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

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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,

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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