Population structure of lake trout (*Salvelinus namaycush*) in a large glacial-fed lake inferred from microsatellite DNA and morphological analysis

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Abstract: Understanding the structure of intraspecific genetic and morphological diversity within and across habitats is a fundamental aspect of biodiversity research with conservation value. Atlin Lake is the largest lake in British Columbia, Canada, and contains relatively pristine populations of lake trout (*Salvelinus namaycush*) that are key components of the lake's fish community and local fisheries. Lake trout from Atlin Lake were examined for genetic and phenotypic variation using eight microsatellite DNA loci, body form, and colouration. Genetic assays were also conducted on lake trout from the adjoining Tagish Lake and from 17 other localities to provide spatial context for the variation within Atlin Lake. The genetic data suggested that there were three genetic subpopulations within the Atlin–Tagish lake system. Morphological analysis identified two morphological groups of lake trout within Atlin Lake. Genetic and morphological groupings in Atlin Lake were not associated with each other. A mixed-stock analysis of samples collected from Atlin Lake commercial and recreational fisheries suggested that all genetic subpopulations contributed to the fishery and that there was some contribution from fish originating from within Tagish Lake.

Résumé : La compréhension de la structure de la diversité intraspécifique génétique et morphologique au sein des habitats et entre les habitats est une composante essentielle de la recherche sur la biodiversité reliée à la conservation. Le lac Atlin est le plus grand lac de la Colombie-Britannique, Canada, et il contient des populations de touladis (*Salvelinus namaycush*) encore relativement dans leur état originel qui forment une composante essentielle de la communauté de poissons du lac et de la pêche locale. Nous avons examiné la variation génétique et phénotypique des touladis du lac Atlin à l'étude de huit locus microsatellites d'ADN, de la forme corporelle et de la coloration. Nous avons aussi procédé à des analyses génétiques des touladis du lac Tagish voisin et de 17 autres localités pour obtenir un contexte spatial pour étudier la variation au sein du lac Atlin. Les données génétiques indiquent qu'il existe trois sous-populations génétiques dans le réseau des lacs Atlin et Tagish. L'analyse morphologique a permis d'identifier deux groupes morphologiques de touladis dans le lac Atlin. Les regroupements génétiques et morphologiques dans le lac Atlin ne sont pas associés l'un à l'autre. Une analyse de stock mixte d'échantillons récoltés dans les pêches commerciales et sportives au lac Atlin montre que toutes les sous-populations génétiques contribuent à la pêche et qu'il y a une certaine contribution des poissons provenant du lac Tagish.

[Traduit par la Rédaction]

Introduction

The origin, extent, and patterns of genetic diversity within species are central issues for evolutionary biologists (Futuyma 1997) and are increasingly important for conservation because genetic diversity can influence the persistence of populations (Frankham et al. 2002). Consequently, there is a growing need to study genetic diversity of contemporary populations to help understand how population viability might change in response to environmental fluctuations (Schwartz et al. 2007). One aspect of genetic variation is population subdivision, the partitioning of a species into two or more independent or semi-independent genetic subpopulations that may exist in the same or different habitats (e.g., Waples and Gaggiotti 2006).

In many fishes, including salmonids (salmon, trout, char, whitefish, and grayling), assays of population distinctiveness have been employed in a variety of conservation contexts such as separating native from introduced fish (e.g., Taylor et al. 2007) or to identify distinct populations to facilitate population-specific management initiatives (Gunn et al. 2003). As dominant predators of north-temperate freshwater lakes in the Nearctic, lake trout (*Salvelinus namaycush*) are

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important members of aquatic communities across their range. Lake trout are exploited, to varying degrees, in traditional, commercial, and recreational fisheries and declines in abundance and loss of specific populations are occurring in a number of areas (Guinand et al. 2003; Gunn et al. 2003). In addition, lake trout populations are vulnerable to other human activities, including the introduction of non-native species (Smith and Tibbles 1980; Guinand et al. 2003; Dextrase and Mandrak 2006). In an effort to better manage exploitation-based and environment-based stressors on lake trout populations, the species has been the subject of a number of studies to resolve population distinctiveness. For instance, the study of sympatric and allopatric lake trout populations has resolved genetic differences in survival, morphology, early development rate, physiology, allozymes, and microsatellite allele frequencies (Brown et al. 1981; Ihssen et al. 1988; Guinand et al. 2003). Genetic "inventories" may help to identify populations of conservation significance; populations with low levels of genetic variability might be compromised in their ability to persist in current or changing environments, and populations with high levels of diversity might be of great value for opposite reasons (Frankham et al. 2002; Piller et al. 2005).

Many north-temperate fish species consist of two or more major genetic lineages thought to have arisen from divergence during isolation in distinct glacial refugia — divergence perhaps enhanced by postglacial adaptation to their present environments (Bernatchez and Wilson 1998; Power 2002). The many episodes of isolation while in separate refugia followed by secondary contact between divergent lineages have probably contributed to the tremendous morphological, ecological, and genetic variability observed within lake trout that makes them an excellent model for studying evolutionary process of divergence (Magnan et al. 2002).

Atlin Lake is located in northwestern British Columbia (BC), Canada, and is the province's largest natural lake ecosystem, with a surface area of 792 km^2. Atlin Lake forms one of the headwater lakes of the Yukon River system and interconnects with Tagish Lake. Lake trout are thought to have colonized this area from both the Nahanni and Beringian refugia using freshwater dispersal corridors (no anadromous populations of lake trout are known) beginning about 10 000 years ago (Wilson and Hebert 1998). In addition, because of its large size and diversity of habitats, Atlin Lake has the potential to contain multiple genetically distinct populations of lake trout. There have, in fact, been anecdotal accounts of different lake trout phenotypes (based on observations of shape, size, and colour) occurring in different parts of the lake (N. DeGraaf, Taku River Tlingit First Nation, Atlin, BC, unpublished data). In addition to the importance of lake trout from Atlin Lake to the local community, the lake is of interest to a broader understanding of lake trout biology and conservation. This is because relatively few populations in the western part of the geographic range of lake trout have been examined in detail genetically, especially for pristine areas such as Atlin Lake. Furthermore, there are longstanding accounts of variation in lake trout in body shape and colour, variability initially thought to be restricted to the Laurentian Great Lakes (Dehring et al. 1981; Gunn et al. 2003; Bronte and Moore 2007). Subsequent investigations have found morphological variants in other northern lakes (Blackie et al. 2003; Alfonso 2004; Zimmerman et al. 2006). How geographically widespread such variants are and how they originate or are maintained in their respective environments, however, remain unknown.

Despite the fact that lake trout from different areas of Atlin Lake appear to spawn at the same time of year, variation in food source, water depth, and water turbidity may promote local differentiation. If more than one morphotype exists in Atlin Lake, it would be important to determine if these morphotypes signal genetically distinct populations such that a better understanding of the basis of within-lake biodiversity can be obtained (Keeley et al. 2007). For instance, should distinct morphotypes be a purely phenotypic response to variable environments within the lake, then management might focus on maintaining a minimum population size and a diversity of habitats across the entire lake so that this plasticity is sustained. Alternatively, if morphotypes are associated with genetically distinct populations of lake trout, then these morphotypes provide a means to monitor genetic diversity and efforts should be directed towards understanding the biological processes that sustain such genetic variation (spatial or ecological segregation) such that a plan for the maintenance of within-lake biodiversity can be designed. Maintenance of such intraspecific genetic biodiversity is considered important for the long-term persistence of species (Bowen 1999; Allendorf and Luikart 2007) and their productivity within specific habitats (Hilborn et al. 2003), as well as for ecosystems as a whole (Booth and Grime 2003; Reusch et al. 2005). Further, a common problem in fisheries management is that sometimes the management of only fish occurs when it should include assessment of fishers and their effort (Hilborn 2007). Lake trout in Atlin Lake are exploited in commercial and recreational fisheries but have not been subjected to fish stocking programmes. Consequently, if Atlin Lake contains genetically distinct subpopulations of lake trout, an understanding of the relative levels of exploitation of each component through mixed-stock fishery analysis would help guide a conservation plan for each subpopulation integrated across the whole lake.

To contribute to a greater understanding of the structure of diversity in Atlin Lake *S. namaycush* and provide guidelines for more effective management and conservation, we conducted a survey of microsatellite DNA and morphological variation in collections of lake trout from Atlin Lake, as well as from several other localities in BC and Yukon. Although investigations on lake trout from Lake Mistassini, Québec, and Great Bear Lake, Northwest Territories, are recent exceptions (Blackie et al. 2003; Zimmerman et al. 2007), the vast majority of data on variability in lake trout have been collected for highly perturbed populations of lake trout in the Laurentian Great Lakes. Consequently, our data contribute to much needed comparative data from relatively understudied and less perturbed northwestern ecosystems.

Materials and methods

Sample collections

We obtained lake trout tissue samples from adult fish that were collected between 2000 and 2006 from 19 lakes in BC, Yukon, and Northwest Territories using a variety of gillnet

gear (1.5- to 3-inch mesh sizes) that captured fish ranging from 192 to 887 mm fork length. The lakes sampled varied in surface area (determined from topographic maps) from 1.1 to 792 km^2 (Table 1; Fig. 1). Three lakes (Trapper, Arctic, and Muncho) had sample sizes <10 individuals, and we used them only in general descriptions of variability. We sampled two lakes from the upper Yukon River watershed in northwestern BC and adjacent Yukon Territory (Atlin and Tagish lakes) more extensively. These two lakes are interconnected by the Atlin River, are very large (>300 km^2 in surface area), and are characterized by very low levels of development and, presumably, human disturbance. For about one-half of its length, Atlin Lake is surrounded by Atlin Provincial Park. In Atlin Lake, we collected fish during general lake surveys (N = 186) using small-mesh gillnets deployed at 1 km intervals for 1 h, as well as by sampling catches landed at the Great Northern Fish Company (GNFC, N = 101). We also sampled tissue from adults obtained in the recreational fishery (N = 33). Fish from Atlin Lake were weighed and measured for fork length, and adipose fin tissue was removed and stored in 95% ethanol. We calculated condition factor of each fish as (weight (in grams) $\times$ 100)/length^3 (in cm). In 2005 and 2006, samples from the Atlin Lake survey were photographed for morphological and colouration analyses (see below). In Tagish Lake, we collected adult fish using gillnets from known spawning reefs (N = 175), as well as from nonspawning areas (N = 52), analogous to the whole-lake surveys conducted in Atlin Lake.

Genetic data collection and analyses

We extracted total genomic DNA using the QIAgen DNeasy kit system, and DNA was precipitated and resuspended in Tris–EDTA (pH 8.0) buffer and then stored at –20 °C. Polymerase chain reactions (PCR) were performed across eight loci in 10 μL volumes in MJ PTC 100 thermal cyclers using fluorescently labeled primers and assayed on a Beckman-Coulter CEQ 8000 automated capillary sequencer/genotyper (Supplemental Table S1[2]). The loci used were Sco2, Sco102, Sco107, Sco19, Sco215, Sfo18, Ssa197, and Smm22 (Supplemental Table S1[2]). We routinely re-ran individual samples across all loci to check for consistency of scoring; in no case did these re-runs produce results that differed from the original analyses.

We tested for departures from Hardy–Weinberg equilibrium (HWE) and linkage equilibrium using Fisher's exact test in which P values were estimated using the Markov chain method as implemented in GENEPOP (version 3.1; Raymond and Rousset 1995). We used the sequential Bonferroni correction method (Rice 1989) to adjust critical type-I error rates when making multiple simultaneous hypothesis tests. For tests within populations, we adjusted alpha levels sequentially by the number of loci for tests of HWE and by the number of pairwise comparisons between loci for tests of linkage disequilibrium. Basic descriptive statistics of sample size (N), number of alleles (A), observed heterozygosity (H_O), and expected heterozygosity (H_E) were compiled using TFPGA (version 3.2; Miller 1997), whereas allelic richness (A_R) was determined using FSTAT (version 2.9; Goudet 2001). Weir and Cockerham's (1984) F_{ST} (θ) values were calculated using ARLEQUIN (version 3.0; Excoffier et al. 2005) to test population differentiation via pairwise comparisons. There was no evidence from permutation tests in SPAGeDi (Hardy and Vekemans 2002) that mutation contributed to population differentiation, so we limited our genetic distance inferences to those based on F_{ST}. Next, we used PCAgen (Goudet 1999) to conduct a principal component analysis (PCA) on allele frequency data to visualize genetic differentiation among the sample localities. Microsatellite variation was also partitioned into its components by performing an analysis of molecular variance (AMOVA) following Excoffier et al. (1992) and as implemented in ARLEQUIN. Different hierarchical arrangements of samples such as watershed groupings, PCA groupings, and Atlin Lake and Tagish Lake subpopulations (see below) were tested.

We tested for a correlation between geographic distance and genetic distance (F_{ST}) and its significance to determine if the observed genetic structure could be explained by the isolation-by-distance model (IBD; Slatkin 1993). We used TFPGA to perform the Mantel test (Mantel 1967), and significance of any correlation was determined using the default setting of 999 matrix permutations. We used Google Earth (http://earth.google.com/download-earth.html) to calculate the distances between localities.

We adopted several approaches to estimating the population structure of lake trout within Atlin and Tagish lakes. First, the number of subpopulations, K, within Atlin Lake and Tagish Lake was estimated by employing the program STRUCTURE (version 2.2; Pritchard et al. 2000). Because Atlin and Tagish lakes are interconnected by the Atlin River and lake trout are present in this waterway, we did not conduct STRUCTURE analyses separately by lake. A Bayesian clustering algorithm, Markov chain Monte Carlo based approach assumes a model of K subpopulations, characterized by allele frequencies at each locus and assigns individuals probabilistically to each of these subpopulations. This analysis then uses a likelihood approach to determine the most probable number of K subpopulations using a general genetic inheritance model to minimize Hardy–Weinberg and linkage disequilibrium within the subpopulations. We ran multiple trials following Evanno et al. (2005), with a "burn-in" period of 10 000 iterations followed by 10 000 iterations to estimate K and subpopulation membership; longer runs with more iterations did not alter the basic results. Initial analyses examining values of K from 1 to 30 suggested that the true value of K was ~5 within Atlin and Tagish lakes. Consequently, we conducted more detailed simulations employing the admixture model with correlated allele frequencies at each K from 1 to 8 using a burn-in of 50 000 iterations followed by 450 000 iterations and replicated each five times per K value. The model of K with the highest log-likelihood value was chosen as the most probable level of population subdivision. We did not employ the ad hoc statistic, ΔK (Evanno et al. 2005), because of the generally low levels of subdivision resolved where this statistic performs poorly at resolving the true K (Waples and Gaggiotti 2006). After the most likely number of subpopulations was deter-

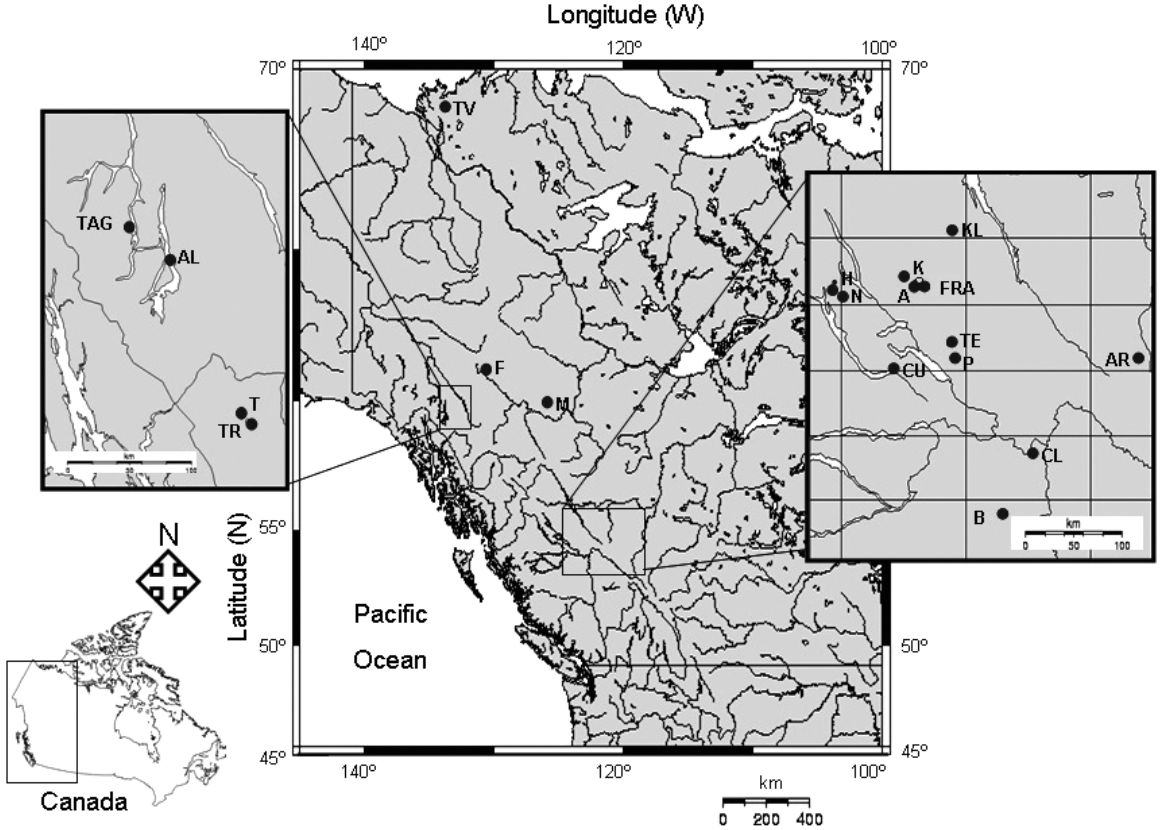
[2] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca).

Table 1. Sample sites, sample size (*N*), locations, watersheds, elevations, and surface area of lakes sampled for lake trout (*Salvelinus namaycush*).

Lake	Location	*N*	Latitude (N)	Longitude (W)	Watershed	Elevation (m)	Area (km^2)
1. Atlin (AL)	BC/Yukon	186	59°29′57.14″	133°44′12.07″	Yukon	886	792
2. Tatsemerie (T)	BC	35	58°20′47.47″	132°19′14.49″	Taku	1270	~57
3. Tagish (TAG)	BC/Yukon	227	59°46′21.87″	134°14′29.46″	Yukon	1109	354.6
4. Trapper (TR)	BC	9	58°27′27″	132°37′32″	Taku	1457	~24
5. Muncho (M)	BC	6	58°59′38″	125°46′57″	Mackenzie	1494	~50
6. Frances (F)	Yukon	19	61°23′	129°35′	Mackenzie	804	99.4
7. Travaillant (TV)	NWT	17	69°39′16.95″	131°5l’03.75″	Mackenzie	27	~102
8. Bednesti (B)	BC	25	53°22′41″	123°51′44″	Fraser	1201	2.61
9. Hautete (H)	BC	32	55°11′14″	126°8′50″	Fraser	989	2.3
10. Tezzeron (TE)	BC	23	54°42′52″	124°27′59″	Fraser	910	78.1
11. Arctic (AR)	BC	8	54°25′17″	121°40′36″	Fraser	1327	1.1
12. Cluculz (CL)	BC	24	53°52′23″	123°34′20″	Fraser	860	19.9
13. Fraser (FRA)	BC	27	55°4′56″	124°45′3″	Fraser	880	54.6
14. Nakinlerak (K)	BC	36	55°14′52.512″	125°14′54.134″	Fraser	1092	7.3
15. Natowite (N)	BC	32	55°5′11″	125°56′46″	Fraser	938	16
16. Cunningham (CU)	BC	30	54°35′31″	125°19′20″	Fraser	863	29.9
17. Airline (A)	BC	28	55°7′43″	125°3′47″	Fraser	839	4.5
18. Klawli (KL)	BC	28	55°21′0″	124°21′43″	Fraser	1006	4.6
19. Pinchi (P)	BC	26	54°36′43″	124°24′28″	Fraser	830	55.5

Note: BC, British Columbia; NWT, Northwest Territories.

Fig. 1. Locations in western Canada where lake trout (*Salvelinus namaycush*) were collected in this study. Population codes are given in Table 1. Inset at the top left shows Atlin and Tagish lakes in northwestern British Columbia and Yukon; inset on the right shows the upper Fraser River, British Columbia; inset at the bottom left shows the whole study region in relation to Canada.



mined, each fish was assigned to the subpopulation for which its inferred ancestry coefficient, q, was at least 0.5 (i.e., the proportional contribution to its genome across the eight loci from a specific subpopulation was estimated to constitute at least 0.5; Pritchard et al. 2000).

We partitioned Atlin Lake into four large geographic regions that corresponded to areas that are partially isolated from each other or that had distinct limnological conditions: north arm (northernmost 50 km of lake), central arm (next 50 km of lake), west arm (distinct restrictions between this area and central and south arms), and south arm (southernmost 25 km of lake headed by the Llewellyn Glacier field). Contingency tests were performed to assess statistical significance of differences in the frequencies of the STRUCTURE-generated genetic subpopulations among these geographic regions within Atlin Lake using PAST, a general spreadsheet-based package of statistical analyses (Hammer et al. 2001). Similar tests were also performed to assess whether the genetic subpopulations within Tagish Lake were associated with distinct spawning locations.

We next calculated individual pairwise identity indices (Mathieu et al. 1990) in the Atlin Lake and Tagish Lake subpopulations identified by STRUCTURE with IDENTIX (Belkhir et al. 2002). The identity index varies from 0 to 1.0 and provides a measure of relatedness because closely related individuals are more likely to produce homozygous offspring and IDENTIX estimates the expected proportion of loci that are homozygous in the offspring of any pair of individuals. IDENTIX also implements a permutation test of the null hypothesis of no relatedness by comparing the distribution of the observed pairwise relatedness coefficients in a population sample with its null expectation. The identity values were calculated because the sampling was not based on actual spawning locations, at least in Atlin Lake, and examining this estimate of individual relatedness may resolve finer patterns of genetic differentiation especially because kin groups have been resolved in other char at postjuvenile life stages (Fraser et al. 2005).

The composition of baseline populations defined by genetic data for Atlin and Tagish lakes and for use in subsequent fisheries mixture analyses was determined using STRUCTURE by identifying the K most likely subpopulations for both Atlin and Tagish lakes' survey samples and assigning each individual to its most likely population (i.e., $q > 0.5$) as described above. These baseline subpopulations were used in conjunction with genetic mixture analysis to determine if the commercial and recreational fisheries in Atlin Lake were sampling proportionately from each population using the Bayesian approach to mixed-stock analysis implemented in ONCOR (Anderson et al. 2008). This analysis utilizes the conditional maximum likelihood algorithm to estimate mixture proportions (Miller 1987) and the Rannala and Mountain (1997) method for estimating the probability of observing a genotype in series of baseline populations, each of which was characterized across a number of genetic loci. We estimated the robustness of our analyses in two ways. First, 95% confidence intervals were generated by bootstrap resampling with replacement in both the mixture samples (across individuals) and the baseline samples (alleles across genotypes) during 5000 replicate analyses. Second, we produced simulated mixtures ($N = 200$) in which we fixed the proportional contribution of each subpopulation in turn to 1.0 and then estimated the mixture proportions for all baseline subpopulations contributing to the simulated mixtures. In this case, "perfect" performance of the mixture analysis would return an estimated proportional contribution for each subpopulation of 1.0. Initially, this analysis compared the commercial and angling samples with all 19 lakes sampled to determine the general performance of our data and ONCOR (i.e., no contributions from lakes other than Atlin and Tagish should be observed). Next, commercial and recreational fishing samples were analysed, both separately and in a combined analysis, to assess the contributions of the various genetic subpopulations from Atlin and Tagish lakes.

Morphological analyses

We photographed individual fish from Atlin Lake using a digital camera with a measuring board as a consistent background for light standardization. Body shape was measured with the aid of TPSDIG software (Rohlf 1997). The digital images were imported into TPSDIG and x and y coordinates were established using 16 (each with an x and y coordinate) landmarks to measure the shape of each fish (Zimmerman et al. 2006; Bronte and Moore 2007; Supplemental Fig. S1[2]). These data were then imported into a companion program TPSRELW (Rohlf 1997) that centers, scales, and aligns the coordinates using the Procrustes method and calculates an average configuration to describe the consensus body shape among a sample of fish. The program then compares each set of coordinates with the consensus body shape using thin-plate spline analysis (Bookstein 1991). The method produces a value for each fish that represents its deviation at each x and y coordinate from the consensus shape, generating 32 such deviation scores for each fish (x, y coordinates across 16 landmarks). The TPSDIG software was also used to measure head depth, midbody depth, and caudal peduncle depth and to confirm body length, which had been measured in the field. To account for variation in body size related (isometric) differences, head depth, midbody depth, and caudal peduncle depth were size-corrected to the overall sample mean fork length (521 mm) by employing the residuals from the linear relationship between fork length and individual trait in subsequent statistical tests (Thorpe 1976). These latter three measurements, in combination with condition factor and the 32 deviation scores from the landmark analysis, resulted in 36 body shape measurements for each fish.

We quantified body colouration, or more accurately brightness, of each lake trout image with the aid of Image J software (version 1.32j; http://rsb.info.nih.gov/ij) following the methodology of Zimmerman et al. (2006). All images were converted to black and white and the measurements were taken on a 0 (black) to 256 (white) scale and averaged for a total overall "brightness factor". Brightness was measured at six positions along the lateral surface of each fish as defined by Zimmerman et al. (2006): midoperculum, two equidistant points along the body above the lateral line, two below the lateral line, and one at the midpoint of the caudal peduncle. We performed ANOVAs to assess the level of brightness differentiation among (*i*) the genetic subpopulations resolved via STRUCTURE, (*ii*) body shape based mor-

phological clusters (see below), and (*iii*) within-lake geographical units.

To summarize morphology, condition factor, and brightness into a measure of overall phenotypic variation, we conducted principal component analysis (PCA) on the correlation matrix among all variables using PAST. To determine the number of phenotypic clusters that could be resolved among all lake trout, a model-based clustering method was employed without a priori designation of populations using the program MCLUST as implemented within the R Statistical Software Project (Fraley and Raftery 2003; R Development Core Team 2008). The method fits the observed frequency distribution of PCA scores obtained from the analysis above to alternative models of structure in terms of the number, shape, and variability within phenotypic clusters. In the first model, a single morphological cluster is assumed to best represent the data. In subsequent models, two or more (up to eight in our case) clusters are assumed to exist. The model with the highest Bayesian information criterion (BIC) is selected as the model best describing morphological variation, with differences in BIC values between alternative models that are greater than 6 indicating "strong" evidence and those greater than 10 indicating "very strong" evidence in favour of the model with the highest BIC value (Fraley and Raftery 2003). The MCLUST analysis includes a function allowing classification of individual observations (fish) to the resolved morphological groups.

We conducted a discriminant function analysis (DFA) with jackknifing in PAST to measure the reliability of classification of individual fish to morphological groups resolved in the MCLUST analysis. A contingency test was also performed using PAST to determine (*i*) if the morphological groups resolved above were associated with the four geographic regions in Atlin Lake and (*ii*) if there was a relationship between the STRUCTURE-defined genetic subpopulations and the morphological clusters. Finally, we estimated the number of genetic groups within the sample of fish examined morphologically using STRUCTURE as detailed above, testing among potential K values of 1–5.

Results

Intrapopulation genetic variation

We assayed microsatellite variation across 818 individuals at eight loci. The number of alleles ranged from four (Sco102) to 30 (Smm22) across all populations, with an average of 14.8 alleles per locus for the entire study region and an average of 5.3 alleles per locus per lake (Supplemental Tables S1 and S2[2]). Observed heterozygosity ranged from 0.17 to 0.91, with an average of 0.51 across all loci and populations (Supplemental Table S2[2]). The two most variable loci were Smm22 and Ssa197, with 30 and 24 alleles globally, respectively. Fourteen out of 152 (8 loci × 19 populations) tests showed statistically significant deviations from HWE, and all were heterozygote deficiencies. Seven such deficiencies were from Atlin Lake (four loci) and Tagish Lake (three loci); the remaining samples were largely in HWE. Tests for linkage disequilibrium resulted in none of the 532 tests being significant. Consequently, we considered each locus to represent an independent measure of genetic variation and divergence.

The level of variation within populations varied greatly. Expected heterozygosity over all eight loci ranged from a low of 0.30 (Bednesti Lake) to a high of 0.75 (Atlin Lake) (Supplemental Table S2[2]). Fourteen populations were found to have at least one of the eight loci fixed, with Sco102 being the most commonly fixed locus. No more than two loci, however, were fixed in any one population. Between watersheds, there were no fixed allele differences among the eight microsatellites studied; however, there were several alleles found to be unique to individual watersheds, especially within the Yukon and Fraser rivers' watersheds. There was a strong, positive correlation between lake surface area and allelic richness ($r = 0.68$, $P = 0.001$).

Population structure among lakes

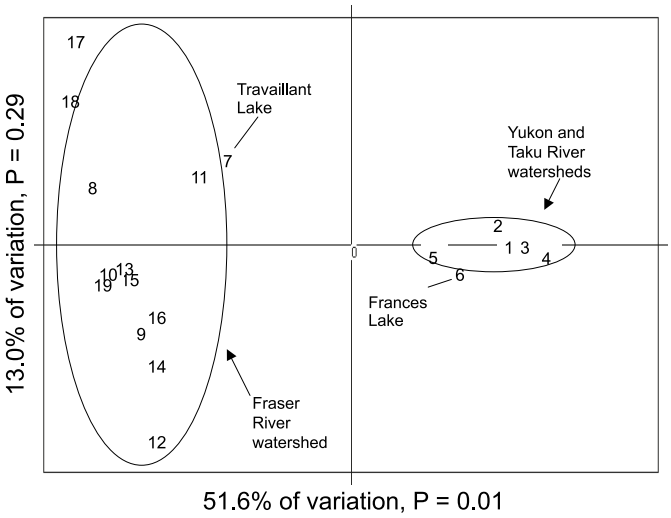
There were 120 pairwise comparisons of F_{ST} calculated across the eight loci between populations with sample sizes greater than 10 ($N = 16$), and all but two of these were significant at $\alpha = 0.05$ (Supplemental Table S3[2]). The average F_{ST} across all comparisons was 0.258 and ranged from 0.009 to 0.616. A principal component analysis (PCA) of allele frequency for all lakes revealed two groupings, with PCA 1 being the major (and only significant, $P < 0.01$) axis of differentiation between groupings (Fig. 2). Lakes in the Fraser River watershed and Travaillant Lake (Mackenzie River, Northwest Territories) formed one group that was separate from the remaining samples (Yukon, northwestern BC). We next examined genetic differentiation by grouping the various populations by watershed; AMOVA indicated that variation among the watershed groups (19.6%, $P < 0.001$) exceeded that among populations within watersheds (13.8%, $P < 0.001$; Table 2). Further tests were conducted based on the groups suggested by the principal component analysis (Fraser River – Travaillant Lake vs. all others; Fig. 2). Here, there was a considerable narrowing of the difference between the two PCA groups (3.3%, $P < 0.001$), as well as that among populations within PCA groups (6.0%, $P < 0.001$).

When all populations were included, a test for IBD was not significant ($r = 0.27$, $P = 0.09$). The populations were then grouped according to the two PCA clusters and tested for IBD within each group, which was also not significant within either group ($r = 0.34$, $P = 0.25$, and $r = 0.24$, $P = 0.22$, respectively). When lakes from the Fraser River watershed were analyzed separately, however, we detected a significant pattern of IBD ($r = 0.47$, $P = 0.025$).

Population structure within lakes

Four hundred and thirteen individuals from Atlin and Tagish lakes were genotyped at eight microsatellite loci: 186 from Atlin Lake and 227 from Tagish Lake. The most variable loci were Smm22 and Ssa197, with 26 and 18 alleles, respectively. STRUCTURE analysis indicated the presence of genetic substructure, with three clusters (ATL-A, $N = 94$ fish; ATL-B, $N = 123$ fish; ATL-C, $N = 196$ fish) being the most likely number of subpopulations within these interconnected lakes (Supplemental Table S4[2]). When we pooled individual fish by membership within a subpopulation, only three of the 24 (eight loci × three subpopula-

Fig. 2. Principal component analysis of allele frequency variation at eight microsatellite loci among all sample lakes. The numbers correspond to the population codes listed in Table 1.



tions) tests showed statistically significant HWE deviations. Tests for linkage disequilibrium resulted in none of the 84 pairwise tests being significant. Within any of the three subpopulations, the number of alleles ranged from three (Sco102, subpopulations ATL-A and ATL-B) to 24 (Smm22, subpopulation ATL-A), with the three subpopulations averaging 10.6, 10.1, and 11.8 alleles, respectively (Supplemental Table S5[2]). Observed heterozygosity ranged from 0.18 (subpopulations ATL-A and ATL-B, Sco102) to ~0.90 (subpopulations ATL-A and ATL-C, Smm22), with an average of 0.65 across all loci and populations (Supplemental Table S5[2]).

Estimates of F_{ST} between the three subpopulations averaged 0.022 and ranged from 0.005 between subpopulations ATL-A and ATL-C to 0.027 between ATL-A and ATL-B to 0.034 between ATL-B and ATL-C (all $P < 0.001$). Pairwise identity values averaged 0.25, 0.38, and 0.27 in subpopulations ATL-A, ATL-B, and ATL-C, respectively, and none was significantly different from that expected under random mating within subpopulations (all $P > 0.20$).

Contingency tests failed to find any geographic pattern in the distribution of the three genetic groupings across the four areas of Atlin Lake ($P = 0.25$; Fig. 3). In contrast to Atlin Lake, however, there was a highly nonrandom association between membership of individual fish in one of the three genetic subpopulations and area of capture (spawning bed) within Tagish Lake ($P < 0.0001$; Fig. 3). Furthermore, there was a strong difference in the frequency of the three genetic subpopulations between Atlin and Tagish lakes (contingency test, $P < 0.0001$; Fig. 3). In particular, Tagish Lake possessed a higher frequency of genetic subpopulation ATL-B than did Atlin Lake. We performed an analysis of molecular variation by pooling all STRUCTURE-generated subpopulations from Atlin and Tagish lakes by geographic region within lake. The AMOVA indicated that only a small, but significant, percentage of the total variation found was attributable to differences between lakes (1.2%, $P = 0.04$), but slightly more was attributable to differences among regions within lakes (2.3%, $P < 0.001$), and most variation was attributable to differences among individual fish within subpopulations (96.6%, $P < 0.001$).

Mixed-stock fisheries analysis

Initial comparisons of lake trout commercial and angling samples ($N = 134$) from Atlin Lake with baseline samples from all 19 lakes in this study estimated that 79.6% of these samples were from Atlin Lake, while 20.4% originated from Tagish Lake. As expected, no other lakes were identified as contributing to the Atlin Lake fisheries. Lake trout from commercial fisheries and those caught by local anglers were compared with the three genetic subpopulations identified both in Atlin and Tagish lakes. Commercial fisheries appeared to draw most heavily from subpopulation ATL-C (82.7%), followed by subpopulation ATL-B (15.1%), and finally from subpopulation ATL-A (2.2%) (Table 3). The angling catch also appeared to sample most heavily from subpopulation ATL-C, followed by ATL-B, but few fish from ATL-A were apparently exploited (Table 3). When we partitioned the baseline and commercial samples by lake, mixture analysis suggested that about 75% of all fish exploited originated from Atlin Lake (Table 3), with the remainder from Tagish Lake. The angling catch was even more dominated by fish from Atlin Lake (91% vs. 9%; Table 3). Finally, all samples were partitioned by lake region (Atlin) or spawning ground (Tagish). In this analysis, 75% of commercially exploited fish were estimated to have originated from Atlin region 4 and Tagish Lake region SW (Table 3). The angling catch was also dominated by fish from Atlin Lake region 4, but other Atlin Lake regions and Tagish Lake region DB also appeared to contribute to the recreational fishery (Table 3). Interestingly, both in commercial and angling analyses, Tagish Lake region W did not appear to contribute any fish (Table 3). Confidence intervals for real and simulated mixture analyses by genetic subpopulation and lake were largely nonoverlapping and associated with minimum baseline sample sizes of about 100 (Table 3). This was in marked contrast to the poor performance of mixture analyses conducted by region where simulated proportions were highly variable with broad confidence intervals that all included 0. A baseline sample size of at least 50 appears to be required for simulated mixtures to approach 1.0 with relatively narrow confidence limits (Table 3).

Morphological analysis

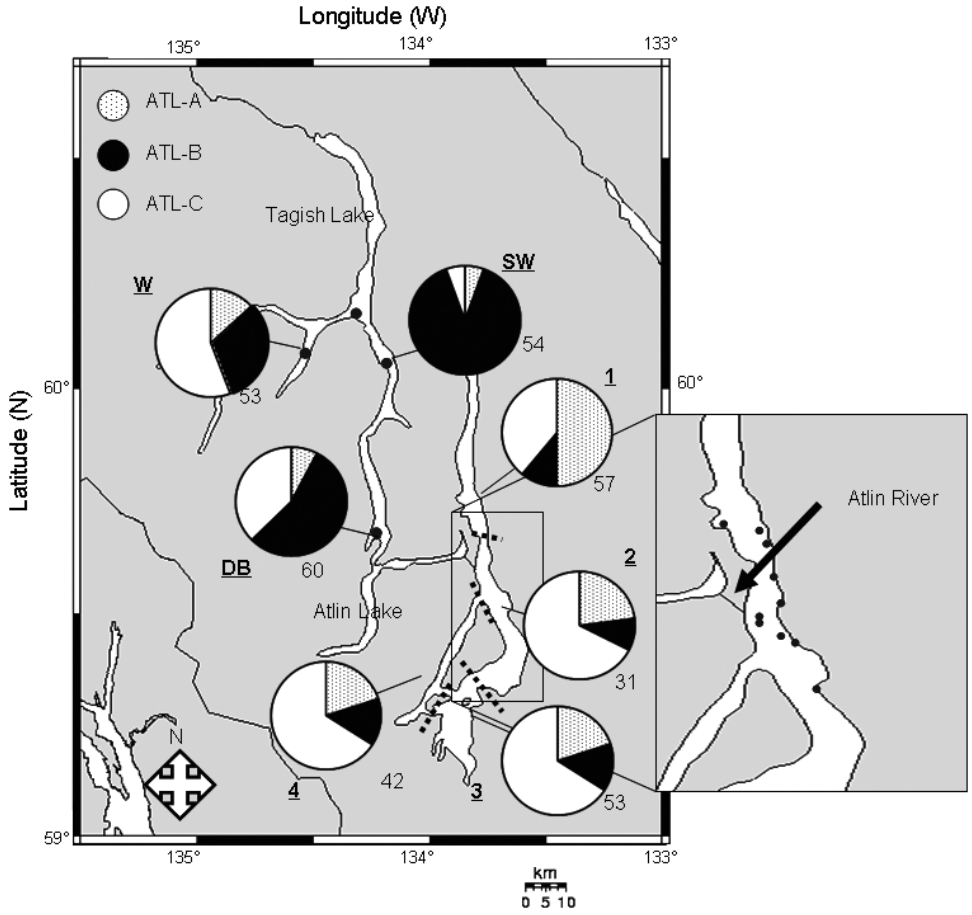
We examined 104 lake trout morphologically and in terms of body brightness. Two components in the PCA accounted for 56.2% of total variation and axes 1 and 2 (PC1 and PC2) made up 40.7% and 15.5% of the total variation, respectively (Supplemental Table S6[2]). Fish length was uncorrelated, with scores along PC1 or PC2 (both $P > 0.1$) suggesting the lack of an allometric effect on body shape differences. A model describing two distinct morphological clusters with unequal variances within them had the highest BIC of –1080 (Fig. 4). Alternative models had BIC values at least 20 points lower than the model describing two populations: MCLUST reported a BIC of –1110 for models with one morphological cluster and a minimum BIC of –1100 for models with three or more clusters. The separation of the two clusters was evident, however, only along PC2, which appeared to differentiate fish with long heads and

Table 2. Analysis of molecular variance within and among western lake trout (*Salvelinus namaycush*) populations based on allele frequency variation across eight microsatellite DNA loci.

	Yukon vs. Taku vs. Mackenzie vs. Fraser watersheds			Between PCA groups			Atlin vs. Tagish		
Variance component	$F_{statistic}$	% variation	*P*	$F_{statistic}$	% variation	*P*	$F_{statistic}$	% variation	*P*
Between regions or lakes	0.196	19.58	<0.001	0.149	14.97	<0.001	0.012	1.2	0.04
Among populations within regions or lakes	0.333	13.76	<0.001	0.137	11.64	<0.001	0.023	2.2	<0.001
Within populations among regions or lakes	0.171	66.66	<0.001	0.26602	73.40	<0.001	0.034	96.6	<0.001

Note: Comparisons between Atlin and Tagish lakes incorporated variation among three genetic subpopulations resolved within each lake, respectively.

Fig. 3. Map of the distribution of lake trout (*Salvelinus namaycush*) genetic subpopulations within Atlin and Tagish lakes according to STRUCTURE analysis. The bold dashed lines indicate the separation between geographical regions in Atlin Lake. Shading within each pie chart depicts the frequency of each of the three genetic subpopulations at that locality, with the sample size shown as the numeral to the right of each pie chart. Solid circles represent known or suspected spawning locations in Atlin and Tagish lakes (M. Connor, Taku River Tlingit First Nations Fisheries Department, unpublished data). Locality codes are given as underlined numbers for each of the four regions in Atlin Lake or abbreviations for the three spawning reefs in Tagish Lake (DB, Deep Bay; W, Windy Arm; SW, Squaw Point).



caudal regions from those with deeper heads and bodies overall (Supplemental Table S6[2]; Fig. 4). The five top-ranked positive coefficients along PC2 were for x1, x3, x4, x10, and x11 associated with head, eye, and caudal region landmarks (Supplemental Fig. S1 and Supplemental Table S6[2]). The most negative coefficients for PC2 were for x6, x7, x14, x15, and y5 associated with fin position and head landmarks (Supplemental Fig. S1 and Supplemental Table S6[2]). Condition factor and "brightness" score had low PC coefficients along PC2, typically in the order of five or six times lower than for the landmarks with the highest coefficients (Supplemental Table S6[2]). In general, fish that scored

Table 3. Genetic mixture analysis results for 101 commercial samples and 33 recreational angling samples of lake trout (*Salvelinus namaycush*) from Atlin Lake as generated using ONCOR (Anderson et al. 2008) and variation at eight microsatellite DNA loci.

		Sample mixture		
Group	*N*	Commercial	Angling	Simulated mixture
Genetic				
ATL-A	94	0.022 (0.000–0.110)	0.258 (0.034–0.462)	0.990 (0.971–1.000)
ATL-B	123	0.151 (0.053–0.267)	0.002 (0.000–0.262)	0.979 (0.954–0.999)
ATL-C	196	0.827 (0.688–0.924)	0.741 (0.456–0.927)	0.995 (0.977–1.000)
Lake				
Atlin Lake	186	0.773 (0.587–0.884)	0.908 (0.612–0.999)	0.953 (0.898–0.999)
Tagish Lake	227	0.227 (0.116–0.410)	0.092 (0.001–0.385)	0.964 (0.917–0.999)
Region				
Atlin Lake 1	18	0.068 (0.000, 0.193)	0.152 (0.000, 0.357)	0.225 (0.149–0.297)
Atlin Lake 2	21	0.121 (0.018, 0.276)	0.119 (0.000, 0.375)	0.284 (0.169–0.379)
Atlin Lake 3	30	0.001 (0.000, 0.184)	0.148 (0.000, 0.408)	0.361 (0.278–0.446)
Atlin Lake 4	56	0.503 (0.248, 0.606)	0.269 (0.000, 0.602)	0.630 (0.527–0.728)
Tagish Lake W	55	0.000 (0.000, 0.062)	0.000 (0.000, 0.006)	0.763 (0.665–0.848)
Tagish Lake SW	55	0.252 (0.044, 0.379)	0.080 (0.000, 0.370)	0.633 (0.545–0.697)
Tagish Lake DB	61	0.056 (0.000, 0.201)	0.233 (0.001, 0.457)	0.969 (0.921–0.999)

Note: Tagish Lake: W, Windy Arm; SW, Squaw Point; DB, Deep Bay. Mixture values represent the estimated contribution of each genetic subpopulation within Atlin and Tagish lakes, for lakes, and for geographic regions (Atlin Lake) or spawning localities (Tagish Lake) within lakes averaged (95% confidence intervals (CI) in parentheses) over 5000 bootstrap replicates. Subpopulations or lakes or regions with the highest average proportional contributions are indicated in bold. Simulated mixtures indicate average (95% CI) proportional contribution of each subpopulation or lake or region in simulated mixtures of 200 fish, where the true contribution of each is 1.0 under conditions of original baseline sample sizes (*N*).

most positively on PC2 were "stockier" with shorter and shallower heads, bodies, and caudal regions relative to the more elongated shapes of fish that had high negative scores along PC2 (Supplemental Fig. S2[2]).

The jackknifed DFA resulted in 75.9% correct morphological assignment, with two-thirds of the miss-assignments occurring owing to fish from morphological group 1 (MG1) assigned to group 2 (MG2). There was slight, but significant, association between geography and morphological group; MG1 predominated in the south arm of Atlin Lake ($P = 0.046$; Fig. 4). By contrast, there was no significant association ($P = 0.35$) between morphotype composition and the three STRUCTURE-generated genetic subpopulations identified earlier.

The brightness scores ranged from a low of near 100 (representing dark shading with distinct white spotting) to over 120 (representing silver–white shading with indistinct light spotting; Fig. 5). Average brightness scores were not statistically different among the three Atlin Lake genetic subpopulations ($P = 0.13$) or the two morphological clusters ($P = 0.15$). The observed geographical distribution of brightness scores was, however, found to be significant ($P = 0.016$), with the higher mean scores (lighter variety) being more common in the south arm (Fig. 5). An F_{ST} of 0.004 (95% CI of –0.001 to 0.009) was calculated between the two morphological clusters, six times lower than the value of the global F_{ST} of 0.022 among the three genetic subpopulations. Finally, analysis using STRUCTURE provided no evidence of more than one genetic population within the group of fish examined morphologically; log-likelihood was highest at $K(1) = –2731$, with $K(2)$ having the next highest likelihood at –2939.

Discussion

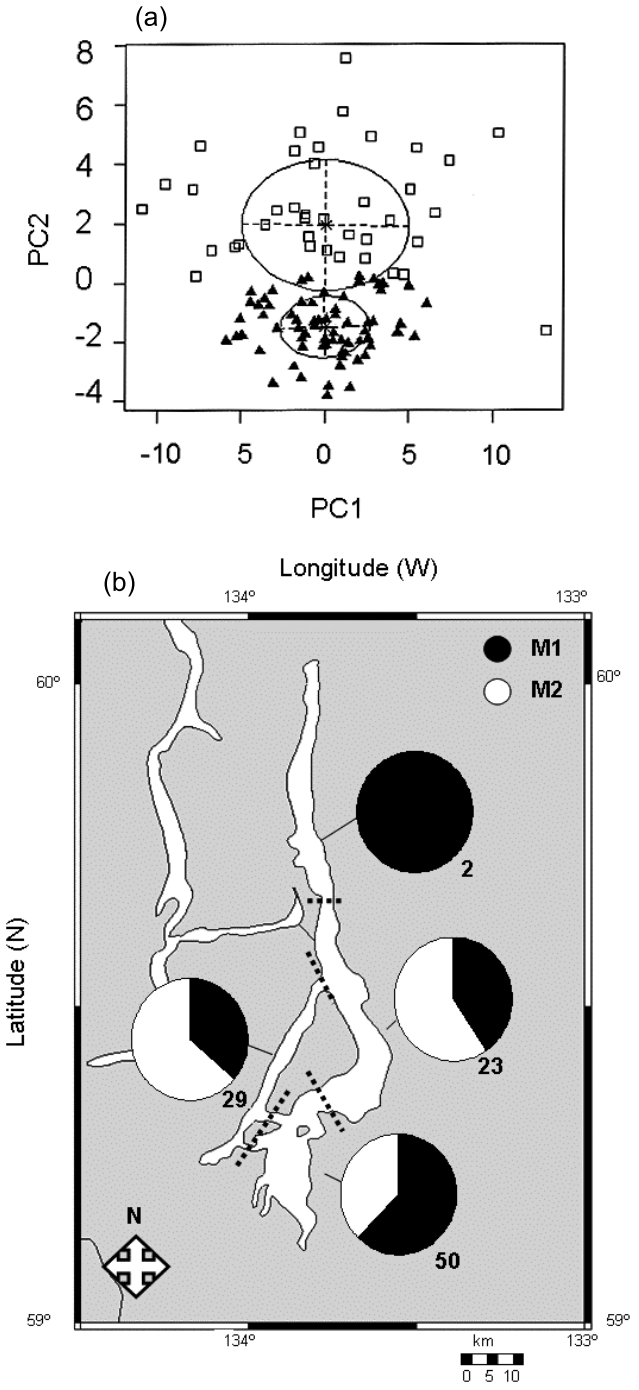
The ability to detect genetic substructure within populations has greatly increased over the last decade, which can provide information to promote effective management (Schwartz et al. 2007). Our study examined the level of genetic diversity and subdivision of lake trout within Atlin Lake and other western lake trout populations to help facilitate comparisons with other populations in more heavily studied and perturbed systems of eastern North America. These data can be used as baseline levels of genetic diversity for more effective conservation and management decisions for lake trout both in Atlin Lake and more generally.

Genetic variability across lakes and regions

Our study yielded significant F_{ST} values that ranged from 0.014 between two lakes within the same watershed and connected by a short river (Atlin and Tagish lakes) to 0.616 between two lakes (Trapper Lake, Taku River watershed, and Bednesti Lake, Fraser River watershed) that are separated by about 780 km, located in different watersheds, and are thought to have been colonized by fish from distinct glacial refugia (Wilson and Hebert 1998). The overall mean pairwise F_{ST} of 0.25 is well within the range reported among populations of freshwater resident salmonid fishes that, as a group, tend to have higher levels of genetic subdivision than anadromous salmonids (Hendry et al. 2004). Substantial subdivision among freshwater populations of salmonids, despite the excellent dispersal potential of salmonid fishes, is consistent with the generally greater constraints on interlocality dispersal imposed by the underlying landscape in freshwater environments.

Where opportunities for dispersal do exist such as be-

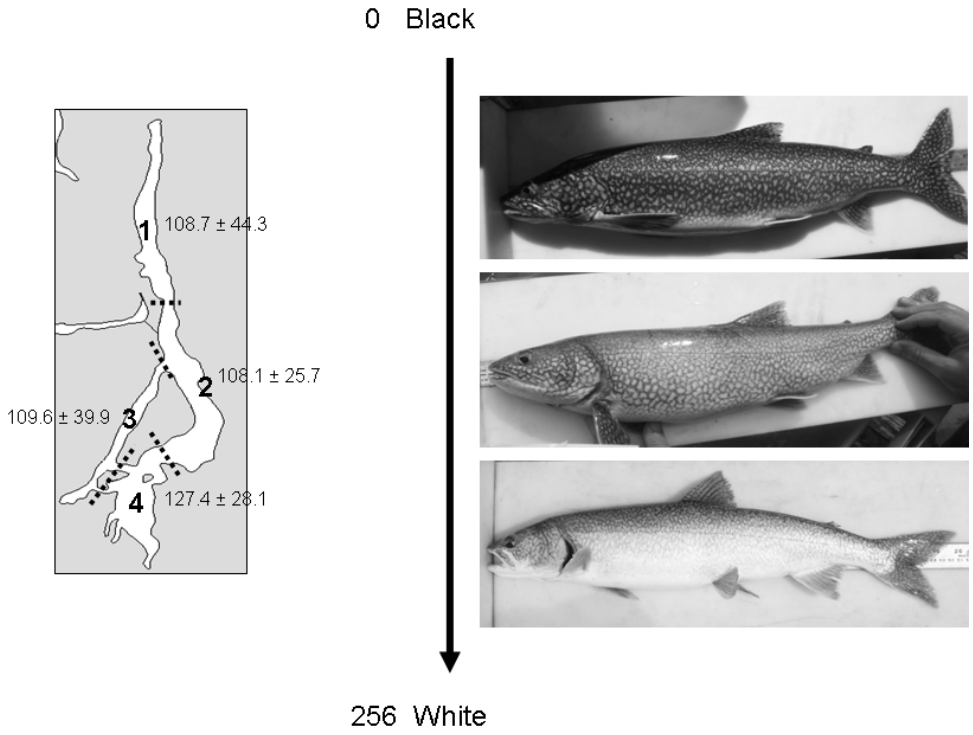
Fig. 4. (*a*) Morphological variation among Atlin Lake lake trout (*Salvelinus namaycush*) along the first and second principal components (PC1 and PC2). Ellipses represent the covariances of the values along each component, solid triangles correspond to morphological group 1 (MG1), and open squares correspond to morphological group 2 (MG2). (*b*) Map of the distribution of the two morphological groups among four geographic regions within Atlin Lake. The value to the right of each pie chart is the sample size for each region of the lake.



tween Atlin and Tagish lakes, genetic subdivision is lower. More modest levels of subdivision (expressed as G_{ST} and F_{ST}) of between 0.024 and 0.058 were reported among historical and contemporary wild and hatchery populations of lake trout within Laurentian Great Lakes Huron and Superior (Guinand et al. 2003; Page et al. 2004). Measures of subdivision are expected to be lower when more continuously distributed populations (e.g., within lakes Huron and Superior) are compared relative to widely separated or completely isolated populations (e.g., our study lakes, among Lakes Superior and Huron, and upper Mississippi River drainage lakes; DeWoody and Avise 2000; Page et al. 2004; Piller et al. 2005). Consequently, the large spatial scale of our study (Northwest Territories to central BC) probably accounts in large part for the strong differentiation that we observed. These results reinforce the importance of geographic proximity as an important factor influencing genetic structure among populations (Slatkin 1993; reviewed by Bohonak 1999). It is, however, more surprising that a signature of isolation-by-distance was detected among lake-dwelling populations within the Fraser River basin because these populations are strongly isolated currently. Consequently, there is almost surely very little contemporary movement between these lakes (i.e., although lake trout may occur in rivers and occasionally spawn there, they are generally associated with deepwater, lacustrine habitats; Scott and Crossman 1998). These results suggest that historical patterns of recolonization (timing, direction, sequence of events) may play some role in influencing current patterns of genetic structure even among relatively isolated populations (Crispo and Hendry 2005).

Analyses based on morphology and mtDNA have suggested that the Atlin–Tagish lakes area was colonized postglacially by fish from two refugia: the Nahanni Refuge and the Bering Refuge. By contrast, the lower Mackenzie River (e.g., Travaillant Lake) and the Fraser River watersheds are thought to have been colonized by fish from the Bering Refuge only (Bodaly and Lindsey 1977; Wilson and Hebert 1998). Our results are consistent with these hypotheses given that the Atlin–Tagish samples were very distinct from the Travaillant Lake and Fraser River samples, suggesting that they have had distinct origins postglacially. Further, our microsatellite data suggested that the Yukon and Taku rivers' watershed lakes are as similar to each other as are the lakes within the Fraser River watershed. The similarity between the Yukon (draining to the Bering Sea) and Taku (Pacific drainage) rivers is consistent with a common origin for these fish and (or) with suspected historical drainage connections between watersheds of these major river systems in the southern Yukon Territory (Lindsey 1975). By contrast, the sources of lake trout in the Mackenzie River watershed appear to be more variable. Travaillant Lake (lower Mackenzie River, Arctic drainage) lake trout were more similar to lake trout from the Fraser River watershed (Pacific drainage) than to fish from a lake tributary to the upper Mackenzie River (via the upper Liard River, Arctic drainage) — Frances Lake. Fish from Frances Lake, in turn, were more similar to lake trout from the Yukon and Taku rivers. The latter result is consistent with the shorter distance between Frances Lake and the upper Yukon River, even though Frances Lake is part of the upper Liard River, which ultimately flows to the Mackenzie River via the Peace River. In addition, Frances Lake lies just west of the area thought to have encompassed the Nahanni Refuge, which probably provided some postglacial colonists to the southwestern Yukon

Fig. 5. Results of comparisons of average (± standard deviation, SD) brightness values for lake trout (*Salvelinus namaycush*) sampled from the four geographic regions within Atlin Lake. Sample sizes are given in Fig. 2, and images illustrate the extremes of colouration of lake trout found within Atlin Lake.



(Wilson and Hebert 1998; Stamford and Taylor 2004). These patterns highlight the importance of considering historical patterns of connectivity in the interpretation of genetic affinities among contemporary populations in lake trout, a phenomenon shared with other fishes from geologically active areas (Currens et al. 1990; Waters and Wallis 2000; Zemlak et al. 2008).

Genetic variability within and between Atlin and Tagish lakes

Atlin and Tagish lakes were the only two populations that displayed deviations from HWE across multiple loci, which suggested that some substructure might be present, an inference supported by the STRUCTURE analysis. Genetic subdivision within single geographic localities is common in salmonids (summarized by Hendry et al. 2004), including lake trout (Ihssen et al. 1988; Page et al. 2003), and might have been expected in Atlin and Tagish lakes as they are by far the largest lakes in our survey. The level of subdivision found among the three genetic subpopulations in Atlin and Tagish lakes (F_{ST} = 0.022) was greater than between Atlin and Tagish lakes when the fish were simply grouped by lake and not accounting for genetic substructure within lake (F_{ST} = 0.014). Atlin Lake and Tagish Lake are connected by the Atlin River where lake trout do occur (M. Connor, Taku River Tlingit First Nation Fisheries Department, Atlin, BC, unpublished data). Interlake dispersal via this river probably explains the finding of the same genetic groups between the lakes and, therefore, that fish found in either Atlin Lake or Tagish Lake may be more similar to fish found in the other lake. In addition, the generally higher levels of subdivision observed in Tagish Lake relative to Atlin Lake may result from our sampling of actual spawning reefs in Tagish Lake, sampling that should yield a better estimate of genetic structure as a function of reproductive isolation than the more general lake survey sampling of nonreproductive adults completed in Atlin Lake.

Subpopulations within Atlin and Tagish lakes appeared to exhibit comparable levels of subdivision (F_{ST} = 0.005–0.034) with those reported among populations separated over similar distances within individual Laurentian Great Lakes (Ihssen et al. 1988; Page et al. 2004). By contrast, these values are somewhat lower than the maximum values reported from a study of spawning reefs within Lakes Huron, Michigan, and Superior (F_{ST} = >0.1) (Page et al. 2003), but these latter samples represented spawning populations that had been repopulated after stocking of fish from diverse hatchery sources.

Biochemical genetic and tagging data both strongly suggest that some lake trout exhibit very precise homing, returning to their spawning site year after year (Ihssen et al. 1988). These studies also indicated that the typical dispersal range of most lake trout is about 30 km but can be upwards of 50 km, but usually no more than 100 km, from their spawning shoals (Ihssen et al. 1988). Lake trout populations may also utilize environmental cues to select spawning beds, suggesting that distance is not the sole isolating factor (Perkins et al. 1995). In Atlin Lake, the spawning shoals identified thus far are all within the central portion of the lake. Consequently, the location of these spawning beds in close proximity to each other may be one of the reasons for such apparent high gene flow in Atlin Lake, but large areas of the lake remain unsurveyed for spawning areas. In Tagish Lake, three spawning bed locations had sufficiently high sample

numbers to determine if they were primarily from one genetic subpopulation or not. Genetic subpopulation ATL-C dominated the spawning bed furthest west, while the spawning beds in the eastern and southern portions of the lake were characterized by higher proportions of subpopulation TAG-B, suggesting some independence among spawning areas.

Phenotypic vs. genetic diversity

Local residents have reported multiple colour and shape morphs of lake trout in Atlin Lake for generations, but whether the different morphotypes were discrete populations or represented a continuous range of variation was not known (M. Connor, Taku River Tlingit First Nation Fisheries Department, Atlin, BC, unpublished data). Our study suggests the presence of two morphological kinds of lake trout within Altin Lake that appear to differ primarily in head shape, body depth, and fin position. The three morphotypes found within the Laurentian Great Lakes exhibit significant differences in head shape (Moore and Bronte 2001) and the Great Bear Lake "redfin" and "normal" morphotypes differ in head size and dorsal fin position (Alfonso 2004). Independent from these morphotype differences was variation in brightness in fish from across Atlin Lake. Zimmerman et al. (2006, 2007), in contrast, found brightness differences to be associated with different body forms and that they tended to be collected from different water depths. Similarly, geographic segregation of colour morphs of lake trout has also been reported in Great Bear Lake (i.e., light in the south, dark in the north), but the basis of this pattern is unknown (Blackie et al. 2003).

The striking difference in brightness of fish observed in Atlin Lake is most likely promoted by variability in the lake's environment, especially in terms of turbidity and possibly water colour. The high level of turbidity caused by the influx of sediment from glacial meltwater characterizes the lake's south arm where the distinctive bright "silver" shading of the lake trout predominates. In fact, locally, these fish are known as the "glacial" variety. Colouration differences found in Lake Mistassini (Zimmerman et al. 2007) showed individuals in the deeper waters to be lighter-shaded than those in shallow water. Although all fish captured in the Atlin Lake survey were found in depths of less than 50 m, the increased turbidity caused by the glacial meltwater in the south end of the lake may be simulating the conditions in deeper waters. By contrast, lake trout in Great Slave Lake (Zimmerman et al. 2006) exhibited the reverse pattern (i.e., light in shallow water, and dark in deep water). Perhaps if fish in Atlin Lake were caught in a greater range of water depths, an association between depth and shading–colour–brightness might also be found. The extent to which such brightness variants are the result of genetic differences or result from an environmentally induced phenotypic response is, however, unknown (Miller et al. 2007; Stuart-Fox and Moussalli 2009). Indeed, there is a long history of morphological investigation in salmonids, and such variation can be primarily environmental, genetic, or both in origin (e.g., Pakkasmaa and Piironen 2001; Imre et al. 2002; Keeley et al. 2007). The three main morphotypes of lake trout found in some portions of the Laurentian Great Lakes (so-called "humper", "siscowet", and "lean" varieties) explained a greater percentage of total microsatellite variation than locality, suggesting that morphological groups may also represent distinct genetic groups in lake trout (Page et al. 2004; Bronte and Moore 2007). Furthermore, lake trout raised in hatcheries maintain morphological differences among parental groups, suggesting that a genetic component is involved (Moore and Bronte 2001). As yet, however, no genetic information has been provided for the other morphologically diverse lake trout in the Mackenzie Great Lakes. Whether that would be the case of Atlin Lake lake trout and whether or not such differences are associated with fitness are uncertain as the necessary common garden, performance, or selection experiments have not been performed (Grewe et al. 1994; Perkins et al. 1995; Bronte and Moore 2007).

Despite the identification of two morphotypes (independent of brightness differences), there was considerable overlap between the types, which was confirmed by a level of miss-classification of approximately 26% by discriminant function analysis. Because of the overlap and subtle differences between the morphotypes, it would be difficult to assign an individual fish to one type or the other in the field by visual identification. This presence of different morphotypes in Atlin Lake could be driven, in part, by sexual dimorphism, and we were unable to discount this possibility by sexing all of our fish. Sexual dimorphism in lake trout, however, is much less distinct than in other char, even when spawning (McPhail 2007), and there was no obvious way for us to sex our prespawning samples externally. Also, Zimmerman et al. (2007) found no association between sex and either morphological or brightness variation in Lake Mistassini lake trout. In addition, in the few mortalities that did occur during our sampling, sex appeared to be randomly distributed between morphotypes (two females and one male were in MG1 and one male and one female were in MG2). The brightness differences are unlikely to be explained solely by sexual dimorphism given the clear association of brightness scores with lake water colour (clear vs. glacial flour colour in the south arm) and associated crypsis via background matching (sensu Stevens and Merilaita 2009). Finally, because the morphotypes–brightness differences showed significant geographic associations in Atlin Lake, if such variation was driven by sexual dimorphism, one would need to invoke sexual differences in habitat use to explain the association between phenotype and geography.

The morphological subpopulations resolved in Atlin Lake did not correspond to the subpopulations defined through microsatellite analysis. This could result from environmental differences among localities that control the expression of the phenotypes among members of different genetic subpopulations that use a range of environments during nonspawning periods. Alternatively, there could be considerable gene flow at neutral microsatellite loci among lake trout subgroups characterized by adaptive differences in morphology, and there are many theoretical and empirical grounds for expecting adaptive divergence in phenotype in the presence of some gene flow at neutral loci (e.g., McKay and Latta 2002; Saint-Laurent et al. 2003; Hendry and Taylor 2004).

Our data are too few for a definitive analysis of any association between the established morphotypes from the Lau-

rentian Great Lakes and those found in Atlin Lake. Casual visual identifications based on the morphological descriptions suggest, however, that Atlin Lake contains both a lean (streamlined shape and generally inhabiting water < 70 m deep) and a siscowet-like variety (deeper-bodied inhabitants of waters 70–150 in depth; Page et al. 2004). Unfortunately, none of the siscowet type could be included in our morphological analysis because of poor quality photographs. A more comprehensive comparative analysis across lakes could determine if the recognized morphotypes such as the siscowet reported from the Laurentian and Mackenzie Great Lakes are morphologically similar to those in Atlin Lake and if such similarity results from parallel evolution. Regardless of their exact identity or affinities, Atlin Lake is relatively small compared with other lakes where multiple morphotypes and genetic populations have been reported. This morphological diversity may be promoted by the physical connectivity between Atlin and Tagish lakes and the increasing habitat complexity that this represents.

Fisheries contributions and conservation

It is not surprising that some percentage (~22% on average) of the samples collected from Atlin Lake recreational and commercial fisheries was estimated to constitute fish from Tagish Lake owing to known incidences of dispersal of lake trout through the Atlin River, which connects Atlin and Tagish lakes. Lake trout caught by angling have a lower proportional contribution from Tagish Lake than do those from the commercial fisheries. This seems reasonable because the Atlin River is closer to the commercial fisheries locations than areas that receive the greatest angling effort.

Genetic mixture analysis estimated that genetic subpopulation ATL-C was most frequently sampled by both commercial operators and anglers. This subpopulation also possessed the largest estimated effective population size, which might explain its high estimated greater contribution to fisheries if this reflects a higher census size (Northrup 2008). In fact, baseline fish assigned to ATL-C were the most or second most abundant of all fish sampled in both Atlin (60%) and Tagish (42%) lakes. The contribution of ATL-C to fisheries in Tagish Lake is unknown due to the lack of samples from fisheries in Tagish Lake. In addition, given its high relative frequency, ATL-B probably contributes substantially to the Tagish Lake fisheries. Proximity of fishery areas to main feeding or reproductive habitats of subpopulations may contribute to unequal fishery contribution. In fact, the known spawning locations in Atlin Lake are found in its central portion and this area is the most heavily used by anglers. Therefore, if the genetic subpopulations are driven largely by homing to specific spawning beds, then those subpopulations closer to popular angling locations could show a higher contribution. In terms of regions within lakes, however, our mixture estimates had broad confidence intervals and thus interpretations of differential exploitation of the fish sampled from particular regions of either lake must be interpreted cautiously. Our simulated mixture results suggested that sufficient microsatellite variability was assayed across the eight loci to generate resolution consistent with previous studies of mixture analyses of lake trout, at least at the level of lake or genetic subpopulation (Page et al. 2003; DeKoning et al. 2006). Our simulations, however, also indicated that increasing baseline sample sizes would improve confidence for mixture analyses at smaller spatial scales within lakes.

Our study has resolved microsatellite allele frequency differences between samples across a broad geographic scale in British Columbia, Yukon, and the Northwest Territories and within single lakes of the upper Yukon River watershed. We also found evidence of morphological and colouration differences between localities within Atlin Lake, BC, indicating that such phenomena are general ones for lake trout across their geographic range. It is well known that the extent of genetic substructure within a population assemblage linked by gene flow and its influence on effective population size can have important effects on the time to fixation or loss of alleles, as well as which alleles are more likely to fix (e.g., Whitlock and Barton 1997; Whitlock 2003). Following these principles, the lake trout substructure of Atlin and Tagish lakes is an important attribute that can impact the rate of evolution of lake trout and hence influence their persistence in the face of environmental change (Whitlock 2003). Consequently, to sustain lake trout and their fishery potential within the large and complex ecosystem of Atlin–Tagish lakes, managers should consider and maintain the morphological and molecular biocomplexity that we have documented and the interaction of subpopulations within and between these two lakes (cf. Hilborn et al. 2003). The evolutionary potential of lake trout, and ultimately their persistence, in this large lake ecosystem and others like it most likely depends on the survival of many subpopulations across a diversity of habitats within such large lake basins. More broadly, previous conceptions of lake trout ecology such as a narrow ecological niche and limited tolerance to temperature and oxygen levels, based largely on the Laurentian Great Lakes populations, have been revised based on new information (Wilson and Mandrak 2003). If the Laurentian Great Lakes are quantitatively different, as some have suggested (Wilson and Mandrak 2003), then obtaining knowledge of populations outside this area such as we have provided offers a more complete understanding of the biology of lake trout.

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