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# Genetic stock identification and mixed-stock fishery analysis of Arctic Char (Salvelinus alpinus) in Darnley Bay, Northwest Territories

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#### **Foreword**

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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#### **ABSTRACT**

Genetic mixed-stock fishery analysis (MSFA) is applied to resolve the proportional contribution of populations to a fishery where a mixture of populations is known to be harvested. Here, we examine the genetic composition of 882 Arctic Char (*Salvelinus alpinus*) collected from two important coastal subsistence fisheries (mouths of Hornaday River and Lasard Creek) in eastern Darnley Bay, Northwest Territories. Specifically, we assayed genetic variation at 18 microsatellite loci to determine whether:

- 1) stock components (baseline) can be genetically identified;
- 2) population structure is temporally stable;
- 3) stock components are always separated and, if not, where they mixed; and
- 4) the proportional stock contributions to areas identified as mixed-stock fisheries can be estimated.

The results indicated population subdivision was low (Global FST;  $\theta$  = 0.010). Estimates of genetic structure ( $\theta$ ) revealed few significant differences between coastal harvesting locations with little interannual variation (with the notable exception of char collected from Lasard Creek in 2010). According to the realistic fishery simulations using empirical baseline sizes, between the years 2009 and 2012 the Hornaday River contributed 84.4%, 80.9%, 92.7%, and 88.8%, respectively, to the Hornaday River fishery, and between the years 2010 and 2012 it contributed 64.3%, 89.9%, and 81.5%, respectively, to the Lasard Creek fishery. Furthermore, these simulations indicated that both harvest locations were dominated by the Hornaday River stock (88.9% and 87.7%, respectively and pooled across years). The co-management partners for Arctic Char in Darnley Bay can utilize the results from the genetic MSFA to build upon previously established management plans.

# Analyse d'identification génétique des stocks et de la pêche de stocks mélangés d'omble chevalier (*Salvelinus alpinus*) dans la baie Darnley, dans les Territoires du Nord-Ouest

#### RÉSUMÉ

Les auteurs appliquent l'analyse génétique de stocks mélangés (AGSM) dans le but de régler la question de la contribution proportionnelle des populations à une pêche dans le cadre de laquelle un mélange de populations est capturé. Dans le cadre de la présente étude, les auteurs examinent la composition génétique des 882 ombles chevaliers (*Salvelinus alpinus*) prélevés lors de deux importantes pêches de subsistance côtières (embouchures de rivière Hornaday et ruisseau Lasard) dans l'est de la baie Darnley, dans les Territoires du Nord-Ouest. Plus précisément, ils ont testé les variations génétiques de 18 loci microsatellitaires afin de déterminer si :

- 1) les composantes des stocks (niveau de référence) peuvent être identifiées génétiquement;
- 2) la structure des populations est stable sur le plan temporaire;
- 3) les composantes des stocks sont toujours séparées et, si non, où elles se sont mélangées;
- 4) les contributions proportionnelles des stocks aux zones désignées comme étant des pêches de stocks mélangés peuvent être estimées.

Les résultats ont indiqué que la subdivision de la population était faible (FST global;  $\theta$  = 0,010). Les estimations de la structure génétique ( $\theta$ ) ont révélé peu de différences importantes entre les sites de pêche côtière où il y a peu de variation interannuelle (à l'exception notable de la pêche de l'omble chevalier dans le ruisseau Lasard en 2010). Selon les simulations réalistes de la pêche effectuées à l'aide de tailles de référence empiriques, entre 2009 et 2012, la rivière Hornaday a fourni 84,4 %, 80,9 %, 92,7 % et 88,8 %, respectivement, de la pêche dans la rivière Hornaday; entre 2010 et 2012, elle a fourni 64,3 %, 89,9 %, et 81,5 %, respectivement, à la pêche du ruisseau Lasard. En outre, ces simulations ont indiqué que les deux lieux de pêche étaient dominés par le stock de la rivière Hornaday (88,9 % et 87,7 % respectivement et en combinaison pour toutes les années). Les partenaires de cogestion de l'omble chevalier de la baie Darnley peuvent s'appuyer sur les résultats de l'AGSM pour développer les plans de gestion établis précédemment.

#### INTRODUCTION

#### **ANADROMY AND MIXED-STOCK FISHERIES**

Anadromy is a life history tactic whereby fishes undergo migration from fresh water to marine environments for the purpose of feeding then eventually undergo a return migration to freshwater for subsequent spawning and/or over-wintering (McDowall 1997; Hendry et al. 2004; Quinn 2005). The life-history tactic of anadromy presents unique challenges to fisheries managers concerned with the harvest of potentially mixed populations of species while feeding at sea or during downstream and upstream migrations. Mixed-stock fisheries occur when a composite of populations (i.e., several distinct populations) are harvested at discrete locations. It is often not known which specific populations are being harvested and to what extent. This can severely complicate fisheries management and conservation strategies aimed at conserving biodiversity and ensuring long-term sustainability (Utter and Ryman, 1993). Anadromous fishes, particularly salmonids, have received considerable attention in mixed-stock fisheries research due to their propensity to migrate and variability in seasonal movements (e.g., Atlantic Salmon, Salmo salar, Galvin et al. 1995, Kolionen 1995, Kolionen and McKinnell 1996; Broad Whitefish, Coregonus nasus; Harris and Taylor, 2010; Pacific salmon: Coho, Oncorhynchus kisutch; Millar 1987, Miller et al. 1996; chum, O. keta; Fournier et al. 1984, Beacham et al. 1985; Sockeye, O. nerka: Grant et al. 1980, Wood et al. 1987; and Chinook, O. tsawytscha: Smouse et al. 1990, Waples and Teel 1990, Beacham et al. 1996).

The level of genetic heterogeneity within a fishery is especially pertinent to population demography and management where respective populations of origin differ in biological characteristics and/or abundance (Bekkevold et al. 2011) and can be assessed by a mixed-stock fishery analysis (MSFA). For example, genetic MSFA comprises a number of statistical methods developed to assign individual specimens to their population of origin and to calculate the probability of a particular genotype arising in any potential source population (reference or baseline population) (reviewed in Utter and Ryman 1993). Genetic stock identification (GSI) uses maximum likelihood or Bayesian techniques to estimate stock contributions of a fishery and is advantageous when there is uncertainty in individual assignment (reviewed by Manel et al. 2005). The potential for the application of MSFA to most marine species has been regarded as limited (Utter and Ryman 1993, but see Ruzzante et al. 2000 for an example) because the level of population substructuring is typically low relative to other species (Ward et al. 1994). However, the advent of highly polymorphic microsatellite DNA loci used for MSFA has enabled more fine-scale resolution of genetic population structure (e.g., Beacham et al. 2012; Ruzzante et al. 2000).

In the Canadian Arctic, there are many documented mixed-stock fisheries among anadromous fishes (e.g., Krueger et al. 1999; Harris and Taylor 2010), yet efforts to resolve the proportional contributions of discrete populations to these fisheries are relatively rare (but see Harris and Taylor 2010). The lack of detailed information on which populations are being harvested, and to what extent, has important management implications for most species given their commercial, subsistence and recreational importance (Roux et al. 2011a). Thus, information pertaining to mixed-stock fisheries in arctic environments will be important for guiding future management and conservation efforts.

#### **ARCTIC CHAR**

The Arctic Char, Salvelinus alpinus (Linnaeus), has a coastal distribution across the Holarctic, and is highly sought after for subsistence food among northern residents. These fish represent the northern-most freshwater species in the world and are commonly found in the absence of

other fish species (Johnson 1980). Arctic Char are often anadromous, however solely lacustrine populations exist. Spawning occurs in autumn over gravel or rocky shoals in lakes or calm pools in rivers. Young anadromous char spend between three to nine years in freshwater before undergoing smoltification, a process consisting of physiological, morphological, and behavioural changes in preparation for marine adaptation. The downstream migration of smolts and adults occurs at ice break-up in spring or early-summer. Feeding occurs throughout the summer for periods of 30 to 100 days, before migrating back to freshwater to spawn and/or overwinter (Johnson 1980; Dempson and Kristofferson 1987).

Coastal and riverine fisheries of anadromous char typically occur during these migrations as either the char move into the sea to feed in the spring or return to freshwater in the fall to overwinter. Stocks of Arctic Char will not only mix together during the summer while feeding in the marine environment, but could also mix when returning to freshwater habitat as a result of dispersal or straying. Although there is ample evidence to support site fidelity of Arctic Char to their natal streams (Johnson 1980; Hendry et al. 2004), there is evidence that suggests rates of dispersal and straying may be relatively high (Dempson and Kristofferson 1987; Gyselman 1994; Moore et al. 2013). Fidelity is an imperative assumption to fisheries management in the Canadian Arctic that has traditionally considered char within each river as a discrete stock (Kristofferson et al. 1984). Accordingly, quotas for fish harvest are typically assigned on a riverby-river basis (Roux et al. 2011b). Anadromous salmonids, like char, utilize a series of behavioural and physiological attributes that allow them to home to their natal freshwater habitats (Hendry et al. 2004; Quinn 2005). This homing strategy results in low amounts of gene flow among proximate populations and should lead to high levels of genetic differentiation and local adaptation (Taylor 1991; Fraser et al. 2011). Despite the homing abilities of salmonids, some individuals stray and spawn elsewhere (Quinn 1993; Moore et al. 2013). Evolutionarily, this is critical to ensure the persistence of the species through the colonization of new habitats (Milner and Bailey 1989), the avoidance of unfavourable local conditions (Leider 1989; Hendry et al. 2004) and for avoiding inbreeding and competition among kin (Hendry et al. 2004).

In the Canadian Arctic, tagging studies suggest various degrees of site fidelity and that the phenomenon of straying is relatively high in Arctic Char populations compared to other salmonids (Gyselman 1994). Furthermore, recent genetic data also confirm the potentially high rates of straying in comparison to other salmonids (Moore et al. 2013). In such instances, riverspecific harvest quotas have limited biological relevance without some indication of the level of dispersal of fish between river systems.

#### PAULATUK AND DARNLEY BAY

Paulatuk is a small community in Northwest Territories that borders Darnley Bay, a southerly arm of the Amundsen Gulf. Many of the residents participate in subsistence fishing of Arctic Char during their seasonal migrations within the two major and adjoining river systems flowing into the east side of Darnley Bay: Hornaday and Brock rivers. Adult fish are harvested using gill nets set in the marine waters in proximity to the mouths of these rivers. These fish are predominantly caught from early/mid-July to late-August prior to upstream migrations. These coastal fishing sites present an opportunity for researchers to study the potential mixed-stock fishery among Arctic Char in this region.

#### Study Area

The headwaters of the Hornaday River are located within the western Kitikmeot Region of Nunavut. The more than 300 km of meandering river travels westerly along the southern border of the Melville hills before entering Northwest Territories, and transitioning North-westerly entering Darnley Bay approximately 14 km from the community of Paulatuk. Both anadromous

and nonanadromous (land-locked, resident) char are suggested to occur in the Hornaday River system (MacDonell 1996, 1997; Reist et al. 1997). The anadromous form occurs as far upstream as La Roncière Falls (approximately 45 km from the mouth of the river) which present a barrier to further upstream migration. The headwater lake of the Brock River, Brock Lake, is located in the Melville Hills region of Tuktut Nogait National Park, NT. The length of the Brock River from the main outlet of Brock Lake to its confluence in Darnley Bay is approximately 100 km. Brock Lake is known to contain unexploited populations of nonanadromous and anadromous Arctic Char that have been captured as far as the mouth of the Hornaday River (Roux et al. 2011b).

The present day fishery is exclusively a local subsistence fishery, and has been monitored by the community since 1990. Paulatuk, in collaboration with project stakeholders, has implemented their Charr Management Plan 1998-2002 (PHTC 1999) to conserve its local char stocks and ensure their long-term wellbeing. Coastal char fisheries in Darnley Bay and the associated harvest monitoring program have mainly been carried out in the estuary of the Hornaday River to capture char during their annual upstream migration to overwintering and/or spawning areas in the upper reaches of this river. In recent years however, the dominant fishing locations of local residents has been shifting from sites at the mouth of the Hornaday River to sites closer to the Brock River estuary in the Lasard Creek area. To date, nothing is known about the contributions to harvest or mixing of populations from these two river systems in Darnley Bay coastal fisheries.

#### GENETIC POPULATION STRUCTURE AND MIXED-STOCK ANALYSIS

The shift in fishing locations may have implications for how the coastal harvest monitoring program is conducted depending on whether or not char from the Hornaday and Brock river systems are genetically distinct and on the genetic composition of the harvested component of the stocks in the two estuaries. If char from the two systems are genetically distinct and the mixed-stock composition differs between fishing areas, the monitoring program may need to be adjusted accordingly.

The resolution of population genetic structure can be important for the delineation of genetically distinct management units, and for informing the relative contributions of these unit stocks to potential coastal mixed-stock fisheries. Microsatellite loci have been used effectively to test genetic structure in Arctic Char (e.g., Bernatchez et al. 1998; Englbrecht et al. 2002; Moore et al. 2013, Harris et al. 2013), and for mixed-stock analysis in other fish species (Beacham et al. 2012; Ruzzante et al. 2000).

#### **OBJECTIVES OF THE PRESENT STUDY**

Fisheries and Oceans (DFO), Paulatuk Hunters and Trappers Committee, and Paulatuk Charr Working Group had requested science advice on the current stock status and sustainable harvest level of Arctic Char from the Hornaday River, and information on the contribution of putative stocks to the harvests at important fishing locations during the summer as it relates to the current coastal harvest monitoring programs. The following objectives are addressed within the context of this study:

- 1. Examine the genetic structure of Arctic Char from the Brock and Hornaday rivers using polymorphic microsatellite loci to determine if these systems are genetically distinct.
- 2. Determine the short-term (3–4 years) stability of the structure as well as the relative contributions of char from the Hornaday and Brock river systems to annual harvests in the estuary fishing areas within Darnley Bay.

The results of this work will provide an important basis for future decisions by the Paulatuk Charr Working Group regarding the management of the fishery and the approach to future monitoring of the fishery.

#### **MATERIALS AND METHODS**

#### SAMPLE COLLECTION AND MOLECULAR METHODS

To conduct the genetic MSFA fin clips were collected from char;

- 1) inhabiting the Brock and Hornaday rivers to use as a baseline (sample of origin) to evaluate whether genetic differences exist between rivers (i.e., determine if they are separate stocks), and
- 2) harvested at the mouths of Hornaday and Lasard Creek presumably in a mixed-stock fishery between 2010 and 2012 (Table 1; Figure 1).

The baseline sample from the Brock River were immature (<300 mm) char presumed to have not undergone anadromous migrations. These char were captured by electrofishing in two locations in the river in August 2010 (n = 70). Attempts were made to electrofish for juveniles in the Hornaday River in several locations (including the Coalmine area and other tributaries such as George and Rummy creeks) in August 2010, however no juveniles were captured. Because of a lack of juveniles, a sample of anadromous adults that were not current-year spawners (resting) were taken from the winter fishery at the Coalmine area in November 2011 (n = 80) and used as the baseline for the Hornaday River with the assumption that these char were natal to this system.

Fin clips of char taken from both coastal fishing locations during the summer were preserved in 95% ethanol prior to DNA extraction using Qiagen DNeasy tissue extraction kits (Qiagen Inc., Valencia, CA) following manufacturer protocols.

Individual Arctic Char genotypes were obtained at 18 microsatellite loci combined in four multiplex reactions. Details of the primers and PCR reactions used for each of the four multiplexes follow Moore et al (2013). For each locus, the forward primer was labeled with a fluorescent dye, and the reverse primer was PIG-tailed to reduce stutter and facilitate genotyping (Brownstein et al. 1996). Each polymerase chain reaction (PCR) was performed in a 10 µL volume with 1 µL of genomic DNA. The PCR cycles were as follows: an initial denaturation step of 5 minutes at 95°C, 35 cycles of denaturation (30 seconds at 94°C), annealing (30 seconds at 55°C) and extension (45 seconds at 72°C), and a final extension cycle of 30 minutes at 72°C. Amplified microsatellite fragments were analyzed using an automated sequencer (ABI 3130 Genetic Analyzer; Applied Biosystems, Foster City, CA) with the LIZ 600 size standard. All genotypes were scored using GeneMapper (ver. 4.0, Applied Biosystems) software and then manually inspected to ensure accuracy.

#### GENETIC ANALYSES AND ESTIMATION OF STOCK COMPOSITION

For each sampling location and year, MICRO-CHECKER (ver. 2.2.0.3; van Oosterhout et al. 2004) was used to test for genotyping errors in the form of null alleles, large allele dropout and allele scoring errors. Basic descriptive statistics of microsatellite variation, including number of alleles ( $N_A$ ), expected heterozygosity ( $H_E$ , Nei's unbiased gene diversity), and observed heterozygosity ( $H_O$ ) were calculated using Microsatellite Toolkit (Park 2001).

Tests for deviations from Hardy–Weinberg equilibrium (HWE) of observed genotypes were performed using GENEPOP (ver. 4.2; Raymond and Rousset 2003) for each locus–population

combination using an exact test in which two-tailed P values were estimated using a Markov chain method (Guo and Thompson 1992). GENEPOP was also used to test for genotypic linkage disequilibrium for all combinations of locus pairs within populations and to test for population differentiation between all pairs of populations (refer to Table 1) over all loci combined using log-likelihood (G) based exact tests (Goudet et al. 1996) using default values. The results from all tests were compared with an adjusted alpha ( $\alpha$  = 0.05) using the sequential Bonferroni procedure (Rice 1989).

Genetic differentiation among sample pairs was estimated using Weir and Cockerham's (1984)  $\theta$  statistic used to calculate pairwise  $F_{ST}$ . Pairwise  $F_{ST}$  among all samples was compared in ARLEQUIN (ver. 3.5; Excoffier et al. 2005) and significance was assessed using 10 000 permutations. The overall level of population subdivision based on average pairwise estimates was calculated in FSTAT (version 2.9.3.2; Goudet 2002).

The genetic stock identification program ONCOR (Kalinowski et al. 2007), which implements a conditional maximum likelihood approach (Millar 1987), was used to estimate the stock composition of populations (Brock and Hornaday rivers) that contribute fish to those caught in the subsistence fisheries along the east coast of Darnley Bay. Mixture proportions (including 95% confidence intervals) for each fishery, and each year sampled, were estimated by bootstrapping baseline (as per Rannala and Mountain 1997) and fishery (mixture) samples 1000 times. Next, the data were subjected to simulations to assess the accuracy, and to account for sample size effects, of the mixture analysis. The original mixture proportions were used to perform realistic fishery simulations by randomly sampling 1000 fish from the fishery. To assess how differences in baseline population sizes may potentially impact the mixed-stock estimates, the realistic simulations were performed first with the empirical baseline population sizes and then with simulated baseline population sizes of 50, 100, and 500. For analyses involving empirical baseline population sizes, ONCOR uses the method of Anderson et al. (2008) to simulate mixture genotypes and to estimate their probability of occurrence. When non-empirical baseline population sizes were used, mixture genotypes were simulated following Kalinowski et al. (2007). Each realistic fishery analysis was simulated 1000 times. To test the temporal stability of the fisheries, samples collected at the same location over multiple years (e.g., Lasard Creek and mouth of the Hornaday River) were initially treated independently before being combined to increase fishery sample sizes for the ONCOR analysis. To further assess the accuracy of the estimated stock contributions, fishery samples were simulated (N = 1000 and data were bootstrapped 5000 times) in which all of the individuals in the fishery sample are from the same baseline population. Using these data, mixture proportions for all baseline populations contributing to the simulated mixtures were then estimated. Sample size effects on our estimates were assessed by performing simulations initially with our empirical baseline sample size and then using simulated baseline sample sizes of 50, 100, and 500 fish.

#### RESULTS AND DISCUSSION

#### INTRAPOPULATION GENETIC VARIATION

A total of 882 samples were collected from four locations (Table 1; Fig. 1). The results of the MICRO-CHECKER analysis identified three loci potentially suffering from null alleles or other scoring errors: Sco109, Sco212, and OMM1128. Those loci were therefore eliminated, including monomorphic Smm21, from all subsequent analyses, leaving a total of 14 informative loci. The  $N_A$  per locus ranged from 2 (Sfo18, SSOSL456, and Sco216) to 38 (Sco216) and  $H_E$  ranged from 0.22 (Sfo18) to 0.96 (Sco216). Within sample locations,  $N_A$ , averaged across all loci, ranged from 8.14 in Lasard Creek sampled in 2010 to 15.86 in Lasard Creek sampled in 2011 (Table 2). Mean  $H_E$ , averaged across all samples and all loci, was 0.74, ranging from 0.69 in

Brock River baseline samples (i.e., BRK-B) to 0.77 in Hornaday River baseline samples (i.e., HORN Pooled).

Conformation to HWE was rejected in 14 of 139 tests using the sequential Bonferroni correction (minimum adjusted alpha = 0.00036). All significant differences from HWE involved deficits of heterozygotes. Significant genotypic linkage disequilibrium was detected in 27 of 885 tests after sequential Bonferroni corrections for multiple comparisons (minimum adjusted alpha = 0.00006). Although departures from HWE can result from factors such as selection, inbreeding, non-random mating, and/or the presence of null alleles, population subdivision is thought to be the most important of these factors for microsatellite loci (Lander 1989). It is therefore not surprising that some level of substructure may be detected when examining a mixed-stock fishery.

#### INTERPOPULATION GENETIC STRUCTURE

Log-likelihood (G) based exact tests of genic differentiation revealed that 37 of 45 population pairwise comparisons were significantly differentiated from each other after sequential Bonferroni adjustment of alpha 0.05 (minimum adjusted alpha = 0.0011; Table 3). Baseline populations from Brock and Hornaday rivers were significantly different from each other, as were annual coastal collections from Lasard Creek. Three of six pairwise comparisons from the annual coastal collections from Hornaday River were significantly differentiated from each other. Only those samples collected in 2009 were not significantly differentiated from the remaining collection years. Similarly, baseline Hornaday River samples were not significantly differentiated from coastal Hornaday River collections in 2010 and 2011.

FST ( $\theta$ ) values ranged from -0.0003 (LES-2012 and HORN-2012) to 0.0646 (BRK-B and HORN-2009) and the overall level of population subdivision based on average pairwise estimates was low ( $\theta$  = 0.010, 95% confidence interval = 0.007–0.012) among all localities and annual samples (Table 3). Though significant, the degree of genetic structure among baseline char is relatively weak (0.0100–0.0607). Among the coastal Lasard Creek fishery samples, pairwise FST ranged from 0.0028 (between collection years 2011 and 2012) to 0.0252 (between collection years 2010 and 2011) (Table 3). Among the coastal Hornaday River fishery samples, pairwise FST ranged from 0.0008 (between collection years 2009 and 2012) to 0.005 (between collection years 2011 and 2012) (Table 2). Between coastal sample locations pairwise FST values ranged from -0.0003 (LES-2012 and HORN-2012) to 0.052 (LES-2010 and HORN-2009). Differences in  $\theta$  were small, but the comparisons were statistically significant in 25 of the 45 comparisons following sequential Bonferroni adjustment (minimum adjusted alpha = 0.0011).

The results of this study indicate various levels of genetic divergence among samples. The significant genetic differences between both baselines imply the existence of genetically differentiated populations where gene flow has been restricted, and mutation and drift may act independently to enhance genetic divergence. However, the FST values observed among baseline samples were relatively low, suggesting only slight genetic differentiation among char from the Brock and Hornaday rivers. Low levels of interpopulation genetic divergence is common in anadromous fishes (Ward et al. 1994) and most likely reflect the long-term combined effect of their general propensity for homing to natal sites and the potential of straying in absence of firm physical barriers to gene flow (Bernatchez et al. 1998). Additionally, large effective population sizes (Ne) may prevent the accumulation of large genetic differences through drift, or ongoing gene flow (Whiteley et al. 2010; Moore et al. 2013). Furthermore, the constraint of using resting adult char from the Hornaday River as baseline samples is not ideal as there is no way to identify their system of origin. Including char of mixed-origin as a baseline in MSFA may lead to spurious genetic results.

Generally, the FST values among coastal sampling sites were lower than those reported in baseline populations. This is not surprising in a purported mixed-stock fishery that supports more than one genetic stock and is due to the lack of apparent genetic structure. The notable exception, however, are the coastal fishery samples collected from Lasard Creek in 2010 in which pairwise FST values were, in some instances, greater than those of baseline char. These results could indicate that baseline samples of Arctic Char collected from Brock and Hornaday rivers are not a complete representation of the genetic composition of the char fishery in Darnley Bay, but represent only part of that stock component. However, this seems unlikely given the great distance to other known suitable overwintering/spawning streams outside of Darnley Bay (e.g., ~300 km from Sachs River, NT ~350 km from Kuujjua River, NT, and ~580 km from Coppermine River, NU).

#### MIXED-STOCK FISHERY ANALYSIS (MSFA)

The MSFA revealed that both baseline populations contribute to the Darnley Bay subsistence fishery and that baseline populations appear to contribute proportionately more to coastal fisheries that are proximate to the baseline system (i.e., Hornaday River baseline to coastal Hornaday River, and Brock River baselines to coastal Lasard Creek) regardless of the year in which samples were collected. However, the Hornaday River contributed more char to both fisheries than the Brock River. When coastal fishery samples were combined across all collection years, baseline Hornaday River samples contributed 88.9% to the coastal Hornaday River fishery and 87.7% to the coastal Lasard Creek fishery. The results of the realistic fishery simulations were based on the estimated empirical mixture proportions generated using ONCOR for each fishery and year collected, as well as pooled data across collection years.

There was considerable concordance in contributions to fisheries across collection years (Table 4). According to the realistic fishery simulations using empirical baseline sizes, between the years 2009 and 2012 the Hornaday River contributed 84.4%, 80.9%, 92.7%, and 88.8%, respectively, to the Hornaday River fishery, and between the years 2010 and 2012 it contributed 64.3%, 89.9%, and 81.5%, respectively, to the Lasard Creek fishery (Table 4). The Brock River source population contributed the least to fisheries (Table 4), with the largest contribution 32.4% occurring in Lasard Creek in 2010. The possibility that there are intermittent partial migratory char within the Brock River, in which one part of the population stays as residents while the other part feeds elsewhere (Klemetsen et al. 2003), cannot be ruled out. This is partially supported by low stock contributions to coastal fisheries, and consistently higher pairwise F<sub>ST</sub> values in comparison to baseline Hornaday River char. The data herein, however, do not permit this clarification. Abundance estimates for these systems are presently lacking and the unequal contributions to coastal fisheries may also be the result of drastic differences in population sizes between the Hornaday and Brock systems that could contribute to the coastal fishery. Indeed, the Brock River system is approximately a third of the size of the Hornaday River system and several studies have documented associations between habitat size and population size (Frankam 1996; Hanfling and Brandl 1998; Castric et al. 2001). Variation in baseline sample sizes affected the point estimates of fishery contributions, but even at simulated baseline sample sizes of 500 fish for each population, the estimated contributions were remarkably similar to empirical baseline sample sizes.

Simulated mixtures (i.e., 100% of each baseline population examined in turn) were used to assess the accuracy and power of our mixture estimates and also indicated that increasing baseline sample sizes would increase the power of our analysis (data not shown). For example, a baseline sample size of 100 resulted in 94%, 99%, and 96% mixture estimations for the baseline populations Brock A, Brock B, and Hornaday River, respectively, compared with 89%,

98%, and 92% when only 50 fish comprised each baseline sample. These data suggest high power to accurately estimate stock contributions.

#### CONCLUSIONS

To our knowledge, this study represents the first genetic mixed-stock fishery analysis of Arctic Char. We documented the significant genetic distinction between char stocks located within the two primary, and adjacent, river systems to Darnley Bay - Hornaday and Brock rivers - and conclude that both of these stocks contribute to coastal fisheries. Generally, temporal variation in the proportional stock contributions to coastal fisheries appears negligible. The Hornaday River consistently contributed larger proportions of char annually to both proximate and distal fisheries within the bay. The results indicate that future management could be designed around the small-scale genetic differences among baseline sampling locations at the scale of the river system (i.e., Hornaday and Brock rivers). A similar management technique for Arctic Char has been proposed in Cumberland Sound (see Harris et al. 2013). Moreover, Dempson and Kristofferson (1987) have proposed the idea of "local stock complexes" which recognizes that a fishery may be harvesting fish from a number of river systems. Here, recognizing Hornaday River and Brock River char as local stock complexes is warranted since they have been identified genetically as discrete stocks, and the composition of the catch from the coastal fishery at different geographic locations has been determined with sufficient power to support a mixed-stock fishery analysis. Stock contributions to Darnley Bay's mixed-stock fishery should be carefully considered when developing management measures and coastal harvest monitoring programs.

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# **TABLES**

Table 1. Sampling locations, sample sizes (N), data type (as it pertains to the mixed-stock fishery analysis), and the year sampled for microsatellite analyses.

Water body	Abbreviation	N	Data type	Year Sampled
Brock River	BRK-A	40	Baseline	2010
	BRK-B	30	Baseline	2010
Hornaday River	HORN-A	30	Baseline	2011
	HORN-B	50	Baseline	2011
Lasard Creek	LES-2010	22	Mixture	2010
	LES-2011	287	Mixture	2011
	LES-2012	95	Mixture	2012
Hornaday River	HORN-2009	24	Mixture	2009
	HORN-2010	59	Mixture	2010
	HORN-2011	89	Mixture	2011
	HORN-2012	156	Mixture	2012

Table 2. Number of alleles ( $N_A$ ), observed (Ho) and expected (He) heterozygosities and significance (following sequencial Bonferroni corrections; minimum adjusted  $\alpha = 0.00036$ ) of unbiased estimates of type 1 error for Hardy–Weinberg (HW) departure proportions per locus and population. Shaded values are those loci that differed significantly from HW equilibrium. Abbreviations for waterbody and years sampled are given in Table 1.

Locus	BRK- A	BRK- B	HORN (Pooled) <sup>a</sup>	LES- 2010	LES- 2011	LES- 2012	HORN- 2009	HORN- 2010	HORN- 2011	HORN- 2012
Sco220										
N <sub>A</sub>	16	17	24	14	28	23	15	23	24	24
Но	0.83	0.83	0.91	0.75	0.88	0.92	0.77	0.93	0.96	0.91
He	0.91	0.84	0.95	0.91	0.94	0.94	0.93	0.94	0.93	0.94
Sco200										
N <sub>A</sub>	12	10	15	10	19	14	7	13	15	14
Но	0.74	0.60	0.71	0.78	0.76	0.75	0.82	0.79	0.82	0.69
He	0.73	0.87	0.83	0.88	0.80	0.78	0.75	0.82	0.79	0.77
Sco215										
$N_A$	4	3	4	5	4	4	3	4	4	4
Но	0.43	0.43	0.43	0.56	0.34	0.45	0.41	0.44	0.32	0.43
He	0.43	0.45	0.47	0.61	0.36	0.49	0.41	0.46	0.34	0.43
Smm22										
$N_A$	14	12	16	14	18	16	10	15	13	19
Но	0.92	0.77	0.89	0.81	0.85	0.94	0.77	0.86	0.88	0.82
He	0.89	0.88	0.91	0.94	0.90	0.89	0.82	0.90	0.87	0.90
Sco202										
$N_A$	9	8	11	8	11	10	9	10	11	11
Но	0.68	0.80	0.71	0.76	0.71	0.71	0.70	0.78	0.78	0.77
He	0.78	0.80	0.80	0.85	0.74	0.75	0.76	0.75	0.79	0.78
Sco218										
$N_A$	9	7	13	7	16	12	9	13	12	16
Но	0.86	0.43	0.75	1.00	0.73	0.73	0.80	0.73	0.69	0.72

Locus	BRK- A	BRK- B	HORN (Pooled) <sup>a</sup>	LES- 2010	LES- 2011	LES- 2012	HORN- 2009	HORN- 2010	HORN- 2011	HORN- 2012
He	0.81	0.67	0.84	0.79	0.86	0.84	0.86	0.89	0.85	0.84
Sfo18										
$N_A$	2	2	2	2	2	2	2	2	2	2
Но	0.46	0.24	0.36	0.19	0.41	0.42	0.52	0.36	0.35	0.43
He	0.51	0.22	0.42	0.27	0.46	0.43	0.51	0.40	0.45	0.45
OMM1105										
$N_A$	9	7	14	9	15	9	8	11	14	13
Но	0.75	0.77	0.77	0.74	0.70	0.69	0.86	0.66	0.80	0.74
He	0.76	0.65	0.82	0.83	0.77	0.76	0.81	0.76	0.79	0.80
OtsG253b										
$N_A$	9	6	10	7	13	10	7	10	11	10
Но	0.65	0.43	0.72	0.70	0.65	0.77	0.59	0.66	0.70	0.66
He	0.72	0.56	0.73	0.78	0.70	0.73	0.69	0.70	0.70	0.72
Smm24										
$N_A$	12	10	18	11	20	13	8	17	15	21
Но	0.85	0.87	0.84	0.79	0.85	0.84	0.95	0.81	0.75	0.85
He	0.87	0.81	0.88	0.89	0.86	0.84	0.80	0.90	0.83	0.88
SSOSL456										
$N_A$	5	2	6	4	8	5	5	5	6	6
Но	0.44	0.53	0.64	0.40	0.66	0.40	0.43	0.54	0.75	0.36
He	0.40	0.47	0.61	0.59	0.57	0.43	0.40	0.55	0.61	0.43
Sco216										
$N_A$	19	16	31	2	38	30	21	27	29	37
Но	0.80	0.97	0.90	0.33	0.91	0.80	0.83	0.79	0.88	0.84
He	0.90	0.91	0.96	0.33	0.95	0.95	0.96	0.95	0.95	0.96
Smm17										

Locus	BRK- A	BRK- B	HORN (Pooled) <sup>a</sup>	LES- 2010	LES- 2011	LES- 2012	HORN- 2009	HORN- 2010	HORN- 2011	HORN- 2012
N <sub>A</sub>	4	5	6	7	8	5	6	6	5	8
Но	0.65	0.63	0.66	0.50	0.70	0.75	0.61	0.59	0.81	0.67
He	0.61	0.72	0.70	0.53	0.73	0.74	0.76	0.76	0.74	0.75
OtsG83b										
N <sub>A</sub>	18	11	15	14	22	14	12	13	19	13
Но	0.97	0.90	0.87	0.84	0.90	0.84	0.91	0.75	0.87	0.88
He	0.93	0.85	0.88	0.91	0.89	0.89	0.88	0.89	0.88	0.89

<sup>&</sup>lt;sup>a</sup>Baseline Hornaday River Arctic Char samples (Table 1) were subsequently pooled based on post-hoc microsatellite analyses in ARLEQUIN ver. 3.5 (Excoffier et al. 2005) revealing low population structure ( $F_{ST} = 0.0007$ ; P = 0.4144).

Table 3. Above diagonal: genetic differentiation among pairs of populations (ns, nonsignificant; asterisks, significant; sequential Bonferroni-corrected minimum alpha, 0.0011). Below diagonal: pairwise  $F_{ST}(\theta)$  comparisons among all pairs of populations. Bold values are those that differed significantly after sequential Bonferroni correction (minimum alpha, 0.0011). Abbreviations for waterbody and years sampled are given in Table 1).

	BRK-A	BRK-B	HORN (Pooled)	LES- 2010	LES- 2011	LES- 2012	HORN- 2009	HORN- 2010	HORN- 2011	HORN- 2012
BRK-A	_	*	*	*	*	*	*	*	*	*
BRK-B	0.0607		*	*	*	*	*	*	*	*
HORN (Pooled)	0.0100	0.0399	_	*	*	*	*	ns	ns	*
LES-2010	0.0256	0.0504	0.0099	_	*	*	*	*	*	*
LES-2011	0.0092	0.0493	0.0005	0.0252	_	*	ns	*	ns	*
LES-2012	0.0054	0.0442	0.0022	0.0204	0.0028	_	*	*	*	ns
HORN-2009	0.0073	0.0646	0.0053	0.0520	-0.0027	0.0018	_	ns	ns	ns
HORN-2010	0.0084	0.0403	-0.0021	0.0175	0.0018	0.0010	-0.0025	_	*	*
HORN-2011	0.0106	0.0575	0.0004	0.0344	-0.0008	0.0056	-0.0023	0.0042	_	*
HORN-2012	0.0058	0.0467	0.0008	0.0186	0.0037	-0.0003	0.0008	0.0013	0.0050	_

Table 4. Results of the genetic mixture analysis generated in ONCOR (Kalinowski et al. 2007) showing the annual estimated percent contributions of source populations of Arctic Char to coastal fisheries in Darnley Bay, Northwest Territories. The values represent the mean estimated percent contributions (lower, upper 95% confidence intervals) estimated from realistic fishery simulations under a variety of simulated population sizes (i.e., n = 50, n = 100, n = 500) including the empirical baseline sizes (E).

			Hornaday Coastal		Lasard Coastal			
	50	100	500	E	50	100	500	E
	2009			24				N/A
Brock A	19.5 (13.7, 26.5)	17.7 (13.9, 22.1)	16.5 (13.7, 19.3)	14.6 (12.0, 17.3)				
Brock B	1.9 (0.8, 3.4)	1.3 (0.5, 2.3)	1.1 (0.4, 1.8)	1.1 (0.4, 1.9)				
Hornaday	78.6 (71.2, 84.9)	81.0 (76.3, 84.9)	82.5 (79.5, 85.3)	84.4 (81.6, 87.0)				
	2010			59				22
Brock A	25.4 (19.2, 32.5)	23.8 (19.4, 28.5)	23.3 (20.2, 26.8)	18.9 (16.2, 21.8)	41.4 (34.5, 48.3)	42.0 (37.2, 47.3)	43.6 (39.8, 47.5)	32.4 (29.3, 35.6)
Brock B	0.7 (0.0, 1.9)	0.2 (0.0, 0.9)	0.1 (0.0, 0.3)	0.2 (0.0, 0.6)	4.4 (2.9, 6.2)	4.2 (2.9, 5.5)	4.1 (2.8, 5.4)	3.3 (2.2, 4.5)
Hornaday	74.0 (66.4, 80.3)	76.0 (71.5, 80.3)	76.6 (73.2, 79.7)	80.9 (77.9, 83.7)	54.2 (47.1, 61.1)	53.8 (48.3, 58.9)	52.4 (48.3, 56.1)	64.3 (60.9, 67.4)
	2011			89				287
Brock A	7.5 (3.4, 13.3)	4.4 (1.9, 7.8)	1.7 (0.4, 3.0)	5.4 (3.5, 7.2)	12.4 (7.5, 18.3)	10.0 (6.8, 13.9)	7.7 (5.6, 9.9)	9.0 (7.0, 11.0)
Brock B	3.1 (1.6, 4.9)	2.5 (1.3, 3.7)	2.1 (1.2, 3.1)	2.0 (1.0, 3.0)	2.0 (0.7, 3.6)	1.4 (0.5, 2.5)	1.1 (0.4, 1.9)	1.1 (0.4, 1.9)
Hornaday	89.4 (83.4, 94.4)	93.1 (89.4, 96.1)	96.2 (94.6, 97.9)	92.7 (90.6, 94.8)	85.7 (79.4, 90.9)	88.6 (84.6, 92.1)	91.2 (88.8, 93.5)	89.9 (87.8, 92.0)
	2012			156				95
Brock A	14.1 (9.0, 19.8)	11.7 (8.3, 15.3)	9.7 (7.5, 11.9)	10.1 (7.9, 12.3)	24.2 (18.7, 30.9)	23.0 (18.8, 27.2)	22.3 (19.4, 25.3)	18.3 (15.6, 21.0)
Brock B	2.0 (0.8, 3.6)	1.4 (0.6, 2.3)	1.1 (0.4, 1.9)	1.1 (0.4, 1.9)	0.7 (0.0, 1.9)	0.2 (0.0, 0.9)	0.1 (0.0, 0.2)	0.2 (0.0, 0.6)
Hornaday	84.0 (77.9, 89.2)	87.0 (83.0, 90.4)	89.2 (87.0, 91.6)	88.8 (86.5, 91.1)	75.1 (68.0, 80.8)	76.8 (72.5, 81.1)	77.7 (74.7, 80.6)	81.5 (78.7, 84.2)
(	Combined			328				404
Brock A	14.2 (9.0, 20.5)	11.7 (8.5, 15.5)	9.7 (7.4, 12.0)	10.0 (7.8, 12.4)	15.5 (10.5, 21.0)	13.4 (9.9, 17.1)	11.7 (9.4, 14.3)	11.3 (8.9, 13.5)
Brock B	2.0 (0.8, 3.8)	1.4 (0.5, 2.5)	1.1 (0.4, 1.9)	1.1 (0.4, 1.8)	1.9 (0.8, 3.6)	1.4 (0.5, 2.4)	1.1 (0.4, 1.9)	1.1 (0.4, 1.8)
Hornaday	83.8 (77.1, 89.3)	86.9 (82.9, 90.2)	89.3 (86.7, 91.6)	88.9 (86.5, 91.2)	82.6 (76.5, 87.9)	85.2 (81.2, 88.9)	87.2 (84.5, 89.5)	87.7 (85.3, 90.1)

### **FIGURES**

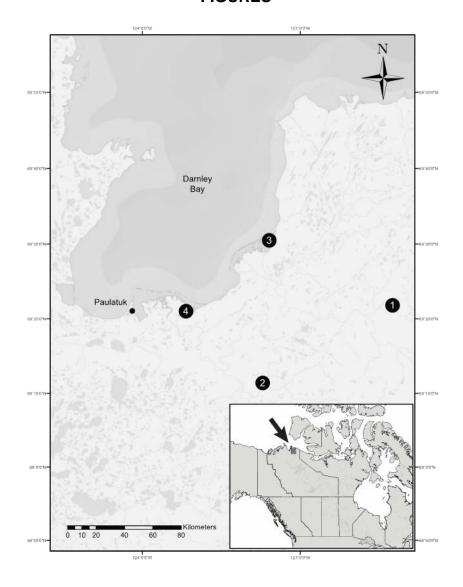


Figure 1. Map of the study area showing sample locations within and in proximity to Darnley Bay, Northwest Territories, Canada. Numbers refer to the locations: 1) Brock River – baseline; 2) Hornaday River – baseline; 3) Lasard Creek – mixed fishery; 4) Hornaday River - mixed fishery.