Migration harshness drives habitat choice and local adaptation in anadromous Arctic Char: evidence from integrating population genomics and acoustic telemetry

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Running head: Migratory ecology of Arctic Char

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Abstract

Migration is a ubiquitous life history trait with profound evolutionary and ecological consequences. Recent developments in telemetry and genomics, when combined, can bring significant insights on the migratory ecology of non-model organisms in the wild. Here, we used this integrative approach to document dispersal, gene flow and local adaptation in anadromous Arctic Char from six rivers in the Canadian Arctic. Telemetry data from 124 tracked individuals indicated asymmetric dispersal, with a large proportion (72%) of fish tagged in three rivers migrating up the shortest river in the fall. Population genomics data from 6,136 SNP markers revealed weak, albeit significant, population differentiation (F_{ST} = 0.011) and population assignments confirmed the asymmetric dispersal revealed by telemetry data. Approximate Bayesian Computation simulations suggested the presence of asymmetric gene flow but in the opposite direction than that observed from the telemetry data, suggesting that dispersal does not necessarily lead to gene flow. These observations suggested that Arctic Char home to their natal river to spawn, but may overwinter in rivers with the least harsh migratory route to minimize the costs of migration in non-breeding years. Genome scans and genetic-environment-associations identified 90 markers putatively associated with local adaptation, 23 of which were in or near a gene. Of those, at least four were involved in muscle and cardiac function, further highlighting the potential importance of migratory harshness as a selective pressure. Our study illustrates the power of integrating genomic and telemetry to study migrations in non-model organisms in logistically challenging environments such as the Arctic.

Introduction

Migrations are a common feature of the life history of many animal species (Dingle 2014). Despite their obvious evolutionary and ecological consequences, however, migrations have been a challenging topic of study. Quantifying migratory phenotypes can be difficult because they can occur over vast distances. Furthermore, while some migratory traits are amenable to laboratory studies and more classical quantitative genetic approaches (e.g. Berthold & Querner 1981; Roff & Fairbairn 1991), many migratory traits are impossible to recreate in experimental settings. Recent technological developments in telemetry techniques both on land (Kays *et al.* 2015) and underwater (Hussey *et al*. 2015), now allow observations of animal movement in the wild over broad spatial scales and with unprecedented levels of detail. Parallel to these developments is the exponential increase in the availability of genomic technologies for non-model organisms (Davey *et al.* 2011, Andrews *et al.* 2016). These new genomic tools make genome-wide assessments of genetic variation possible, thus offering novel ways to link genotypes to migratory phenotypes in the wild for non-model organisms (Liedvogel *et al.* 2011; Shafer *et al.* 2016; Franchini *et al.* 2017). The integration of telemetry and genomic datasets, therefore, provides a powerful approach to document the population level consequences of migrations and to better understand the genetic basis of migratory traits (Shafer *et al.* 2016).

One population level consequence of migration is that it can redistribute genetic diversity. Indeed, while homing to reproduction sites is commonly associated with migrations (Dingle 2014), it is rarely perfect, thus leading to dispersal and gene flow. Consequently, the precision of homing and the spatial scale over which migrations take place influence the scale over which gene flow, and thus genetic structure, can be observed (e.g., Castric & Bernatchez 2004). Furthermore, inter-individual differences in migratory behaviour are common and can influence gene flow (e.g., Turgeon *et al.* 2012; Shafer *et al*. 2012; Delmore *et al*. 2015). Therefore, the study of how migratory behaviour, dispersal, and gene flow interact to determine the genetic structure of populations and their capacity to locally adapt can greatly benefit from integrating genomic and telemetry data.

The diversity of migratory life histories in salmonids makes them excellent model systems for the study of migration ecology (Hendry *et al*. 2004; Quinn 2005). Anadromy, a trait shared by many salmonid species, refers to a migratory life cycle whereby individuals are born in freshwater, feed and grow in saltwater, and return to freshwater to reproduce. In most salmonids, return migrations to freshwater occur exclusively for the purpose of reproduction, but some species, notably in the genus *Salvelinus*, must also return to freshwater to overwinter (e.g., Johnson 1980; Moore *et al.* 2013; Bond *et al*. 2015). The Arctic Char (*Salvelinus alpinus*) is a facultative anadromous, iteroparous salmonid with a circumboreal distribution (Johnson 1980; Klemetsen *et al*. 2003; Reist *et al*. 2013). Anadromous individuals undergo annual feeding migrations to the ocean and must return to freshwater in the fall because winter conditions in the Arctic Ocean are not favorable (Johnson 1980; Klemetsen *et al*. 2003; see Jensen & Rikardsen 2012 for an exception). The summer feeding and growth period is thus limited by the ice-free period on the rivers used for migrations, which can be as short as a month at higher latitudes (Johnson 1980). This restricted feeding period limits energy gains, and results in skipped reproduction such that in most populations, spawning occurs once every two to four years (Dutil 1986). The migratory behaviour of anadromous Arctic Char is therefore best understood as three distinct migrations: (1) spring feeding migrations to the ocean; (2) fall spawning migrations to freshwater spawning sites; and (3) fall overwintering migrations to freshwater overwintering sites when the individual is not in breeding condition (Fig. 1). Given that optimal spawning habitats likely differ from optimal overwintering habitats, we can predict that individuals might select different habitats in different years depending on their maturity status. Accordingly, there is evidence that Arctic Char home to their natal sites to spawn, but often utilize non-natal overwintering sites in years when they are not in breeding condition (Johnson 1980; Gyselman 1994; Moore *et al*. 2013; Gilbert *et al*. 2016). Natal homing to reproduction sites, but use of alternative overwintering sites, may lead to temporary mixing of different stocks in freshwater, making both population-specific sampling and fisheries management challenging. The integration of telemetry and genomic datasets is therefore particularly promising for studying Arctic Char migrations.

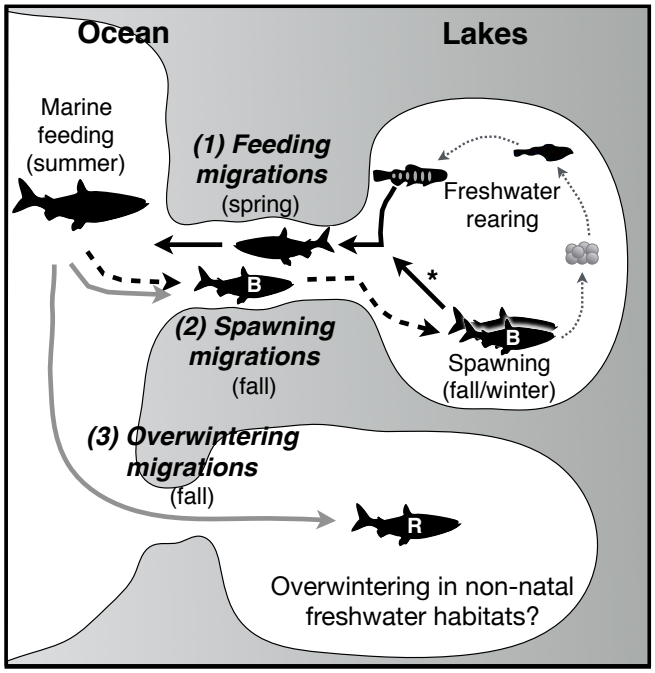


Figure 1. The anadromous Arctic Char life cycle highlighting three distinct migrations. (1) Feeding migrations (black arrows): after hatching and rearing in freshwater for several years (grey dotted arrows), individuals smoltify and begin to migrate to saltwater to feed during the summer when the rivers and ocean are free of ice. (2) Spawning migrations (dashed black arrows): individuals in breeding condition (B) return to their natal freshwater habitat to spawn. Because individuals are not in breeding condition every year, spawning migrations occur only once every 2-4 years. The arrow with the asterisk indicates that Arctic Char are iteroparous and can resume feeding migrations the following spring after spawning (3) Overwintering migrations (full grey arrows): in the years when individuals are not in breeding condition (resting (R)), they must still return to freshwater to overwinter to avoid lower temperatures and increased salinity in the marine environment. Because optimal conditions for spawning and overwintering may differ, use of non-natal overwintering sites might be more frequent than use of non-natal spawning sites. We hypothesized that individuals would favor less harsh migrations for overwintering, as indicated by the shorter river. Silhouette of adult fish from PhyloPic.org.demographic

A key variable driving the evolution of migrations and associated traits is migration distance and harshness. Indeed, migration distance greatly influences the costs of migrations, and strategies that minimize these costs will tend to be favored (Roff 2002). An example is the rapid evolution of a new migratory route in the blackcap (*Sylvia atricapilla*), a migratory warbler from continental Europe, in response to changing environmental conditions (Berthold *et al*. 1992). Prior to the 1970s, this bird migrated to Africa to overwinter, but milder conditions and the presence of bird feeders led to an increase in the frequency of individuals overwintering in Britain, thus benefiting from much shorter migrations (Berthold *et al*. 1992). Freshwater migration lengths and

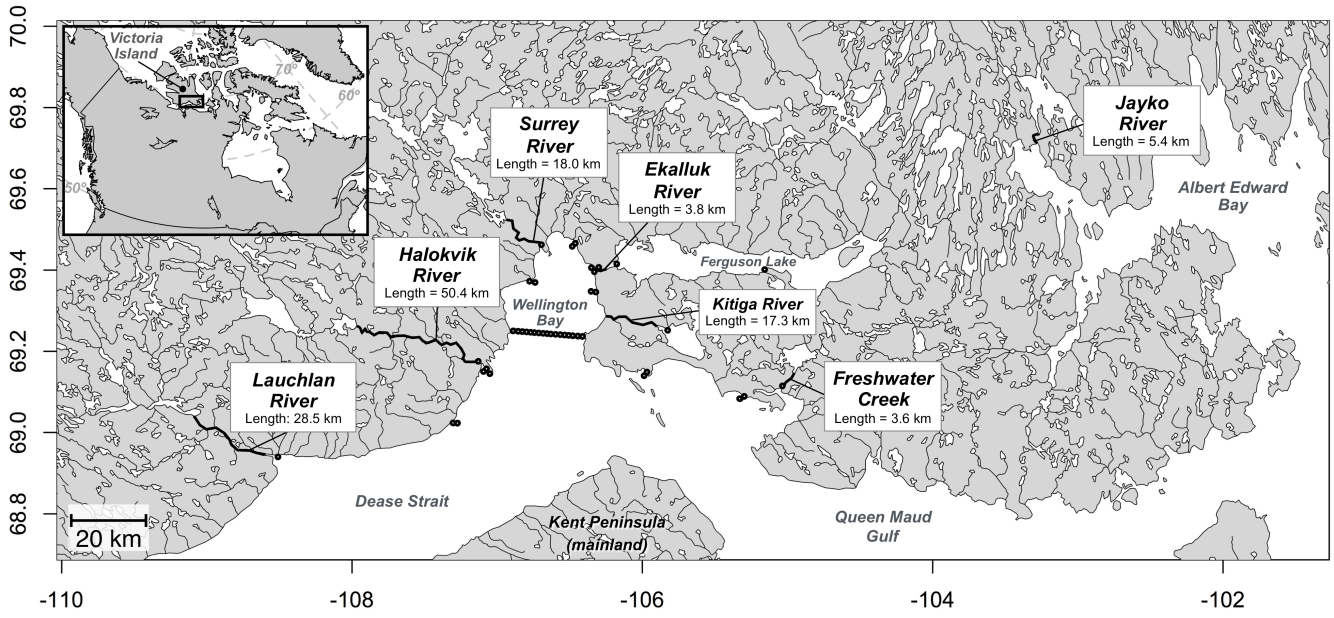
131 elevation gain (i.e. their 'harshness') are also well known drivers of the bioenergetics
132 costs of migrations in anadromous fishes, including salmonids (e.g., Bernatchez &
133 Dodson 1987), and can drive local adaptation of their life history (e.g., Schaffer & Elson
134 1975), morphological (e.g., Crossin et al. 2004), and physiological traits (e.g., Eliason *et*
135 *al*. 2011). The extent to which migration harshness drives habitat choice (particularly
136 during overwintering migrations) and local adaptation in anadromous Arctic Char,
137 however, remains largely unknown (but see Gilbert *et al*. 2016).
138 In order to investigate the possible role of migration harshness in driving habitat
139 choice and local adaptation, we combined genotyping-by-sequencing (GBS) and acoustic
140 telemetry data to study the migrations of anadromous Arctic Char from southern Victoria
141 Island in the Canadian Arctic Archipelago (Fig. 2). The largest commercial fishery for
142 Arctic Char in Canada has been operating in this region for over five decades (Day &
143 Harris 2013) and the local Inuit people rely on this resource for economic, subsistence,
144 and cultural purposes (Kristofferson & Berkes 2005). At least seven rivers in this region
145 support populations of Arctic Char, five of which are commercially fished. Previous
146 studies in the region have demonstrated that many of the stocks mix at sea (Dempson &
147 Kristofferson 1987; Moore *et al.* 2016), and that genetic differentiation estimated from
148 microsatellite markers is low (Harris *et al*. 2016). Here, we aimed to better understand
149 patterns of homing and dispersal of Arctic Char in the region. Particularly, we are
150 interested in how variation in the harshness of freshwater migrations observed among
151 rivers in the region (Fig. 2; Table 1) influences freshwater habitat choice and local
152 adaptation. We addressed four empirical objectives related to this question. First, we used
153 acoustic telemetry data to show that many individuals used different freshwater habitats
154 in different years, and fish from all tagging locations preferentially used the shortest river
155 in the Wellington Bay region (Ekalluk River). Second, we used population genomic data
156 to document population structure and infer the natal origins of migrating individuals, thus
157 confirming that dispersal to non-natal habitats occur. Third, we used an Approximate
158 Bayesian Computation (ABC) framework (Csilléry *et al.* 2010) to determine whether the
159 asymmetric dispersal towards Ekalluk River is associated with asymmetric gene flow.
160 Finally, we used genome scans to test for evidence of local adaptation among
161 populations, and we characterized the chromosomes containing these markers and the

proximity of the markers to known genes in a related salmonid genome. This analysis provided new insights on patterns and consequences of migrations in this ecologically, economically, and culturally important species.

Materials and Methods

Telemetry data and sampling

Detailed methods for the acoustic telemetry data collection can be found in Moore *et al*. (2016). In short, 42 moored Vemco VR2W passive acoustic receivers were deployed between July 13 and September 8, 2013 (Fig. 2) along the southern shore of Victoria Island, Nunavut, near the community of Cambridge Bay. Detections were continually recorded until receivers were last retrieved between July 27 and August 31 2016. There are at least seven watersheds in the region that support runs of anadromous Arctic Char, and these watersheds are drained by rivers that vary in length and elevation gain (Fig. 2 & Table 1). We placed a receiver in six of these seven rivers to detect migrating fish (the Jayko River was excluded from the telemetry study because of the large distance between this site and the others). A total of 124 Arctic Char were surgically implanted with Vemco V16 acoustic transmitters at three tagging locations (Table 1). In all cases, the fish were collected at the river mouth near the ocean, and for the Ekalluk and Surrey River, tagging occurred during the downstream migration (early July), whereas tagging at the Halokvik River targeted upstream migrants (mid/late-August). Because tagging occurred well before spawning, no information is available about the breeding status of migrating individuals. In the absence of this information, we refer to the use of non-natal freshwater systems as ‘dispersal’ throughout the manuscript, while we use the term ‘homing’ to refer to the use of natal habitat regardless of the purpose of the migration. We also refer to all migrations back to freshwater in the fall as ‘return migrations’ regardless of whether an individual dispersed or homed. A fin biopsy was taken from each tagged fish for genomic analysis. Additional baseline samples for genetic analysis were taken from sampling conducted during the upstream migrations to monitor commercial stocks or were collected from commercial fishery catches (Table 1). Samples from the Kitiga River (N69.29° W106.24°), which harbors an Arctic Char population, were also obtained, but DNA extractions for these samples failed.



193 **Figure 2.** Map of the study area highlighting the names and lengths of the rivers sampled. The black circles
194 with a white center represent the locations of the moored acoustic receivers used to track the movements of
195 Arctic Char surgically implanted with transmitters at the Ekalluk, Surrey and Halokvik rivers.

196

197 *Library preparation, sequencing, and SNP calling*

198 A salt-extraction protocol adapted from Aljanabi & Martinez (1997) was used to extract
199 genomic DNA. Sample quality and concentration were checked on 1% agarose gels and a
200 NanoDrop 2000 spectrophotometer (Thermo Scientific). Concentration of DNA was
201 normalized to 20 ng/µl (volume used = 10 µl; 200 ng total) based on PicoGreen assays
202 (Fluoroskan Ascent FL, Thermo Labsystems). Libraries were constructed and sequenced
203 on the Ion Torrent Proton platform following a protocol modified from Mascher *et al.*
204 (2013). In short, genomic DNA was digested with two restriction enzymes (*PstI* and
205 *MspI*) by incubating at 37°C for two hours followed by enzyme inactivation by
206 incubation at 65°C for 20 minutes. Sequencing adaptors and a unique individual barcode
207 were ligated to each sample using a ligation master mix including T4 ligase. The ligation
208 reaction was completed at 22°C for 2 hours followed by 65°C for 20 minutes to inactivate
209 the enzymes. Samples were pooled in multiplexes of 48 individuals, insuring that
210 individuals from each sampling location were sequenced as part of at least 6 different
211 multiplexes to avoid pool effects. One randomly chosen replicate per sampling location

was also run on a different multiplex to evaluate sequencing errors (Mastreta-Yanes *et al.* 2015). Libraries were size-selected using a BluePippin prep (Sage Science), amplified by PCR and sequenced on the Ion Torrent Proton P1v2 chip. The obtained reads were first aligned to the closely related Rainbow Trout (*Oncorhynchus mykiss*) genome (Berthelot *et al.* 2014) using GSNAP v2016-06-09 (Wu *et al.* 2016). The detailed methods for SNP identification, SNP filtering and genotyping using STACKS v.1.40 (Catchen *et al.* 2013) are presented in online supplementary materials.

Genomic data: basic statistics, population structure, and identification of putative dispersers

Observed (H_O) and expected heterozygosity (H_E), and the inbreeding coefficient (G_{IS}) were estimated using GenoDive v2.0b27 (Meirmans and Van Tienderen 2004). Effective population size (N_E) was estimated for the baseline samples using the linkage disequilibrium method in NeEstimator V2.01 (Do *et al.* 2013) with a critical value for rare alleles of 0.05. Pairwise population differentiation was quantified using F_{ST} (Weir & Cockerman 1984) and their significance values estimated with 1000 permutations also in GenoDive. A Discriminant Analysis of Principal Components (DAPC; Jombart *et al*. 2010) in the R package adegenet (Jombart 2008) was used to describe population structure. First, the 'find.clusters' function was used with the number of clusters *K* varying from 1 to 12. PCA scores of individuals were then plotted and the 'compoplot' function was used to calculate their proportion of membership to the genetic clusters identified. ADMIXTURE (Alexander *et al.* 2009) was also run varying the number of clusters *K* between 1 and 12. Putative dispersers were identified on the basis of cluster membership probabilities in DAPC and ADMIXTURE. Putative dispersers were defined as having a >50% probability of membership to a different cluster than the majority (>50%) of other sampled individuals at that location. These putative dispersers, as well as individuals that had less than 75% probability of membership to any genetic clusters (i.e. putatively admixed individuals) were removed from some analyses (when noted) to avoid biases. Note that both of these thresholds are purposefully low to ensure all putative dispersers are removed.

Population assignments

The software *gsi_sim* (Anderson *et al*. 2008) implemented in the R package *AssigneR* (Gosselin *et al*. 2016) was used to perform population assignments and test the power of the genetic baseline. We first simulated the assignment power of the baseline samples (see Table 1) and evaluated the impact of the number of markers used for assignment on power. Markers were ranked based on their F_{ST} values computed from a 'training' set of individuals comprising 50% of the total sample size per population and assignment success was computed for the remaining 'hold-out' individuals to avoid high-grading bias (Anderson 2010). The analysis was repeated for ten randomly generated training sets, and with ten randomly generated subsets of 30 individuals per population to evaluate the sensitivity of the analyses. We used the package *RandomForestSRC* (Ishwaran & Kogalur 2015) implemented in *AssigneR* to impute missing data based on allele frequencies per population using 100 random trees and 10 iterations and compared assignment success with and without imputations. We repeated these analyses on a dataset from which we removed the putative dispersers identified with the clustering analyses (see previous section for details) since their presence could bias assignment power downward. Next, we treated the acoustically tagged individuals as samples of unknown origin and assigned them back to the baseline samples using the function *assignment_mixture* in *AssigneR*. To avoid biasing the accuracy of the assignment with the missing data imputation, for each individual we only used the allele frequencies of the loci for which genotypes were present – the number of markers used for the assignments in this case therefore varied among individuals (min = 4,633; max = 5,966).

Demographic simulations

To better understand the relative contributions of recent divergence and current gene flow on observed population structure, and to verify whether the observed patterns of dispersion translated into realized gene flow, an ABC pipeline using coalescent simulations was used (Csilléry et al. 2010). Following other approaches (e.g., Illera *et al*. 2014), three demographic models were compared and demographic parameters for the best model were estimated. The analyses focused on comparing the Ekalluk and Lauchlan samples and then the Ekalluk and Halokvik samples to limit computational demands.

Furthermore, these comparisons are the most relevant to test our hypotheses as they are representative of the eastern (Ekalluk) and western (Lauchlan and Halokvik) group of populations identified as the first level of hierarchical structure (see Results). We tested three models: (1) a null hypothesis of panmixia; (2) a model of population divergence with gene flow (i.e. the isolation with migration model, IM); and (3) a model of population divergence without gene flow (strict isolation, SI). The simplest model of panmixia was controlled by a single parameter, $\theta = 4\ N_1\mu$, where N_1 is the effective size of the panmictic population and μ is the mutation rate per generation. The SI model is characterized by six demographic parameters, namely $\theta_1=4N_1\mu$, $\theta_2=4N_2\mu$, and $\theta_A=4N_A\mu$, with N_1 and N_2 the effective population size of the two daughter populations and N_A the effective size of the ancestral population respectively. All these parameters are scaled by $\theta_1=4N_{ref}\mu$ where N_{ref} is the effective size of a reference population. The two daughter populations diverged from the ancestral T_{split} generations ago with $\tau = T_{split}/4N_{ref}$. The IM model was further characterized by the migration rates $M_1 = 4Nm$ (migration into population 1 from population 2) and $M_2 = 4Nm$ (migration into population 2 from population 1) sampled independently and where Nm is the effective migration rate. Large prior distributions following those commonly applied were used and drawn from uniform distributions (e.g., Sousa & Hey 2013). Coalescent simulations ($n = 10^6$) were performed under each model using msnam (Ross-Ibarra *et al*. 2008), which is a modified version of the widely used ms simulator (Hudson 2002), under the infinite site model of DNA mutation. The pipeline of Roux *et al*. (2013) was used with modifications to test for panmixia, and priors were computed with a Python version of priorgen (Ross-Ibarra *et al*. 2008). Details of the summary statistics used, the model selection procedure, analysis of robustness, parameter estimations and posterior predictive checks can be found in the online supplementary materials. Values were not transformed into biological units since the mutation rate (μ) was unknown. Instead coalescent units were kept, and ratios of θ and M_1/M_2 are interpreted only.

Genome scans and functional annotation of outliers

Genomic studies of local adaptation using different methods to identify outlier loci often only detect partially overlapping sets of loci (Gagnaire *et al*. 2015). A common practice

to partially circumvent these problems is to combine genome scans with genetic-environment-association (GEA) methods as a way to more reliably identify the most likely targets of selection (De Villemereuil *et al.* 2014). Therefore, we identified markers putatively under selection using two genome scan methods and a GEA method. First, Bayescan v1.2 (Foll & Gaggiotti 2008) was used to detect outlier loci with elevated F_{ST} among the baseline populations (Table 1) with 5,000 iterations, and a burn-in length of 100,000. To increase our chances of finding outliers while also limiting type-two errors, we tested values of 100, 1,000 and 5,000 for the prior odds (Lotherhos & Whitlock 2014). This analysis was run on the dataset from which putative dispersers were removed to avoid biases (n = 273 after dispersers removed). Second, we used PCAdapt (Luu *et al*. 2017), which uses PCA to control for population structure without *a priori* population definition, a particularly useful feature in the current study where we expect significant admixture among populations. Because of this feature, we ran the analysis on the full dataset including the putative dispersers, which also increased the analytical power associated with increased sample size (n = 318). Simulations have also shown that PCAdapt is less prone to type-2 errors than Bayescan, and to be less affected by the presence of admixed individuals in the samples (Luu *et al*. 2017). The optimal number of principal components necessary to describe population structure was determined using the graphical method described in Luu *et al.* (2017) and varying *K* from 1 to 10. Finally, we also used latent factor mixed models (LFMM; Frichot *et al*. 2013) implemented in the R package LEA to test directly for correlations between allele frequencies at specific loci and migratory difficulty. We also varied K from 1 to 10 to identify the optimal number of latent factors required to describe population structure. Migration harshness was measured as 'work' (Crossin *et al*. 2004), that is the product of river length and altitude gain, standardized to a mean of zero and a standard deviation of one. Because the environmental data had to be assigned to a sampling location, we used the dataset with the putative dispersers removed for this analysis (n = 273). As recommended by the authors in both user manuals, for PCAdapt and LFMM, the missing data were imputed as before using package *RandomForestSRC* (100 random trees and 10 iterations). All three approaches controlled for false discovery rates (FDR) with an α of 0.05.

Given the absence of a reference genome for Arctic Char, we used the recently developed high-density linkage map for the closely related Brook Char (*Salvelinus fontinalis*; Sutherland *et al.* 2016) to determine approximate genomic positions of the markers using the MapComp method (Sutherland *et al.* 2016). In brief, MapComp aligns the flanking sequence of GBS markers of two genetic maps (here the Brook Char genetic map and the anonymous Arctic Char markers) to a reference genome of a closely related species. Here the Atlantic Salmon (*Salmo salar*) genome (Lien *et al.* 2016) was used because it has longer scaffolds than the current Rainbow Trout assembly and thus allowing more markers to be paired (see Sutherland *et al.* 2016). Then, markers from each species that are closest to each other in nucleotide position on the reference genome within a set distance are paired. The pairing was conducted in 10 iterations, removing paired anonymous Arctic Char markers each iteration and rerunning the pairing with the Brook Char genetic map to permit pairing of more than one anonymous marker with a single genetic map marker, as previously described (Narum *et al.* 2017). Here, the distance cutoff between the two markers on the reference genome was within 1 Mbp maximum distance to find approximate locations for the greatest number of markers. All significant outliers were also used in a BLAST query against the Atlantic Salmon genome, which has a full annotation available in NCBI (Lien et al. 2016; NCBI Genome ICSASG_v2 reference Annotation Release 100; GCA_000233375.4). Outlier markers that were found by more than one method, but did not BLAST unambiguously to the Atlantic Salmon genome, were also checked against the Rainbow Trout (*Oncorhynchus mykiss*) genome.

Results

Telemetry data

The receivers located at the mouth of each river allowed the inference of likely freshwater overwintering/spawning sites of 98 acoustically tagged Arctic Char for up to three consecutive years (Table S1 in online supplementary materials). The telemetry data suggested a pattern of asymmetric dispersal. Indeed, many fish from all three tagging locations were detected one or more years in the Ekalluk River (Fig. 3), the shortest and least harsh river around Wellington Bay (Fig. 1 and Table 1), and one of the shortest in

366 the entire area. First, of the 47 fish tagged in both 2013 and 2014 at the Ekalluk River, 44
367 returned to this river each year (94%). Second, 16 of the 21 fish (76%) tagged at the
368 Surrey River returned to the Ekalluk River each year they were detected, while only one
369 fish returned to the Surrey River. We therefore hypothesize that the fish tagged at the
370 Surrey River were likely Ekalluk River fish that we intercepted moving north and west,
371 an interpretation consistent with observations that many Ekalluk River fish visit the
372 Surrey River estuary shortly after their out-migrations to the ocean (Moore *et al*. 2016)
373 and also consistent with the population genomic data presented below. Of the 30 fish
374 tagged at the Halokvik River, 28 returned to this river at least once (93%), but six (20%)
375 returned to the Ekalluk River at least once. Furthermore, of the 12 fish with more than
376 one year of data that were inferred to have returned to the Halokvik River at least once,
377 seven (58%) migrated to the Ekalluk River during other years, demonstrating that an
378 individual might readily utilize different freshwater sites during its lifetime. In summary,
379 we detected a majority of fish from all three tagging locations (72%) migrating to the
380 Ekalluk River at least once.

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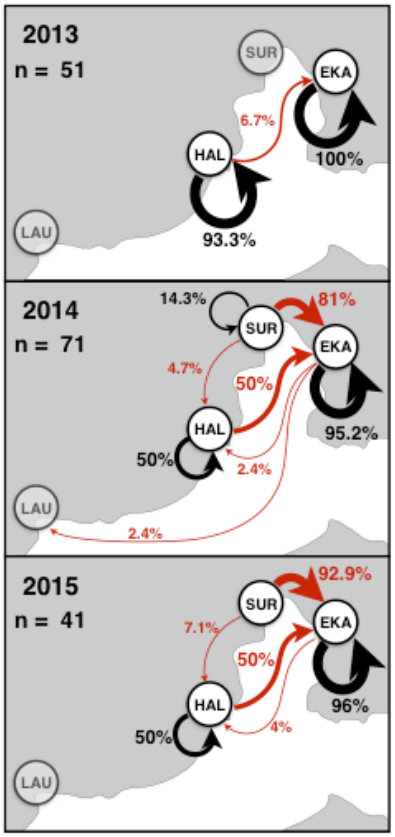


Figure 3. Choice of overwintering/spawning habitat by Arctic Char from three tagging locations inferred from acoustic telemetry data collected over three summers (2013, 2014, and 2015). The proportion of individuals returning to the same location where they were tagged (black) or dispersing to a different location from that where they were tagged (red) are indicated. The thickness of the arrows is roughly proportional to the proportion of individuals observed migrating to this location. The map indicates the approximate locations of Lauchlan (LAU), Halokvik (HAL), Surrey (SUR), and Ekalluk (EKA) rivers (shaded labels indicate no individuals were tagged there that year). The numbers of individuals that were unambiguously assigned to an overwintering location each year (n) are indicated.

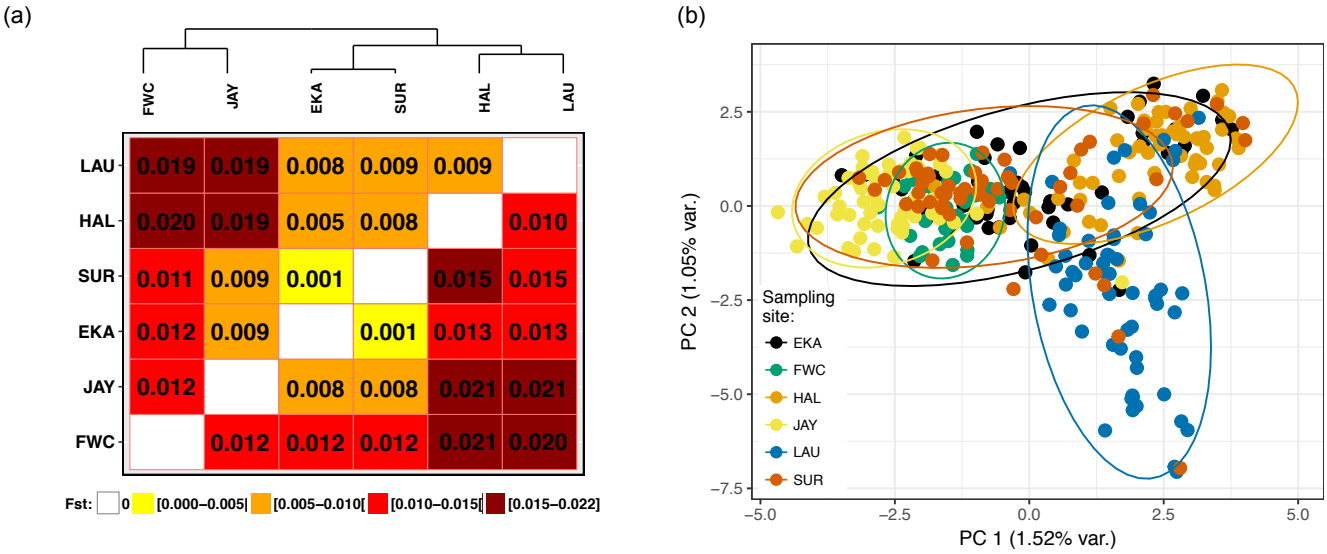
Sequencing and SNP calling

After cleaning and demultiplexing, a total of 1.9 billion reads were left with an average of 4.0 million reads per individual (coefficient of variation: 30.3%). The assembly resulted in a catalog containing 2,963,980 loci and a total of 13,568,653 SNPs (over 1,272,427 polymorphic loci) after the population module of STACKS. Individuals with more than 25% missing genotypes were removed from all analyses (Table 1), and 6,230 high quality SNPs were retained after filters. In addition, 94 putatively sex-linked markers were removed (see Fig. S4 & S5 in supplementary materials and Benestan et al. 2017 for details) leaving a total of 6,136 SNPs used in all subsequent analyses (Table 2). Replicate individuals (n = 11) sequenced twice had identical genotypes at 92.0-97.1% of the markers (mean 94.6%), which was comparable to error rates reported in Mastreta-Yanes et al. (2015).

Genomic data: basic statistics, population structure, and identification of dispersers

Levels of heterozygosity were comparable among populations (H_O: 0.099-0.105; H_E: 0.103-0.107), and although all G_{IS} values were significantly positive, indicating an excess of homozygotes, the values were all small (0.001-0.037) (Table 1). Effective population size estimates varied between 335 (Lauchlan River; 95% c.i.: 318-353) and 1081 (Ekalluk River; 95% c.i.: 935-1280). A weir enumeration study conducted in 1979-1983 found the Ekalluk River to be the most abundant population in the region, and the Lauchlan River the least abundant, but the estimates of census size were much larger than the N_E estimates reported here (183,203 for Ekalluk River and 10,850 for Lauchlan; McGowan 1990). Population differentiation among rivers was weak albeit significant, with an overall F_{ST} of 0.011 (95% c.i. 0.010-0.012; Fig 4a). The F_{ST} value between Ekalluk and Surrey rivers was substantially lower than the other values (F_{ST} = 0.001; 95% c.i. 0.0006-0.0016), which was in agreement with our telemetry observations that most fish captured at Surrey migrated to the Ekalluk River in the fall. It is thus likely that sampling at the Surrey River resulted in the interception of Ekalluk River fish migrating through the area, and those two sampling locations were combined for subsequent analyses unless otherwise noted. When the putative dispersers were removed (*see below*), the overall F_{ST} increased to 0.014 (95% c.i. 0.013-0.015; Fig. 4a). The presence of

415 population structure between most sampling locations was also supported by the PCA
416 analysis, although there was some important overlap among some sampling sites (Fig.
417 4b). The sampling locations that were most separated in the PCA (Jayko and Lauchlan
418 rivers) were also the most geographically distant sampling locations.



419 **Figure 4.** Description of population structure among the Arctic Char sampling sites: (a) heatmap
420 of pairwise F_{ST} values before (above the diagonal) and after (below the diagonal) putative
421 dispersers were removed from the samples (see text for details). All values are significantly
422 different from zero ($p < 0.05$). (b) Results of the principal components analysis showing PC scores
423 of each individual along the first two principal axes (1.52% and 1.05% of the total variance
424 explained respectively). Individuals and the 95% confidence ellipses are colour-coded by
425 sampling location.

426

427 The Bayesian Information Criterion in the DPAC analysis best supported the
428 presence of two genetic clusters (Fig. S6 online supplementary materials) differentiating
429 the two westernmost populations (Lauchlan and Halokvik) from all others (Fig. 5). Based
430 on cluster membership, several putative dispersers and admixed individuals could be
431 identified (Fig. 5). A majority of putative dispersers (38/45; 85.4%) were individuals
432 belonging to the western genetic cluster, but sampled in the Ekalluk or Surrey River. In
433 contrast, many fewer dispersers from the eastern group were identified in the western
434 sampling locations (three in Lauchlan and three in Halokvik; i.e., 13.3% of putative
435 dispersers). In other words, the DAPC analysis supported the conclusion of asymmetric
436 dispersal from the western sampling populations towards the shorter and less harsh

Ekalluk River. Unlike the DAPC analysis, the cross-validation errors in the ADMIXTURE analysis did not support the presence of two genetic groupings (K = 1 had the lowest cross-validation error). Nonetheless, at $K = 2$, the individual Q-values were consistent with the results of the DAPC, and all putative dispersers identified with the DAPC had >50% probability of membership to the alternative genetic cluster in ADMIXTURE as well. Furthermore, at K=5 the ADMIXTURE results suggested that genetic differentiation among rivers was present, albeit weak.

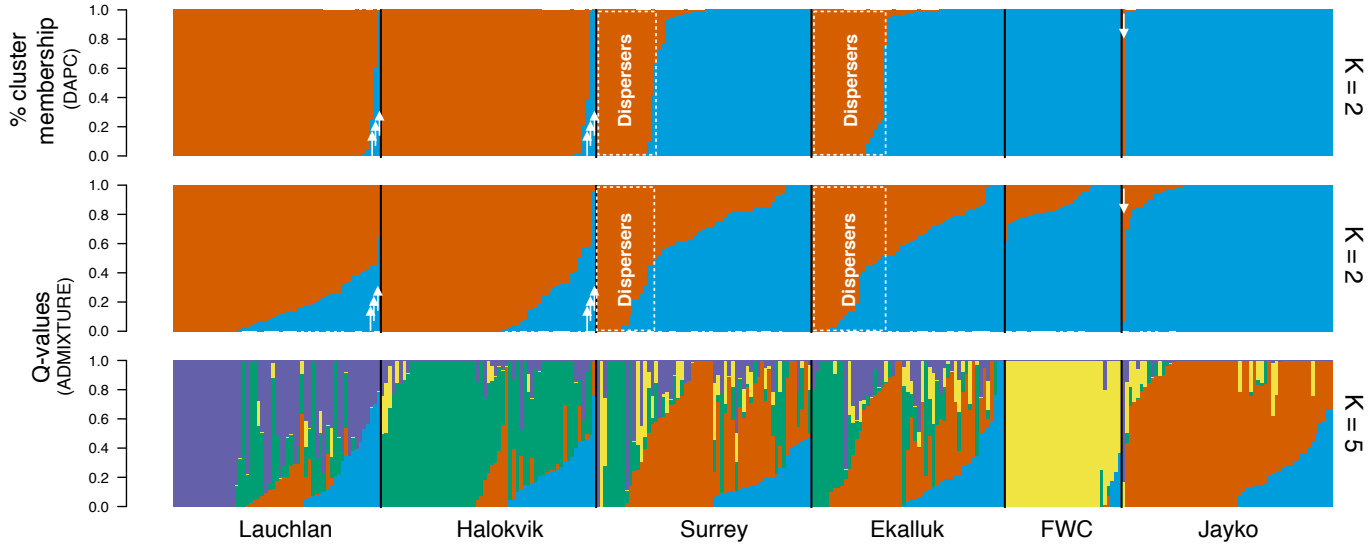


Figure 5. Results of two clustering analyses used to identify putative dispersers. The top panel is a compo-plot generated from a DAPC using two genetic clusters showing the percent cluster membership of each individual in the analysis. The bottom two panels are the individual Q-values from the ADMIXTURE analyses with two (middle) and five (bottom) clusters. The putative dispersers are individuals with Q-values or percent cluster membership greater than 0.5 but assigned to a cluster different from that of most other individuals sampled at the same sampling location and are identified with a white arrow or a white dotted rectangle. Note that the order of individuals varies among panels, but the identity of putative dispersers was the same in the two analyses.

Population assignments

Overall, the results of the assignment tests using all 6,136 markers revealed that there was sufficient power to infer the origin of individuals with high accuracy (84.3%-94.5%). There was limited variation in assignment success when using different subsets of individuals, indicating that the results are stable (*not shown*). Overall assignment

success was maximized when using 3,000 markers both when the putative dispersers were retained or removed (Fig. 6a & 6c). We therefore present pairwise assignment success per population based on 3,000 markers (Fig. 6b & 6d). As expected, when dispersers were present in the dataset, assignment success was lower overall (84.3% without imputations; 88.0% with imputations; range: 71-100%; Fig 6b), and many individuals from Halokvik and Lauchlan River were assigned to Ekalluk and Surrey River, and vice-versa. As expected, assignment success was improved when the putative dispersers were removed from the dataset (92.3% without imputations; 94.5% with imputations; range 81-100%; Fig 6d), and most of the mis-assignments involved fish caught at Lauchlan River but assigned to Halokvik River, or fish caught at Jayko River assigned to Ekalluk and Surrey River.

After confirming that there was sufficient assignment power, we used genomic data to assign tagged individuals to their most likely river of origin (Table S1 online supplementary materials). In this case, we used all the markers to simplify the treatment of missing data (*see Methods*) and because assignment success based on all markers was not substantially lower than that based on a subset of 3,000 markers (Fig. 6a & 6c). Eleven of the 57 fish (19%) tagged at the Ekalluk River in 2013 and 2014 were assigned to the Halokvik River and two to the Lauchlan River. Only two of the 11 putative Halokvik-origin fish were detected overwintering/spawning in the Halokvik River at least once, and all others were detected only in the Ekalluk system one or more years. In contrast, all but one (29/30) of the fish tagged at the Halokvik River were assigned to the Halokvik River itself. Six of those 29 individuals were detected overwintering/spawning in both Ekalluk and Halokvik River in different years. Notably, all but one fish (32/33) detected at least one year at Halokvik River were assigned to the Halokvik River. Finally, fish tagged at Surrey River were assigned to all possible rivers (except Freshwater Creek), but a majority (19/31) were assigned to the grouped Ekalluk-Surrey sample. In short, population assignments also support the prevalence of asymmetric dispersal towards the Ekalluk River.

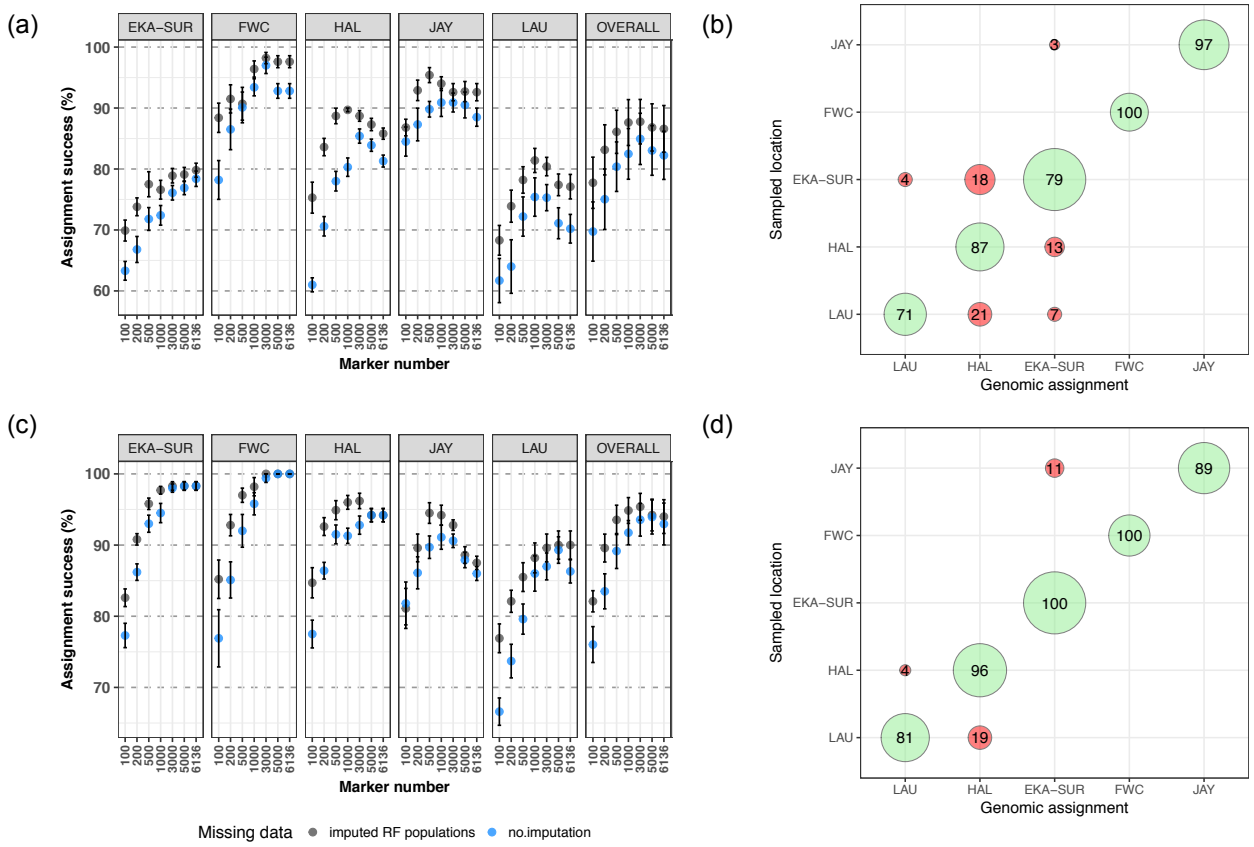


Figure 6. Results of assignment tests with all individuals (a & b) or with putative dispersers removed (c & d). The two left panels (a & c) show how assignment success per sampling location varied according to the number of markers used for the assignment, and on whether missing data were imputed using Random Forest (RF) or not. The two right panels (b & d) show results of the assignments with all markers with missing data imputed. The green circles on the diagonal display successful assignments, and the red circles display mis-assignments (i.e., the sample was assigned to a different river from that where it was sampled). Circle size is proportional to the number of individuals in each category.

Demographic simulations

The model selection procedure unambiguously indicated that the isolation with migrations (IM) model was the best with P(IM) ~ 1, while both the panmixia and the strict isolation models had posterior probabilities close to zero (Table S4 in online supplementary materials). This inference was highly significant with a robustness of 1 (Fig. S8 in online supplementary materials). Estimates of demographic parameters

produced confidence intervals of various widths, and differed most from the posterior in the Ekalluk-Halokvik comparison than in the Ekalluk-Lauchlan comparison. These estimates were mostly informative with regards to estimates of effective populations size, divergence times (except in the Ekalluk-Lauchlan comparisons), and intensity of migration rates, pointing especially to highly asymmetric gene flow (Fig. S9 & S10 in online supplementary materials). This asymmetric gene flow, however, was in the opposite direction of the asymmetric dispersal inferred from both the telemetry and the genetic data. Indeed, gene flow was higher from Ekalluk to Lauchlan, with a ratio of $M_2/M_1 = 3.08$, and from Ekalluk to Halokvik, with a ratio of $M_2/M_1 = 4.06$ (Table 3). Ratios of effective population size (θ_1/θ_A) tended to indicate an expansion of the Ekalluk population relative to the ancestral population in both comparisons (ratios of 9.68 and 3.45). Posterior distributions also indicated an expansion of the Halokvik population ($\theta_2/\theta_A = 2.58$), but the Lauchlan population size was inferred to be smaller than the ancestral population size ($\theta_2/\theta_A = 0.082$), although this should be interpreted cautiously since the estimate of the ancestral population size was not highly accurate. Posterior predictive checks in the Ekalluk-Lauchlan indicated that we were able to accurately reproduce the summary statistics. In the Ekalluk-Halokvik comparison, however, three statistics were significantly different from the observed data, namely the averaged number of shared polymorphic sites and the averaged and standard deviations of the net divergence (Da). In summary, the ABC approach provided robust estimates of demographic parameters, and suggested that the asymmetric dispersal towards Ekalluk River observed with both the telemetry and genomic data did not translate into pronounced gene flow in that direction.

Genome scans and functional annotation of outliers

The three different methods of outlier detection identified a total of 90 markers putatively under selection. The Bayescan analysis identified 30 outlier loci when the prior odds were set at 100 (Fig. S11 online supplementary materials), 10 loci with prior odds of 1000, and 5 loci with prior odds of 5000, all with an FDR of 0.05. The PCAdapt analysis was run assuming two genetic clusters after graphical evaluation of the eigenvalues according to Luu et al. (2017). Consistent with the DAPC results, the first two PC axes

explained most variation and only those were retained to account for population structure (Fig. S12 online supplementary materials). Six outliers were identified with FDR = 0.05 (Fig S13 online supplementary materials). Of those six, four were also identified by the Bayescan run with prior odds of 100 and two with the run at prior odds of 5000. For the LFMM analysis, cross-entropy was also minimal at K = 2 (Fig. S14 online supplementary materials), and two latent factors were thus retained to control for population structure. With an FDR = 0.05, 58 markers were identified as outliers by the LFMM analysis, none of which overlapped with the markers identified by Bayescan or PCAdapt.

A total of 2070 of the 6136 markers were positioned onto the Brook Char linkage map and appeared distributed throughout the genome on many chromosomes (Fig. 7). Of the 90 unique markers identified combining the three outlier detection analyses, 34 provided unambiguous BLAST results against the Atlantic Salmon genome (see Table S5 for full list of successful BLAST results). Of those, 23 were found to be in a gene, while nine were found within 100kb of a gene. Only one of the four outliers that were significant in both the PCAdapt and the Bayescan had unambiguous BLAST results, but three of them were located on the same 914Kb scaffold (*scaffold_363*) on the Rainbow Trout genome. The marker with a successful BLAST result was within the gene *FAM179B*, which codes for a protein required for the normal structure and function of primary cilia, an organelle usually associated with the reception of extracellular mechanical and chemical stimuli (Das *et al*. 2015). Several outlier-linked genes were involved in muscle or cardiac muscle function and development (*Myocyte-specific enhancer factor 2C*, *nebulette*, *glycogen phosphorylase muscle form*) or in gluconeogenesis (*fructose-1,6-bisphosphatase 1-like*). Several other outlier-associated genes have possible functions linked to axon guidance or neuronal development (*neural cell adhesion molecule 2*, *neural cadherin-like isoform X1*; *Arf-GAP with GTPase*; *ANK repeat and PH domain-containing protein 2*; *NEDD8-conjugating enzyme Ubc12*; *CD9 antigen-like isoform X2*). Finally, two outliers were found within immune-response genes (*Interferon regulatory factor 4*; *Ig heavy chain V region-like*).

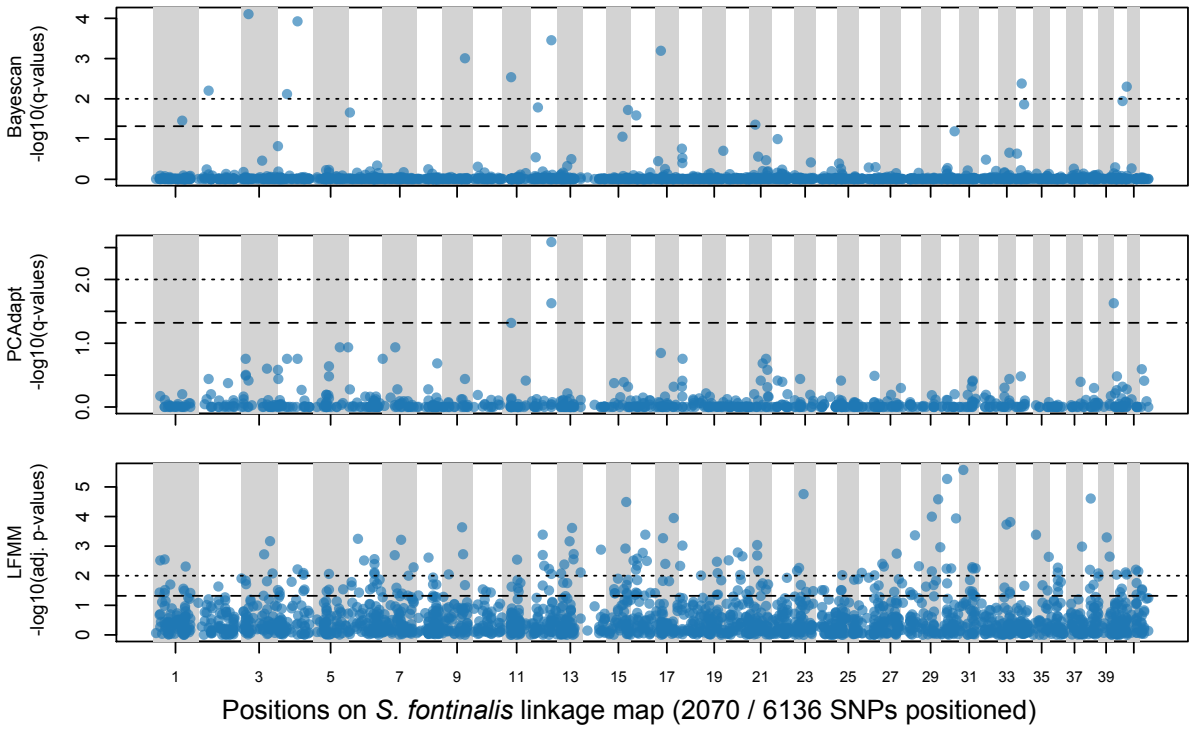


Figure 7. Manhattan plots showing the position of each marker that was successfully mapped to the closely related Brook Char genetic map (grey shading and number on the x-axis show linkage groups) and the test statistics from the two genome scan analyses (top: Bayescan and middle: PCAdapt) and the genetic environment association (bottom: LFMM). On the y-axes are the –log10 transformed values of the test statistics (q-values for Bayescan and PCAdapt and adjusted p-values for LFMM). The dashed lines represent the 0.05 significance threshold (used throughout) and the dotted line a 0.01 threshold (for reference only).

Discussion

Concurrent developments in both genomic and telemetry technologies offer new and powerful ways to study the migratory ecology of animals behaving in their natural environments (Schaffer *et al*. 2016). The present study illustrates how this integrative approach can be especially fruitful for animals displaying complex migratory behaviours and inhabiting logistically challenging regions. Here telemetry data suggested the presence of asymmetric dispersal between high Arctic populations of anadromous Arctic Char, and population genomic data confirmed the river of origin of putative dispersers. Dispersal was predominantly from western sampling locations towards the Ekalluk River, the shortest and least harsh river in the Wellington Bay area. Demographic simulations

582 using an ABC approach also revealed an asymmetry in gene flow, but in the opposite
583 direction, indicating that the observed asymmetric dispersal does not necessarily lead to
584 gene flow. Instead, the ABC simulations suggested that Ekalluk River, the most abundant
585 Arctic char population in the region (McGowan 1990), was a major source of gene flow
586 to surrounding areas. Together, our observations suggested that Arctic Char home to their
587 natal river to spawn, but may overwinter in rivers with the least harsh migratory route,
588 thus potentially minimizing the costs of migrations in non-breeding years. Another
589 finding facilitated by the integration of telemetry and genomic data was the possible
590 identification of local adaptation in migratory traits as indicated by the identification of
591 many outlier markers linked to genes demonstrated to have a role in muscle or cardiac
592 function. Related physiological traits have been demonstrated to evolve in response to
593 migration harshness in other anadromous salmonids (Eliason et al. 2011), thus suggesting
594 that this environmental factor could also drive local adaptation in Arctic Char.
595
596 *Asymmetric dispersal: overwintering habitat choice minimizes cost of migrations*
597 Unlike most other anadromous salmonids, which return to freshwater exclusively
598 to reproduce (Fleming 1998; Quinn 2005), Arctic Char and other *Salvelinus* species also
599 return yearly to freshwater to overwinter (Armstrong 1974; Johnson 1980). Given that
600 optimal habitats for reproduction and for overwintering likely differ, we might expect
601 individuals to migrate to separate locations depending on the purpose of the migration
602 (Dingle 2014). Precise homing to reproduction sites is the norm in salmonids, and it has
603 been suggested that local adaptation to nesting and rearing environments is an important
604 driver of philopatry (Hendry *et al*. 2004). Requirements to home, however, might be
605 relaxed when an individual is returning to freshwater to overwinter instead of to
606 reproduce (i.e., when there are no genetic or evolutionary consequences). Accordingly,
607 there is evidence that Arctic Char (and the closely related Dolly Varden; *Salvelinus*
608 *malma*) home to their natal habitats to spawn, but can use non-natal habitats to overwinter
609 (Armstrong 1974; Gyselman 1994; Moore *et al*. 2013; Gilbert *et al*. 2016). Here, we
610 documented the use of alternative freshwater habitats by the same individuals in
611 consecutive years using acoustic telemetry data. In addition, we used population genomic
612 data to infer the natal origins of individuals, and confirmed that the asymmetric dispersal

observed with the telemetry data was indeed due to fish of western origin (i.e., from Halokvik and Lauchlan) dispersing to the Ekalluk River. This was the case with the Ekalluk and Surrey baseline samples, which clustering analyses suggested contained a large proportion of western-origin fish. In addition, population assignment of the tagged fish showed that all but one fish detected at least once overwintering/spawning in Halokvik River were from Halokvik River, while many of the fish migrating to the Ekalluk River were from Halokvik River. The inference of asymmetric dispersal, therefore, is strengthened by the combination of independent and complementary sources of evidence from telemetry and genomic data.

The population genomic data could also be used to infer whether the observed dispersal resulted in high levels of gene flow. While dispersal was highly asymmetric towards Ekalluk River, the ABC modeling suggested that it did not necessarily translate into realized gene flow. Indeed, the best supported ABC model was the isolation with migration model with asymmetric gene flow, but the direction of the asymmetry was opposite to that observed with direct dispersal. This asymmetry in gene flow, instead, was from the most abundant population (Ekalluk) towards the least abundant populations (Halokvik and Lauchlan), as suggested by our N_E estimates (both from LDNE and ABC) and from field measures of abundance (McGowan 1990), and may thus simply reflect differences in effective gene flow. In short, while the reasons for this asymmetric gene flow remain unclear, it was clear that the observed asymmetry in dispersal does not translate into realized gene flow and suggests that dispersers from the Halokvik and Lauchlan rivers do not regularly reproduce in the Ekalluk River system.

Taken together, the observations of asymmetric dispersal with both genomic and telemetry data, but the lack of realized gene flow in the same direction, are consistent with the hypothesis that individuals in breeding condition home to spawn but may select overwintering habitats that minimize the costs of migrations in the years when they are not in breeding condition. Indeed the Ekalluk River is one of the most easily accessible rivers in the region (measured as work; Table 1). While other rivers are also easily accessible (e.g., Freshwater Creek, Jayko River), they are further away from Wellington Bay, and comparatively fewer fish from the Wellington Bay tagging locations travel there (Moore *et al*. 2016). Other features of the freshwater habitats may also explain the

observed habitat choice. For example, the Ekalluk River drains the largest lake on Victoria Island, Ferguson Lake, which could offer more abundant or better overwintering habitats, perhaps resulting in lower overwintering mortalityNonetheless, other studies of Arctic Char have also found spawning habitat accessibility to be a major constraint to migrations (Gyselman 1994; Gilbert *et al.* 2016), and the easier migrations afforded by the short Ekalluk River constitutes a very plausible explanation for the observed patterns of dispersal.

This hypothesis, however, assumes the ability of Arctic Char to assess which river provides the most readily accessible overwintering habitat. One possibility is that individuals explore a variety of freshwater habitats during their summer migrations and assess their accessibility. Observations of back and forth movement during homing in other salmonids (Quinn 2005), and the regular use of different estuaries by individuals during the summer documented by our telemetry work (Moore *et al.* 2016) suggest this is plausible. Another possibility is that the decision of where to overwinter relies in part on collective navigation (i.e., increased ability to navigate through social interactions), a possibility that has been discussed in relation to salmon homing (Berdahl *et al*. 2014). Given that the Ekalluk River has the most abundant population of Arctic Char in the region (McGowan 1990; Day & Harris 2013), it is also possible that collective navigation biases dispersal towards the river with the greater number of individuals. Continued collection of telemetry data will allow future tests of some of these hypotheses.

In summary, the integration of telemetry and genomic data allowed nuanced insights into the complex migratory behaviour of anadromous Arctic Char. Notably, it allowed circumventing logistical challenges posed by the sampling of baseline samples. In most salmonids, sampling in the freshwater is used to ensure the origin of sampled fish for baseline collections, but here the use of different freshwater habitats by adults within their lifetime precludes any certainty in inferring origins of sampled adults even if collected in freshwater. Sampling juveniles prior to their first marine migration (e.g., Moore *et al*. 2013) would also be an option, but attempts at collecting juveniles in 2010 and 2015 failed. Given the complex migratory behaviour of Arctic Char and the logistical challenges associated with sampling, the integration of telemetry and genomic data provided a powerful tool for understanding the ecology of this species.

675

676 *Evidence for local adaptation in migratory traits*

677 Anadromous salmonids display tremendous diversity in migratory traits both
678 among and within species, and their study has contributed to our understanding of
679 migration ecology (Hendry *et al*. 2004; Dingle 2014). The application of molecular tools
680 has provided important insights to the study of the genetic basis of migratory traits in
681 salmonids. For example, O'Malley & Banks (2008) used a candidate gene approach to
682 link variation in two circadian rhythm genes, *OtsClock1a* and *OtsClock1b*, to variation in
683 run timing in anadromous Chinook salmon (*O. tshawytscha*). Other studies have
684 identified SNP markers linked to migration timing in both Chinook salmon (Brieuc *et al.*
685 2015) and Steelhead Trout (*O. mykiss*; Hess *et al*. 2016). Finally, Hecht *et al*. (2015)
686 linked variation in RAD markers with 24 environmental correlates using redundancy
687 analysis in Chinook salmon, finding that freshwater migration distance was the
688 environmental variable explaining most of the genomic variation. This last result parallels
689 our own conclusions regarding the importance of migratory harshness, and is also
690 corroborated by many classic studies demonstrating the importance of migratory
691 difficulty as a selective factor driving adaptation in morphological, physiological, and
692 life-history traits (Shaffer & Elson 1975; Bernatchez & Dodson 1987; Crossin *et al*.
693 2004; Eliason *et al*. 2011).

694 In the present study, we used genome scans and genetic-environment correlations
695 with migratory difficulty to identify markers putatively under divergent selection. These
696 approaches have been used extensively to identify putative targets of selection, but also
697 have several limitations, which have been amply discussed in the literature (Lotterhos &
698 Whitlock 2014; Haasl & Payseur 2015; Bernatchez 2016; Hoban *et al*. 2016). For
699 example, recent demographic history and patterns of isolation by distance have been
700 shown to lead to high levels of false positives (Lotterhos & Whitlock 2014; Hoban *et al*.
701 2016). The geographical proximity of the populations under study, however, precludes
702 important differences in terms of demographic history since the re-colonization of the
703 area from a single source population in the last 6,500 years (Moore *et al*. 2015). In
704 addition, Lotterhos & Whitlock (2015) concluded that maximum power in genome scan
705 studies could be achieved by examining geographically proximate and genetically similar

706 populations that experience contrasting environments, criteria that our system fulfilled.
707 Nevertheless, few of the outlier markers identified by the three methods overlapped, a
708 common finding (de Villemereuil *et al*. 2014; Gagnaire *et al*. 2015). Interestingly, three
709 of the four markers that overlapped between the PCAdapt and the Bayescan approaches
710 were co-located on the same 914 Kb scaffold (*scaffold_363*) on the Rainbow Trout
711 genome (two of the markers failed to provide unambiguous BLAST results on the better
712 annotated but more distantly related Atlantic Salmon genome). The co-localization of
713 three outliers with evidence from two separate methods therefore suggests the importance
714 of this genomic region, and provides some confidence in the success of the method.
715 In accordance with our *a priori* expectation that migration harshness is a major
716 selective factor differing among rivers, several of the outlier SNPs were linked to muscle
717 and cardiac functions and development. One of the outlier SNPs was located within 75Kb
718 (5' side) of *myocyte enhancer factor 2* (MEF2), a transcription factor acting as an
719 important regulator of vertebrate skeletal muscle differentiation and heart development
720 (Potthof & Olson 2007). Supporting the relevance of this protein for migrating salmonids,
721 cardiac mRNA levels of MEF2 were elevated in Atlantic salmon following 10 weeks of
722 experimental exercise training (Castro *et al*. 2013). An outlier was also located within the
723 gene coding for *nebulette*, a cardiac-specific actin-binding protein essential in the
724 structure of the sarcomere Z-disc (Bonzo *et al*. 2008). Another marker was within the
725 gene coding for *muscle glycogen phosphorylase* (PYGM), the enzyme responsible for
726 breaking up glycogen into glucose subunits to power muscle cells (Kitaoka 2014).
727 Mutations in this gene in humans are linked to McArdle disease, the major symptom of
728 which is exercise intolerance in the form of premature fatigue, myalgia and muscle
729 cramps (Kitaoka 2014). Finally, another marker was within the gene coding for *fructose-*
730 *1,6-biphosphatase*, which is involved in the conversion of glycerol into glucose (i.e.,
731 gluconeogenesis; Lamont *et al*. 2006). These results are consistent with the hypothesis
732 that migration harshness is a major selective force in anadromous Arctic Char, and
733 provide a basis to formulate hypotheses regarding the functional traits underlying long-
734 distance migrations.
735 The annotations of several other genes are also noteworthy in the context of
736 homing, which in salmonids involves memory, learning, and chemosensing (Quinn

2005). First, the protein coded by the previously mentioned MEF2 gene is also enriched in neurons, in the cerebellum, the cerebral cortex and the hippocampus, and its involvement in synaptic plasticity suggests a role in learning and memory (Potthof & Olson 2007). Second, the only marker from Rainbow Trout *scaffold_363* that could be localized is within the gene coding for *FAM179B*, a protein required for the structure and function of the primary cilia, an organelle usually associated with the reception of extracellular mechanical and chemical stimuli, and which has been linked to chemotaxis in *C. elegans* (Das et al. 2015). Note, however, that this scaffold contains 36 predicted ORFs, and other linked genes could easily be the targets of selection instead of *FAM179B*. Finally, several other markers were localized in or near genes coding for proteins that have functions in the nervous system, particularly axon guidance (*neural cell adhesion molecule 2*, *neural cadherin-like isoform X1*; *Arf-GAP with GTPase*; *ANK repeat and PH domain-containing protein 2*; *NEDD8-conjugating enzyme Ubc12*; *CD9 antigen-like isoform X2*; see table S4 for details). There is some evidence from Pacific salmon that homing has both a learned and an innate, genetically determined component (McIsaac & Quinn 1988). The abundance of putative targets of selection identified here linked to the development and function of the nervous system is therefore interesting in that regard, but much additional work would obviously be required to establish causal links (Barrett & Hoekstra 2011). Further, the low density of markers used in the present study did not allow an exhaustive assessment of the targets of natural selection throughout the Arctic Char genome. Nonetheless, the evidence that local adaptation in a variety of biological functions evolved despite low genetic differentiation and in the short time since deglaciation appears fairly robust.

Conclusions

Migration harshness has long been identified as a major driver of adaptation in anadromous salmonids (Shaffer & Elson 1975; Bernatchez & Dodson 1987; Eliason *et al*. 2011; Hecht *et al*. 2015). We here used an integrative approach, linking genome-wide data with telemetry, to provide evidence that migration harshness drives both overwintering habitat choice and local adaptation in anadromous Arctic Char. The genomic data suggested the importance of migratory harshness in driving local adaptation

768 through the identification of several putative targets of selection linked to muscle and
769 cardiac functions. Future work in this system will build on these results to describe
770 physiological differences among populations in relations to migration harshness. These
771 will be particularly relevant in the context of adaptation to a changing Arctic, where
772 migratory environments could exert an important selective pressure on populations (e.g.,
773 Eliason *et al*. 2011). Many of our conclusions would have been difficult, if not impossible
774 to reach with genomic or telemetry data alone, and our study therefore illustrated the
775 synergies made possible by combining the two types of data (Shafer *et al*. 2016). Such
776 integrative approaches will continue to increase our understanding of how migratory
777 behaviour interacts with gene flow to influence the spatial scale at which local adaptation
778 can evolve, and will advance the study of the genetic basis of migratory traits of species
779 behaving in their wild habitats.
780
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Table 1. Sample information, river characteristics, and basic genetic diversity estimates for the six sampling locations used in the present study. Work is the product of river length and elevation gain, standardized to a mean of zero and a standard deviation of one. Sample type refers to whether the individuals were collected specifically for DNA analysis ('Baseline') or were individuals that were equipped with an acoustic tag as part of the telemetry study ('Tagged'). N_{tot} is the total number of individuals sampled, $N_{<0.25\ NAs}$ the number of individuals with less than 25% missing data retained for all analyses, H_O the observed heterozygosity, H_S the expected heterozygosity, and G_{IS} is the inbreeding coefficient. All G_{IS} values are significant (<0.001).

Sampling location	Coordinates	River length (km)	Elevation gain (m)	Work (std.)	Sampling year	Sample type	N_{tot} / $N_{<0.25\ NAs}$	H_O	H_S	G_{IS}	N_e (95% CI)
Lauchlan River	N68.94° W108.52°	28.5	64	0.175	2012	Baseline	57/57	0.102	0.105	0.03	335 (318 – 353)
Halokvik River	N69.16° W107.09°	50.4	118	1.948	2013	Baseline	60/59	0.099	0.103	0.037	823 (736 - 934)
					2013	Tagged	34/30				-
Surrey River	N69.45° W106.68°	18	35	-0.338	2013	Baseline	60/59	0.102	0.105	0.023	892 (798 – 1009)
					2014	Tagged	31/31				-
Ekalluk River	N69.41° W106.31°	3.8	15	-0.584	2013	Baseline	60/53	0.104	0.106	0.017	1081 (935 – 1280)
					2013	Tagged	30/26				-
					2014	Tagged	31/31				-
Freshwater Creek	N69.12° W105.00°	3.6	7	-0.598	1992	Baseline	32/32	0.105	0.105	0.001	389 (351 – 436)
Jayko River	N69.72° W103.28°	5.4	3	-0.602	2014	Baseline	60/58	0.104	0.107	0.022	368 (351 – 387)

Table 2. Summary of the filter steps and number of SNPs or genotypes removed at each step. Allelic imbalance corresponds to the ratio of the number of reads for the major and minor allele in heterozygous genotypes. Note that each filter was run independently and that only SNPs/genotypes that passed all filters were retained, therefore the total number of SNPs/genotypes filtered out does not equal the sum of the number of SNPs filtered out at each step.

Filter [value]	**Number of SNPs/genotypes filtered out**
Depth of coverage [≤ 10000]	3 SNPs removed
Depth of coverage [≥ 5]	6,930,852 genotypes removed
Maximum allelic imbalance [≤ 4]	5,021 genotypes removed
Genotype likelihood [≥ 6]	5,937,959 genotypes removed
Proportion of individuals with a genotype for the SNP [≥ 70%]	153,788 SNPs removed
MAF global [≥0.01]	236,678 SNPs removed
MAF sampling site [≥0.05]	249,951 SNPs removed
Heterozygosity [≤0.6]	3,558 SNPs removed
Minimum Fis [≥ -0.4]	4,237 SNPs removed
Maximum Fis [≤ 0.4]	31,294 SNPs removed
Number of SNPs per locus [≤ 10]	57,694 SNPs removed
Summary	
Total number of SNPs/loci in unfiltered catalogue	286,584 SNPs/81,919 loci
Total number of SNPs filtered out	276,875 SNPs (96.6%)
Total number of SNPs retained	9,709 SNPs
Only one SNP per locus (1st) retained	6,230 SNPs
Sex-linked SNPs removed	6,136 SNPs

Table 3. Parameter estimates for the isolation with migration (IM) model which was determined to have the highest posterior probability among the three models tested (P(IM)≈ 1) for two independent runs comparing Ekalluk and Halovik rivers (EKA-HAL) and Ekalluk and Lauchlan rivers (EKA-LAU). The parameters are θ_A/θ_{ref} for the ancestral population; θ_1/θ_{ref} and θ_2/θ_{ref} for the two daughter populations (θ_1 = Ekalluk in both cases and θ_2 = Halokvik and Lauchlan depending on the analysis), with $\theta_{ref}= 4N_{ref}\mu$ where N_{ref} is the effective size of a reference population and μ is the mutation rate per generation, M_1 is the migration rate into population 1 (Ekalluk) from population 2 (Halokvik and Lanchlan) and vice-versa for M_2, and $\tau = T_{split}/4N_{ref}$ where T_{split} is the time (in generations) since population 1 and 2 diverged from the ancestral population.

		EKA-HAL	EKA-LAU
Parameter	**Prior**	**Median [95% Credible Intervals]**	**Median [95% Credible Intervals]**
θ_A/θ_{ref}	Uniform[0-20]	1.21 [0.20 – 2.38]	5.75 [1.40 – 12.83]
θ_1/θ_{ref}	Uniform[0-20]	11.71 [7.77 – 18.05]	19.86 [19.65 – 19.96]
θ_2/θ_{ref}	Uniform[0-20]	3.10 [1.91 – 6.05]	0.47 [0.18 – 2.75]
M_1	Uniform[0-40]	7.91 [3.93 – 16.28]	12.31 [6.18 -28.39]
M_2	Uniform[0-40]	32.15 [28.15 – 36.32]	37.59 [32.03 – 39.82]
τ	Uniform[0-30]	3.80 [2.30 – 5.30]	11.24 [5.96 – 25.65]