al.*, 2000; Theodorakis, 2003). Patterns of genetic variation may subsequently bear signatures of population persistence and colonization relative to habitat quality, connectivity, dispersal geometry and spatial scale. For example, habitat modifications that reduce passage and restrict gene flow (Hebert *et al.*, 2000) can increase genetic drift within subdivided populations and divergence among populations (Pannell & Charlesworth, 1999) at varying spatial scales. Exposure to urban runoff can select against less tolerant genotypes and consequently alter the size, viability, and genetic diversity of populations (Fox, 1995; Cimaruta *et al.*, 2003).

Prior studies have demonstrated that measurement of spatial genetic variance is a useful approach for examining metapopulation dynamics in stream fishes (Fontaine *et al.*, 1997; McElroy *et al.*, 2003), but

examining both spatial and temporal genetic variance can be more informative (Tessier & Bernatchez, 1999; Garant, Dodson & Bernatchez, 2000; Lundy, Rico & Hewitt, 2000; Hansen *et al.*, 2002; Heath *et al.*, 2002; Alo & Turner, 2005). In addition to validating estimates of population genetic structure (Nielsen *et al.*, 1999), spatially explicit analysis of temporal genetic variation allows more accurate estimation of effective population size (N_e) and immigration rates (m) (Waples, 1989). From a conservation standpoint, N_e and m are two of the most significant parameters influencing genetic variation and therefore the sustainability of populations in heterogeneous environments (Allendorf & Leary, 1986; Pulliam, 1988; Frankham, 1995; Newman & Pilson, 1997; Marr, Keller & Arcese, 2002; Vila *et al.*, 2003). To monitor and assess populations, estimates of N_e and m can elucidate the present condition and project future vulnerabilities of populations (Bagley *et al.*, 2002). Thus, an understanding of the role of N_e and m in maintaining population genetic structure can be critical to management and conservation, especially for small populations inhabiting marginal habitats that are likely to be affected by stochastic events (Pulliam, 1988; Alo & Turner, 2005).

We undertook this study of a moderately pollution-tolerant stream fish within a heavily urbanized catchment to improve basic understanding of metapopulation dynamics in environmentally heterogeneous river–stream networks. Utilizing spatially explicit analysis of temporal genetic variance, we tested whether population persistence reflects habitat quality and location within the catchment. We first examined whether levels of genetic diversity were lower at impaired sites compared to unimpaired locations. Since genetic differences should reflect influences of selection, gene flow or genetic drift, we then assessed whether the distribution of genetic variance was related to habitat quality, geographic proximity of sites, or stream connectivity patterns. We also estimated N_e and m at sites that were sampled multiple times over an 8-year period, with the expectation that estimates of N_e would be smaller and estimates of m would be higher in degraded areas. Finally, we examined spatial and temporal patterns of population subdivision as well as spatial variation in estimates of N_e and m to infer patterns of connectivity and dispersal at different spatial scales.

Methods

Study location and sample collection

The Mill Creek catchment covers 274 km² in south-western Ohio, USA. The basin crosses approximately 45 km of the Cincinnati (OH) urban corridor from its headwaters to the confluence at the Ohio River (Fig. 1). Over 500 000 people live in the drainage area and development is continuing throughout the basin. Mill Creek has been designated a highly impaired waterbody for Aquatic Life Use, Recreation (Primary Contact) and Fish Consumption Advisories (Ohio EPA, 2004a). The system is heavily impacted by industrial and municipal point source discharges, uncontrolled storm water runoff, sewer overflows, and contaminated sediment from industrial discharges, landfills and toxic waste sites (American Rivers, 1997). High pollutant loads, including nutrients, unionized ammonia, metals, surface runoff, organic enrichment, coliform bacteria, pesticides, priority organics and contaminated sediments have been found in Mill Creek water (Ohio EPA, 2004a,b). Many of the waterways also exhibit physical modifications and impaired habitat due to siltation and suspended solids (Ohio EPA 2001, 2004b).

Land use and impairment status is highly variable within the drainage (Ohio EPA, 2004a,b). The upper head waters are dominated by mixed agricultural and residential land use. Riparian corridors in the upper

catchment are mostly intact and in-stream habitats are characterized by coarse substrates and moderate cover. Biotic diversity and abundance are highest in these upper tributaries and head waters. Downstream of the head waters, the catchment contains more residential, urban and light industrial development; fine substrates, stream embeddedness and entrenchment increase, channel sinuosity decreases, and in-stream cover is reduced due to narrow riparian borders. Further downstream, sections of the catchment are heavily industrialized. Former municipal and industrial landfills, including five Superfund sites (abandoned hazardous waste sites which pose a threat to local ecosystems or people and have been identified for priority clean-up), as well as more than 100 combined sewer overflows and 50 sanitary sewer overflows occur in lower areas of the basin. The 13 km segment of the main stem just upstream of the confluence has been channelized and armoured by the US Army Corp of Engineers for flood abatement. The lowest reaches of the catchment typically lack riparian canopy and are characterized by fine, sandy, highly-embedded substrate (Ohio EPA, 2001) and very low fish species diversity and abundance (Ohio EPA, 2004a,b).

The stream fish assemblage in Mill Creek is dominated by pollution-tolerant species, including the central stoneroller (*Camptostoma anomalum*, Rafinesque, 1820), a moderately tolerant cyprinid that is common

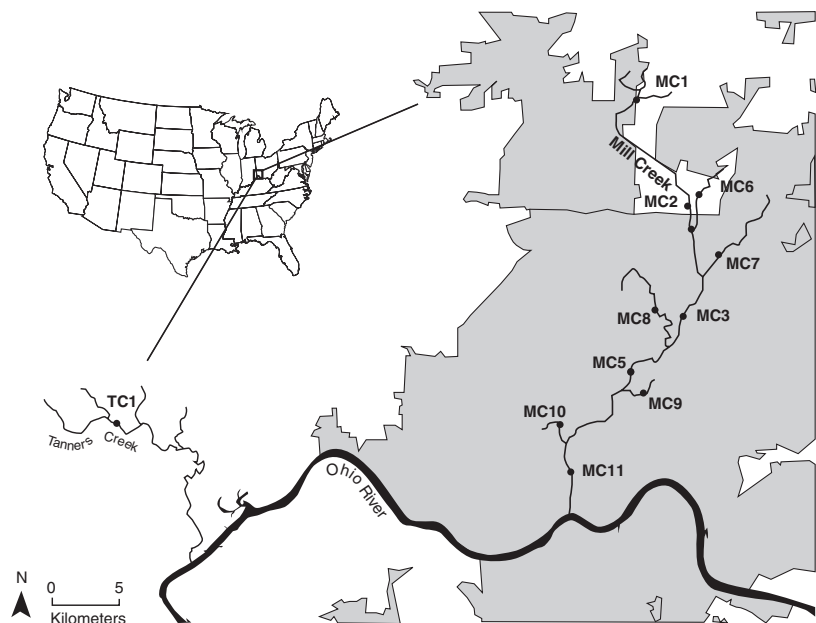


Fig. 1 Location of collection sites in the Mill Creek and Tanners Creek catchments in relation to the Cincinnati metropolitan area (in grey).

in streams of central and eastern North America. Central stonerollers favour runs or riffles in algae-rich streams with clear water, and can dominate stream communities in both numbers and biomass (Lennon & Parker, 1960). As a bottom feeder that prefers filamentous algae, diatoms and detritus, central stonerollers can exert strong direct and indirect effects on stream biota (Power & Matthews, 1983; Power, Matthews & Stewart, 1985). The evolutionary lineage of stoneroller that occurs in Mill Creek is common and relatively abundant in drainages within the Ohio River basin (Blum *et al.*, 2008).

We collected central stonerollers from seven sites in the Mill Creek catchment. Individuals were sampled from four sites along the length of the main stem and from three tributaries. Several attempts to sample stonerollers at three additional sites located in the lower half of the catchment were unsuccessful, one main stem (MC11) and two tributaries (MC9, 10), as these sites were devoid of central stonerollers (Fig. 1, Table 1). For further comparison, we collected samples from a main stem site in Tanners Creek catchment (Dearborn County, Indiana) located to the west of the Mill Creek basin. We categorized the upper headwaters site on Mill Creek (MC1), the most northern tributary site (MC6) and the Tanners Creek (TC) site as unimpaired due to the relatively low urban-industrial activity around these waterways (Fig. 1, Table 1). We categorized the three sites farther downstream on the main stem Mill Creek (MC2, MC3 and MC5) and the two other tributary sites (MC7 and MC8) as degraded. Each site was sampled using minnow traps and backpack electrofishing methods (McCormick & Hughes, 1998). Caudal fins were removed at the caudal peduncle and preserved at

–70 °C for future analysis. We collected samples at sites MC1, MC2, MC3, MC5 and TC during the summer months of 1994, 1995, 2001 and 2002. Sites MC6, MC7 and MC8 were only sampled in 2001 and 2002.

DNA extraction and microsatellite genotyping

Genomic DNA was extracted from 504 fish using a commercial kit (DNeasy; Qiagen, Valencia, CA, U.S.A.) and quantified using a fluorescently-labelled nucleic acid stain (PicoGreen; Molecular Probes). Each individual was genotyped using ten polymorphic microsatellite markers. Nine of the markers (CA2, CA6, CA9–CA14) were previously described (Dimsoski, Toth & Bagley, 2000), and a 10th, CA29 (TAGA₁₁; GenBank accession DQ360110) is described herein (forward primer: 5'-CCTTGCCAGGTGAGAGAAC-TGC-3', reverse primer: 5'-TGGATGGGTTTGCTT-CAGTGG-3'). Thermal cycler (Dyad; MJ Research) parameters were modified from Dimsoski *et al.* (2000) as follows: 1.0 min at 95 °C (1 cycle), 30 s at 95 °C, 30 s at 64 °C, 1.5 min at 72 °C (repeat for 11 cycles decreasing annealing temperature by 0.8 °C/cycle), 30 s at 95 °C, 30 s at 54 °C, 1.5 min at 72 °C (22 cycles), 15 min at 72 °C (final extension). Amplified microsatellites were characterized using a MJ Basestation Genetic Analyzer (MJ Research).

Genetic data analysis

Due to small sample sizes at some of the sites/years, data for 1994 and 1995 were pooled, as were data for 2001 and 2002. An exact test of sample differentiation based on allele frequencies (Raymond & Rousset,

Site	Latitude	Longitude	River segment	Habitat quality
Mill Creek-1 (MC1)	N39°22.720	W84°28.716	Mainstem	Moderate
Mill Creek-2 (MC2)	N39°18.371	W84°26.159	Mainstem	Poor
Mill Creek-3 (MC3)	N39°13.987	W84°26.533	Mainstem	Poor
Mill Creek-5 (MC5)	N39°11.739	W84°29.388	Mainstem	Poor
Mill Creek-6 (MC6)	N39°18.807	W84°25.602	Tributary	Moderate
Mill Creek-7 (MC7)	N39°16.384	W84°24.683	Tributary	Poor
Mill Creek-8 (MC8)	N39°14.204	W84°27.960	Tributary	Poor
Mill Creek-9 (MC9)	N39°10.939	W84°29.449	Tributary	Poor*
Mill Creek-10 (MC10)	N39°09.562	W84°34.188	Tributary	Poor*
Mill Creek-11 (MC11)	N39°08.897	W84°32.803	Mainstem	Poor*
Tanners Creek (TC)	N39°10.172	W84°55.819	Mainstem	Moderate

*Sites devoid of central stonerollers.

Table 1 Collection site coordinates with reference to river segment and habitat quality

1995a,b) performed prior to pooling found no significant differences ($P > 0.05$) between years. For each sample location and composite time period, we determined observed (H_O) and expected (H_E) heterozygosity using GENEPOP version 3.1 (Raymond & Rousset, 1995a,b). GENEPOP version 3.1 was also used to evaluate deviations from Hardy–Weinberg equilibrium (Guo & Thompson, 1992). We used FSTAT version 2.93 (Goudet, 1995) to estimate allelic diversity (A_D) and Nei's (1987) unbiased diversity estimator (H_s). FSTAT version 2.93 was also used to determine whether estimates of genetic diversity (A_D , H_O , H_s) differed among sample sites when grouped by basin and time period. Further comparisons were made among Mill Creek sites grouped by habitat quality, by proximity (grouping sites based on whether they are in the upper or lower catchment), and by connectivity (assuming main stem sites have greater connectivity than tributary sites). The significance of the outcome of each test was determined by comparison of the observed value to 10 000 permutations of samples between groups, with α set at 0.05. We performed spatial and temporal hierarchical analyses of genetic diversity following the analysis of molecular variance model (AMOVA) described by Michalakis & Excoffier (1996) that is implemented in ARLEQUIN 2.001 (Schneider *et al.*, 2000). The spatial AMOVA assessed components of genetic diversity attributable to variance among basins, variance among sites within basins, and variance among individuals within sites for the pooled 2001–02 data only. The temporal AMOVA assessed components of genetic diversity attributable to variance among sites, variance between temporal samples (1994–95 versus 2001–02) within sites, and variance among individuals within sites during each temporal period. We also examined the distribution of genetic variation among groups of sites within Mill Creek categorized by impairment, proximity and connectivity.

ARLEQUIN version 2.001 was used to compute pairwise θ_{st} values (Weir & Cockerham, 1984) under an infinite alleles model to estimate the extent of genetic differentiation among samples from different sites and years. In order to evaluate spatial and temporal genetic differentiation without *a priori* assumptions of genetic subdivision, we used the Bayesian clustering method implemented by STRUCTURE version 2 (Pritchard, Stephens & Donnelly, 2000). This approach probabilistically assigns individuals to populations based on their

genotypes while simultaneously estimating population allele frequencies. We chose a burn-in period of 30 000 iterations and collected data from an additional 10^6 iterations for five sets of replicate runs where K (the number of populations) was set from one to eight. Each run followed a model of no admixture and correlated allele frequencies.

We tested for isolation by distance of central stonerollers within the Mill Creek catchment by comparing estimates of genetic and geographical distances. Pairwise θ_{ST} values were used to represent genetic distances. Geographical distance was expressed as river-kilometres between sampling locations, determined using the Geographic information systems program ARCMAP 8.3 (ESRI, Redlands, CA, U.S.A.). The significance of the correlation was evaluated by a Mantel test (1000 randomizations) with the aid of IBDWS version 3.02 (Jensen, Bohonak & Kelley, 2005).

We determined effective population size (N_e) and immigration rate (m) using the pseudo-maximum likelihood method of Wang & Whitlock (2003). N_e is the size of an idealized population that would lose genetic diversity at the same rate as the actual population, and m is the proportion of non-resident individuals in a population. Unlike other methods for estimating N_e based on temporal changes in allele frequencies (Waples, 1989; Jorde & Ryman, 1995; Anderson, Williamson & Thompson, 2000; Berthier *et al.*, 2002), the temporal method of Wang & Whitlock (2003) does not assume closed populations and allows for migration. The method estimates N_e and m jointly from temporal and spatial data derived from genetic markers including microsatellite loci. The maximum-likelihood method assumes an infinitely large source population providing immigrants into the focal population in which N_e and m are to be estimated. These methods are robust to violations of the assumption and can be applied approximately to a finite source population composed of one or more small subpopulations (Wang & Whitlock, 2003). Using the analysis software, MLNE, we estimated N_e and m for all contemporary sample sites (2001–02) where archived samples were available (1994–95). Allele frequencies for potential immigrants to each focal population in Mill Creek were estimated by pooling genetic data for all samples collected from other sites in the catchment. No estimates were attempted for Tanner Creek as allele frequency data for potential immigrant source populations were not available. In all cases, an

average generation length of 1.5 years was assumed, which translates to the passing of roughly five generations over the time interval investigated (Smith, 1979).

Results

Measures of genetic diversity

All 10 microsatellite loci were polymorphic at each of the sample sites analysed (Table 2). The number of alleles detected at each locus (*A*) varied from 4 at CA12 to 34 at CA10. Within Mill Creek, the mean number of alleles ranged from 7.3 ± 3.7 at MC3 in 1994–95 to 13.1 ± 8.9 at MC1 in 2001–02 (Table 2). Values of H_E ranged from 0.69 ± 0.08 at site MC8 in 2001–02 to 0.75 ± 0.06 at MC5 in 1994–95 (Table 2). All comparisons ($k = 130$) conformed to Hardy–Weinberg

equilibrium after Bonferroni correction except CA9 at site MC3 for 1994/1995 ($F_{IS} = +0.45$, $\alpha = 0.05/130 = 0.0003$). Estimates of genetic diversity (H_S and A_D) did not differ by catchment ($P = 0.10$ and $P = 0.75$, respectively) or by time period ($P = 0.07$ and $P = 0.59$). Estimates of genetic diversity among Mill Creek sites also did not differ by habitat quality ($P = 0.11$ and $P = 0.43$, respectively), upper–lower catchment proximity ($P = 0.11$ and $P = 0.92$), or main-stem-tributary connectivity ($P = 0.19$ and $P = 0.32$).

Temporal versus spatial components of genetic differentiation

Based on the AMOVA of 2001–02 data, an estimated 1.2% ($P < 0.001$) of molecular variance is attributable to differences among catchments (Table 3). Approximately 0.6% of genetic variance occurred among sites

Table 2 Sample sizes (*n*) and genetic diversity statistics for each sampling location

	<i>n</i>	CA2	CA6	CA7	CA9	CA10	CA11	CA12	CA13	CA14	CA29	Mean ± SE
1994–95												
MC1	22	0.84	0.8	0.64	0.6	0.97	0.91	0.39	0.54	0.9	0.94	0.74 ± 0.07
		7	7	7	6	24	12	2	4	18	17	10.4 ± 7.1
MC2	13	0.86	0.8	0.62	0.6	0.96	0.9	0.41	0.22	0.9	0.96	0.7 ± 0.09
		10	5	6	5	18	11	2	2	15	16	9.0 ± 5.9
MC3	28	0.88	0.69	0.59	0.59	0.96	0.91	0.46	0.23	0.88	0.92	0.7 ± 0.08
		10	7	7	6	26	13	2	4	14	17	10.6 ± 7.2
MC5	22	0.87	0.74	0.39	0.63	0.97	0.92	0.47	0.46	0.92	0.95	0.72 ± 0.07
		11	5	5	6	23	13	2	5	18	19	10.7 ± 7.3
TC	23	0.82	0.72	0.53	0.74	0.96	0.89	0.54	0.53	0.91	0.94	0.75 ± 0.06
		8	6	7	10	23	13	3	7	16	15	10.8 ± 6
2001–02												
MC1	80	0.85	0.71	0.59	0.68	0.96	0.91	0.37	0.27	0.9	0.94	0.71 ± 0.08
		12	6	8	9	27	15	3	4	26	21	13.1 ± 8.9
MC2	10	0.74	0.8	0.68	0.74	0.97	0.9	0.43	0.1	0.86	0.92	0.73 ± 0.07
		6	5	6	7	14	9	3	2	10	11	11.7 ± 7.4
MC3	57	0.86	0.75	0.62	0.6	0.95	0.9	0.52	0.31	0.89	0.91	0.7 ± 0.09
		12	6	7	7	26	15	3	5	18	18	7.3 ± 3.7
MC5	45	0.89	0.73	0.58	0.55	0.94	0.91	0.5	0.3	0.89	0.94	0.72 ± 0.07
		13	6	7	9	27	15	3	4	18	21	12.3 ± 7.9
MC6	48	0.86	0.7	0.58	0.7	0.96	0.91	0.38	0.25	0.92	0.94	0.72 ± 0.08
		11	6	8	10	30	15	3	4	23	20	12.9 ± 8.8
MC7	24	0.84	0.69	0.55	0.63	0.97	0.92	0.45	0.3	0.9	0.93	0.71 ± 0.08
		12	6	8	9	28	16	2	4	23	21	10.1 ± 7
MC8	28	0.86	0.76	0.57	0.55	0.94	0.89	0.48	0.23	0.89	0.9	0.69 ± 0.08
		10	6	5	5	23	14	2	3	17	16	9.0 ± 5.2
TC	103	0.83	0.75	0.5	0.69	0.95	0.86	0.4	0.49	0.91	0.93	0.73 ± 0.06
		9	5	8	8	19	9	2	3	13	14	13.0 ± 8.9
<i>A_T</i>		13	8	9	11	34	21	4	9	31	24	

For each locale, the top number under each locus represents the expected heterozygosity (H_E) and the bottom number is the observed number of segregating alleles (*A*). The arithmetic mean and SE for all 10 microsatellite loci also is provided for each site. *A_T* is the number of segregating alleles observed in the total sample.

Table 3 Hierarchical analysis of molecular variance (AMOVA) based on microsatellite allele frequencies for contemporary (2001–02) samples. This analysis includes main stem and tributary sites of Mill Creek

Variance component	d.f.	% Total variance	P-value
Among catchments	1	1.17	0.00012
Among sites within Mill Creek	6	0.59	0.00000
Within sites	782	98.23	0.00000

within Mill Creek ($P < 0.001$) while 98.2% ($P < 0.001$) of genetic variance occurred within sites. A hierarchical analysis of genetic variance based on temporal comparisons of MC1, MC2, MC3, MC5 and TC attributed 1.0% of genetic variance to differences among sites ($P = 0.007$), whereas only 0.1% ($P = 0.21$) of genetic variance was attributed to differences between 1994–95 and 2001–02 (Table 4). This suggests that differences in allele frequencies among sites have been stable over the time interval studied. Additional analyses indicated that consideration of proximity (0.65%, $P < 0.001$) or connectivity (0.25%, $P = 0.97$) did not increase the amount of genetic variance

Table 4 Hierarchical analysis of molecular variance (AMOVA) based on microsatellite allele frequencies for baseline (1994–95) and contemporary (2001–02) samples

Variance component	d.f.	% Total variance	P-value
Among sample sites	4	1.05	0.00683
Among years within sample sites	5	0.13	0.21139
Within site	796	98.82	0.00000

The three Mill Creek tributary sites that were not sampled in 1994–95 were excluded from this analysis.

Table 5 Pair-wise θ_{st} estimates of genetic differentiation among central stonerollers collected at seven sites in Mill Creek (MC) and one site in Tanners Creek (TC)

Samples	MC1	MC2	MC3	MC5	MC6	MC7	MC8	TC
MC1	0.00000							
MC2	0.00707	0.00402						
MC3	0.01053*	0.01473	–0.0009					
MC5	0.01111*	0.01301	–0.002	0.00126				
MC6	–0.0012	0.00339	0.00687*	0.00941*	–			
MC7	0.00873*	0.01064	0.00327	–0.0007	0.00862	–		
MC8	0.00905*	0.01561*	–0.0005	–0.0003	0.00757	0.01017	–	
TC	0.01319*	0.01688*	0.02052*	0.01998*	0.01368*	0.02139*	0.02207*	0.0042

See Fig. 1 for location of sample sites. Diagonal: Pair-wise θ_{st} comparing temporal data where available (1994–95 versus 2001–02). Below diagonal: Pair-wise θ_{st} comparing 2001–02 spatial data.

*Significant value after Bonferonni correction ($P < 0.05$).

attributable to among-site differences within Mill Creek. Consideration of habitat quality only marginally increased the amount of variance attributable to differences among Mill Creek sites (0.7%, $P < 0.001$).

Pair-wise θ_{ST} estimates confirmed that gene flow between basins is lower than gene flow within the Mill Creek catchment. All estimates of genetic differentiation between sites in separate catchments were significantly greater than zero (Table 5). Pair-wise estimates among Mill Creek sites are indicative of weak genetic differentiation rather than panmixia within the catchment. Comparison of pair-wise estimates suggests that sites in the upper catchment (e.g. MC1 and MC6) are slightly differentiated from sites in the lower catchment (Table 5). The observed population structure within the Mill Creek catchment may be partially explained by isolation by distance, as a weak but significant relationship between genetic distance (as measured by pairwise θ_{ST} estimates) and geographical distance (river-kilometres) was found (Fig. 2; $r = 0.4016$, $P_{one-sided} = 0.0378$; slope = 0.0008). However, genetic distances appeared bimodal (Fig. 2) suggesting that the significance of the isolation by distance test may be due to slight substructure between the upper and lower catchment.

Bayesian analysis of subdivision recovered a maximum $\Pr(X | K)$ value at $K = 2$ (Fig. 3a), which suggests that the sampled individuals fall into two populations. Assignment values for each individual at $K = 2$ indicates that the two populations correspond to Mill Creek samples and Tanner Creek samples (Fig. 3b). Although some variation in assignment values was observed among individuals across Mill Creek sample sites, no support was found for

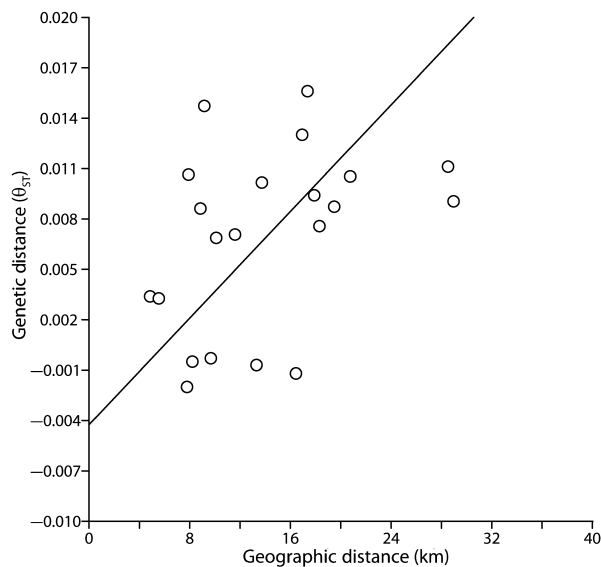


Fig. 2 Genetic distance versus geographic distance among Mill Creek sites sampled during 2001–02. The fitted line represents the associated Reduced Major Axis regression ($y = 7.934 \times 10^{-4} + 4.251 \times 10^{-3}x$, $R^2 = 0.161$).

further spatial or temporal subdivision within either catchment.

Local effective population sizes and immigration rates

Overall, estimates of local effective population sizes for central stonerollers in Mill Creek were relatively low, ranging from 12.4 to 85.2 individuals. In contrast, estimates of immigration rates per generation were moderate to high, ranging from 0.29 to 0.99 (Table 6). Sites MC1 and MC5 supported the largest effective

Table 6 Estimates of effective population size (N_e) and migration rate (m) for main stem Mill Creek sites with corresponding 95% confidence intervals

Site	N_e (95% CI)	m (95% CI)
MC1	85.2 (55.3–139.6)	0.29 (0.21–0.46)
MC2	12.4 (8.7–20.6)	0.99 (0.53–1.00)
MC3	38.7 (28.1–104.7)	0.99 (0.24–1.00)
MC5	86.4 (47.3–111.9)	0.99 (0.43–1.00)

population sizes. Inspection of associated confidence intervals indicates that only one site, MC2, had a significantly smaller effective population size than other sites.

Differences in immigration rate among sample sites in Mill Creek coincide with differences in habitat quality. A relatively low immigration rate of 0.29 was found at MC1, which exhibits moderate quality habitat. In contrast, estimates of immigration rates into the three degraded sites (MC2, MC3 and MC5) were all 0.99, suggesting that these sites are comprised almost entirely of immigrants. However, confidence intervals for immigration rates were large and only those for sites MC1 and MC2 were non-overlapping.

Discussion

Population persistence and habitat quality

The likelihood of population persistence can depend on the quality of available habitat (Hanski & Gilpin,

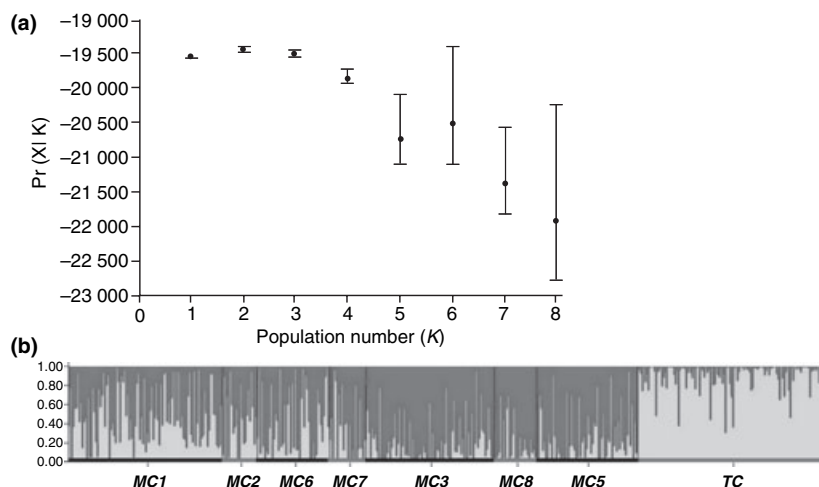


Fig. 3 Bayesian analysis of population structure among Mill Creek and Tanners Creek central stonerollers. (a) Mean and variance of $\Pr(X | K)$ at values of K from 1 to 8. The maximum $\Pr(X | K)$ value was found at $K = 2$. (b) Individual assignment scores resulting from an exemplar run at the maximum $\Pr(X | K)$ value of $K = 2$ distinguishes central stonerollers sampled from Mill Creek from those collected in Tanners Creek.

1997). In an urban catchment like Mill Creek, conditions in highly degraded habitat may result in local extirpation of all but the most tolerant species. Moderately degraded habitat that can support less pollution-tolerant species may still lower individual fitness or select against less tolerant genotypes. Populations of moderately tolerant species, like central stonerollers, might therefore be expected to exhibit lowered genetic diversity in degraded habitats. Low genetic diversity can be indicative of lowered individual fitness (due to inbreeding depression) or lowered population resiliency in the face of environmental change (due to loss of favourable alleles and small effective population size) (Bonnell & Selander, 1974; Allendorf & Leary, 1986; Frankham, 1995; Lynch, 1996; Newman & Pilson, 1997; Saccheri *et al.*, 1998; Westemeier *et al.*, 1998). A prior study of central stonerollers in catchments near Mill Creek found lower genetic diversity within populations occupying poor habitat compared to populations in less impaired habitat (Silbiger, Christ & Leonard, 1998). Gillespie & Guttman (1989) suggested that the allele and genotype frequency shifts they observed in central stoneroller populations were due to selection induced by environmental contaminants. In contrast, we found no relationship between genetic diversity and habitat quality for central stonerollers sampled throughout Mill Creek and from a main stem site in nearby Tanners Creek. Nor did estimates of genetic diversity differ with respect to any other variable, including basin of origin, time, proximity or connectivity. Lack of statistical significance may be due to low power in our study, an unfortunate consequence of low abundances. Attempts to increase the number of sample sites were unsuccessful due to the apparent absence of central stonerollers throughout much of the catchment. Even if levels of genetic diversity do not reflect habitat quality, our results do not exclude the possibility that the likelihood of population persistence is lower in areas of degraded habitat. Immigration and accompanying gene flow from areas with more productive habitat can overwhelm forces that would otherwise result in lower levels of genetic diversity. Even under severe conditions (e.g. when selection is strong or when local populations undergo episodic or cyclical bottlenecks), levels of genetic diversity can be buoyed by high rates of immigration (Ratnaswamy *et al.*, 1999; Keller *et al.*, 2001; McMillan *et al.*, 2006).

Genetic structure of central stonerollers within Mill Creek suggests weak but stable population substructure, as evidenced by the lack of temporal divergence between 1994 and 2002. Pair-wise comparisons reveal slight genetic differentiation between sites located in the upper and lower catchment. While the existing structure coincides with differences in habitat quality, and therefore is consistent with a potential role for selective forces of degraded habitat in the lower catchment, it is also consistent with a simple isolation-by-distance model (Wright, 1943). The high rates of immigration observed in Mill Creek could overwhelm signatures of habitat induced selection. Even if selection for tolerant genotypes generates allele or genotype frequency differences among populations (Gillespie & Guttman, 1989; Bickham *et al.*, 2000; Theodorakis, 2003), levels of genetic differentiation will be small when immigration is high (Gaggiotti, 1996; Gaggiotti & Smouse, 1996; Balloux & Lugon-Moulin, 2002). Further studies on the fitness of central stonerollers from impaired and unimpaired sites would be necessary to determine whether selection is a factor contributing to the genetic differentiation within the catchment.

Estimates of N_e suggest that habitat quality does not strongly inhibit site occupancy (the presence of individuals at a site). The range of the values estimated for central stonerollers in Mill Creek is low, but similar to those derived for populations of brown trout and Rio Grande silvery minnow (Ostergaard *et al.*, 2003; Alo & Turner, 2005) using the same method. While we expected that estimates of N_e would be reduced at impaired sites, we found that N_e was significantly smaller at only one (MC2) of three degraded sites for which we could obtain estimates. It should be noted that relative to other sites within the Mill Creek catchment, multiple, extensive sampling efforts were required here to achieve minimal sample sizes. The small estimated N_e and apparently small census population size at site MC2 is likely due to excessive sedimentation (Ohio EPA, 2004a) as central stonerollers are especially intolerant of silt (Smith, 1979), although other factors could limit carrying capacity or depress N_e at this site as well.

Central stonerollers appear to have little difficulty occupying moderately impaired waterways but differences in immigration rates indicate that poor habitat may limit residency (establishment and persistence of individuals at a site reflecting a sustained

population). Estimates of m were consistently higher for the three impaired sites in Mill Creek relative to unimpaired site MC1. The estimated values of m (e.g. 0.99) at the three impaired sites suggest that nearly all of the central stonerollers at these sites are recent immigrants. Although confidence intervals on these estimates were wide, considered together, estimates of N_e and m at degraded sites in Mill Creek suggest that site occupancy is being sustained under conditions of continuous immigration. Besides habitat modification and siltation, impairment at degraded sites in Mill Creek results from industrial and municipal pollution originating from landfills, hazardous waste sites, combined sewer overflows, raw sewage discharges and urban runoff. Water and sediment studies have detected elevated concentrations of heavy metals, organic compounds such as PCBs, pesticides, ammonia, nutrients and bacteria from sewage contamination (Ohio EPA, 2004a,b). Fish in these areas frequently exhibit external anomalies and have high levels of PCBs in soft tissues (Ohio EPA, 2004a,b). Persistent organic pollutants such as PCBs are potent developmental and reproductive toxicants, with fish being the most sensitive during their early life stages (Peterson, Theobald & Kimmel, 1993). The large N_e and m at degraded sites is consistent with adult survival being relatively less impacted by such toxicants in the Mill Creek catchment.

Specifically, if larval fish are unable to establish residency and persist in the degraded habitat of the lower catchment, high rates of immigration from upstream may be inflating estimates of N_e at these sites.

Connectivity and dispersal geometry

Aquatic systems can exhibit several different patterns of connectivity. Large rivers are linear (Gotelli & Taylor, 1999), whereas ponds distributed across a terrestrial landscape conform to a two-dimensional landscape (Spear *et al.*, 2005). Patterns of connectivity can also be more complex. For example, the connectivity of marshes and levee-canal systems in the Florida Everglades varies over time due to annual dry-down cycles (McElroy *et al.*, 2003). Connectivity in river-stream systems has been characterized as hierarchical and dendritic (Fagan, 2002; Lowe *et al.*, 2006). For biota constrained to aquatic habitats, like stream fishes, patterns of connectivity

can have great effect on population persistence by facilitating or constraining colonization (Fagan, 2002).

Genetic signatures of linear and dendritic connectivity are nearly indistinguishable if stream fishes are equally likely to move upstream and downstream along dispersal corridors. Genetic signatures of linear and dendritic landscapes can also be difficult to distinguish when dispersal among sites is high. Signatures differ most when dispersal is directional and when dispersal among sites is attenuated by distance or selection (Fagan, 2002).

Central stoneroller movement rarely exceeds 135 m and home ranges are on the order of 35 m (± 14 m) of stream length (Mundahl & Ingersol, 1989). Lonzarich, Lonzarich & Warren (2000) concluded from their observation of 862 marked-recaptured stonerollers that movement within two different streams was a 'diffusive process' with equivalent rates of upstream and downstream movement. Schaefer (2001) also found no directional bias in movement among central stonerollers residing in a large outdoor experimental stream system. In contrast, when considering genetic differences among sites alongside estimates of N_e and m , our study suggests that Mill Creek central stonerollers disperse directionally downstream. For example, N_e at site MC2 is lower than at comparable impaired sites farther down in the catchment, but immigration to site MC2 is similar to the high levels observed for other impaired sites. These findings, together with a lack of differentiation between sites MC1 and MC2 (and the presence of differentiation between MC1 and degraded sites in the lower catchment) suggest that site MC2 draws migrants from site MC1 and other sources further upstream whereas lower catchment sites potentially receive immigrants from all upstream sources in the catchment. The relatively high estimate of N_e and low estimate of m at site MC1 is also suggestive of net downstream movement into impaired sites lower in the catchment. Due to the limited number of sample sites, such interpretations remain preliminary and therefore warrant further comparisons of N_e and m relative to genetic differentiation among Mill Creek waterways, especially between additional impaired and unimpaired sites in the upper catchment.

Hierarchical analysis of spatial genetic variance and Bayesian estimates of population structure indicate that gene flow is much lower between catchments

than within catchments. Low gene flow among drainage basins suggests that between-catchment dispersal is uncommon, likely as a result of physical or ecologically impassable barriers. Movement between Mill Creek and Tanners Creek is most likely restricted by the Ohio River, which could be an ecologically impassable barrier due to a lack of suitable habitat (e.g. riffles and runs). Estimates of gene flow among drainage basins for central stoneroller populations in eastern Indiana and western Ohio have also been shown to be very low (Bagley *et al.*, 2002). Further comparison of gene flow estimates among drainages which all empty into the Ohio River, including Mill Creek and Tanners Creek, could demonstrate whether between-drainage dispersal is attenuated by distance or environmental degradation. Comparison of dispersal patterns among drainage basins might also demonstrate whether colonization patterns (e.g. propagule-pool versus migrant-pool) of stream fishes differ across spatial scales (Pannell & Charlesworth, 1999; Fagan, 2002).

Assessment of aquatic environmental condition and conservation of stream fishes

Considering patterns of both spatial and temporal genetic variance, we hypothesize that the persistence of central stonerollers within Mill Creek is largely attributable to spatially variable reproductive success and migration rates that are driven by habitat heterogeneity and dispersal abilities. The weak genetic structure, location-dependent effective population sizes, and variable rates of immigration among sites relative to habitat quality lend support to this scenario. Conditions in the lower catchment with the most degraded habitats may not be suitable for establishment and maintenance of sustained central stoneroller populations, creating a source-sink metapopulation. This type of metapopulation is characterized by habitat of varying quality and asymmetric effective gene flow, with a large fraction of the metapopulation occurring in 'sink' habitats where within-habitat reproduction is insufficient to balance local mortality and emigration. Nevertheless, these habitats are locally maintained by continued immigration from more productive 'source' populations (Pulliam, 1988). Current levels of genetic diversity and slight genetic structure also support the hypothesis that central stonerollers in this catchment represent a

source-sink metapopulation. Population genetic models of source-sink metapopulations have shown that under high rates of immigration a collection of interconnected sinks can maintain levels of genetic variability similar to those observed in source populations (Gaggiotti, 1996; Gaggiotti & Smouse, 1996). When migration from source areas is continuous over time, we should expect only minimal divergence between source and sink populations. Because impaired habitat primarily occurs in the lower portion of the Mill Creek catchment, it is difficult to determine from our data the relative influences of habitat heterogeneity and asymmetric downstream dispersal on source-sink phenomenon and associated population genetic patterns. However, we note that prior studies in different catchments have observed no bias in either upstream or downstream dispersal (Lonzarich *et al.*, 2000; Schaefer, 2001). Additionally, our inability to find central stonerollers in the lower third of the catchment, an area which would benefit most from downstream dispersal but contains the most degraded habitat, suggests that habitat differences play a large role in driving this population dynamic.

Evidence of source-sink metapopulation dynamics in a pollution tolerant stream fish would have considerable implications for biological assessments of aquatic environmental condition. Impairment is often characterized by low species diversity and the presence of pollution tolerant species. From this perspective, our study suggests that assessments may underestimate levels of impairment if consideration is not given to the conditions which sustain pollution-tolerant species in degraded waterways. For example, if residency at a site is low, and occupancy is sustained by continuous immigration, site impairment is likely more severe than would be estimated by standard assemblage-level assessment approaches (McCormick and Peck 2000). The approaches which we have utilized here, including spatially explicit analysis of temporal genetic variation, could improve estimates of impairment by providing information on population persistence as well as the dispersal geometry of stream biota (e.g. enabling discrimination between site occupancy and residency). Monitoring efforts which permit measurement of N_e and m over multiple time intervals could also demonstrate the extent and rate at which biotic integrity recovers from impairment.

Evidence of a metapopulation in a heavily urbanized catchment also has implications for conservation and management of stream fishes. Contemporary loss of freshwater biological diversity is outpacing loss of terrestrial and marine biodiversity (Xenopoulos & Lodge, 2006). Conservation efforts often involve reducing habitat fragmentation because metapopulation models predict that increasing connectivity can improve site occupancy and therefore reduce the probability of extinction (Gotelli & Kelley, 1993; Gotelli & Taylor, 1999; Alo & Turner, 2005; Spear *et al.*, 2005). However, our results suggest that traditional conservation priorities, such as maintaining or improving connectivity across fragmented dispersal corridors, may not be sufficient to sustain at-risk populations. Habitat fragmentation that impedes dispersal can subsequently increase genetic drift and possibly lead to high variance in productivity and reproductive success (Alo & Turner, 2005), but productivity and reproductive success may also vary in relation to habitat quality (Hanski & Gilpin, 1997). In catchments where source–sink dynamics may be occurring, efforts to ameliorate the effects of habitat fragmentation would likely present less net benefit than assigning priority conservation status to unimpaired ‘source’ locations (Pulliam, 1988; Saunders, Meeuwig & Vincent, 2002). Additional benefit might also come from targeted environmental remediation at impaired sites that enhances residency of fishes that are prone to extinction due to low effective population size (Lowe *et al.*, 2006). Efforts are underway to restore riffle habitat and reduce point source discharges into Mill Creek (Butler County Department of Environmental Services). The approaches that we have used here could help assess the success of this effort and facilitate development or implementation of balanced strategies to ensure the long term persistence of at-risk populations, especially small populations inhabiting marginal habitats that are more likely to be affected by stochastic events. Such efforts are also warranted to improve basic understanding of source–sink dynamics within and among river–stream networks (Lowe *et al.*, 2006).

Acknowledgments

We would like to thank John Darling for insightful comments that greatly improved this manuscript. This work was funded by the US EPA Ecological

Exposure Research Division. Although this work was reviewed by the US EPA and approved for publication, it may not necessarily reflect official Agency policy.

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(Manuscript accepted 23 April 2008)