Population Structure, Genetic Diversity, and Dispersal of Anadromous Arctic Char (*Salvelinus alpinus*) in Frobisher Bay, Nunavut, Inferred from Microsatellite Markers

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by

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ABSTRACT

The genetic population structure of anadromous Arctic char from two river systems flowing into Frobisher Bay, Nunavut (the Sylvia Grinnell River and the Bay of Two Rivers) was investigated. A total of 495 individuals were genotyped at twelve microsatellite markers. Four different analytical methods provided no evidence of genetic differentiation between sampling locations within the Sylvia Grinnell River, suggesting that there is no within-river population structure. There was weak but significant genetic differentiation between the Arctic char sampled at the Sylvia Grinnell River and the Bay of Two Rivers. This conclusion was supported by two separate complementary analyses. An analysis of molecular variance (AMOVA) and Bayesian clustering as implemented in STRUCTURE, however, did not fully support the presence of population structure. A genetic assignment procedure suggested the presence of a few dispersers between the Sylvia Grinnell River and the Bay of Two Rivers. The power of this analysis, however, was found to be low and results should be interpreted with caution. Finally, an analysis using the software BOTTLENECK suggested that the recent population declines in the Sylvia Grinnell River have lead to significant reduction in genetic diversity, something that was not observed in the Bay of Two Rivers dataset. Overall, our results support the presence of weak population structure among rivers in the Frobisher Bay region and suggest that the use of molecular tools to inform fishery management in this region may be helpful if appropriate sampling designs are implemented.

RÉSUMÉ

La structure de population de l'omble chevalier anadrome de deux rivières de la Baie de Frobisher (les Rivières Sylvia Grinnell et Bay of Two Rivers) a été investiguée. Un total de 495 spécimens furent génotypés à douze marqueurs microsatellites, dont onze étaient polymorphes et donc informatifs. Quatre méthodes analytiques différentes n'identifient pas de différentiation génétique entre trois sites d'échantillonnage à l'intérieur de la Rivière Sylvia Grinnell, suggérant qu'il n'y a pas de structure de population au niveau de la rivière. La différentiation génétique entre les ombles chevaliers échantillonnées dans la Rivière Sylvia Grinnell et la Bay of Two Rivers était quant à elle significative mais faible. Cette conclusion est supportée par les résultats de deux analyses, pas supportée par ceux d'une troisième (AMOVA), et une quatrième analyse (STRUCTURE) a donné des résultats ambigus. Une procédure d'assignation populationnelle à l'aide des données génétiques suggère la présence d'individus disperseurs entre les deux rivières. Le pouvoir de cette analyse était cependant faible, et ces résultats devraient être interprétés avec soin. Finalement, une analyse avec le programme BOTTLENECK suggère que les récents déclin de population dans la Rivière Sylvia Grinnell ont résultés en une réduction significative de la diversité génétique, une réduction qui n'est pas observée dans la population de la Bay of Two Rivers.

INTRODUCTION

Commercial fisheries for anadromous Arctic char (*Salvelinus alpinus*) in Nunavut are currently managed on a river-by-river basis (Roux et al. 2011). This management strategy rests on the assumption that Arctic char home to their natal river to spawn and overwinter, and thus that each river is comprised of a single distinct stock (Johnson 1980). This assumption, however, remains largely untested. Indeed, what we know about Arctic char dispersal behavior comes from a few tagging studies that have shown that (1) dispersal can be high between river systems (Gyselman 1994) and (2) that dispersal can vary tremendously between locations (Dempson and Kristofferson 1987).

The Sylvia Grinnell River (Fig. 1) is a traditional Inuit fishing site and its Arctic char stock is an important subsistence resource for the residents of Iqaluit. The population of char from the river, however, was reported to be depleted in comparison to historical levels (Gallagher and Dick 2010). In 2010, a tagging study was initiated in the Sylvia Grinnell River (63°44.5' N 68°34.3' W) and the Bay of Two Rivers (63°36.8' N 68°50.7' W) in an effort to generate an estimate of stock abundance for the Sylvia Grinnell population and to better understand stock structure among char populations in Frobisher Bay (VanGerwen-Toyne et al. 2013). A secondary goal was to evaluate the application of genetic techniques for providing input into the management of Arctic char in the area. More specifically, there was interest in determining whether genetic data would support the application of a river-by-river management strategy.

The present report summarizes the findings of a microsatellite DNA assessment of samples collected from the Bay of Two Rivers and from three sampling locations in the Sylvia Grinnell River system. The study had four main goals: (1) to test whether the Arctic char population from Sylvia Grinnell River is genetically distinct from char in the Bay of Two Rivers, located about 30 km away (2) to examine the potential for genetic differentiation between different sampling locations within the Sylvia Grinnell River system, (3) to determine whether genetic assignment tests could be used as a tool to study dispersal of Arctic char between rivers in Frobisher Bay, and (4) to evaluate whether fishery-induced population declines have lead to the loss of genetic diversity in the Sylvia Grinnell population. Overall, the results of the present study will be used for guiding the management of this Arctic char subsistence fishery.

METHODS

Samples

Arctic char fin clips were collected in 2010 and 2011 at a variety of sampling sites on the Sylvia Grinnell River and the Bay of Two Rivers (Table 1). In 2010, samples from adult individuals were collected at one sampling site at the Bay of Two Rivers and at three sites in the Sylvia Grinnell River. Three tagged

individuals were also recaptured that year and used in the genetic analyses. Samples from adult individuals were again collected at two of the Sylvia Grinnell River sites in 2011, and juveniles were collected from a site on the Sylvia Grinnell.

Genotyping of microsatellite markers

Total DNA was isolated from the fin tissue samples using Qiagen (Valencia, CA, USA) DNeasy Blood and Tissue Kits. Individual genotypes were obtained at twelve microsatellite loci: *Smm17, Smm22*, *Smm24* (Crane et al. 2004), *Sco200*, *Sco215*, *Sco216, Sco220* (DeHaan and Ardren 2005), *OtsG83b*, *OtsG253b* (Williamson et al. 2002), *Omm1105*, *Omm1128* (Rexroad et al. 2001), *SSOSL456* (Shaklee 2003), Table 2).

The twelve loci were combined in three different PCR multiplexes: (mpAC1) *Sco200* with *Smm22*, *Sco220*, and *Sco215*; (mpAC3) *Omm1128* with *Smm24*, *OtsG253b*, *SSOSL456*, and *Omm1105*; and (mpAC4) *Smm17* with *Sco216* and *OtsG83b*. For each locus in mpAC2 and mpAC4, the reverse primer was PIG-tailed (Brownstein et al. 1996). Qiagen (Valencia, CA, USA) Multiplex PCR Kits were used for the PCR, and multiplex reactions used the following cycling conditions: an initial denaturation period of 15 min at 95℃, followed by 35 cycles of denaturation (94℃ for 30 sec), annealing (55℃ for 1m30s) and elongation (72℃ for 1min), a final elongation period of 30 min at 60℃ was implemented. Concentrations of each reagent followed the guidelines provided by the manufacturer. The PCR products were run on an Applied Biosystems (Carlsbad, CA, USA) 3100 Genetic Analyzer. GeneMapper Software version 3.7 (Applied Biosystems) was used to automatically score microsatellite alleles, and all scores were manually checked for quality.

Analysis of microsatellite polymorphisms

Each locus was tested for departures from Hardy-Weinberg equilibrium (HWE) within each sampling location. Departures from HWE are indicative of non-random mating (e.g., presence of population structure) or of natural selection acting on allele frequencies, and can thus reveal interesting biological processes. Potential for linkage disequilibrium (LD) between loci was also examined. Departures from LD indicate that different loci are either physically linked in the genome (and thus inherited together) or are linked through other biological process. Loci that are in LD cannot be used as independent information sources for population genetic analyses. FSTAT version 2.9.3.2 (Goudet 2001) was used to test for departures from HWE and LD. In both cases, the nominal significance level was set at 0.05 (a Bonferroni correction for multiple comparisons (Rice 1989) was implemented) and the program selected the appropriate number of permutations. FSTAT was also used to calculate observed heterozygosity (H_O), Nei's (1987) unbiased expected heterozygosity (H_S) and allelic richness (A_R). The later statistic was calculated with a rarefaction approach to control for differences in sample size. Heterozygosity and allelic richness provide indices of genetic diversity, low genetic diversity providing evidence for small population size.

To ensure that samples from different years, but from the same location, did not differ, I calculated the significance of F_{ST} values between 2010 and 2011 or the two sampling locations with samples from those two years: the 'Falls' site and the 'Metal Dump' site (there is only juvenile samples from the 'End of Runway' site in 2011 which were not included in the present analysis). FSTAT was used to calculate F_{ST} values (Weir and Cockerham 1984) between each sampling year, and significance was assessed with 10,000 permutations at a nominal significance level of 0.05 (using a Bonferroni correction for multiple comparisons).

Population structure

Multiple complimentary approaches were used to test for the presence of population structure at two hierarchical levels: (1) among sampling sites within the Sylvia Grinnell River, and (2) between the Bay of Two Rivers and the Sylvia Grinnell River.

First, GENETIX (Belkhir et al. 2004) was used to perform Factorial Correspondence Analysis (FCA), a multivariate exploratory data analysis tool that allows the visualization of individual genotypes in multivariate space. The results of the FCA were plotted in 3D using R (R Development Core Team 2010).

Second, FSTAT was used to generate pairwise F_{ST} between each sampling location, as well as between rivers (i.e., all sampling locations within Sylvia Grinnell River combined together). Statistical significance of pairwise F_{ST} values was assessed with 10,000 permutations at a nominal significance level of 0.05 (using a Bonferroni correction for multiple comparisons).

Third, ARLEQUIN (Excoffier et al. 2005) was used to perform an Analysis of Molecular Variance (AMOVA) and test for the presence of hierarchical population structure in the dataset. An AMOVA is similar to an ANOVA in that it partitions the variance among different treatments (here the geographical locations of the sampling sites) and determines whether there are significant differences between them. Two hierarchical levels of groupings were defined: between rivers, and among sampling sites within the Sylvia Grinnell. The level of significance of the genetic structure exhibited in each grouping was assessed using 10,000 permutations.

Last, STRUCTURE (Pritchard et al. 2000) was used to identify distinct genetic clusters in the dataset. Contrary to all previous analyses, STRUCTURE does not use *a priori* population information and instead identifies distinct clusters based solely on the information contained in the genotypes of individuals by maximizing Hardy-Weinberg equilibrium and linkage equilibrium. We ran STRUCTURE on the entire data set under the admixture model with independent allele frequencies. We varied K (i.e., the number of genetic clusters) from 1 to 10 and ran 20 independent runs for each value of K with a burn-in of 250,000 followed by 500,000 MCMC replicates per run. The results were visualized using STRUCTURE HARVESTER (Earl 2011), which implements the ΔK method of

Evanno *et al.* (2005) to infer the most likely number of clusters. Results from multiple independent runs were combined using CLUMPP (Jakobsson and Rosenberg 2007) using the 'greedy' algorithm with 1,000 repeats. Results from CLUMPP were used in DISTRUCT (Rosenberg 2004) to generate the bar plot of individual Q-values.

Genetic assignment tests

The program GENECLASS2 (Piry et al. 2004) was used to conduct assignment tests and identify putative dispersers in the dataset. Because the other analyses showed that there was no population structure within the Sylvia Grinnell River (see next section), we combined all samples from the three Sylvia Grinnell River sampling locations. Samples were assigned or excluded using the Bayesian computation method of Rannala and Mountain (1997) and the Monte-Carlo resampling algorithm of (Paetkau et al. 2004) to simulate 100,000 individuals with a 0.05 type I error rate. In order to avoid type-I errors (i.e., individuals identified as dispersers who are actually not), the criterion for confident assignment used by Hauser et al. (2006) was used here. The assignment score of individuals (i.e., $score_{i,l} = L_{i,l} / \Sigma L_{ij}$ where $L_{i,l}$ is the likelihood of individual *i* belonging to sample *l*) as calculated by GENECLASS2 was used to eliminate all individuals that had a score lower than 95%. The individuals that did not fit this criterion were labeled 'un-assigned'. Such non-assignment can result from the presence of immigrants from un-sampled rivers, or from low resolving power to assign individuals (see below).

Power to confidently assign individuals was also evaluated following the guidelines provided by Paetkau et al. (2004). First, reciprocal likelihood of assignment to the two putative populations of origins were plotted for each individual in the two samples. If there is sufficient power to assign individuals, the two populations should be separated on each side of the one-to-one line. Furthermore, values of D_{LR} (mean genotype likelihood ratios) for each population were computed. Simulations performed by Paetkau et al. (2004) showed that D_{LR} values provided the best mean to predict the power of an assignment test, with values above three providing satisfactory power, and values above five providing maximal power.

Demographic bottleneck and genetic diversity

Biological assessments suggest that recent (1970s) fishing pressure on the Sylvia Grinnell River led to a population decline (Gallagher and Dick 2010). Such demographic bottlenecks can leave a genetic signature in terms of reduced genetic diversity. More specifically, bottlenecks are expected to lead to rapid loss of allelic diversity, while heterozygosity is lost at a slower pace (Luikart and Cornuet 1998). The discrepancy between these two measures of genetic variation can then be used to investigate the genetic effects of demographic bottlenecks. The software BOTTLENECK (Cornuet and Luikart 1996) allows such investigations through a comparison of observed genetic diversity to that generated using simulated data evolving *in silico* under models assuming stationary population sizes. If a recent population bottleneck has occurred, we

expect the observed heterozygosity (H_e) to be higher than that expected (H_{eq}) under the null model given the number of alleles (k) in the sample. This program was used on both the Bay of Two Rivers and the Sylvia Grinnell River samples. If fishing pressure left genetic signatures, we would expect the Sylvia Grinnell samples to display evidence of genetic bottlenecks, while the Bay of Two Rivers samples should not. The coalescent simulations in BOTTLENECK were run according to a two-phase mutation model (TPM) with a 90% proportion of stepwise mutations (SMM), a variance of 30, and for 10,000 iterations. The sign test and the two-tailed Wilcoxon signed rank test implemented in the program were used to determine significance, based on the recommendations made in the software manual (Cornuet and Luikart 1996).

RESULTS

Microsatellite polymorphism

All but one of the twelve microsatellite loci screened showed high levels of polymorphism. *Omm1128*, was monomorphic and thus removed from all subsequent analyses. Overall, the average number of alleles per locus was high (23.45) and ranged from 4 (*Sco215*) to 48 (*Sco216*) (Table 3).

Twelve locus-site combinations showed significant departures from HWE (all heterozygote deficits) at $\alpha = 0.05$. Only one, however, remained significant after correcting for multiple comparisons with a Bonferroni correction (α after correction = 0.00069): *OtsG83* in the Sylvia Grinnell Metal Dump (SGMD) site. Nine pairs of loci showed significant linkage disequilibrium at $\alpha = 0.05$ but none remained significant after Bonferroni correction ($\alpha = 0.0014$). Indices of genetic diversity appear generally similar between the different sampling sites (Table 4).

There was no significant genetic differentiation between samples collected in different years but at the same sampling location. At the 'Falls' site, F_{ST} between the 2010 and 2011 samples was 0.0021 (P = 0.4917). At the 'Metal Dump' F_{ST} between the 2010 and 2011 samples was 0.0002 (P = 0.525). Samples collected in different years were therefore combined in all analyses (including the analyses described in the last paragraph).

Population structure

The FCA showed a slight separation between the Bay of Two Rivers samples and the Sylvia Grinnell River samples (Figure 2). However, there was no indication of sub-groupings within the Sylvia Grinnell River sampling locations (not shown). The FCA also identified an outlier (individual: 2-064; Fig. 2B). This individual had missing data at three loci, and is a homozygote for a very rare allele. While it is possible that this sample is biologically meaningful, it is more likely a genotyping artifact, and the sample was removed from all subsequent analyses. Removal of this individual did not change the distribution of samples along axis 1 in the FCA (not shown), but changed their distribution on axis 3 (Fig.

2C). Two of the recaptured samples cluster with the Sylvia Grinnell River, while one individual appears to cluster with the Bay of Two Rivers. The location of initial capture and of recapture of these samples is currently not available, making it difficult to interpret this result.

The F_{ST} value between the Bay of Two Rivers and the Sylvia Grinnell River was small but significant (F_{ST} = 0.0192; P = 0.05), suggesting that the two populations are genetically differentiated. When the Sylvia Grinnell samples were divided into three sub-locations, all three sub-locations showed significant pairwise F_{ST} in comparisons with the Bay of Two Rivers. The pairwise F_{ST} values among sites within the Sylvia Grinnell, however, were not significant (Table 5), suggesting that there is no sub-population structure within this system. Consistent with the FCA analysis (and the STRUCTURE analysis to follow), one of the F_{ST} values between the juvenile samples and the Sylvia Grinnell samples was significant.

The AMOVA confirmed the lack of population structure within the Sylvia Grinnell River (P = 0.74; Table 6). Similarly, but contrary to the results of the other analyses, there was no evidence of population structure between the Sylvia Grinnell and the Bay of Two Rivers (P = 0.248; Table 6).

Genetic assignment tests

GENECLASS2 identified only a few migrants in the samples from both rivers: 4.5% of assigned individuals in the Bay of Two Rivers and 1.8% in the Sylvia Grinnell River (Fig. 4). The criterion for confident assignment used (i.e., assignment score >95%), however, left 14.4% of individuals un-assigned. This high level of un-assignment is also reflected by the low average probabilities of assignment observed. In the Bay of Two Rivers samples, average probability of self-assignment is only 77.1% while probability of assignment to the Sylvia Grinnell is as high as 39.2%. In the Sylvia Grinnell, the average self-assignment probability is 64.3% and the probability of assignment to the Bay of Two Rivers is 23.2%.

The power of the genetic assignment test to distinguish between the river of origin of individual fish was found to be minimal. First, visual examination of the reciprocal likelihood plot (Fig. 5) shows a considerable amount of overlap between the two populations. Second, the D_{LR} values of 2.46 and 2.69 for the Bay of Two Rivers and Sylvia Grinnell River respectively) raise further doubts regarding the power of the analysis.

Demographic bottleneck and genetic diversity

The analysis performed in BOTTLENECK provides support for the hypothesis that the Sylvia Grinnell River has suffered from a recent population bottleneck, while the Bay of Two Rivers population has not. For the Sylvia Grinnell samples, 10 of 12 loci showed an excess of heterozygosity (H_e) compared to expectations (H_{eq}) (sign test: P = 0.039; Wilcoxon: P = 0.006). For the Bay of Two Rivers sample, only 7 of 12 loci showed an excess of H_e (sign test: P = 0.53; Wilcoxon: P = 0.092).

DISCUSSION

The population structure of anadromous Arctic char from two rivers flowing into Frobisher Bay was investigated using microsatellite markers. Genetic differentiation between the two rivers was found to be minimal, but significant. Because of low amounts of genetic differentiation, analyses of dispersal using assignment tests had low power. Most individuals, however, were assigned to their river of origin albeit with low assignment probability. Finally, there is evidence that recent population declines in the Sylvia Grinnell River had negative consequences on genetic diversity, which may have long-term consequences for the capacity of this population to respond to environmental change. In conclusion, the use of genetic tools for the study of anadromous populations of Arctic char highlighted the potential usefulness of such an approach in providing insights for management. The sometimes equivocal results obtained, however, also highlight the importance of sampling design and sample size for such studies. We briefly discuss some recommendations for future studies using genetic tools in this region.

Population structure within the Sylvia Grinnell River

Four separate analyses were unable to identify genetic differentiation among the three sampling locations within the Sylvia Grinnell River. This result, however, should be interpreted with caution since it is based on samples of adults collected during the upstream migration. The sampling of adults during their movement through the river does not necessarily reflect the final spawning location of adults. Their location of capture may thus poorly reflect the spatial distribution of spawning aggregations.

In fact, the patterns observed with the juvenile samples suggest that this may be the case. Indeed, in both the FCA and the STRUCTURE analysis, the juveniles appear to cluster together and away from the other samples of that population. There are at least three possible explanations for this pattern. First, it could be a methodological artifact. The juvenile samples had more missing data at several loci, most likely because of lower DNA quality. The most obvious cause for this would be the preservation method of the juvenile samples, which were frozen instead of preserved in 95% ethanol like the other samples. Because of this potential problem, all results from the juvenile samples should be cautiously interpreted. Second, sampling juvenile salmonids for the purpose of describing population genetic parameters can be problematic because it can lead to an increased likelihood of sampling related individuals (individuals from the same clutch, for example). This phenomenon is referred to as the Allendorf-Phelps effect (Waples 1998). It should be noted, however, that this problem is likely to be less of an issue with Arctic char, whose long freshwater residence ensures that multiple cohorts are usually sampled. While we do not have age data for the juvenile fish used in this analysis, their varying length does indeed suggest that they are of differing age. Third, it is possible that the genetic differentiation observed between the juvenile and adult samples reflects the presence of multiple spawning aggregations in the Sylvia Grinnell River. However, we did not

find evidence of such clustering in the adult samples. As mentioned earlier, the sampling of adults during the upstream river migration does not necessarily reflect the final spawning location of adults. Sampling of adults in the winter (assuming they are spawners and not simply overwintering), or sampling of juveniles from more locations, would provide a better test of the hypothesis of genetic differentiation among spawning aggregations within the Sylvia Grinnell River.

Population structure between the Sylvia Grinnell and Bay of Two Rivers

There was evidence for weak, but generally significant, genetic differentiation between the Sylvia Grinnell River and the Bay of Two Rivers. This was supported by the FCA analysis and by the F_{ST} values. Not all analyses, however, unambiguously support this conclusion: the AMOVA rejected the presence of population structure and Bayesian clustering resulted in ambiguous results. For instance, there appears to be a clear clustering of the Bay of Two Rivers individuals, but in the Sylvia Grinnell many individuals appear to cluster in the Bay of Two Rivers genetic cluster, while others appear to have shared ancestry between the two clusters. Even when K is assumed to equal 3, the genetic clustering of the Sylvia Grinnell fish is imperfect, with large contributions of the other genetic cluster. This could again reflect low overall genetic divergence, or perhaps even the presence of multiple genetic groups within the Sylvia Grinnell River (see discussion above). The present data set is not sufficient to answer this question.

It should also be noted that the F_{ST} values observed in this study were generally smaller than those observed among populations in Cumberland Sound (global F_{ST} = 0.038; Moore et al. 2013). This could reflect the closer geographical proximity of the two sites studied here, compared with the farther typical distances between sites in Cumberland Sound (where each river is typically found at the end of long fiords) (Moore et al. 2013). Bernatchez et al. (1998) also used microsatellite data to examine genetic populations structure of three river populations of anadromous Arctic char flowing in the same bay in Labrador (Voisey's Bay, near Nain). Despite the geographical proximity of the sampling sites, they still detected F_{ST} values that were generally higher than in the present study (between 0.0161 and 0.0471).

Genetic assignment tests

The genetic assignment tests identified a small number of putative migrants between the Sylvia Grinnell and Bay of Two Rivers populations. Straying is regarded as a fairly widespread phenomenon in salmonids (Hendry et al. 2004) and Arctic char in particular (Gyselman 1994; Dempson and Kristofferson 1987; Moore et al. 2013). Consistent with the results of the genetic assignment tests, VanGerwen-Toyne et al. (2013) reported that two fish that were tagged in the Bay of Two Rivers were recaptured by fishers at the mouth of the Sylvia Grinnell River. The number of migrants identified with the genetic assignment method was, however, significantly smaller than that reported by Moore et al. (2013) for the Cumberland Sound region. Although they used slightly different criteria for

the identification of migrants, Moore et al. (2013) report that between 15.8% and 25.5% of the genetically assigned individuals are identified as strays. The genetic differentiation among populations in that region was generally greater than that observed in the two Frobisher Bay populations, and therefore cannot explain this discrepancy. It is also possible that the number of populations investigated in the Moore et al. (2013) study made confident assignment more difficult than with only two populations. Finally, it is also possible that the differences in the number of migrants identified actually reflect a difference in the biology of the two population complexes, a fascinating possibility that may be worth further investigation.

It should also be noted that a substantial number of individuals remained unassigned given the criterion we used for confident assignment. Such un-assigned individuals can be interpreted in one of two ways. First, they could be individuals coming from un-sampled rivers (ghost populations) near the two sampled rivers. This is certainly a possibility given that only two of the many char stocks distributed around Frobisher Bay were sampled in this study. Such ghost populations can bias assignment tests because the individuals are necessarily assigned to one of the rivers included in the data set (i.e., even if the individual does not come from one of the rivers sampled, it will still have a higher likelihood of being assigned to one of the rivers). Second, low power to assign individuals can result in low assignment scores and assignment probabilities. The power analysis presented here suggests that low power is an issue with the present genetic assignment procedure.

There are several potential reasons for such low assignment power. First, power increases with genetic differentiation between populations (Paetkau et al. 2004). The results of the analysis in the previous section have shown that while there is significant genetic differentiation between the two populations examined here, it is relatively low. Second, the absence of reference samples for the assignment procedure can be problematic. Indeed, in the current analysis, the genotypes of the very individuals to be assigned were used to characterize the genetic makeup of the two populations. If those samples contain a large number of migrants from the other population, the two populations will appear artificially more genetically similar, thus reducing power. One potential solution to this problem in the case of Arctic char is to use samples of non-migrating juveniles, which have not had the opportunity to disperse. Third, increasing the number of microsatellite loci scored can increase power. A preliminary study conducted last year on the same populations using eight microsatellite markers had lower power in general, but the increased number of microsatellites used in this study did not lead to a substantial increase in power. It seems doubtful, therefore, that increasing the number of microsatellite markers further would resolve the issue of low power, in this case. It should be noted, however, that the number of dispersers identified in the current analysis is much lower than that identified in the previous analysis utilizing only eight microsatellite markers. This suggests that conclusions regarding the extent of dispersal depend on the number of markers used, and that a small increase in power – as in the current study – can

lead to a large reduction in what can only be interpreted as wrongly identified dispersers.

Demographic bottleneck and genetic diversity

Demographic bottlenecks are well known to result in a loss of genetic variation through increased inbreeding (breeding among related individuals; Allendorf and Luikart 2007). This erosion of genetic diversity affects average heterozygosity in the population, but more importantly, it also leads to loss of allelic diversity (Allendorf and Luikart 2007). There is now substantial evidence that loss of genetic diversity can lead to increased extinction risk (Frankham 2005) or reduce the capacity of population to adapt to changing environmental conditions (Bürger and Lynch 1995; Hoffman and Sgro 2011).

The Sylvia Grinnell River is the target of an important subsistence fishery, and sustained an attempt at the development of a commercial fishery between 1947 and 1966 (Gallagher and Dick 2010). Declining catch per unit effort and a variety of other indicators suggested that the stock was over-fished and the commercial fishery was closed in 1967 (Gallagher and Dick 2010). Evidence suggests that the stock is only recently showing signs of recovery and that the population remains below historical levels (Gallagher and Dick 2010).

The genetic analysis performed in BOTTLENECK revealed that the Sylvia Grinnell River population underwent a recent demographic bottleneck that led to the erosion of genetic diversity. This result is consistent with the hypothesis that over-fishing is at least in part responsible for the decline in genetic diversity in the previous decades. This interpretation is supported by the fact that the analysis performed on the Bay of Two Rivers population did not identify a recent bottleneck. This population was never targeted by a commercial fishery and sustains only a minor subsistence fishery. It should also be noted, however, that the Sylvia Grinnell River, because of its close proximity to Iqaluit, may also suffer from more anthropogenic impact than the more isolated and pristine Bay of Two Rivers. The contribution of other stressors can therefore not be ruled out. Regardless of the cause, however, the observed decline in genetic diversity could have important consequences for the long-term persistence and viability of this stock (Frankham 2005).

Management implications and future work

The present study, given its limited sampling effort (both in terms of individuals and number of populations sampled), should probably be regarded as a preliminary study and using its results as a basis for management decisions without additional data would be precarious. Despite this caveat, the results suggesting significant population differentiation between rivers do support the current ‘river-by-river’ management paradigm. The genetic assignment tests, however, also detected a non-trivial number of migrants between the two rivers. As discussed above, the results of the assignment tests have to be interpreted cautiously, but they are in line with other tagging studies (VanGerwen-Toyne et al.

2013). Regular dispersal between the two rivers does not necessarily mean that the current river-by-river management approach should be abandoned, but is a fact that should be kept in mind by managers. For example, in extreme cases where a stock suffers a dramatic decline requiring a fishery closure, this measure may be more effective if fishing is reduced in other nearby rivers as well. The finding that the Sylvia Grinnell suffered a recent demographic bottleneck does not suggest any specific management action, but does reinforce the importance of setting harvest limits on this recovering population.

Future studies utilizing molecular markers in this region should attempt to improve the sampling design of the present study in a few important ways. First, to get an accurate picture of the levels of genetic differentiation among rivers of the Frobisher Bay area and to improve our capacity to utilize genetic assignment tests, more rivers would need to be sampled. Second, accurate estimation of allelic frequencies require a minimum number of individuals per sample (at least 30, ideally 50 or more, depending on the number of alleles at a locus). Some of the sample sizes in the current study (e.g., 'end of runway' and 'falls' sites in 2010) did not reach this minimum. Third, the samples available for this study were not sufficient to appropriately test the hypothesis of within-river genetic differentiation in the Sylvia Grinnell River. Some of the results of our analysis do suggest that such within-river structure may be possible, and future studies may want to explore this possibility. To do so, sampling of juveniles and/or of spawning fish on the spawning grounds would be more appropriate because they would better reflect the location of spawning aggregation than adult fish sampled as they undergo their upstream migrations.

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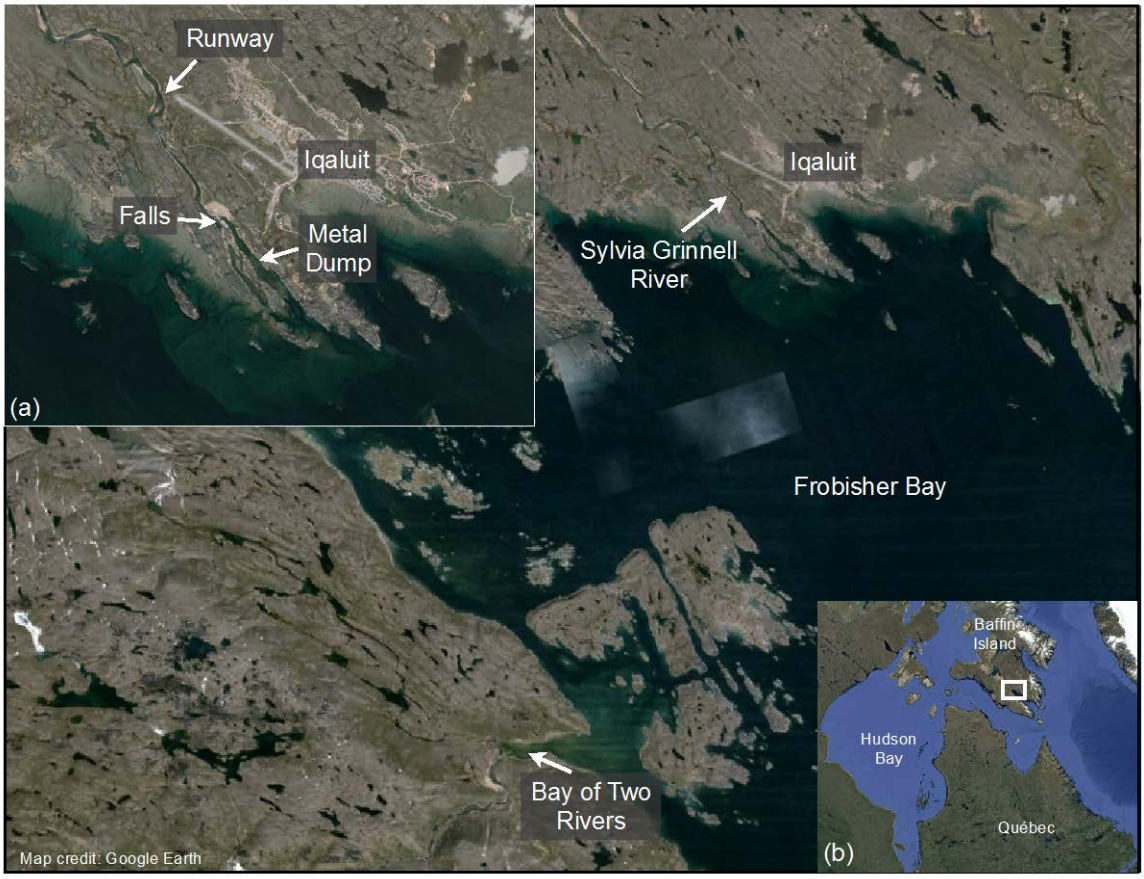

Figure 1. Map of Frobisher Bay close to the city of Iqaluit showing the location of the two rivers sampled in this study. Inset (a) shows the Sylvia Grinnell River in more detail and shows the location of the three sampling sites along the river. Inset (b) shows the location of the study area in Northern Canada.

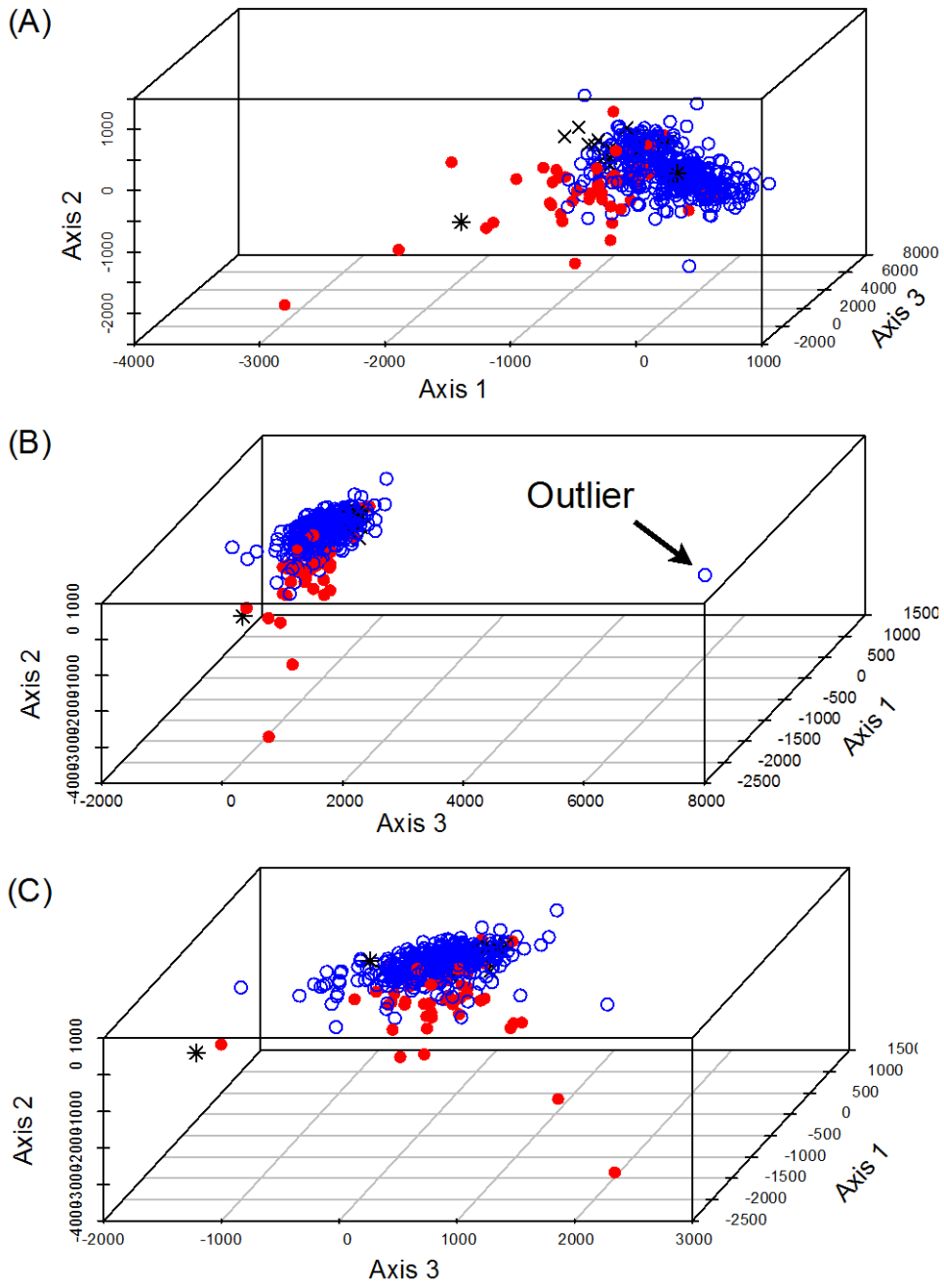

Figure 2. Results of the factorial correspondence analysis performed in GENETIX for Arctic char captured in the Sylvia Grinnell River (open blue circles) and the Bay of Two Rivers (red filled circles). The black 'X' are juvenile char from the Sylvia Grinnell, and the asterisks are char recaptured from a previous mark-recapture study. (A) Results of the first FCA including all samples. (B) Same analysis as in A, but changing the axis perspective, to show the presence of an outlier. (C) Results of the analysis performed again without the outlier. The presence of the outlier had a negligible effect on the distribution of the samples along Axis 1.

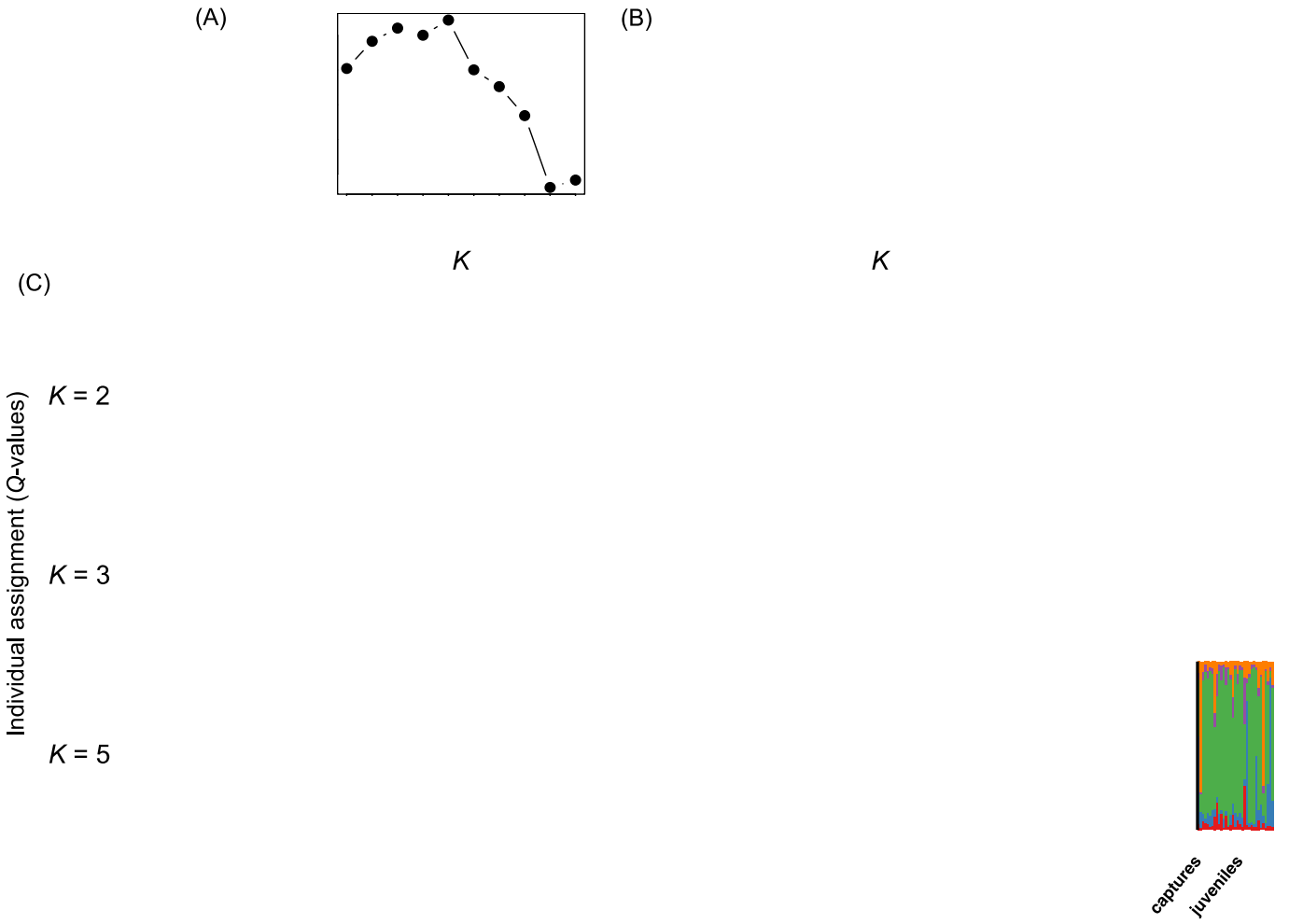

Figure 3. Results of the STRUCTURE analysis for Arctic char captured in the Sylvia Grinnell River and Bay of Two Rivers. (A) Likelihood profile showing that the lnP(D) value peaks at a K of 5, and to a lesser extent at K = 3. (B) Distribution of ΔK values, an alternative method for the selection of the most likely K value, indicating that the most likely number of genetic clusters is 2. (C) Probability of assignment (Q-values) to the different genetic clusters (represented by different colors) for the analysis assuming K = 2, K = 3, and K = 5.

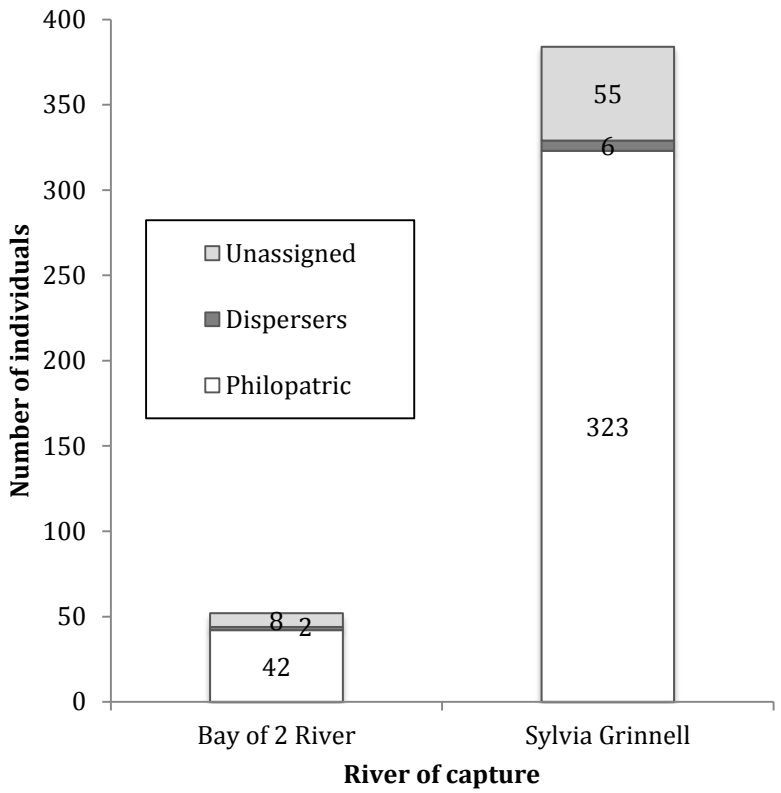

Figure 4. Summary of the results of the genetic assignment tests for Arctic char captured in the Sylvia Grinnell River and Bay of Two Rivers. The Bay of Two Rivers sample appears to contain proportionally more dispersers and un-assigned individuals than the Sylvia Grinnell River sample.

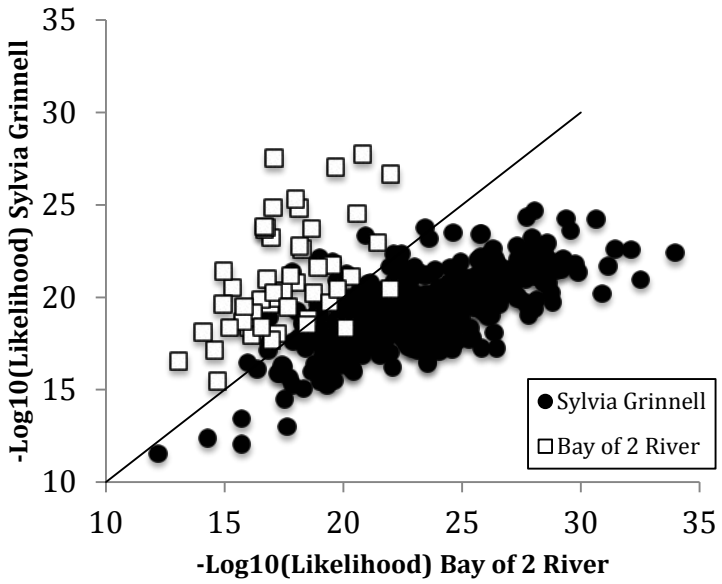

Figure 5. Plot of reciprocal likelihood of assignment for individual Arctic char captured in the Sylvia Grinnell River (hollow squares) and at the Bay of Two Rivers (filled circles). The line is the one-to-one relationship. Note how individuals from both rivers overlap over the one-to-one line, which indicates that power to discriminate between rivers of origin for individuals is minimal.

Table 1. Summary of Arctic char samples used in this study.

River	Site	Year	N
Bay of Two Rivers		2010	52
Sylvia Grinnell River	End of runway	2010	8
Sylvia Grinnell River	Metal dump	2010	152
Sylvia Grinnell River	Metal dump	2011	125
Sylvia Grinnell River	Falls	2010	12
Sylvia Grinnell River	Falls	2011	110
Juveniles (SGR)	End of runway	2011	33
Recaptures		2010	3

Table 2. Sequences of the forward and reverse primers used for the 12 microsatellite loci screened in this study, and fluorescent dyes used. The loci are grouped according to the multiplex reaction in which they were screened.

Multiplex	Primer	Forward sequence	Reverse sequence	Dye**
mpAC1	*Sco200*	GTGCCTTGGTGGAGATTAC	CCTTTATGTGTCCCTGTATGA	VIC
	Smm22	CCC AAT GCA GAT AAG ACC TT	TCT ATA GGC TTA TTT GAA TGG AAT	NED
	Sco220	AAC GAG TTC TAA TGA CTC CAA C	ATC ATG CTC ATC ATC ACT CTC	6-FAM
	Sco215	GAG AGA GAG AGA TGG GTG ACA	ATC CAC AAA ACA AGA TTG CTA	PET
mpAC3	*Omm1128*	CCACATCCTAGAACCGTTGA	CAATACACAGCACCAACAACC*	VIC
	Smm24	CAT TGA TCA AGA AGC CAG TGC	TGT ATT TGG CCA ATA TAA CAC AGC*	NED
	OtsG253b	GAG CAG GCC GAG CAG GTG TCT	AAT TGG GTC ATT AAG GCT CTG TGG*	VIC
	SSOSL456	CTT CCC AGG AGT CAT CAT AAA TCT	GTT TAA ACC CCA CTG CTT GTT GAG TGT*	6-FAM
	Omm1105	GCA CAC TGT CTG GGT AAG AGA	GCA GAG CCA CAC TAA ACC A*	PET
mpAC4	*Smm17*	AAG GAT GGT GAG GAC AAT ACA	ACC TTG AGA AAT CTA TAT GTG GTCTA*	NED
	Sco216	CCT TGT GAG AGC TAA GGT AGT G	GGA GGA CAT ATT CCA ACT TTG*	PET
	OtsG83b	TAG CCC TGC ACT AAA ATA CAG TTC	CAT TAA TCT AGG CTT GTC AGC AGT*	6-FAM

* Reverse primers were PIG-tailed. See text for details.
** For mpAC1, the reverse primers were labeled with fluorescent dye; for mpAC2 and mpAC4, the reverse primers were labeled.

Table 3. Number of alleles, observed heterozygosity (H_O), and Nei's (1987) expected heterozygosity (H_S) for each locus screened in this study.

Locus	No of alleles	H_O	H_S
Sco200	27	0.89	0.89
Sco215	4	0.69	0.64
Sco220	35	0.96	0.95
Smm22	25	0.82	0.92
Omm1105	20	0.68	0.78
Omm1128	1	0	0
OtsG253b	13	0.77	0.83
Smm24	26	0.91	0.93
SSOSL456	24	0.75	0.84
OtsG83b	26	0.89	0.85
Sco216	48	0.97	0.95
Smm17	10	0.73	0.78
Average	21.58	0.75	0.78
Average without OMM1128	23.45	0.82	0.85

Table 4. Indices of genetic diversity for Arctic char from Sylvia Grinnell River and the Bay of Two Rivers. The shaded area indicates the results for when the Sylvia Grinnell sample is divided in the three sampling locations. The total number for the pooled Sylvia Grinnell samples is less than the sum of the three sampling locations because individuals with too much missing data were removed for the final analysis.

Population	Sub-locations	N	Unbiased Hz	Obs Hz	No Alleles	Allelic richness
Bay of Two Rivers		52	0.7807	0.7716	8.73	13.21
Sylvia Grinnell		385	0.7818	0.7510	20.92	13.65
	End of runway	8	0.7873	0.8021	3.77	
	Metal Dump	277	0.7831	0.7498	11.89	
	Falls	122	0.7772	0.7495	11.90	
Juveniles		33	0.7577	0.7564	5.04	
Recaptures		3	0.7833	0.6944	1.51	

Table 5. Semi-matrix of pairwise F_{ST} values between Arctic char from the Sylvia Grinnell River and Bay of Two Rivers. (Bo2R = Bay of Two Rivers; SGER = Sylvia Grinnell End of Runway; SGF = Sylvia Grinnell Falls; SGERjuv = Sylvia Grinnell End of Runway juveniles)

	Bo2R	SGER	SGMD	SGF	Recap	SGERjuv
Bo2R	0	0.013	0.0157*	0.0166*	0.0227	0.0192
SGER		0	-0.0076	-0.0058	0.0152	0.0191
SGMD			0	0.0006	0.0157	0.0153
SGF				0	0.0204	0.0142*
Recap					0	0.0485*
SGERjuv						0

* Statistically significant values after Bonferroni correction for multiple comparisons.

Table 6. Results of the analysis of molecular variance conducted in program ARLEQUIN testing for hierarchical population structure for Arctic char captured in the Sylvia Grinnell River and Bay of Two Rivers.

Source of Variation	df	Sum of squares	% variation explained	*P* value
Among groups	1	7.502	1.95	0.248
Among populations within group	2	2.848	-0.07	0.7397
Within population	914	1501.5	98.12	< 0.0005