



Challenge to the model of lake charr evolution: shallow- and deep-water morphs exist within a small postglacial lake

LOUISE CHAVARIE¹, ANDREW M. MUIR², MARA S. ZIMMERMAN³, SHAUNA M. BAILLIE⁴, MICHAEL J. HANSEN⁵, NANCY A. NATE¹, DANIEL L. YULE⁶, TREVOR MIDDEL⁷, PAUL BENTZEN⁴ and CHARLES C. KRUEGER¹

¹Center for Systems Integration and Sustainability, Michigan State University, 115 Manly Miles Building, 1405 South Harrison Road, East Lansing, Michigan, USA

²Great Lakes Fishery Commission, 2100 Commonwealth Blvd., Suite 100, Ann Arbor, Michigan, USA

³Washington Department of Fish and Wildlife, 600 Capitol Way N., Olympia, Washington, USA

⁴Department of Biology, Dalhousie University, 1355 Oxford St., Halifax, Nova Scotia, Canada

⁵United States Geological Survey, Hammond Bay Biological Station, 11188 Ray Road, Millersburg, Michigan, USA

⁶United States Geological Survey, Lake Superior Biological Station, 2800 Lakeshore Drive, Ashland, Wisconsin, USA

⁷Harkness Laboratory of Fisheries Research, Ontario Ministry of Natural Resources and Forestry, Trent University, 2140 East Bank Drive, Peterborough, Ontario, Canada

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All examples of lake charr (*Salvelinus namaycush*) diversity occur within the largest, deepest lakes of North America (i.e. > 2000 km²). We report here Rush Lake (1.3 km²) as the first example of a small lake with two lake charr morphs (lean and huronicus). Morphology, diet, life history, and genetics were examined to demonstrate the existence of morphs and determine the potential influence of evolutionary processes that led to their formation or maintenance. Results showed that the huronicus morph, caught in deep-water, had a deeper body, smaller head and jaws, higher eye position, greater buoyancy, and deeper peduncle than the shallow-water lean morph. Huronicus grew slower to a smaller adult size, and had an older mean age than the lean morph. Genetic comparisons showed low genetic divergence between morphs, indicating incomplete reproductive isolation. Phenotypic plasticity and differences in habitat use between deep and shallow waters associated with variation in foraging opportunities seems to have been sufficient to maintain the two morphs, demonstrating their important roles in resource polymorphism. Rush Lake expands previous explanations for lake charr intraspecific diversity, from large to small lakes and from reproductive isolation to the presence of gene flow associated with strong ecological drivers. Published 2016. This article has been contributed to by US Government employees and their work is in the public domain in the USA, *Biological Journal of the Linnean Society*, 2016, **00**, 000–000.

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INTRODUCTION

Two important themes predominate research on speciation: the mechanism(s) (e.g., ecological vs. non-ecological divergence) and its geographic context (e.g., allopatry vs. sympatry) (Rundle & Nosil, 2005;

Hendry, 2009; Hendry *et al.*, 2009). Processes creating population divergence leading to speciation are usually identified after species have formed (e.g., sister species) (Beheregaray & Sunnucks, 2001; Bolnick & Fitzpatrick, 2007). The processes involved are complex and can comprise many different scenarios (Rundle & Nosil, 2005). One way to investigate such complexity is to study processes that occur in systems

*Corresponding author. E-mail: chavarie@msu.edu

in which speciation may be in progress (Orr & Smith, 1998). In this context, scenarios from incipient ecological species represent opportunities to investigate the framework of adaptive divergence and gene flow (Bush, 1994; Skúlason & Smith, 1995; Hendry, 2009).

Where intraspecific diversity is observed, the various levels of ecological and genetic divergence involved have been difficult to disentangle and the mechanisms at work have been challenging to identify correctly (Rundle & Nosil, 2005; Hendry, 2009). Understanding the role of ecological drivers in the formation of new species while concurrent selective pressures operate within populations was thought to be straight forward, but empirical evidence has confounded the development of a generalized conceptual model for speciation (Hendry, 2009). In this context, the investigation of ongoing evolutionary processes on populations that have recently invaded novel habitats or when ecological shifts have occurred (e.g., variation in climate or resource availability), provides opportunities in which adaptive divergence and initial speciation mechanism(s) can be identified. The observation of ecological divergence within a recent time scale (Thompson, 1998; Kinnison & Hendry, 2001; Hairston *et al.*, 2005; Kinnison & Hairston,

2007) provides examples defined as ‘contemporary’ or ‘rapid evolution’ adaptations to novel environments (Hendry, Nosil & Rieseberg, 2007).

Geologically young lakes on receding glacial fronts tend to be fish species poor and typically have diverse habitats available for colonization (Skúlason & Smith, 1995; Smith & Skulason, 1996; Pielou, 2008). Such semi-isolated and physically variable environments often act to select for generalist genotypes that can adjust their morphology, physiology, and behaviour to exploit the wide range of available environmental conditions (Bamber & Henderson, 1988; Beheregaray & Sunnucks, 2001). This adaptability allows populations to invade and use multiple niches within newly emerging post-glacial freshwater systems, shifting from generalists to multiple specialists (Kawecki, 1998; Bush & Butlin, 2004; Elmer, 2016). In northern hemisphere post-glacial lakes, fish taxa show some of the most remarkable examples of sympatric resource polymorphisms among vertebrates (Tables 1 and 2). Resource polymorphism theory seeks to explain how variation generated by differences in resource use in the presence of divergent selection can ultimately explain the evolution of species (Bush, 1994). Within post-glacial lake

Table 1. Polymorphism in native lake charr populations (Canada and USA), known to date, and the nature of their freshwater diversification

Localities	Surface area (km ²)	Max. depth (m)	No. of morphs	Nature of divergence	Ecological drivers strength	Reproductive isolation
Superior	82 100	406	4	Depth segregation: shallow-water vs. profundal; insectivory, piscivory	Strong	Present
Great Bear Lake	31 153	446	4	No depth segregation; weak benthic vs pelagic and insectivory, piscivory	Unknown	Present
Great Slave Lake	27 200	614	2	Depth segregation: shallow-water vs. profundal	Strong	Unknown
Mistassini	2335	183	2	Depth segregation: shallow-water vs. profundal	Strong	Partial
Rush	1.3*	~86	2	Depth segregation: shallow-water vs. profundal; insectivory, piscivory	Strong	Not detected*

*Represents the present study and the novelty of Rush Lake when compared with lake charr diversification model (i.e., first case of lake charr diversification within small lakes).

Table is a synthesis of the following sources: **General:** Eshenroder (2008), Muir *et al.* (2015), **Superior:** Krueger & Ihsen (1995), Moore & Bronte (2001), Harvey, Schram & Kitchell (2003), Goetz *et al.* (2010, 2011), Muir *et al.* (2014), **Great Bear Lake:** Blackie, Weese & Noakes (2003), Alfonso (2004), Chavarie *et al.* (2013, 2015, 2016a, b), Harris *et al.* (2015), **Great Slave Lake:** Zimmerman *et al.* (2006, 2009), Hansen *et al.* (2016), **Mistassini:** Zimmerman *et al.* (2007), Hansen *et al.* (2012), Marin (2015).

Table 2. Polymorphism in a selected list of post-glacial fishes to compare the nature of their sympatric divergence in terms of trophic ecology or movement patterns. Small lakes were defined by surface area < 500 km² and large lakes as ≥ 500 km² (Tilzer & Serruya, 2012); small and large lakes categories represent the presence ($n \geq 1$ = Yes) or absence (No) of intraspecific divergence within those habitats

Species	Localities (native range)	Small lakes	Large lakes	No. of lacustrine morphs	Habitat types	Nature of divergence
Lake charr	Canada, USA	No*	Yes	2–4	Lakes, sea (barely present)	Insectivory, piscivory, profundal, and migration
Arctic charr	Holoarctic	Yes	Yes	2–4	Lakes, rivers, sea	Benthic, pelagic, profundal, insectivory, piscivory, and migration
Brook charr	Canada, USA	Yes	Yes	2	Lakes, rivers, sea	Benthic, pelagic, and migration
Brown trout	West Palearctic	Yes	No	2	Lakes, rivers, sea	Benthic, pelagic, insectivory, piscivory and migration
Lake whitefish spp.	Holoarctic	Yes	Yes	2–4	Lakes, rivers, sea (barely present)	Benthic, pelagic, profundal insectivory, piscivory, and migration
Threespine stickleback	Holoarctic	Yes	No	2	Lakes, rivers, sea	Benthic and pelagic, Migration
Sunfish spp.	Canada, USA, Non-native (e.g., Japan)	Yes	No	2	Lakes, rivers	Benthic and pelagic
Perch spp.	Holoarctic	Yes	Yes	2	Lakes, rivers	Benthic and pelagic

*indicates that the present study of Rush Lake was not included in this table. Lake charr were unusual in contrast with other post-glacial fishes in regards to the lack of reported divergence in small lakes until the present study.

Table is a synthesis of the following sources: **2 + spp review**: Robinson & Wilson (1994), Skúlason & Smith (1995), Smith & Skulason (1996), Robinson & Parsons (2002), Klemetsen *et al.* (2003), Klemetsen (2013), **lake charr** (*Salvelinus namaycush*): Zimmerman *et al.* (2006, 2007), Eshenroder (2008), Goetz *et al.* (2010), Swanson *et al.* (2010), Chavarie *et al.* (2013, 2016a, b), Muir *et al.* (2014, 2015), Harris *et al.* (2015), Marin (2015), **Arctic charr** (*Salvelinus alpinus*): Snorrason *et al.* (1994), Skúlason *et al.* (1999), Jonsson & Jonsson (2001), Adams & Huntingford (2002), Alekseyev *et al.* (2002), Sinnatamby, Reist & Power (2013), Woods *et al.* (2013), **brook trout** (*Salvelinus fontinalis*): Imre, McLaughlin & Noakes (2002), Proulx & Magnan (2004), Fraser & Bernatchez (2005), Bertrand, Marcogliese & Magnan (2008), Morinville & Rasmussen (2008), **brown trout** (*Salmo trutta*): Jonsson (1985, 1989), Pakkasmaa & Piironen (2001), Stelkens *et al.* (2012), **lake whitefish spp.** (*Coregonus lavaretus* & *Coregonus clupeiformis*): Amundsen, Bøhn & Våga (2004), Bernatchez (2004), Kahilainen & Østbye (2006), Østbye *et al.* (2006), Harrod, Mallela & Kahilainen (2010), Præbel *et al.* (2013), Kahilainen *et al.* (2014), **threespine sticklebacks** (*Gasterosteus aculeatus*): Taylor & McPhail (1986), Schluter & McPhail (1992), Schluter (1995), Østbye *et al.* (2016), **sunfish spp.** (*Lepomis macrochirus* & *Lepomis gibbosus*): Robinson & Wilson (1996), Parsons & Robinson (2006), Berchtold *et al.* (2015), and **perch spp.** (*Perca fluviatilis* & *Perca flavescens*): Svanbäck & Eklöv (2002, 2006), Kocovsky & Knight (2012), Faulks *et al.* (2015), Stepien, Behrmann-Godel & Bernatchez (2016).

ecosystems, the most common mode of resource polymorphism in fishes is associated with benthic vs. pelagic habitats, although other mechanisms of intraspecific divergence are possible, but less common (e.g., littoral–profundal resource axis, Tables 1 and 2) (McPhee, Noakes & Allendorf, 2012; Præbel *et al.*, 2013; Hooker *et al.*, 2016).

In contrast with the highly variable Arctic charr (*Salvelinus alpinus*), which have readily radiated into benthic and pelagic morphs in many small and large lakes throughout the holarctic (Snorrason *et al.*, 1994; Jonsson & Jonsson, 2001; Klemetsen, 2010), all reported examples of lake charr (*Salvelinus namaycush*) diversity occur within the largest,

deepest lakes of North America (i.e., > 2000 km²; Table 1) (Goodier, 1981; Krueger & Ihssen, 1995; Zimmerman, Krueger & Eshenroder, 2006, 2007; Chavarie, Howland & Tonn, 2013). Distances among habitats in bathymetrically complex large lakes, in association with divergent reproductive behaviours (e.g., spawning time), can function as sympatric semi-isolating barriers to gene flow (Nosil, 2008; Muir *et al.*, 2012a), allowing over time the accumulation of adaptations within diverging populations. Typically, depth is the main niche axis defining lake charr differentiation, thought to have occurred in sympatry post-glacially, with individuals diverging to occupy shallow and profundal environments, and partitioning prey resources within and between those habitats (Hubbs & Lagler, 1949; Zimmerman *et al.*, 2006, 2007; Eshenroder, 2008; Chavarie *et al.*, 2013; Harris *et al.*, 2015). Cases of polymorphism in lake charr independent of depth exist, with radiation within shallow waters involving a weak benthic–pelagic gradient combined with differential resource use (e.g., piscivorous vs. insectivorous), nonetheless, these are known only from large lakes (Brown *et al.*, 1981; Goodier, 1981; Chavarie *et al.*, 2013, 2016a, b, c; Harris *et al.*, 2015). Differences in phenotypic plasticity, behaviour, assortative mating, and philopatry have been hypothesized to account for why Arctic charr diversify in small lakes whereas lake charr do not (Eshenroder, 2008).

One potential exception that challenges the hypothesis that freshwater lake charr only differentiates (i.e., either originate or be maintained) in large lakes (Eshenroder, 2008) comes from a brief historical account that suggested that co-existing shallow- and deep-water morphs (Fig. 1) occur in a small (1.3 km²) postglacial lake, Rush Lake, situated 5 km south of Lake Superior, near the southern edge of the species range (Hubbs, 1929). Thus, to determine whether two

lake charr morphs occur within this small lake, we revisited Rush Lake more than 75 years later to compare the morphology, life history, habitat use, abundance and distribution, trophic ecology, and neutral genetic diversity of lake charr. Our aim was to address: (1) if divergence exists between lean (shallow-water) and huronicus (deep-water) consistent with the theory of resource polymorphism and with Hubbs (1929, 1930) original description. We also extended our work to investigate: (2) whether the morphs were ecologically and genetically distinct units to determine the influence of the evolutionary processes that led to their formation or maintenance. A species, such as lake charr, in which past cases of intraspecific diversity cases have been constrained to large lakes, would be expected to show strong mechanistic separation to originate and maintain polymorphism in such a geographically small system (Gavrilets & Vose, 2005; Gavrilets *et al.*, 2007). Finally, we examined: (3) the genetic origin of Rush Lake morphs in an attempt to evaluate if the two morphs originated from its nearest neighbour, Lake Superior, or in sympatry post colonization.

METHODS

STUDY SITE AND FIELD COLLECTIONS

Rush Lake (Fig. 2), is elevated 12 m above and located < 5 km from Lake Superior. Rush Lake is small (1.31 km²; ESRI ‘dtl_wat’ series, Redlands, CA, USA) and contains the deepest habitat among neighbouring lakes in the Huron Mountains in Michigan’s upper peninsula, USA (max depth ~86 m; data from this study). The introduction of smallmouth bass (*Micropterus dolomieu*) and rainbow smelt (*Osmerus mordax*), and the translocation of brook charr (*Salvelinus fontinalis*) into several lakes were the primary faunal changes that have occurred (Christy, 1929). Sample size and methods were restricted due to the size of the lake and the uniqueness and rarity of the huronicus population. During 2006, an expedition was mounted to investigate the lake using non-lethal sampling (i.e., angling). Nine of the captured lake charr were typical of the lean morph from Lake Superior, and an additional four lake charr caught in deep-water corresponded to Hubbs’ (1929, 1930) description of *S. huronicus*. During 2007, the lake charr morphs were targeted with short-duration (< 4 h), bottom-set gillnets (lethal sampling; *n* = 68). Two types of gillnets were deployed from 10 to ~86 m to ensure collection of lake charr from all depths. Two sets were made using 183-m long by 1.8-m high nylon gangs with stretch-mesh sizes ranging from 50.8 to 114.3 mm, in 12.7-mm increments (Hansen *et al.*, 2012). Twelve sets were made

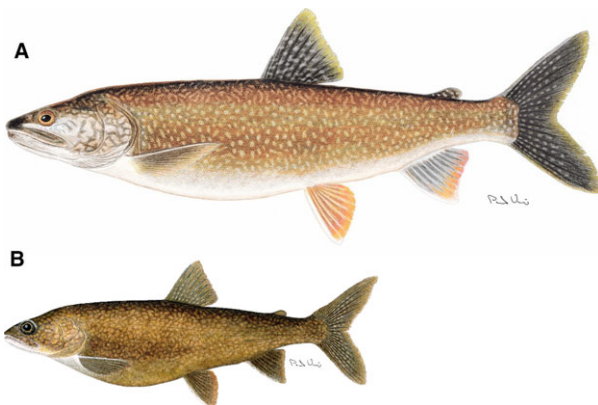


Figure 1. Lean (A) and huronicus (B) lake charr morphs sampled from Rush Lake within the present study. Illustration by P. Vecsei.

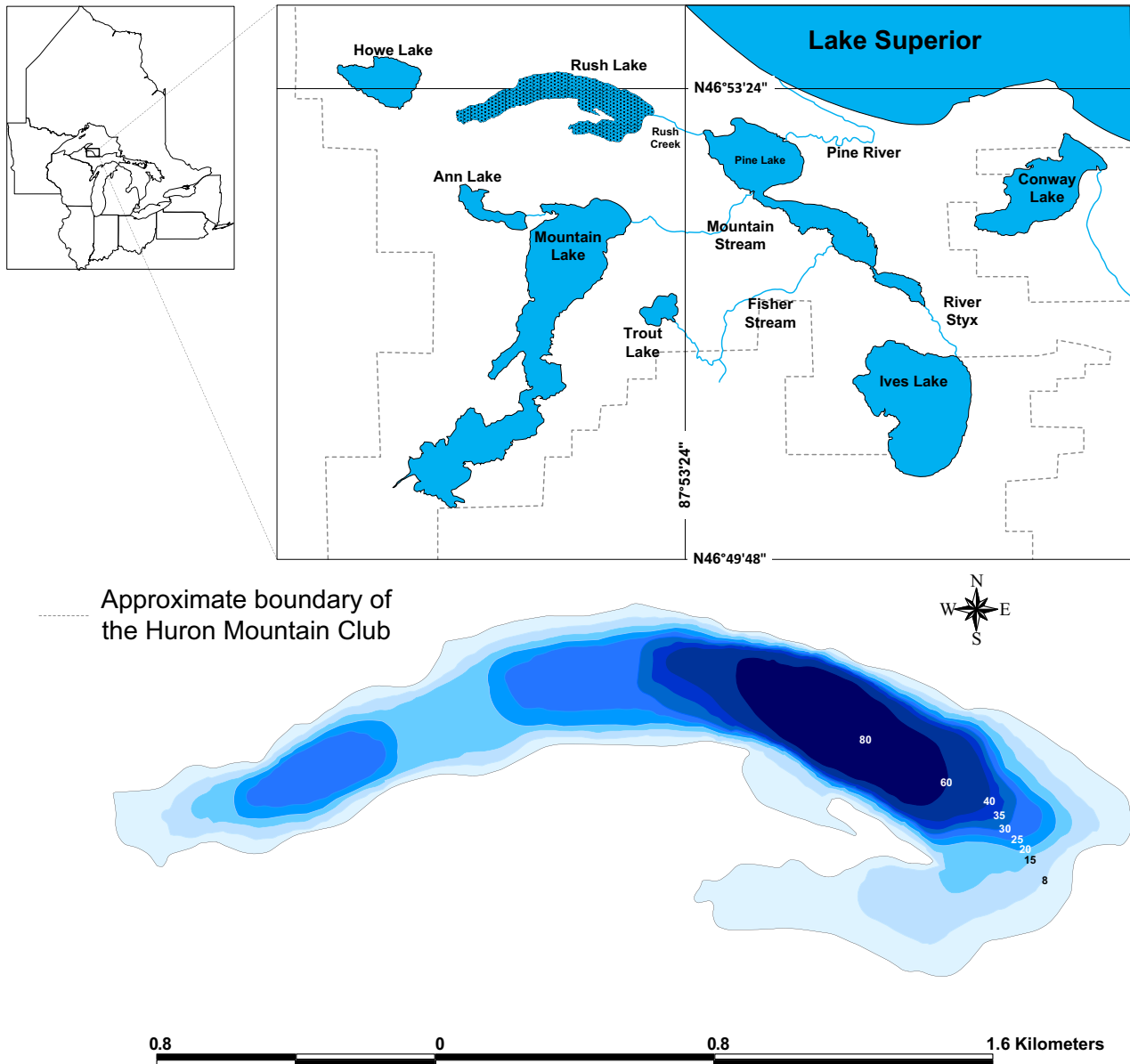


Figure 2. Map of the study area showing Rush Lake. Bathymetric contours in metres; data from this study.

using 64-m long by 1.8-m high monofilament gillnets consisting of stretch-mesh sizes from 57.15 to 127 mm, in 6.35-mm increments (Sandstrom & Lester, 2009). Date, time, GPS location, and minimum and maximum water depth read from a sounder were recorded for each net set. During spring 2011, angling yielded an additional eight lake charr.

Live sampling included collecting a calibrated digital image of the left side of each fish according to Muir, Vecsei & Krueger (2012b), measuring total length (T_L mm), and collecting a fin clip stored in 95% non-denatured ETOH for genetic analysis. All gillnetted fish were photographed, and sampled for sagittal otoliths and dorsal muscle tissue. Biological

data collected included sex, maturity, weight of the fish in air (W_A g), and in water (W_w g) with the swim bladder deflated (for estimating buoyancy). Stomachs were removed and fixed in 10% formalin for gut content analysis.

TREATMENT OF DATA

Sexes were pooled for all analyses because sexes did not differ in TL ($F = 0.001$; $P = 0.99$), buoyancy ($F = 0.052$; $P = 0.82$), or body shape ($F = 0.002$; $P = 0.97$). Kolmogorov–Smirnov tests were used to assess normality of error distributions for all variables and a Levene’s test assessed homogeneity of

variance. When transformations were ineffective, non-parametric tests were used.

MORPHOLOGY

Lake charr ($n = 81$) were morphologically assessed and identified according to the methods used by Muir *et al.* (2014). Size-free head and body shape (using centroid sizes; a robust measure of fish size) were quantified using geometric morphometric methods (TPS; State University of New York at Stony Brook; <http://life.bio.sunysb.edu/morp>; Zelditch *et al.*, 2004). Twenty sliding semi-landmarks and eight homologous landmarks were digitized on images to characterize head shape and 16 homologous and four sliding semi-landmarks were digitized on whole-body images to characterize body shape (see Muir *et al.*, 2014 for landmarks and semi-landmarks). Landmark data were used to scale each individual and obtain centroid size and partial warp scores using TPSrelw (<http://life.bio.sunysb.edu>). Principal component analysis (PCA), using singular value decomposition on the correlation matrix, was used to reduce the 26 head shape and 18 body shape variables; the first four principal components (PCs) from each of the analyses were retained for assignment analyses and to summarize morphological variation using IMP software (<http://www3.canisius.edu/~sheets/morphsoft.html>).

A combination of Bayesian cluster analyses (MCLUST; Fraley & Raftery, 2009) and a visual identification by two experienced lake charr biologists (A. M. Muir & C. C. Krueger) assigned each individual to a morph. Disagreement between the visual and model assignments were settled using decision rules described in Muir *et al.* (2014). Reconciled assignments of lake charr to morphs is referred to as the *overall group assignment*, for all subsequent analyses. Canonical variate analyses and Jackknife validation procedures were conducted on both body and head shape data with CVAGen V. 8 from the IMP software (<http://www3.canisius.edu/~sheets/>). Single-factor permutation multivariate analysis of variance (MANOVA) with 10 000 permutations was performed with CVAGen to test whether body and head shape differed between morphs.

GENETIC DIVERSITY

Total genomic DNA was extracted using a silica-based method (Elphinstone *et al.*, 2003) and 20 microsatellite loci were genotyped for population genetic analyses (Supporting Information, Table S1). MICRO-CHECKER 2.2.3 (Van Oosterhout *et al.*, 2004) was used to assess scoring errors and the presence of null alleles for each lake charr morph. Hardy-Weinberg Equilibrium (HWE) was tested using ARLEQUIN 3.5

(Excoffier & Lischer, 2010). FSTAT 2.9.3.3 (Goudet, 1995) and ARLEQUIN were used to measure the number of alleles (A), allelic richness (A_R), the number of private alleles, observed and expected heterozygosity (H_O and H_E , respectively). Statistical significance for differences in A , H_O , H_E , and A_R among morphs was determined using the independent samples median test in SPSS 21.0 (IBM SPSS Statistics Inc.). Private allelic richness, PA_R , the number of private alleles standardized by sample size for each population, was calculated in HP-RARE (Kalinowski, 2004). Genetic diversity estimates were compared among lean and huronius morphs at Rush Lake, and lean and humper (due to their morphological similarity, see Discussion for more details) morphs in Lake Superior (sampling sites Isle Royale, Grand Marais, Stannard Rock, Superior Shoal, Big Reef, and Klondike Reef) and Lake Mistassini, Québec (S.M. Baillie & P. Bentzen, unpubl. data). Additionally, we compared Rush Lake lake charr diversity to four small lakes of similar size ($< 6 \text{ km}^2$) in the Kogaluk River watershed, Labrador, Canada, as reported by McCracken *et al.* (2013) to provide context in terms of genetic diversity expected in small vs. large lakes. Standardized sets of common microsatellite loci were used for all inter-lake comparisons (Supporting Information, Table S2).

POPULATION GENETIC STRUCTURE

To compare genetic differentiation among lean and humper morphs from several lakes, we generated pair-wise F_{ST} (Wright, 1965) and R_{ST} (Slatkin, 1995) estimates with 10 000 permutations in ARLEQUIN. The null hypothesis that alleles were drawn from the same frequency distribution in both morphs was tested using the genic differentiation test in GENEPOP 4.2 (Rousset, 2008). Neighbor-joining (NJ) trees assessed population clustering (POPULATIONS v1.2.32; available from <http://bioinformatics.org/~tryphon/populations/>). Unrooted trees were based on Cavalli-Sforza and Edwards chord distance matrix (Cavalli-Sforza & Edwards, 1967) and visualized using TREEVIEW v1.6.6 (Page, 1996). To determine the number of genetic populations, K , we used the Bayesian clustering method implemented in program STRUCTURE 2.3.4 (Pritchard, Stephens & Donnelly, 2000; Falush, Stephens & Pritchard, 2007; Hubisz *et al.*, 2009) and a principal co-ordinates analysis (PCoA) in GENALEX 6.5 (Peakall & Smouse, 2012). The program STRUCTURE was used with the admixture model and *a priori* morph assumptions to estimate the number of genetic populations, K . Burn-in length was set at 5.0×10^5 , followed by 3.0×10^6 randomization steps and ten independent runs were conducted for each value of K (from $K = 1$ to $K = 10$). The estimated natural log probability of K ($\ln P(K)$)

was generated in STRUCTURE HARVESTER Web 0.6.92 (Earl & vonHoldt, 2012).

LIFE HISTORY

Methods for estimating life history parameters followed those described by Hansen *et al.* (2012), and summarized here. Sagittal otoliths were used to estimate lake charr ($n = 68$) age because otolith thin sections were validated for age estimation of lake charr to an age of at least 50 years (Campana, Casselman & Jones, 2008). Length, weight, and age were compared between lean and huronicus morphs using single-factor ANOVA (Zar, 1999). Log-transformed weight-length relationships were compared between morphs using a general linear model with weight (grams) as the dependent variable, length (mm) as the independent variable, morph as a class variable, and the interaction between length and morph to compare slopes (Zar, 1999). Growth in length with age was modelled using two versions of the Von Bertalanffy length-age model fit to back-calculated length-at-age of individual fish (Mooij, Van Rooij & Wijnhoven, 1999; Quinn & Deriso, 1999):

$$L_t = L_\infty \left(1 - e^{-K(t-t_0)}\right) + \varepsilon$$

$$L_t = L_\infty - (L_\infty - L_0) \left(1 - e^{-(\omega/L_\infty) \times t}\right) + \varepsilon$$

These models described back-calculated length, L_t , at-age t as a function of theoretical maximum length ($L_\infty = \text{years}$), instantaneous rate at which L_t approaches L_∞ ($K = 1/\text{year}$), theoretical age-at-zero length ($t_0 = \text{years}$), early growth rate ($\omega = L_\infty \times K = \text{mm/year}$; Gallucci & Quinn, 1979), length-at-age = 0 ($L_0 = \text{mm}$), and additive error (ε). Model parameters, L_∞ , K , t_0 , L_0 , and ω and their asymptotic standard errors were compared between morphs using a non-linear mixed-effect model (NLME) with a fixed population effect to compare between morphs, and random individual effects (Vigliola & Meekan, 2009). Maturity status (dependent variable; immature = 0, mature = 1) at length and age (independent variables) was estimated using logistic regression (Hosmer, Lemeshow & Sturdivant, 2000). Length and age at 50% maturity of each morph was estimated as the ratio of the absolute value of the intercept, $|b_0|$, to the slope, b_1 .

HABITAT USE

A Mann–Whitney analysis compared median depth of capture between morphs to test for differences in habitat use. Buoyancy between the two lake charr morphs was compared using a t -test. Buoyancy was

calculated as $B = [(W_a - W_w)/W_a] \times 100$ according to Muir *et al.* (2014). Buoyancy was also compared between morphs with the approach used in the weight-length analysis described above, in which morph is a class variable, with mass in water as the dependent variable, and mass in air as the independent variable. Fish with high lipid content weigh less in water at a given mass, a characteristic associated with reduced swimming costs in fishes without swim bladders (Corner, Denton & Forster, 1969; Bone, 1972; Eastman, 1988). High lipid content also occurs in vertically migrating fishes whose swim bladders are compressed after rapid descents (Alexander, 1972; Krause, Eshenroder & Begnoche, 2002).

ABUNDANCE AND DISTRIBUTION

Abundance of lake charr morphs was estimated by a combination of acoustic methods, gillnet sampling, and angling. The acoustic approach assumed all large acoustic targets below the thermocline were lake charr and strata surface areas were measured without error. The first assumption is likely valid given that Rush Lake contains no other large-bodied, deep-water, pelagic fishes (Hubbs, 1930). Two whole lake night acoustic surveys were conducted on 27 and 29 August 2007. In total, 18 and 20 parallel transects, separated by 150 m, were completed per survey. Based on bathymetric depth of capture of morphs by angling and gillnetting ($\leq 45 \text{ m} = 74\%$ lean, 26% huronicus; $> 45 \text{ m} = 3\%$ lean, 97% huronicus), we stratified Rush Lake into two zones – shallow ($\leq 45 \text{ m}$) and deep ($> 45 \text{ m}$) – for the purpose of estimating abundance (Supporting Information, Table S3).

Acoustic data were collected with a Simrad EY500 split-beam echo sounder, equipped with a 120 kHz, 7.2° (half-power beam width) split-beam transducer, field calibrated with a standard 23-mm tungsten carbide sphere. The pulse duration and ping rate were fixed at 0.1 ms and 4–5 pings per second, respectively. The on-axis mark threshold during collection was -70 dB . Survey data were analyzed using Sonar-Data Echoview software (Version 4.40.71.11366; Sonar-Data Ltd). An automatic bottom tracking algorithm was used to define a bottom line 0.5 m above the lake bed to exclude bottom echoes. Further, echoes not meeting single target detection criteria (Rudstam *et al.*, 2009) were also excluded. Accepted fish echoes were amalgamated into fish tracks using the Echoview 4D fish tracking algorithm. Density calculations were limited to fish tracks having mean target strength greater than -35.7 dB ($n = 61$ and 59 for August 27 and 29, respectively), equivalent to a TL of 300 mm and larger (Middel, 2005). We also excluded acoustic information in which bathymetric depths were $< 7 \text{ m}$

under the assumption that lake charr would avoid the warm epilimnion of Rush Lake (water temperature exceeded 20 °C during late August).

Sample volume of an acoustic beam increases with depth. To standardize fish density estimates for increasing sample volume, detected fish were weighted back to a 1-m wide swath at the surface using a formula provided by Yule (2000):

$$F_w = 1/[2 \cdot R \cdot \tan(3.6^\circ)]$$

where F_w equals weighted fish, R equals range or distance beneath the transducer, and 3.6° equals one-half the nominal transducer beam width. For example, at 8 m below the 7.2° transducer, the cone diameter $2 \cdot R [\tan(3.6^\circ)]$ is 1.0 m. It follows that a fish tracked at 8.0 m of range equaled one weighted fish at the surface (all fish were normalized to a 1-m transect width). At 40 m below the transducer the cone diameter is 5.0 m and a fish tracked at this range equaled 0.20 weighted fish. We summed F_w in each zone along each transect. Densities (number·m⁻²) in the shallow and deep zone of each transect were estimated by dividing these sums by the distances traversed (m) within the two bathymetric strata (depths of 7–45 m and > 45 m, respectively). Multiplying these densities by 10 000 provided density estimates with units number·ha⁻¹. Average densities were calculated using transects as sample units.

Observed morph densities in each stratum were calculated by multiplying average densities for each survey by morph proportions from gillnet and angling surveys. Observed morph abundance by strata was calculated by multiplying observed morph densities by the surface area of Rush Lake where bathymetric depths were 7–45 m (92.05 ha) and > 45 m (19.7 ha), estimated from a hypsographic curve. A bootstrap approach ($n = 1000$ iterations) was used to estimate 95% confidence intervals (95% CI) of morph density estimates by incorporating uncertainty in acoustic density estimates and morph proportions (*sensu* Yule *et al.*, 2009). Finally, observed morph abundance estimates (and 95% CIs) were summed over both zones for each survey.

TROPHIC ECOLOGY

Stomachs

Prey were dissected from each stomach, sorted, and weighed (± 0.001 g). Prey were pooled into one of three categories: terrestrial insects, aquatic invertebrates, and fish (fish species + fish remains). Due to their abundance, *Mysis diluviana* (opossum shrimp), *Diporeia* spp. (amphipods), and rainbow smelt were defined as separate prey categories. Diet composition was reported by morph using frequency of occurrence

(%O), abundance (%N), and mass (%M) of prey (Supporting Information, Table S4). To scale the importance of each prey category, %O, %N, and %M were used to calculate the relative importance index (RI) at the morph level (lean vs. huronicus) but also at the individual level, in which %O was excluded (George & Hadley, 1979). Dietary differences between lake charr morphs were tested with a one-way ANOSIM with 9999 permutations and a SIMPER analysis (Anderson & Braak, 2003; see Chavarie *et al.*, 2016a, b) using PAST 3 (Hammer, Harper & Ryan, 2001). Within each morph, diet differences were tested, between depth strata that lake charr were caught (20–30 m, 30–40 m, and 60–90 m) using ANOSIM and SIMPER analysis.

Isotopes

Dorsal muscle tissue from lake charr were collected for isotopic analysis in August 2007. Tissue samples were frozen at -20°C prior to isotopic analysis. Thawed tissues collected for isotope analysis were oven-dried at 60°C for 2 days and homogenized to a fine powder. Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes were analyzed at the University of California-Davis Stable Isotope Facility on a Europa Hydra 20/20 continuous-flow isotope-ratio mass spectrometer. Isotopes were reported as δ values representing a deviation in parts per thousand (‰) from a standard, Pee Dee belemnite (PDB) limestone for $\delta^{13}\text{C}$ and atmospheric nitrogen for $\delta^{15}\text{N}$. Nineteen percent of the samples were analyzed in duplicate; one standard error of the mean difference between replicates was 0.39‰ for $\delta^{13}\text{C}$ and 0.14‰ for $\delta^{15}\text{N}$.

To account for variability in carbon signature due to lipid content, lipids were extracted from a subset ($n = 21$) of fish tissue samples using a modified Folch method (Sweeting, Polunin & Jennings, 2006) and $\delta^{13}\text{C}$ was reanalyzed. The $\delta^{13}\text{C}$ values for the fish samples were compared between lipid-extracted samples and bulk tissue samples. A non-linear relationship between $\Delta \delta^{13}\text{C}$ ($\delta^{13}\text{C}_{\text{lipid-extracted}} - \delta^{13}\text{C}_{\text{bulk}}$) and C:N ratio of untreated samples closely resembled the Kiljunen *et al.* (2006) model. To improve the fit, we re-estimated the parameters D (difference in $\delta^{13}\text{C}$ between protein and lipids) and I (a constant) with a non-linear least squares regression (nls function in R 2.15.1; www.r-project.org). The revised model ($R^2 = 0.99$, $F_{1,19} = 5397$, $P < 0.0001$) was used to normalize all untreated fish samples for lipid content:

$$\delta^{13}\text{C}' = \delta^{13}\text{C} + 6.112 * [0.005 + (3.9/(1 + 287/L))],$$

in which $\delta^{13}\text{C}'$ is the lipid-corrected value of the sample, $\delta^{13}\text{C}$ is the observed untreated value of the sample, and L is the proportional lipid content of the sample calculated by:

$$(L = 93/[1 + ((0.246 * C : N) - 0.775^{-1})].$$

Two separate *t*-tests compared $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between the lean and huronicus morphs. To test for form-function relationships in trophic adaptations (Bock & Von Wahlert, 1965; Lauder, 1981; Cooke & Terhune, 2015), head shape and body shape PC1, and body size (centroid size) were regressed against $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ using linear regression and slope values were tested to determine if they differed from 0. To test for the pertinent relationships, $\delta^{13}\text{C}$ values were selected because they are known to distinguish littoral vs. pelagic or nearshore vs. offshore food webs and $\delta^{15}\text{N}$ are known to distinguish trophic position (Fry, 2007).

RESULTS

MORPHOLOGY

Two morphs were identified within our lake charr collection on the basis of body and head shape (Supporting Information, Fig. S1). Of the 81 lake charr captured, 43 were classified as huronicus, and 38 were classified as lean. Agreement between body and head model classifications and experts were between 84 and 86%. For the body shape measures, the first four PCs accounted for 67% of lake charr shape variation (Fig. 3a). PC1 (33% variation) primarily separated lean and huronicus morphs on the basis of body depth, peduncle depth and length, and head shape and length. The lean morph tended to have a much more narrow body, a pointier and longer head, and a longer narrower caudal peduncle than the huronicus morph. PC2 (16% variation) discriminated more subtle variations in anterior-posterior variation in fin insertions and eye position.

For the head shape measures, the first four PCs accounted for 79 % of lake charr shape variation (Fig. 3b). Similar to the body shape model, PC1 (42% variation) accounted for gross differences in head shape, with leans having a longer, but less deep and more pointy head, longer maxillae, and a lower more posterior positioned eye than huronicus. Overall, huronicus had a deep, short head, with a greater slope in head profile from the eye to the snout than the lean morph. Overall, huronicus was more variable in PC2 (19% variation) than leans, with subtle differences in the position of the eye, maxilla, and snout shape.

CVA indicated variation in body shape between huronicus and lean (Axis 1: $\lambda = 0.41$, $P = 0.03$; Supporting Information, Fig. S2a) with 65.4% correct assignments. Permutation MANOVA confirmed body shape differences between huronicus and lean

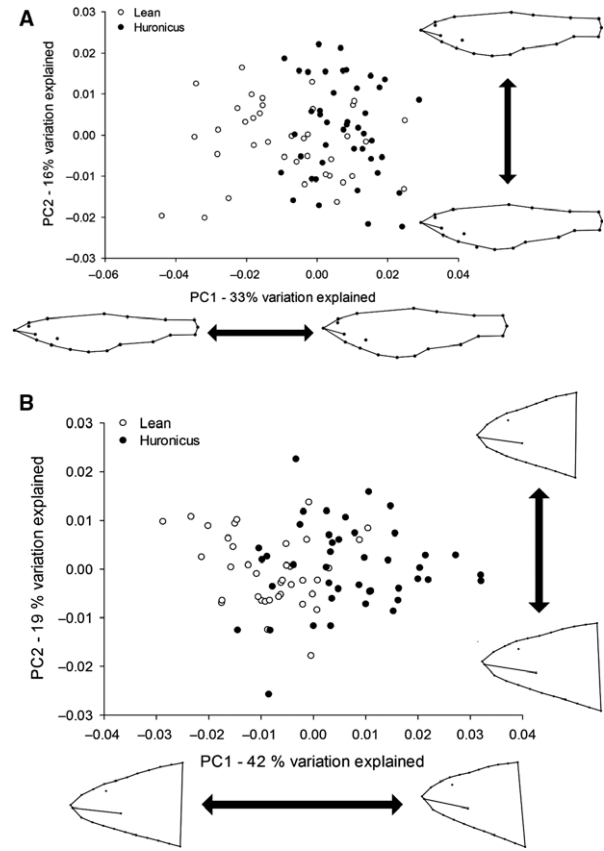


Figure 3. PCA ordination of lake charr body shape (A) and head shape (B) with percentage representing the variation explained by that component. Outlines drawn from vector plots represent the body shape variation on each axis. Morphs were identified by McCluster analysis (Fraley & Raftery, 2009) and overall group assignment.

($F = 9.62$, d.f. = 1, $P < 0.01$) whereas grouping explained 81.2 % of total variance. CVA suggested variation in head shape between huronicus and lean (Axis 1: $\lambda = 0.30$, $P = 0.01$, Supporting Information, Fig. S2b) with 65.3 % correct assignments. Permutation MANOVA confirmed head shape differences between huronicus and lean ($F = 16.4$, d.f. = 1, $P < 0.01$) whereas grouping explained 88.5 % of total variance.

GENETIC DIVERSITY

Eighteen of 20 microsatellite loci were polymorphic (two to nine alleles per locus) within each morph (Supporting Information, Table S2). Lake charr populations were in Hardy-Weinberg Equilibrium except for locus Ssa85 and one instance of a possible null allele apparent at locus Omm1105 in the huronicus morph, but not the lean morph (Supporting

Table 3. Comparison of mean allelic diversity and genetic equilibria in lean and huronicus lake charr morphs from Rush Lake to lake charr from (A) Lake Superior based on 18 microsatellite loci common to both datasets, and (B) Lake Superior and Mistassini based on ten microsatellite common loci. Columns indicate the number of individuals genotyped (n), mean number of alleles (A), observed heterozygosity (H_O), expected heterozygosity (H_E), and the P -values of Hardy–Weinberg Equilibrium tests (HWE). Allelic richness (A_R) and private allelic richness (PA_R) estimates were based on a minimum sample size of 34 diploid individuals

	Lake – Morph	n	A	A_R	H_O	H_E	PA_R	HWE
Based on 18 microsatellite loci common	Rush lean	38	3.6	3.2	0.39	0.40	0.15	0.60
	Rush huronicus	51	3.3	2.9	0.41	0.40	0.12	0.58
	Lake Superior lean	201	10.6	7.8	0.55	0.58	1.48	0.36
	Lake Superior humper	71	8.6	7.4	0.52	0.57	1.17	0.39
Based on ten microsatellite loci common	Rush lean	38	3.3	3.2	0.36	0.38	0.11	0.63
	Rush huronicus	51	2.8	2.7	0.39	0.37	0.00	0.64
	Lake Superior lean	201	8.1	6.0	0.48	0.50	0.41	0.44
	Lake Superior humper	71	7.1	6.0	0.46	0.51	0.57	0.47
	Mistassini lean	42	7.7	7.2	0.58	0.60	0.80	0.47
	Mistassini Humper	37	7.4	7.3	0.60	0.60	1.09	0.43

Information, Table S2). A , A_R , H_E , and H_O estimates did not differ between morphs (all $p \approx 0.8$) (Table 3). Of 69 alleles sampled, 12 (17%) were private. The lean morph had nine private alleles at seven loci with frequencies ranging from 0.013 to 0.054, whereas the huronicus morph had three private alleles at two loci ranging in frequency from 0.010 to 0.011 (Supporting Information, Table S2). Frequencies of private alleles for Rush Lake leans were < 0.016 (rare alleles), with the exception of one allele which was 0.054 (Fig. 4). The average frequency of private alleles for leans was 0.018 and for huronicus was 0.010 (Fig. 4). Lake charr in Rush Lake have 2.5 and 1.5 times lower allelic richness and expected heterozygosity estimates, respectively, than conspecifics in nearby Lake Superior (Table 3). Rush Lake genetic diversity was less than half that of Lake Mistassini, which was more diverse than Lake Superior (Table 3). Furthermore, PA_R estimates suggested that less genetic differentiation occurred between morphs in Rush Lake than in lakes Superior and Mistassini. The genetic diversity in Rush Lake was comparable to small Labrador lakes (Supporting Information, Table S5).

POPULATION GENETIC STRUCTURE

Both the Rush Lake lean and huronicus were genetically differentiated from Lake Superior and Lake Mistassini morphs according to F_{ST} and R_{ST} estimates, with the exception of three paired-comparisons of R_{ST} : Stannard Rock leans and Rush Lake leans, Stannard Rock leans and Rush Lake huronicus, and Grand Marais leans and Rush Lake leans

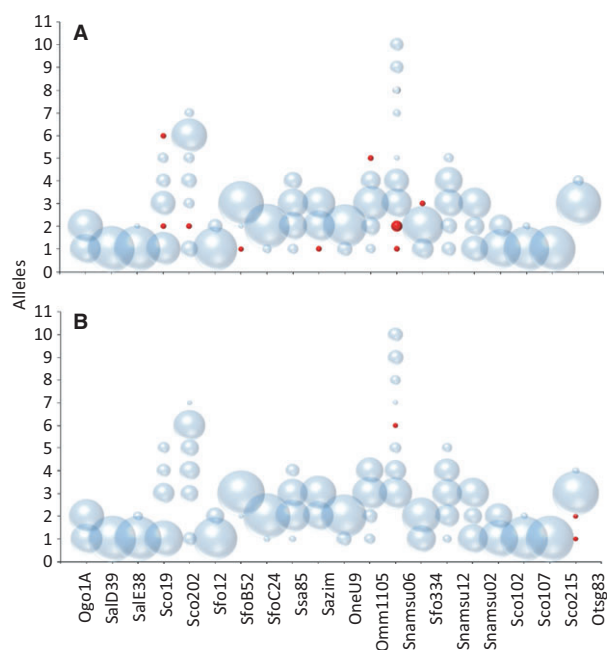


Figure 4. Allelic frequency bubble chart for 20 microsatellite loci genotyped for (A) lean and (B) huronicus lake charr morphs in Rush Lake calculated using ARLEQUIN (Excoffier & Lischer, 2010). Bubble diameter corresponds to allelic frequency and alleles shown in red are private.

(Supporting Information, Table S6). Similarly, the NJ tree indicated that Rush Lake morphs were distinct from the other two lakes (Fig. 5). The Bayesian clustering analysis result of $P(K)$ at $K = 1$ (mean $P(K) = -2159.3$; Supporting Information, Fig. S3) on Rush Lake morphs indicated one genetic population

of lake charr occurred in Rush Lake (Pritchard *et al.*, 2000; Supporting Information, Fig. S4). This result was supported by PCoA, in which multilocus genotypes of lean and huronicus lake charr overlapped in multivariate space (Supporting Information, Fig. S5). The genic differentiation test revealed two loci (Sco202 and Sfo334) with different allelic frequency distributions between morphs, although these differences were neither significant after sequential Bonferroni correction for multiple tests (Peres-Neto, 1999) nor when loci were pooled.

LIFE HISTORY

The lean morph was longer, younger, lighter in weight (when shorter than 500 mm), less buoyant, with a higher early growth rate to a longer average asymptotic length than huronicus. Leans were significantly longer and younger than huronicus, but the two morphs did not differ in overall mean weight (Table 4; Supporting Information, Figs S6 and S7). When shorter than 500 mm, leans were lighter than huronicus because the intercept of the weight-length

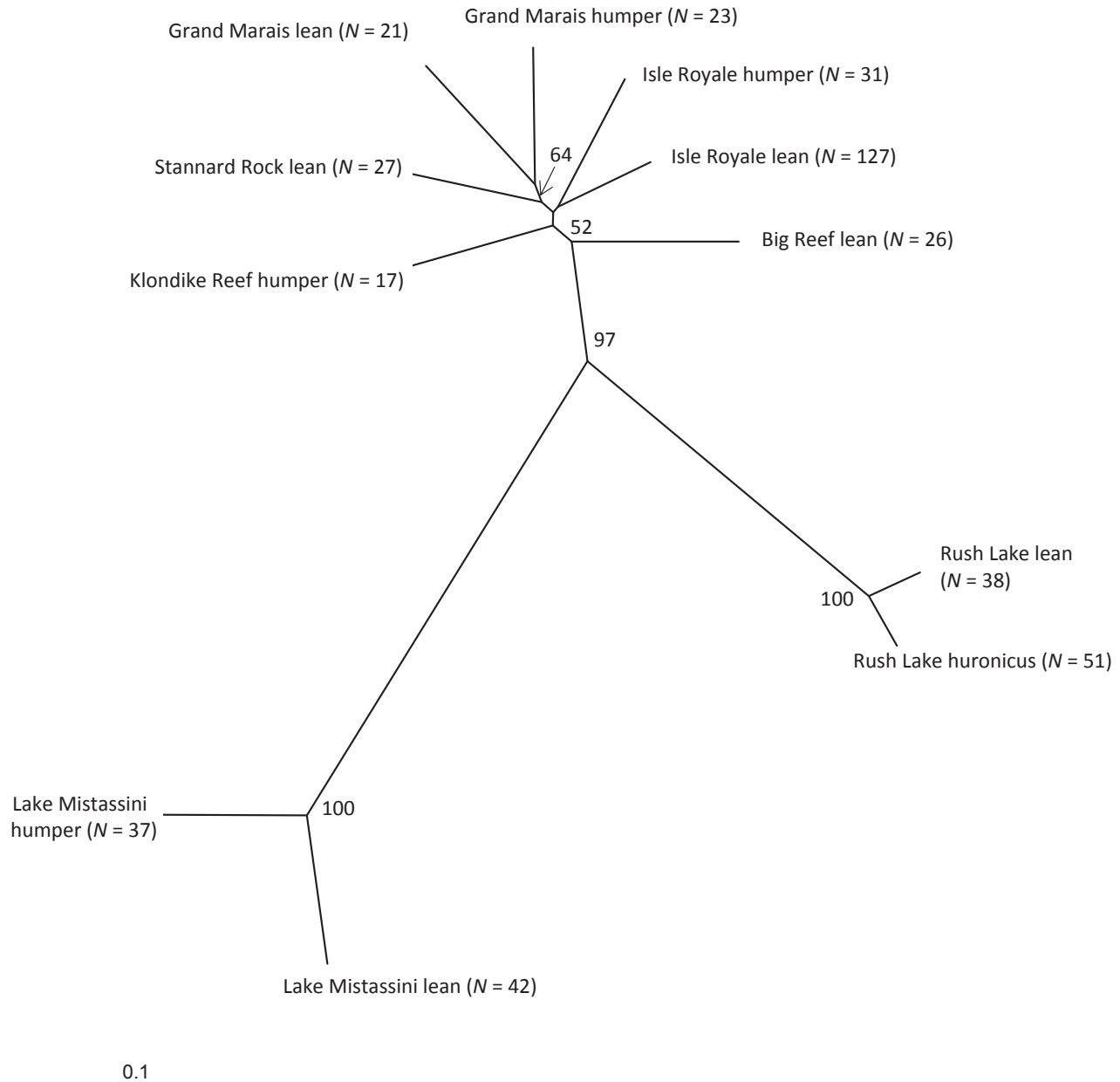


Figure 5. Neighbor-joining tree based on Cavalli-Sforza & Edwards (1967) chord distance model with 100 bootstraps on ten microsatellite loci from leans and huronicus/humpers from Rush Lake, Lake Superior, and Lake Mistassini. Note: Perfect agreement with UPGMA tree.

relationship was greater for huronicus than for leans (Fig. 6a; Table 4). Leans had higher early growth rate and reached a longer average asymptotic length than huronicus (Fig. 6b; Table 4). Only four of 24 leans were immature, so maturity was not significantly related to age ($Z = 1.58$; $P = 113$) or length ($Z = 1.59$; $P = 112$). Similarly, only one of 44 huronicus were immature, so maturity was not significantly related to age ($Z = 0.14$; $P = 0.89$) or length ($Z = 0.68$; $P = 499$). However, mature leans averaged 11.8 years in age and 432 mm in length, whereas mature huronicus averaged 16.7 years in age and 393 mm in length (Table 4).

HABITAT USE

Median depth of capture differed between lean and huronicus morphs (Mann–Whitney $U = 23.5$, $P \leq 0.01$; Supporting Information, Fig. S8), with lean charr occurring at shallower depths than huronicus

(median; lean = 28.8 m and huronicus = 85.1 m). Overall, huronicus (mean = 94.33 ± 0.13) were more buoyant than leans (mean = 94.73 ± 0.09 ; $t = -2.65$; d.f. = 66; $P = 0.01$). Huronicus became more buoyant as length increased than leans and slopes of relationships between water mass and air mass differed significantly between morphs (Fig. 6c; Table 4).

ABUNDANCE AND DISTRIBUTION

Huronicus abundance within Rush Lake was less than estimated for the lean lake charr morph (Table 5). Combined lake charr densities in Rush Lake were estimated to be $30.7 \text{ fish}\cdot\text{ha}^{-1}$ (95% CI = $18.7\text{--}42.5\cdot\text{ha}^{-1}$) on 27 August, and $31.3\cdot\text{ha}^{-1}$ (95% CI = $15.2\text{--}54.1\cdot\text{ha}^{-1}$) on 29 August 2007. Point estimates of abundance within each morph were within the 95% CI of the two surveys, indicating that the two estimates did not differ statistically. However, when estimated within depth strata (7–45 m and > 45 m), abundance

Table 4. Total length (TL = mm), weight (g), age (years), intercept and slope of \log_e -transformed weight–length relationships (body condition), intercept and slope of wet-weight vs. dry-weight relationships (buoyancy), and average growth parameters [age-at-length-zero (t_0 = years), length-at-age-zero (L_0 = mm), early growth rate (ω = mm/year), instantaneous growth rate (K = years $^{-1}$), and asymptotic length (L_∞ = mm)] for two lake charr morphs captured in Rush Lake

	Parameter		Lean	Huronicus	F-ratio	P
Demography	TL	Estimate	430	393	7.6	$\leq 0.01^*$
		SE	10.8	8.0		
		Range	333–525	335–641		
	Weight	Estimate	687	580	1.7	0.2
		SE	65.6	48.4		
		Range	250–1,410	340–2,160		
	Age	Estimate	11.7	16.8	22.1	$\leq 0.01^*$
		SE	0.9	0.6		
		Range	7–18	10–31		
Condition	Intercept	Estimate	16.6	11.8	30.2	$\leq 0.01^*$
		SE	1.0	0.620		
	Slope	Estimate	3.8	3.0	16.6	$\leq 0.01^*$
		SE	0.2	0.1		
Buoyancy	Intercept	Estimate	5.2	6.5	13.0	$\leq 0.01^*$
		SE	1.3	1.2		
	Slope	Estimate	0.05	0.04	6.2	0.02*
		SE	0.002	0.002		
Growth	t_0	Estimate	–0.9	0.8	0.02	0.9
		SE	–0.06	0.08		
	L_0	Estimate	47	44	13.3	$\leq 0.01^*$
		SE	3.4	2.4		
	ω	Estimate	68	53	14.7	$\leq 0.01^*$
		SE	3.1	2.2		
	K	Estimate	0.1	0.1	0.1	0.8
		SE	0.008	0.005		
	L_∞	Estimate	590	474	54.2	$\leq 0.01^*$
		SE	19.5	13.4		

Significant results ($P < 0.05$) are highlighted by **.

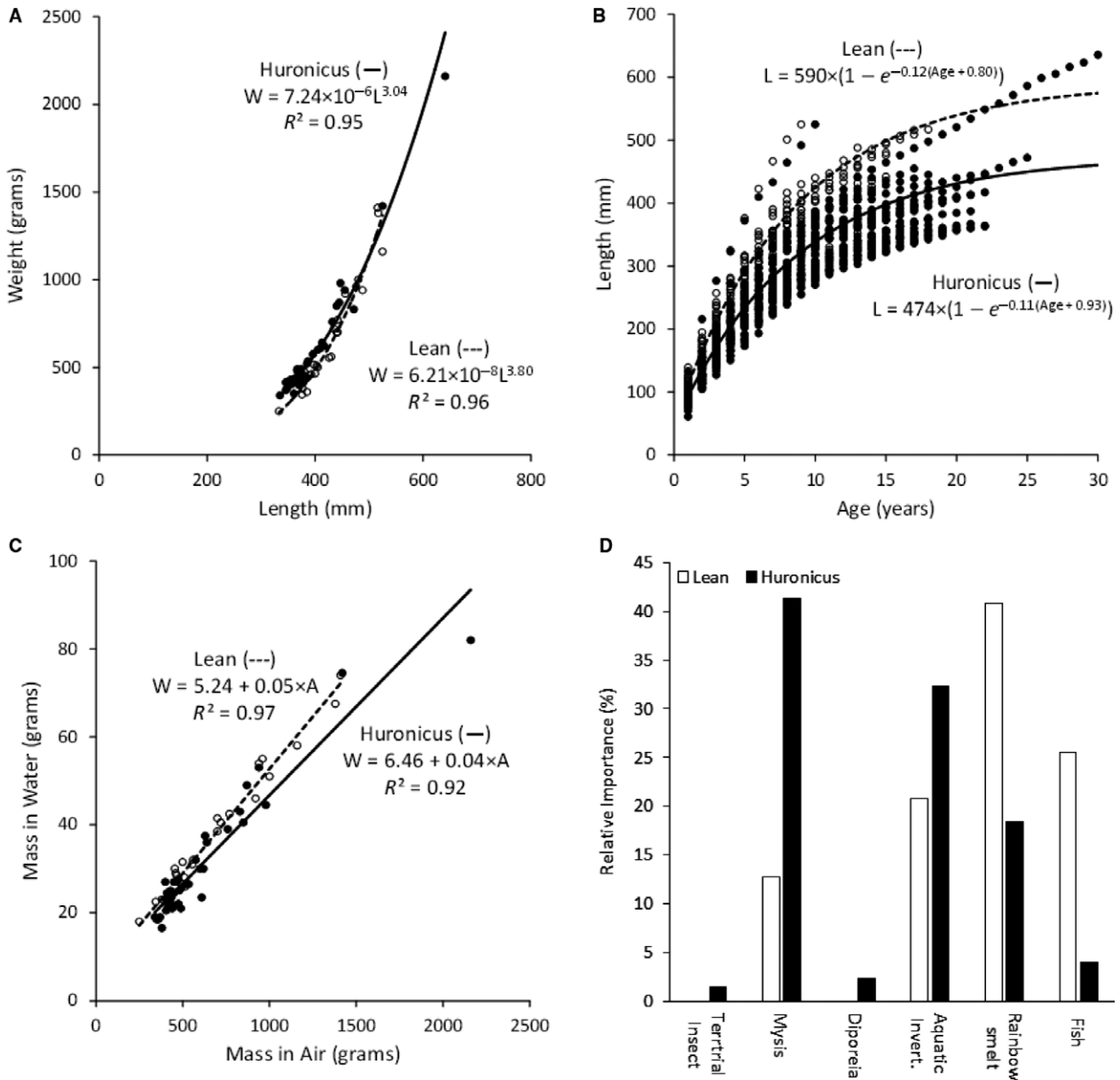


Figure 6. Weight-length relationships (A), length-age relationships (B), and relationships between mass in water and air (i.e., buoyancy) (C) for huronicus (solid dots, solid line) and lean (open dots, dashed line), whereas relative importance (%) of diet composition in non-empty stomachs (D) of lean ($n = 24$) and huronicus ($n = 37$) lake charr morphs in Rush Lake. Aquatic invertebrates (other than *Mysis* and *Diporeia*) included oligochaetes, larval Diptera, and Ephemeroptera and fish included unidentified remains fishes (N.B., list of possible prey fishes available in lake from methods section).

and density significantly differed between morph with lean char more abundant in shallow waters and huronicus more abundant in deep waters.

TROPHIC RESOURCE USE

Relative importance index (using %O, %N, %M of prey) differed between morphs. *Mysis* and aquatic

invertebrates were more important as prey for huronicus whereas fishes were more important as prey for leans (ANOSIM: $R = 0.47$, $P < 0.001$) (Fig. 6d). Stomach contents of the lean and huronicus morphs mainly differed in *Mysis*, rainbow smelt, aquatic invertebrates, and fish with SIMPER-calculated contributions to morph diet differences of 32.51%, 26.57%, 19.76%, and 19.66%, respectively.

Table 5. Density (number·ha⁻¹) and abundance of lake charr morphs in Rush Lake estimated during night time August 2007 acoustic surveys. The 95% confidence intervals (95% CI) were estimated with a bootstrap (see Methods section for details). Lake-wide estimates equal the sum of morph estimates for water depths ≤ 45 and > 45 m calculated for two summer surveys

Date	Stratum	Density (95% CI)		Abundance (95% CI)	
		Lean	Huronicus	Lean	Huronicus
August 27	7–45 m	18.5 (11.3–24.9)	6.5 (3.9–8.9)	1740 (1067–2339)	611 (367–838)
	> 45 m	0.9 (0.1–1.9)	26.1 (16.1–39.6)	18 (2–37)	514 (317–780)
	lake-wide	18.7 (11.4–25.3)	12.0 (7.3–17.2)	1758 (1069–2376)	1125 (684–1618)
August 29	7–45 m	19.4 (9.2–34.1)	6.8 (3.1–12.0)	1824 (868–3209)	640 (289–1132)
	> 45 m	0.8 (0.0–1.7)	23.6 (13.7–36.1)	16 (2–33)	465 (270–710)
	Lake-wide	19.6 (9.3–34.5)	11.7 (5.9–19.6)	1840 (870–3242)	1105 (559–1842)

Relative importance index of stomach contents differed according to the depth strata where huronicus were caught (ANOSIM: $R = 0.62$, $P < 0.001$) whereas leans showed no diet differences related to their capture depth (ANOSIM: $R = 0.07$, $p = 0.17$). Huronicus caught in 60–90 m differed in their relative importance index from huronicus caught in 20–30 m and 30–40 m ($P < 0.03$), but not between depth strata of 20–30 m and 30–40 m ($P > 0.05$). Fish and rainbow smelt were more abundant in huronicus stomachs caught in shallower depth strata whereas *Mysis* and aquatic invertebrates were more important in the deep depth strata. Huronicus stomach contents mainly differed in *Mysis*, rainbow smelt, fish, and aquatic invertebrates with SIMPER-calculated contributions to depth strata diet differences of 37.84%, 23.8%, 20.32%, and 13.39%, respectively.

The lean morph occupied a slightly higher trophic position ($\delta^{15}\text{N} = 9.1$) than the huronicus morph ($\delta^{15}\text{N} = 8.8$; $T = 1078$; $P = 0.007$); although statistically different, the difference may not be biologically significant. The lean morph also had a more littoral signal (mean $\delta^{13}\text{C} = -26.95$) whereas huronicus had a more pelagic offshore signal (mean $\delta^{13}\text{C} = -27.65$; $T = 6.31$; $P < 0.01$) (Fig. 7; Supporting Information, Fig. S9). The slope of the body shape vs. $\delta^{13}\text{C}$ did not differ from zero ($r^2 = 0.14$; $t = 1.54$, $P = 0.13$). In comparison, both head shape ($r^2 = 0.36$; $t = 2.85$, $P = 0.005$) and body size ($r^2 = 0.28$; $t = 2.43$, $P = 0.018$; Fig. 7) were correlated with $\delta^{13}\text{C}$ values. No relationships were found for $\delta^{15}\text{N}$ (Supporting Information, Fig. S10).

DISCUSSION

This study, which integrated information on lake charr morphology, life history, habitat use, population abundance, diet, and genetics, supports Hubbs'

(1929, 1930) contention that two distinct lake charr morphs occur in Rush Lake. Our findings quantify and expand understanding of this unusual, contemporary case in a small lake. As described by Hubbs, lake charr populations in Rush Lake include two co-existing morphs, a large-bodied, shallow-water lean morph and a plump-bodied deep-water morph, the huronicus. Our evidence of morphological and ecological differentiation found between lean and huronicus morphs from Rush Lake was consistent with the hypothesis that foraging selection pressures differ along the niche axis of shallow- vs. deep-water habitats. This niche axis has been identified as a main driver of lake charr differentiation in multiple lakes in North America (Zimmerman *et al.*, 2006, 2007, 2009; Muir *et al.*, 2014, 2015). Although depth is not as common axis of divergence than benthic vs. pelagic for most fish species, morph diversification has been associated with depth and foraging opportunities and observed across a range of fish taxa (e.g., Salmonidae, Cichlidae) and geographic locations (e.g., Africa, North America, Europe) (Jonsson & Jonsson, 2001; Turner *et al.*, 2001; McPhee *et al.*, 2012; Siwertsson *et al.*, 2013a; Skoglund, Knudsen & Amundsen, 2013; Hooker *et al.*, 2016; Turgeon *et al.*, 2016).

Morphological differences between morphs highlight their differential habitat use in association with differing foraging opportunities. Huronicus having a deeper body, higher buoyancy, deeper depth at which the morph occurred, and deeper peduncle stands in contrast to the lean morphology. Body shape and buoyancy variations are probably related to depth distributions and swimming tactics, with the deep-water morph using hydrostatic lift to enhance vertical migration (Videler, 1993; Henderson & Anderson, 2002; Blake, 2004). In contrast, the shallow-water morph likely relies more on hydrodynamic lift, characteristics linked to cruising movements of pelagic

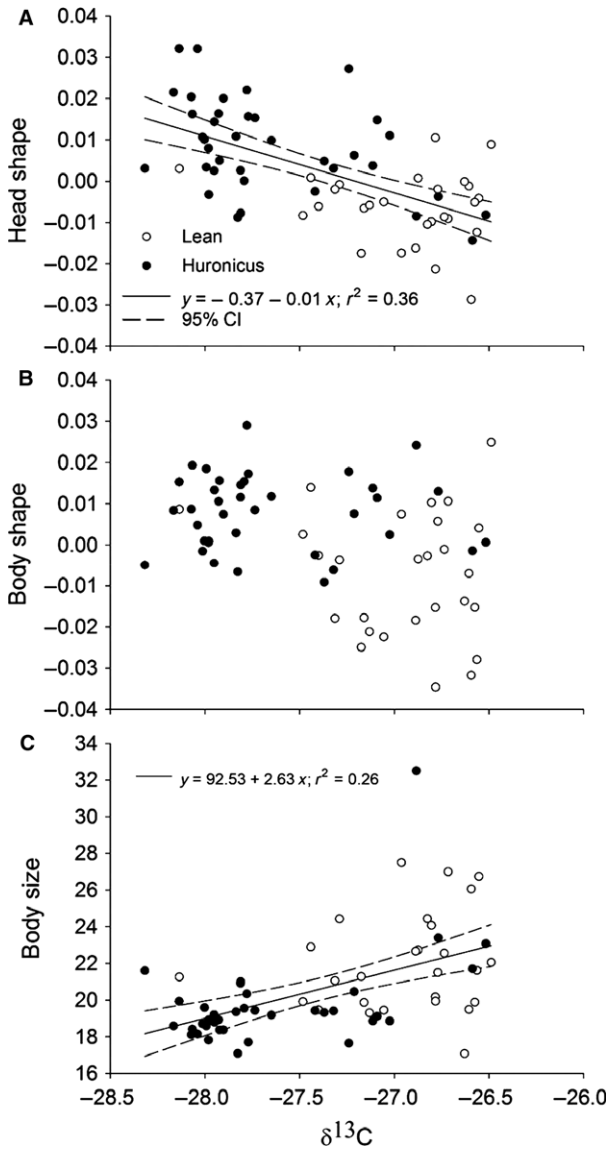


Figure 7. Linear function with a 95% confidence interval for the first principal component of body shape (A) and head shape (B) explaining 33% and 42% of the variation in shape between morphs, respectively, and centroid size (the size component from the geometric analysis of body shape) (C) associated with carbon ($\delta^{13}\text{C}$) isotope signatures for lean (open dots) and huronicus (solid dots) lake charr morphs from Rush Lake. Morphs were identified by McClust cluster analysis (Fraleay & Raftery, 2009) and overall group assignment. Head shape and body size slopes differed significantly from 0 ($P \leq 0.05$) but not the body shape slope ($P > 0.05$).

predators (Webb, 1984; Pakkasmaa & Piironen, 2001; Gillespie & Fox, 2003).

The head shape of each morph matched their feeding strategy as indicated by the $\delta^{13}\text{C}$ signature, implying adaptation associated with feeding activity

(Montaña & Winemiller, 2013; Berchtold *et al.*, 2015; Østbye *et al.*, 2016). Differences in head morphology related to resource use could influence foraging efficiency associated with particular prey and/or environments (Adams, Woltering & Alexander, 2003; Kahilainen *et al.*, 2007; Cochran-Biederman & Wine-miller, 2010; Svanbäck & Schluter, 2012). Longer head, longer maxillae, and a more posterior eye position of the lean morph relative to the huronicus morph should provide a wider lateral field of view, useful for piscivorous feeding (Proulx & Magnan, 2004; Keeley, Parkinson & Taylor, 2005, 2007; Janhunen, Peuhkuri & Piironen, 2009). The huronicus morph with limited gape and eye position should be optimized for low-light vision, and adapted as a vertical migrating predator feeding on *Mysis*, its main prey (Hrabik *et al.*, 2006; Muir *et al.*, 2014).

Differences in life-history strategies among ecologically specialized morphs have been repeatedly observed elsewhere and Rush Lake is no exception (Sandlund *et al.*, 2013; Woods *et al.*, 2013; Chavarie *et al.*, 2016a, b). Rush Lake lake charr displayed trade-offs in degree of juvenile and adult growth rates (i.e. fast vs slow), probably linked to the exploitation of different resources and environments, with differences pronounced to the extent that morphs could be assigned based on their life-history traits alone (Schluter, 1995; Fraser, Huntingford & Adams, 2008; Jonsson & Jonsson, 2014). Huronicus' slower growth, smaller adult size, and older mean age than the lean morph was similar to deep-water lake charr elsewhere and linked to small-sized prey and possibly lower feeding rates (Burnham-Curtis & Bronte, 1996; Hansen *et al.*, 2012; Muir *et al.*, 2015). The piscivorous feeding of the lean morph would likely provide more energy intake for growth resulting in a greater size at maturity than huronicus (Jonsson *et al.*, 1999; Jonsson & Jonsson, 2001; Snorrason & Skúlason, 2004). Age and body size are critical drivers of maturation, and both are linked in a relationship known as the maturation reaction norm (Heino, Dieckmann & Godø, 2002; Hutchings, 2011; Morbey & Shuter, 2013). In salmonids, gonad development is dependent on body size, and consequently, could provide a threshold that differentially triggers development between morphs (Wright, 2007; Johnston & Post, 2009; Morbey & Shuter, 2013). A lack of immature fish in our sample did not allow us to detect differences in length and age-at-maturity between morphs. However, in this instance, the trade-off identified was not in the age- and length-at-maturity but in life span itself. The huronicus morph by having greater longevity than the lean morph, could have a potential fitness, in terms of lifelong egg deposition potential, equivalent to the lean morph despite huronicus' smaller average body size.

Our results indicated that, despite morphological and ecological differences between the lake charr morphs in Rush Lake, reproductive isolation was lacking or incomplete. The apparent lack of genetic divergence at neutral loci examined between lake charr morphs in Rush Lake when compared to the more genetically diverse populations in Lake Superior and Lake Mistassini (Krueger *et al.*, 1989; Page, Scribner & Burnham-Curtis, 2004; Baillie *et al.*, 2015, 2016), leads to two competing explanations for the origin and maintenance of two morphs in Rush Lake: (1) sympatric evolution within Rush Lake, or (2) differentiation elsewhere with subsequent colonization of Rush Lake followed by post-colonization weakening of reproductive barriers. Weak genetic differentiation can be due to the population being in early phases of divergence, porous isolating mechanisms, or a combination of these processes. Although differentiation in the face of gene flow is thought to be difficult and prevents the development of strong reproductive isolation, sympatric differentiation events might be more frequent than expected (Briggs, 1999; Johannesson, 2001; Nosil, 2008). Sympatric differentiation with gene flow is feasible under particular conditions that include assortative mating, divergent selection against hybrids, or habitat isolation, but homogenization is likely to happen unless strongly reinforced by some isolation processes (Gavrilets & Vose, 2005; Niemiller, Fitzpatrick & Miller, 2008; Nosil, 2008).

Nonetheless, both proposed mechanisms (sympatric differentiation and differentiation elsewhere followed by colonization) explaining the origin and maintenance of Rush Lake polymorphism, have a common denominator through phenotypic plasticity. If Rush Lake represents an early stage of sympatric differentiation in which phenotypic plasticity is operating on a single gene pool, the population could be evolving and forming two phenotypic clusters (Skúlason, Snorrason & Jonsson, 1999; Svanbäck, Pineda-Krch & Doebeli, 2009; Bird *et al.*, 2012). In the alternative scenario, that Rush Lake were colonized by two forms and reproductive barriers have weakened or collapsed, the expression of phenotypic plasticity of a single gene pool would explain the maintenance of morphological and ecological differences between lean and *huronicus*. Reproductive barriers can be reversible, especially when a change in ecological conditions happen, precipitating a collapse back to continuous adaptive variation without reproductive isolation or discontinuous adaptive variation with minor reproductive isolation (Hendry, 2009).

A theoretical scenario of the evolution of lake charr divergence in Rush Lake was introduced in the scientific literature by Behnke (1972) and Eshenroder (2008) who both hypothesized that *huronicus*

originated from nearby Lake Superior. Their reasoning arose from the observation that Rush Lake was the only example of a small lake with co-existing morphs and because *huronicus* appears to be analogous to the humper morph found in nearby Lake Superior (Eschmeyer, 1955; Rahrer, 1965; Krueger & Ihssen, 1995). Humper-like morphs in Lake Superior and Lake Mistassini have been described as a deep-water morph that specializes on *Mysis diluviana*, with morphological characteristics such as a small body size, small head, narrow peduncle, moderate eye size situated dorsally on the head, and life-history characteristics such as a long life span (Zimmerman *et al.*, 2007; Eshenroder, 2008; Muir *et al.*, 2015). The combination of these ecological, morphological, and life-history characteristics were consistent with this study's description of the *huronicus* morph in Rush Lake. Eshenroder (2008) presented a conceptual model for the origin of lake charr morphs and offered the hypothesis that lean and *huronicus* morphs of Rush Lake diverged within proglacial Lake Duluth and were isolated from present day Lake Superior during lowering water levels and isostatic rebound.

Despite the uncertainty about the origin (i.e., sympatric vs. allopatric divergence) and mechanism(s) involved in maintenance (i.e., natural selection or sexual selection vs. drift or founder effect) (Taylor, 1999), parallel sympatric pairs of lake charr, associated with ecological divergence, have been maintained in multiple North American lakes (Zimmerman *et al.*, 2006, 2007; Chavarie *et al.*, 2013, 2015; Muir *et al.*, 2015). In the face of gene flow, large lakes, such as Lake Superior, Lake Mistassini, Great Slave Lake, and Great Bear Lake, are more likely to provide reproductive isolating barrier(s) through isolation by geographic distance and lake bathymetry than do small lakes (Krueger & Ihssen, 1995; Page *et al.*, 2004; Goetz *et al.*, 2010; Harris *et al.*, 2015). Rush Lake, with its small size, would seem unlikely to generate and maintain such intraspecific diversity for lake charr, although isolation by distance is not essential for sympatric intraspecific diversity to occur. For example, intraspecific diversity within small systems has been reported for Arctic charr, threespine stickleback (*Gasterosteus aculeatus*), whitefish (*Coregonus* spp.), and cichlids (Schluter & McPhail, 1992; Barluenga *et al.*, 2006; Kahilainen & Østbye, 2006; Garduño-Paz *et al.*, 2012). Consistent with its small lake size, genetic diversity and divergence were comparable with other single-morph lake charr that inhabited small lakes in Labrador (McCracken *et al.*, 2013), and contrasted with the higher genetic diversity and divergence observed in large lakes (i.e., Lake Superior and Lake Mistassini). One of the few empirical examples of sympatric speciation within a small

lake demonstrated that ecological speciation through divergent habitat preferences and resource partitioning was possible if assortative mating through behavioral isolation was present (Barluenga *et al.*, 2006; Gavrillets *et al.*, 2007). Thus, although isolation by distance is not an essential component to generate and maintain intraspecific diversity, reproductive isolation should be sufficient to ensure some level of separation, which emphasizes the unusual character of Rush Lake example of intraspecific diversity. Although Bayesian clustering analysis and distance measures of neutral loci indicated that Rush Lake lean and huronius morphs were not genetically divergent, the lack of non-neutral markers in the analyses may have prevented us from detecting multiple genetic populations (Putman & Carbone, 2014). This uncertainty is reinforced by the statement made by (Hubbs, 1929, 1930) about an apparent temporal reproductive segregation between the two morphs, although no specific detailed information on the morph's spawning behaviour was described by Hubbs. The presence of the private alleles might also indicate weakly restricted gene flow but uncertainty remains as it might also be an artefact of sampling. Genomic sequencing would be an interesting next step to look for areas under selection (i.e., linked to trophic morphologies) combined with a telemetry study to examine timing and location of spawning and reproductive behaviour.

Differences in habitat use associated with variation in foraging opportunities alone could have been sufficient to act as ecological drivers (i.e., via a reduction of intraspecific competition; Skúlason & Smith, 1995) to maintain two morphs in Rush Lake. Ecological and evolutionary processes often act concurrently and their congruent response can be rapid and at a fine scale (Hairston *et al.*, 2005; Kristjánsson *et al.*, 2011; Kristjánsson *et al.*, 2012; Richardson *et al.*, 2014). The direct relationship found herein between morphology and ecological characters provided evidence that local environmental variation contributed to maintaining morphological differentiation of lake charr, with forms related to function (trophic ecology and habitat use related to depth) (see Kristjánsson *et al.* (2011) for another example). In environments where ecological drivers, such as depth in association with foraging opportunities, remain relatively constant, the expression of morphological traits could be stable over time if individual lake charr show high depth fidelity (Svanbäck & Eklöv, 2006; Chavarie *et al.*, 2015; Faulks *et al.*, 2015). Thus, the presence of the two morphs in Rush Lake could be a bimodal response to feeding environments. Morphs appeared to be similarly abundant lake-wide within Rush Lake (Table 5). Elsewhere, co-existing lake charr morphotypes, such as the Lake Superior siscowet (deep-water morphotype) dominates the lean- or shallow-water trout by a

factor of ten in abundance (Bronte *et al.*, 2003). Thus, both shallow and deep habitats in Rush Lake seem to offer 'ecological opportunity' settings that could sustain the population abundances of the morphs in this lake (Wellborn & Langerhans, 2015).

Phenotypic plasticity and/or trait heritability, thus, may play important roles in resource polymorphism, especially in a genus such as *Salvelinus* that shows prominent plasticity (Klemetsen, 2010, 2013; Muir *et al.*, 2015; Elmer, 2016). Patterns of phenotypic variations are repeatedly associated with particular lake environments and selection pressures (Robinson & Parsons, 2002; Schluter *et al.*, 2004; Snorrason & Skúlason, 2004) for which, in lake charr, depth and foraging opportunities appear to be two common drivers. Distinct ontogenetic trajectories or phenotypic plasticity, even in the absence of reproductive isolation, can maintain polymorphisms (Grant & Grant, 1994; Svanbäck & Persson, 2004; Bird *et al.*, 2012). The whole temporal process of differentiation can be generally viewed as a continuum from individual variation without reproductive isolation, to population-wide phenotypic and genotypic differentiation associated with complete reproductive isolation (Hendry *et al.*, 2009; Præbel *et al.*, 2013). However, some convincing recent examples have showed that divergence in the face of continuous or recurrent gene flow is possible (Niemiller *et al.*, 2008; Nosil, 2008). Such circumstances could be applicable to a genus, such as *Salvelinus*, with recurring hybridization among its species (Behnke, 2010) and that does not strictly follow the Linnaean definition of a species. Examples have recently demonstrated that migration between divergent populations acted as both homogenizing and diversifying forces (e.g., walking-stick insect (*Timema cristinae*); Nosil, Crespi & Sandoval, 2003). Such reinforcement was most likely if migration was sufficiently high, but low enough to avoid the erosion of phenotypic differentiation (Nosil *et al.*, 2003). Migration reinforcing a high level of phenotypic variation within a population and promoting maintenance of ecological differentiation (as may be the case in this study) (Nonaka *et al.*, 2015), may explain the 'charr problem' in which phenotypes and genotypes vary considerably within and across localities and even within a life-time (Nordeng, 1983; Michaud, Power & Kinnison, 2008).

The question still remains as to why Rush Lake is the only known small lake that sustains multiple lake charr morphs? If depth and foraging opportunities alone can function as an 'island' promoting and maintaining rapid adaptive divergence post colonization (e.g., Losos *et al.*, 2000; Robinson & Parsons, 2002), why would Rush Lake be the only example that sustains a deep-water morph (to our knowledge) of a small lake among thousands in North America?

For example, 1781 lake charr lakes occur within the Province of Ontario, of which 25 have a maximum depth > ~86 m (Carl *et al.*, 1990); despite intensive sampling across these lakes, no deep-water lake charr morphs have been found to date in these lakes (N. Lester, Pers. Comm.). If the explanation that *huronicus* originated from Lake Superior is correct, then the answer might simply be that Rush Lake was close to a source of a humper-like, deep-water morph and the lake contained deep-water habitats that were suitable for the humper morph with sufficiently strong ecological drivers to maintain the morph as a separate ecological entity. Other small lake charr lakes in North America might have only one or none of those circumstances occurring.

CONCLUSION

Striking examples of intraspecific pairs and parallel adaptive radiation have been observed in post-glacial lakes throughout circumpolar areas (Skúlason & Smith, 1995; Schluter, 1996; Snorrason & Skúlason, 2004). In North America, processes contributing to freshwater sympatric variation have been largely studied for three-spined stickleback (McPhail, 1993; Barrett, Rogers & Schluter, 2009; Taugbøl *et al.*, 2014), lake whitefish (Lu & Bernatchez, 1999; Siwertsson *et al.*, 2013b; Adams *et al.*, 2016), and Arctic charr (Adams, Wilson & Ferguson, 2008; Reist, Power & Dempson, 2013; Knudsen *et al.*, 2016). Examples of sympatric intraspecific diversity in lake charr documented over the past 15 years have illustrated how polymorphism in lake charr has been underestimated and the processes contributing to it remain poorly understood. Recent discoveries, such as this study, are expanding our understanding of the ecology, life history, and genetics of lake charr morphs in recently de-glaciated lakes across North America. The co-existence of deep- and shallow-water morphs in Rush Lake is a new addition and unique exception, that expands previous explanations for lake charr diversity from large to small lakes. Concordant patterns of morphological and life-history differentiation between streamlined lean and plump-bodied *huronicus* from Rush Lake reflected the influence of foraging opportunities along the niche axis of shallow- vs. deep-water habitats, with these elements likely being the main ecological drivers maintaining two lake charr morphs. Trade-offs in shallow vs. deep-water foraging environments may serve to maintain the phenotypic expression of these polymorphisms even in the absence of the reproductive isolation. This study advances our understanding of the conditions that facilitate divergence in the face of

gene flow, which might be more common than previously thought (Nosil, 2008).

Ecological drivers in Rush Lake likely have remained relatively stable over time. If these ecological drivers were associated with high depth-fidelity of individual lake charr, the expression of lean and *huronicus* would be similarly maintained over time. However, the stability of ecological drivers may be jeopardized by the presumed recent invasion of rainbow smelt into Rush Lake. Rainbow smelt were not observed in Hubbs surveys in the 1920s and were unknown in contemporary times to landowners familiar with the fish fauna in the lake. This species was first detected by our study and their invasion is thought to be recent. The invasion of rainbow smelt may ecologically disrupt the partitioning of resources and the ecological barriers by homogenizing lake charr diets with the opportunity to shift from an invertebrate to a fish-feeding ecology by *huronicus* into shallower-water habitats. The potential disruption of the trophic gradient might lead to the collapse of the morph-pair in Rush Lake, as seen in other species after the introduction of an exotic species (e.g., sticklebacks, Taylor *et al.*, 2006). The lean and *huronicus* morphs in Rush Lake are as morphologically and ecologically distinctive as any shallow- and deep-water lake charr pair previously described, and yet are genetically homogenous at selectively neutral genetic loci. Their presence suggests that they are a functionally important component of a diverse food web structure in Rush Lake with unknown consequences for the stability of the lake ecosystem as a whole.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. BIC scores according to number of cluster from McClust for body shape in (a) and head shape in (b).

Figure S2. CVA of lake charr body shape (a) and head shape (b). Morphs were identified by McClust cluster analysis (Fraley & Raftery, 2009) and *overall group assignment*.

Figure S3. Bar plots of Bayesian genetic clustering analysis for two Rush Lake lake charr morphs (STRUCTURE).

Figure S4. The mean of estimated natural log probably of the data ($K [P(K)]$) was calculated in program STRUCTURE HARVESTER Web 0.6.92 (Earl & vonHoldt, 2012).

Figure S5. Population structure in Rush Lake lake charr as visualized through principal components (PC) analysis of multilocus genotypes (GENALEX) (Peakall & Smouse, 2006; Peakall & Smouse, 2012).

Figure S6. Length frequency of huronicus (solid bars) and lean (open bars) lake charr captured in Rush Lake.

Figure S7. Age frequency of huronicus (solid bars) and lean (open bars) lake charr captured in Rush Lake.

Figure S8. Frequency (%) of huronicus (solid bars) and lean (open bars) lake charr captured (nets and angling) in Rush Lake.

Figure S9. Stable isotopes signatures \pm SE of huronicus (solid circle) and lean (open circle) lake charr in (a) and morphs divided by their depth of capture in (b).

Figure S10. First principal component of body shape (a) and head shape (b) explaining 33% and 42% of the variation in shape between morphs, respectively, and centroid size (the size component from the geometric analysis of body shape) (c) associated with nitrogen ($\delta^{15}\text{N}$) isotope signatures for lean (open dots) and huronicus (solid dots) lake charr morphs from Rush Lake.

Table S1. Microsatellite loci ($n = 20$) used in this study.

Table S2. Allelic diversity and genetic equilibria in (a) lean and (b) huronicus lake charr morphs from Rush Lake.

Table S3. Density (number $\cdot\text{ha}^{-1}$) and abundance of lake charr morphs in Rush Lake estimated by day and night August 2007 acoustic surveys.

Table S4. Diet composition of lake charr morphs in Rush Lake collected in late August 2007.

Table S5. Comparison of genetic diversity of lake charr in Rush Lake and lakes of a similar small size in Labrador (McCracken *et al.* 2013).

Table S6. Pair-wise comparisons of F_{ST} (Wright 1965; below diagonal) and R_{ST} (Slatkin, 1995; above diagonal) using ten microsatellite loci among lean and humper lake charr at Rush Lake (RU), Lake Mistassini (MI), and four locations within Lake Superior: Isle Royale (IR), Big Reef (BR), Stannard Rock (SR), Grand Marias (GM). Asterisks mark values that remained significant after correction for multiple tests (SGoF+ 3.8) (Carvajal-Rodriguez & de Uña-Alvarez, 2011).