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Spatial genetic structure and recruitment dynamics of burbot (*Lota lota*) in Eastern Lake Michigan and Michigan tributaries

Danielle M. Blumstein [a,*], Daniel Mays [b], Kim T. Scribner [a,c]

[a] Department of Integrative Biology, Michigan State University, 203 Natural Science, East Lansing, MI 48824, USA
[b] Little River Band of Ottawa Indians, Natural Resources Department, 2608 Government Center Drive, Manistee, MI 49660, USA
[c] Department of Fisheries and Wildlife, Michigan State University, 13 Natural Resources, East Lansing, MI 48824, USA

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ABSTRACT

Burbot (*Lota lota*) are the only freshwater member of the Cod like (Lotidae) family that have a circumpolar distribution and occupy the widest geographic distribution of all Laurentian Great Lakes fish species. Information regarding burbot spatial genetic structure and recruitment dynamics is critical for the development of effective management strategies. Although burbot are a species of conservation concern throughout their range, little demographic or behavioral information exists. We estimated levels of genetic diversity within, and the degree of spatial population structure between samples collected from Lake Michigan and tributaries of the Manistee River, MI. Measures of genetic diversity across 10 microsatellite loci were moderately high. Disparities between adult groups sampled in Lake Michigan and the Manistee River were notable for observed heterozygosity (0.662 vs 0.488) and allelic richness (11.7 vs 6.6). Significant levels of inter-population variance in microsatellite allele frequencies (F_{ST} 0.154 to 0.208) were detected between Lake Michigan and the Manistee River samples. Results indicate reproductive isolation between what plausibly may be riverine and lacustrine spawning life history types. Pedigree analyses for three cohorts sampled in the Manistee River revealed that a sizeable number of adults contributed reproductively to multiple cohorts, indicating spawning philopatry. While data were collected from restricted areas in lacustrine and river habitats, analyses revealing microgeographic genetic structuring, potentially attributed to life history polymorphisms, have significant implications for burbot management in the Great Lakes.

Introduction

Genetic analysis has facilitated the study of spatial patterns, providing insight on the exchange of individuals among historical and contemporary breeding populations in the absence of movement data. Reproductive isolation and accrual of spatial genetic structuring can occur because of historical factors related to glacial events that can result in isolation by distance (Wright, 1946) and adaptive divergence may arise in isolated populations (Bradbury et al., 2013). Structuring can also occur through contemporary mechanisms related to a species ecology, such as kin-biased distribution of juveniles, natal homing with regards to spawning sites (Stepien and Faber, 1998; Gerlach et al., 2001), population differences in timing of reproduction (Hendry and Day, 2005), or life history trait differences, for example, preferences for fluvial or adfluvial habits (Hardy and Paragamian, 2013; Kootenai Valley Resource Initiative (KVRI) Burbot Committee, 2005). Furthermore, isolation could be present because of anthropogenically altered landscapes that result in barriers (Wofford et al., 2005) that could limit dispersal and alter the distances individuals can travel during different life stages. The degree of structuring can occur across macro- and micro-geographic scales. Within the Laurentian Great Lakes, structuring could be present by lake basin, river drainages, or tributaries within a river basin.

Burbot (*Lota lota*) are the only member of the cod-like (Lotidae) family inhabiting and spawning in streams, inland lakes, and the Great Lakes (Nelson, 1994; Stapanian et al., 2008; Jude et al., 2013). Burbot have a circumpolar distribution and occupy the widest geographic distribution of all Great Lakes fish species (Stapanian et al., 2008). The species is a benthic keystone piscivore and an indicator species for cold water ecosystems due to a need for high oxygen levels and unpolluted water (Sanetra and Meyer, 2005; Elmer et al., 2008).

Long term monitoring data from across the species' range, including the Great Lakes, indicates that abundance has declined significantly from historical levels (Stapanian et al., 2007). The decline has been attributed to pollution, habitat change, discharge and barriers from dams, invasive species, and increasing temperatures due to climate change (Stapanian et al., 2010; Underwood et al., 2016). Burbot are rarely prioritized in management programs in the Great Lakes region due to its lack of popularity as a game or commercial fish. Accordingly,

* Corresponding author.
E-mail address: blumstei@msu.edu (D.M. Blumstein).

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there is a lack of data pertaining to the degree of population structure and relative abundance of spawning individuals in Great Lakes and tributaries or of levels of stock recruitment (Stapanian et al., 2008).

Burbot could be genetically structured based on adult movements during the winter, formations of spawning aggregations, and utilization of reefs within the Great Lakes proper (Sanetra and Meyer, 2005; KVRI Burbot Committee, 2005). Spawning occurs under the ice over cobble substrate (Arndt and Hutchinson, 2000) and field observations suggest that aggregations consist of one to two females mating with multiple males (McPhail and Paragamian, 2000). Depending on spawning locations, timing of spawning (winter and spring/summer spawning), and pelagic larvae that exhibit a period of passive dispersal with river currents (O'Gorman, 1983), offspring from different spawning aggregations could disperse to different rearing areas, thereby contributing to population structuring. Furthermore, there is evidence of spawning within tributaries and on open-water reefs in the Great Lakes, indicating location-specific environmental cues may dictate selection of different spawning habitats that can maintain reproductive isolation (Jude et al., 2013).

Previous population genetic research conducted on burbot has focused on levels of diversity in a single population and divergence between populations in multiple locations within the species range. At macro-geographic scales, populations appear to be genetically structured metapopulations due to the species broad continent-wide distribution (Elmer et al., 2008). At micro-geographic scales, isolation has been linked to barriers such as hydroelectric dams (Underwood et al., 2016). However, no genetic work has been conducted on burbot in the Great Lakes.

Our main research objective was to estimate the degree of spatial genetic structure of burbot at regional and local geographic scales. Furthermore, we characterized the recruitment dynamics in terms of the number of breeding adults contributing to juveniles sampled and characterized pedigree relationships among juveniles from multiple year cohorts in the river tributaries.

Material and methods

Field sampling methods

Burbot assessments were conducted by Little River Band of Ottawa Indians (LRBOI) fisheries assessment crews during three consecutive years (2014–2016). Burbot were targeted in river, stream, and lake environments to obtain three different subsets of samples. Caudal fin clips were taken from burbot captured in the Manistee River, two tributaries to the Manistee River, and along the eastern shoreline of Lake Michigan (Fig. 1). In the first subset of samples, adult burbot were collected from the Manistee River during winter spawning migrations using trap and hoop nets near Coho Bend and Rainbow Bend access sites ($n = 44$). In the second subset of samples, juvenile burbot were collected from both Bear and Sickle Creeks during mid-summer electrofishing surveys ($n = 198$). In the third subset of samples, adult burbot were captured from late spring through mid-summer, to target adults migrating to spawning grounds ($n = 44$) in Lake Michigan during biological assessments conducted by LRBOI. A total of 36 sets were conducted annually following standardized gill net assessment methods described in the Lakewide Assessment Plan for Lake Michigan fish communities (Schneeberger et al., 1998), Fishery Independent Whitefish Surveys (MSC, 2002), and Lake Trout Fall Spawning Assessments (Bronte et al., 2007). Samples were collected from adult fish in four general locations in Lake Michigan (Fig. 1; Arcadia, $n = 21$; Muskegon, $n = 12$; Manistee, $n = 14$, and Ludington, $n = 11$; total $n = 58$).

Juvenile burbot from Bear and Sickle Creeks were aged using otoliths collected from a subsample of fish ($n = 10$) and further evaluated using length frequency histograms. Age 0–3 juvenile burbot were assigned to cohorts in both Bear and Sickle Creeks ($n = 16$, 38, and 144 for the 2012, 2013 and 2014 cohorts, respectively).

Genetic analysis

DNA was extracted from fin tissue using Qiagen DNeasy® kits (QIAGEN Inc. Valencia, CA) according to manufacturer's instructions. DNA concentrations were determined using a NanoDrop® ND-1000 spectrophotometer and samples were standardized to a concentration of 20 ng/μl for use in PCR.

Individuals were genotyped with 10 disomic microsatellite loci. Microsatellite markers included *Llo1*, *Llo7*, *Llo11*, *Llo12*, *Llo15*, *Llo16*, *Llo21*, *Llo26*, and *Llo48* and (Sanetra and Meyer, 2005) and *EF139393* (Zhao et al., 2009). PCR reactions were conducted in 25 μl volumes containing 100 ng of template DNA, 0.5 μM of each primer (0.55 μM for *Llo7* and 0.4 μM *Llo15*), 200 μM dNTPs, 1× reaction buffer, 5 U of Taq DNA polymerase (Invitrogen ThermoFisher Scientific Inc. Waltham, MA), and additional deionized water to achieve total reaction volume. PCR conditions were as follows: initial denaturation step of 94 °C for 2 min (3 min for *EF139393*), followed by 30 cycles (35 cycles for *Llo26*, *Llo7*, *Llo12*, *Llo16*; 32 *EF139393*) of 94 °C for 30 s, primer specific annealing temperatures (62 °C for *EF139393*; 59 °C for *Llo21* and *LLo1*; 57 °C for *Llo14* and *Llo12*; 55 °C for *Llo11* and *Llo15*; and 51 °C for *Llo26*, *Llo7* and *Llo16*) for 30 s, 72 °C for 1 min (45 s for *Llo48* and 30 s for *EF139393*), and final extension was for 5 min (7 min for *EF139393*) at 72 °C. Loci were amplified individually in 96-well plates with one negative control per plate and three standards to uniquely identify the plates. Plates were pooled into five different sets. Loci combinations were determined per fluorescently labeled forward primers (FAM, HEX, and NED dyes), and allele size ranges presented by Zhao et al. (2009) and Sanetra and Meyer (2005). Set one pooled Llo26, Llo1, Llo48, and Llo7. Set two pooled Llo11, Llo12, and Llo16. Sets three, four, and five were Llo21, EF139393, and Llo15, respectively. Sets with four loci had two non-overlapping allele sizes ranges labeled with the same fluorescent primer. Fragment lengths were analyzed using an ABI 3730xl at the Genomics Core within the Research Technology Support Facility at Michigan State University.

Electropherograms were analyzed and genotypes scored using GeneMarker software (Softgenetics, State College, PA). Allele sizes for all samples were determined using commercially available size standards (GeneScan™ 500 ROX™, ThermoFisher Scientific Inc., Waltham, MA). All genotypes were independently scored by two experienced laboratory personnel and verified when entered into an electronic database. Any disputed genotypes were reanalyzed and/or reamplified. As an additional measure of quality control and assurance of accurate scoring, ~10% of all individuals were randomly selected and reanalyzed at all loci. The error rate was 0.015.

Measures of genetic diversity and spatial genetic structure among adult and juvenile burbot

Summary measures of genetic diversity including the mean number of alleles per locus, allelic richness, observed and expected heterozygosity, and Wright's inbreeding coefficient (F_{IS}) for burbot sampled from different locales in the Manistee River and open waters of Lake Michigan (Fig. 1) were estimated using the program F-stat (version 2.9.3; Goudet, 2001). Chi square tests were used to quantify the degree of difference in observed heterozygosity (i.e., number of loci heterozygous per individual) between adults from Lake Michigan and Manistee River locales, between Manistee River adults and juveniles, and among Manistee River cohorts.

Estimates of gametic disequilibrium (a measure of lack of independence among loci) and Hardy-Weinberg equilibrium were also estimated using the program F-stat. Statistical significance associated with F-statistics (Weir and Cockerham, 1984) and measures of gametic disequilibria were adjusted to account for multiple testing using sequential Bonferroni corrections (Rice, 1989). We report F-statistics and summary measures of allele frequency and genetic diversity for each of the four Lake Michigan locales (adults) and the two Manistee

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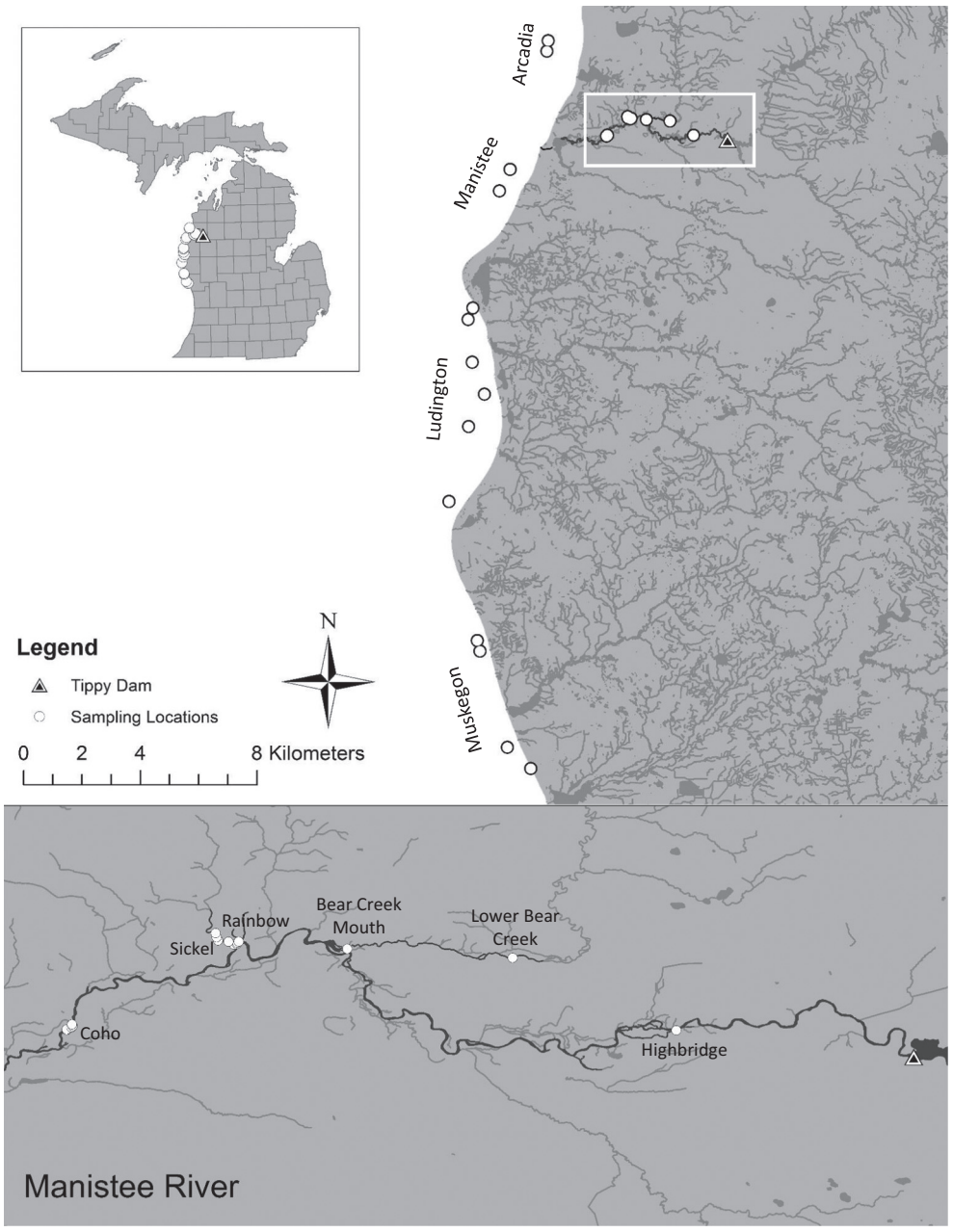

Fig. 1. Study area showing sampling locations in Lake Michigan and the Manistee River (white circles) and Tippy Dam (black triangle). Samples taken in the Manistee River are outlined in a white box and shown in the lower inset.

River sampling locales for adults and for all location/cohort samples. We also report estimates of inter-sample R_{ST} (Slatkin, 1995) which adjusts inter-sample variance in allele frequency based on the difference in allele size (in base pairs). R_{ST} is a measure of evolutionary divergence based the number of assumed mutational events. Estimates of pairwise R_{ST} among sampling groups was conducted using program GENEPOP (Rousset, 2008).

To examine evidence for spatial genetic structure for adults, we used a Bayesian-based method to estimate the number of genetically distinct groups of individuals (K) that were represented in the sample of adults using the program STRUCTURE (version 2.3.4 Pritchard et al., 2000). Analyses were conducted hierarchically. In situations of large spatial variance in allele frequency, sub-structuring within the primary spatial groups of focus (in our case Lake Michigan vs. Manistee River) may go undocumented. We used STRUCTURE to analyze three different datasets, the first was represented by all burbot from Lake Michigan and the Manistee River. Three subsequent analyses were conducted analyzing for any possible sub-structuring within 1) Lake Michigan adults, 2) Manistee River adults, and 3) Manistee River juveniles. Parameters used in the analysis for all fish included a 100,000 replicate 'burn-in' period and 500,000 MCMC replicates following the burn-in. These were based on models assuming allele frequencies across loci were independent and assuming admixture. For each K (1–5) we conducted 10 replicate runs using the aforementioned model parameters. The web based program STRUCTURE HARVESTER (version 0.6.49 Earl and vonHoldt, 2011) was used to summarize estimates of the likelihoods of K. The data for each replicate for each K was used to determine the number of clusters that best fit the data based on the mean likelihood L (K) and variance and estimates of ΔK (Evanno et al., 2005). Based on the most supported K, 100,000 MCMC replicates were run to estimate posterior probabilities of individual assignment for all adults and all juveniles sampled in the

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Manistee River and Lake Michigan to each of the inferred genetically differentiated groups. The program F-stat was then used to estimate variance in allele frequency (F_{ST}) among members from different genetic clusters. The program STRUCTURE was subsequently used for only Lake Michigan fish using the same number of replicates and parameter settings stated above but varying K from one to four. We also conducted unsupervised clustering in STRUCTURE for fish from the Manistee River using the same number of replicates and parameter settings stated above but varying K from one to two.

Pedigree analysis and estimates of numbers and effective numbers of breeding adults in the Manistee River

We used the program COLONY (version 2.0.6.1, Wang, 2009), that implemented a maximum likelihood method described in Wang (2004) and Wang and Santure (2009) to assign juvenile burbot captured in the Manistee River tributaries to full and half sib groups. This method can determine the minimum number of unsampled adults that could contribute to the population without knowledge of the identity of the parents, as in a classic parentage study. Maximum likelihood estimators implemented in the program COLONY can determine if an unsampled adult produced one or more juveniles during the same reproductive event (yearly cohort) based on inferred pedigree relationships among the juveniles. Recent extensions in the program COLONY (Wang and Santure, 2009) allow for polyandry and polygyny (females mating with multiple males and males mating with multiple females, respectively).

Samples were also analyzed in COLONY independently for aged individuals from each of sampling locations, cohorts based on otoliths, and all locations and cohorts combined. Samples were analyzed using a random number reference to prevent the algorithm from converging on a non-optimal local maximum in the likelihood surface. Using different random number seeds for each replicated run may produce slightly different results as seed selection influences searches over the likelihood surface. Two runs were conducted with different number seeds to determine if the results from each run were concordant.

The input parameters used for all runs across all years included (a) male and female polygamy without inbreeding and clonality, (b) dioecious and diploid species parameters, (c) one long run using the full likelihood analysis method with high precision, (d) no updating of allele frequencies, and (e) sibship size scaling with no sibship size prior. Since we only used juveniles from each cohort captured in the Manistee River tributaries, all parameters involving maternity and paternity were unknown. An empirical genotyping error rate of 0.015 was used.

For juveniles from each year and location, we estimated the effective number of breeding adults based on the frequencies of full and half sib progeny arrays using the method of Wang (2009) and implemented in the program COLONY. The general rationale for the estimator is based on the fact that effective breeding population size will vary in proportion to the probability that two random individuals from a year cohort will be siblings that share the same mother, father, or both parents. Effective breeding population size will be small if the cohort of juveniles sampled contains a large number of siblings.

We assessed the accuracy of pedigree assignments based on simulations. We used known adult genotypes to simulate offspring with known full and half-sibling relationships by randomly selecting and "mating" male and female parents from our data base, and randomly selected one allele from each "parent" at each locus to produce simulated offspring ($n = 210$; 35 families) (S. Radak, unpublished program, Michigan State University). To estimate pedigree assignment accuracy, we then used the program COLONY to assign simulated offspring into sib-groups of related (full and half sibs) and unrelated individuals.

Results

Estimation of spatial genetic structure

Estimates of measures of genetic diversity across all microsatellite loci were moderately high though estimates differed significantly between adults sampled from Lake Michigan and the Manistee River (Electronic Supplementary Material (ESM) Table 1). Disparities between adult groups sampled in Lake Michigan and the Manistee River were significant for observed heterozygosity (0.662 vs. 0.488, chi-square 35.42, 6 df, $P < 0.001$). The range of observed heterozygosity was 0.644 to 0.671 across Lake Michigan locales and 0.456 to 0.525 between Manistee samples. Significant differences between adults from Lake Michigan and the Manistee River were observed for allelic richness (11.7 vs 6.6, respectively). Differences in observed heterozygosity between samples of Manistee adults and juveniles and among juvenile Manistee River cohorts were not significant (chi-square 2.92, 7 df, $P > 0.05$ and 17.48, 21 df, $P > 0.05$, respectively).

There was no evidence of significant gametic disequilibrium for any two locus combinations in samples of adults from the four locales in Lake Michigan or two locales in Manistee River. Values of F_{IS} were mostly negative, indicating a general tendency for an excess in observed heterozygosity, relative to Hardy Weinberg expectations. However, F_{IS} values were not significantly different from zero for either Lake Michigan or the Manistee River burbot after Bonferroni correction (ESM Table S1).

Analyses of significant spatial genetic structure based on model likelihood scores and ΔK (ESM Figs. S1A and S1B, respectively) across the adult samples indicated strong support for two genetic groups of individuals ($K = 2$) distinguishing adult burbot sampled in Lake Michigan and those sampled within the Manistee River (Fig. 2). We used the mean posterior probabilities of individual assignment to infer that the genetic cluster was ~0.99. Juvenile burbot from all age classes were also assigned with high posterior probabilities to the Manistee River genetic cluster (Fig. 2). Results from hierarchical unsupervised clustering (i.e., among samples within Lake Michigan and within the Manistee River) failed to detect evidence of sub-structuring (data not shown). Estimates of F_{ST} across the 10 loci between Lake Michigan adults and Manistee River adults and Lake Michigan adults and Manistee River juveniles ranged from 0.170 to 0.201 ($P < 0.01$) and 0.158 to 0.208 ($P < 0.01$), respectively (Table 1). Estimates of R_{ST} were roughly twice the level, from 0.252 to 0.344 ($P < 0.01$) and 0.233 to 0.410 ($P < 0.01$).

Manistee River pedigree analysis

Based on assignment of offspring to half and full sib groups for juvenile fish (age 0–3) ($n = 16$, 38, 58, 86, and 144 for Bear Creek 2012 cohort, Bear Creek 2013 cohort, Bear Creek 2014 cohort, Sickle Creek 2014 cohort and combined 2014 cohorts, respectively), we estimated that the total number of adults that contributed to the juveniles varied over the three juvenile year cohorts from 25 to 176 annually (Table 2). The number of half sib families (kin groups in Fig. 3) varied from 12 to 86, and the number of full sib families varied from 16 to 132 (Tables 2; Fig. 3). The number of adults and families varied among years in accordance with the number of juveniles sampled as did the effective number of breeding adults, which ranged from 60 to 202 (N_b, Table 2). Though the number of juveniles collected and the number of inferred adults (N_s, Table 2) varied similarly across years, the effective number of breeding adults was less variable across years (Table 2).

When juveniles from each of the three cohorts were combined, half sibling pedigree representation by individuals from multiple cohorts indicated repeat spawning. Approximately 25% of inferred adults spawned in two years, while the number of adults spawning in three years was approximately 2%, indicating spawning site fidelity to this river by these individuals.

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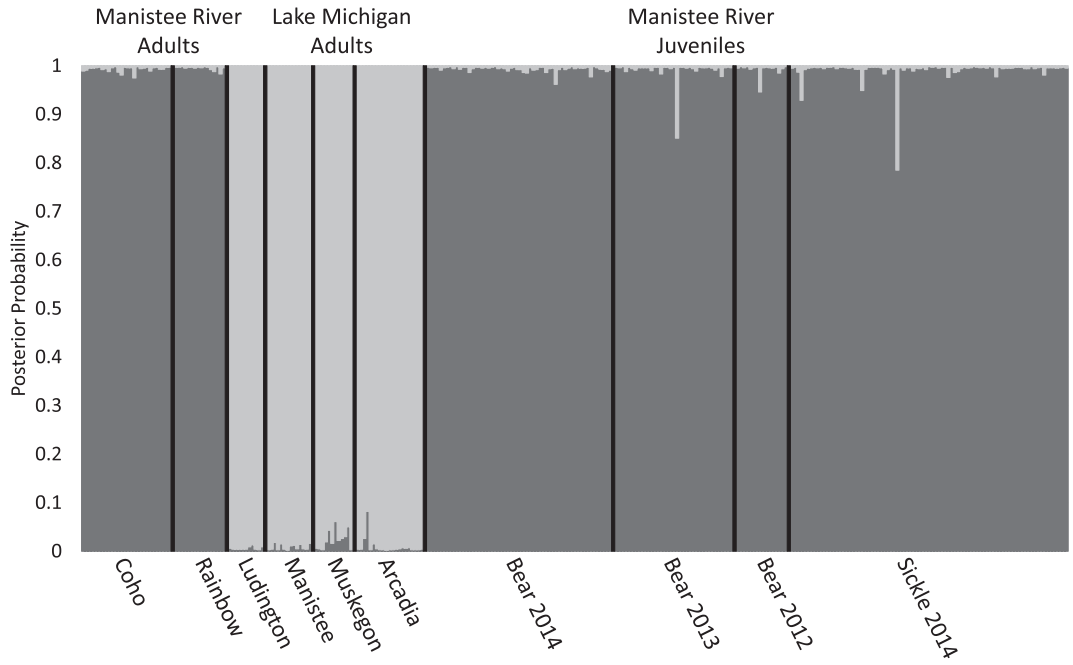

Fig. 2. Posterior probabilities of assignment to each of two inferred genetic clusters for Manistee River adults, Lake Michigan adults, and Manistee River juveniles and their respective sampling locals. Inferred genetic clusters are Manistee River fish (dark grey) and Lake Michigan fish (light grey).

Simulation analyses demonstrated that offspring ($n = 200$) simulated from adult multi-locus genotypes could be assigned with high accuracy to their correct sub groups (0.943 and 1.00, half sibs and full sibs, respectively). The probability of inclusion to correct sib groups was 0.981, 0.991, 0.994, 1.00, and 0.998 (Sickle 2014, Bear 2014, Bear 2013, Bear 2012, and Simulated families respectively).

Discussion

We found strong evidence of spatial genetic structure between Manistee River and Lake Michigan burbot. Bayesian cluster analyses revealed that samples were composed of individuals from two highly genetically differentiated groups. Breeding adults and juveniles had high (mean ~0.99) posterior probabilities of assignment to either the Manistee River or to eastern Lake Michigan (Fig. 2), which is consistent with high levels of inter-group variance in allele frequency (F_{ST} 0.154 to 0.208; Table 1). Genetic homogeneity of adults collected from multiple locations in open waters of Lake Michigan and locations in the Manistee River and the substantial variation in allele frequencies between individuals from Lake Michigan and the Manistee River were unexpected. Based on inter-population levels of spatial genetic variation for lake sturgeon (*Acipenser fulvescens*, Homola et al., 2012) and muskellunge (*Esox masquinongy*, Turnquist et al., 2017), we expected to find fish in Lake Michigan to be a mixture of individuals from genetically differentiated spawning populations. Barluenga et al. (2006) suggests that there could be an admixture of genetically distinct burbot lineages observed. This has been observed in open Great Lakes water on walleye (*Sander vitreus*, Brenden et al., 2015) and lake whitefish (*Coregonus clupeaformis*, VanDeHey et al., 2009). Due to the proximity of Lake Michigan sampling locales to the Manistee River, we expected the open water samples to include fish originating from the Manistee River.

Previous work on burbot (Barluenga et al., 2006; Underwood et al., 2016; Elmer et al., 2008) has shown that populations at varying macro-geographic scales can be genetically differentiated. Elmer et al. (2008) found high levels of population differentiation across North America. In Germany, Barluenga et al. (2006) described a two-step colonization process of burbot from two highly differentiated geographical regions in a single lake. Underwood et al. (2016) described evidence for limited gene flow (significant differences in allele frequency) in the upper and lower Wind River (Wyoming) due to natural and anthropogenic barriers.

Table 1
Pairwise F_{ST} and R_{ST} estimates of inter-sample variance in allele frequency among burbot adults and juveniles sampling in open waters of Lake Michigan and in the Manistee River, MI. F_{ST} is presented above the diagonal and R_{ST} is below the diagonal.

	Adults						Juveniles			
	Manistee River		Lake Michigan				Manistee River cohorts/locations			
Sample	Coho	Rainbow	Lundington	Manistee	Muskegon	Arcadia	Bear 2014	Bear 2013	Bear 2012	Sickle 2014
Coho	–	−0.004	0.196*	0.197*	0.175*	0.201*	0.003	0	−0.002	−0.002
Rainbow	−0.014	–	0.188*	0.195*	0.170*	0.199*	−0.001	−0.009	−0.0062	−0.007
Lundington	0.340*	0.333*	–	−0.016	−0.009	−0.007	0.175*	0.177*	0.193*	0.197*
Manistee	0.341*	0.344*	−0.027	–	−0.009	−0.002	0.178*	0.185*	0.201*	0.202*
Muskegon	0.252*	0.243*	−0.001	0.019	–	0.005	0.154*	0.158*	0.173*	0.178*
Arcadia	0.270*	0.272*	−0.001	0.008	0.019	–	0.185*	0.189*	0.204*	0.208*
Bear 2014	−0.001	−0.021	0.352*	0.359*	0.258*	0.281*	–	−0.002	−0.003	0.001
Bear 2013	−0.015	−0.015	0.320*	0.322*	0.233*	0.253*	0.001	–	−0.008	−0.002
Bear 2012	−0.006	−0.026	0.407*	0.410*	0.322*	0.337*	−0.007	−0.006	–	−0.001
Sickle 2014	−0.006	−0.018	0.358*	0.362*	0.266*	0.283*	−0.005	−0.005	−0.007	–

* Statistical significance ($P < 0.01$).

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Table 2
Summary of burbot samples collected and genotyped and estimates of the total number of adults that contributed to 2012–2014cohorts (N_s) and all years combined and the estimated number of kin groups (N_k). N_b = breeding population; N_s = total number of adults contributing to population; N_k = total number of kin groups and half sib families.

Juvenile year class	Number of juveniles genotyped	Colony replicate	N_b	95% CI of N_b	N_s	N_k	Number of full-sib families
Bear Creek 2012	16	1[a]	60	31–197	25	12	16
		2	60	30–211	25	12	16
Bear Creek 2013	38	1[a]	70	44–122	47	23	32
		2	70	46–114	47	23	32
Bear Creek 2014	58	1[a]	102	72–148	72	36	54
		2	102	71–151	72	36	54
Sickle Creek 2014	86	1	123	92–168	103	51	78
		2[a]	89	62–124	96	43	85
2014 all locations	144	1[a]	202	157–258	174	86	132
		2	215	170–273	176	90	131
All years	342	1			233	117	193
		2[a]			219	111	194

[a] Run with lowest MLE score used for analyses.

While high genetic differentiation has been documented for this species at varying geographic scales, there is no documentation of genetic discontinuities at the geographic scale that is presented by Lake Michigan and the Manistee River. The intergroup variance in allele frequencies is high among samples collected in close proximity (F_{ST} 0.154 to 0.208) while estimates of R_{ST} were roughly twice the level (0.233 to 0.410). R_{ST} can be greater than F_{ST} if the alleles and distributions of allele frequencies at these variants differ between populations. This indicates that populations have been separated for considerable periods, reflecting changes in allele frequencies between Lake Michigan and Manistee River samples.

We also found disparities in measures of genetic diversity including heterozygosity and allelic richness (ESM Table S1) between Lake Michigan and the Manistee River samples. Lower levels of genetic diversity in Manistee River adults and offspring suggest that the adult spawning population size is smaller than the size of the genetic group represented in Lake Michigan samples.

Pedigree analysis revealed that the number of spawning adults producing 198 juvenile burbot genotyped (N_s) was ~226 across three year cohorts. Our estimates of 'effective' adult population size over the entire three year cohort period was ~219 (Table 2). The number of full and half

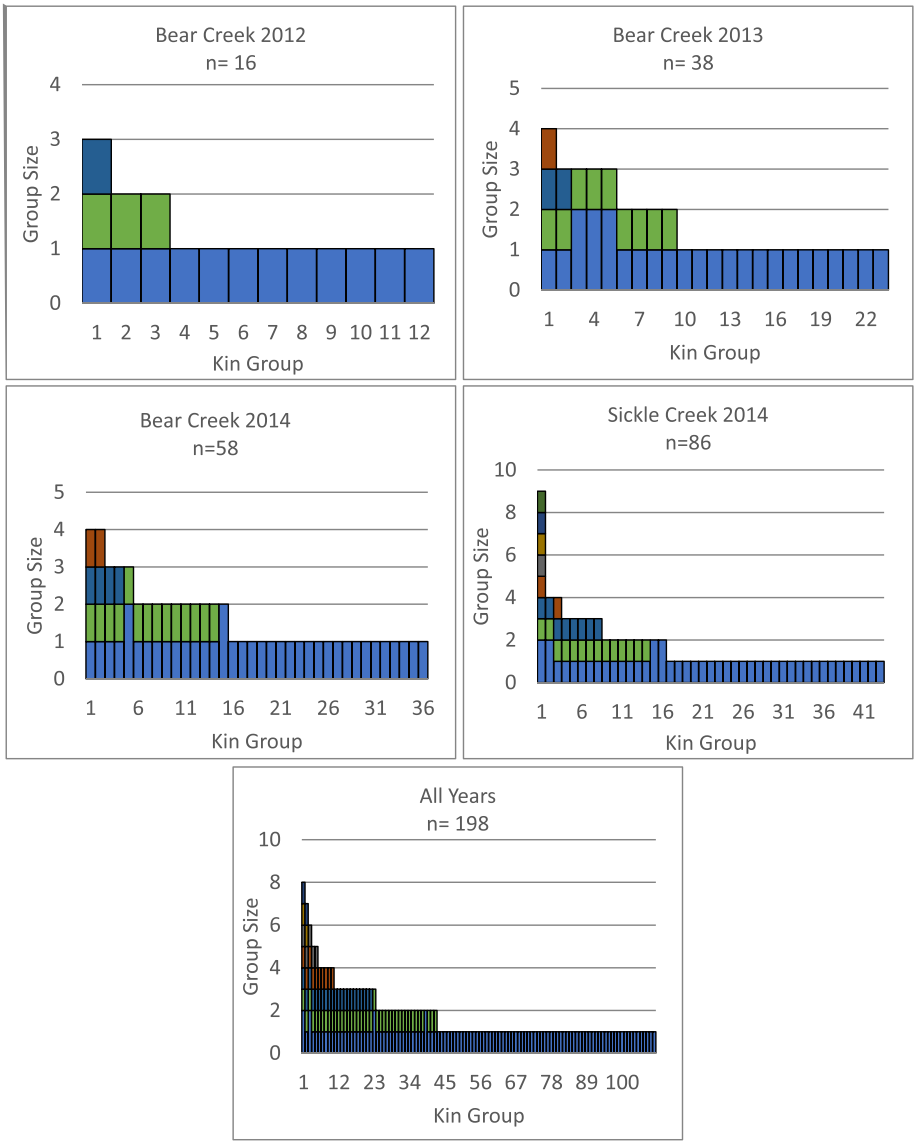

Fig. 3. Distributions of burbot juveniles collected from the Manistee River for each year and location (2012–2014) as well as all years combined into full-sib and half-sib kin groups. Each column is an inferred half-sib kin group comprised of full-sib groups nested within that are identified by color. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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sibling groups represented within each year cohort suggests both polygyny and polyandry as have been previously described (McPhail and Paragamian, 2000), and is consistent with findings of high gonadal investment in male burbot (Cott et al., 2013) that is thought to be associated with intrasexual competition.

With evidence indicating spawning site fidelity to the Manistee River by adults and high similarity between the river sampling sites, we can assume that this population functions as a different population from the Lake Michigan burbot. Given the variability in spawning life history schedules (review in Jude et al., 2013) and high levels of inter-population variance in allele frequency, spawning behaviors may not be plastic but rather may reflect adaptive variation that allow burbot to reproduce at different times and in a variety of locations (Hendry and Day, 2005; Bradbury et al., 2013, respectively). Genetic evidence suggesting a riverine life history is consistent with year round capture histories of juvenile and adult burbot within the Manistee River system. Notably smaller (relative to the Great Lakes) yet mature burbot captured in the Manistee River during winter spawning assessments may suggest that phenotypic differences also exist. Jude et al. (2013) documented burbot spawning in early spring in deep waters of Lake Michigan at nearshore and offshore sites. Additionally, in the current and previous studies, multiple age classes of burbot were captured during the summer electro-fishing surveys. Furthermore, burbot have been captured in the Manistee River during sturgeon larval drift surveys in the spring, as well as during visual surveys in the fall (Mays, pers. obs.). This could indicate that burbot are year-round residents within the Manistee River system, utilizing tributary or backwater systems early on in life and as swimming performance improves, inhabiting portions of the Manistee River. This level of spatial genetic structuring could be observed among other Great Lakes tributary spawning populations and specific Great Lakes spawning regions. Currently, the specific regions of the Great Lakes utilized for spawning by highly differentiated groups of individuals captured in Lake Michigan is unknown and further sampling efforts are needed to understand the structuring across the entire Great Lakes range.

Conclusion

Significant genetic differences between Lake Michigan and Manistee River burbot support the conclusion of two different life history polymorphisms. Though spatially segregated riverine burbot populations are not uncommon, the identification of genetically differentiated populations occurring within the same geographical area with no apparent barrier is unprecedented. To our knowledge, this is the first instance of what may be two sympatric populations of burbot. While physical barriers such as large log jams (pre- and post-settlement) and dams (post-settlement) on the Manistee River may have led to initial separations in gene flow, features that have contributed to the distinctiveness of these populations is uncertain. Preference for riverine or lacustrine life histories seems to be strong enough to reduce gene flow from the Manistee River system into Lake Michigan and vice versa. Pedigree analysis indicates spawning site fidelity of riverine burbot to the Manistee River, further supporting the distinction between lacustrine and riverine life history polymorphisms. Early life history studies as well as movement studies among various life stages may assist in identifying habitat selection and use in riverine specific burbot.

Distinct life history polymorphisms in burbot may have significant implications for burbot management in the Great Lakes. Individual riverine burbot populations may need to be considered independently of one another by managers in order to ensure appropriate protection. Other rivers that have active burbot populations including the Peshekee River (Marquette Co.), Au Train River (Alger Co.), and Sturgeon River (Baraga and Houghton Co.) may need additional studies to determine genetic structure and ensure genetic integrity is maintained. As burbot populations continue to decline, stock specific population analyses are critical to provide requisite data to inform management plans to maintain healthy populations and genetic integrity. Further evaluations of burbot genetic structure among streams and lakes in and around Michigan would provide detailed information to managers.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jglr.2017.10.002.

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