U.S. Fish & Wildlife Service

Genetic Baseline Development for Dolly Varden in Southwestern Alaska

Alaska Fisheries Data Series Number 2014–2

Conservation Genetics Laboratory
Anchorage, Alaska
March 2014

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Genetic Baseline Development for Dolly Varden in Southwestern Alaska

Penelope A. Crane, Mark J. Lisac, Cara J. Lewis, and John K. Wenburg

Abstract

Dolly Varden *Salvelinus malma malma* contribute to subsistence and sport fisheries in Bristol and Kuskokwim bays in southwestern Alaska, especially within the Togiak National Wildlife Refuge. Recent efforts to enumerate this species and determine life history patterns have revealed that they are migratory throughout southwestern Alaska and that the annual return is variable in abundance and stock composition. We collected genetic data from juvenile (N = 1,104) and mature (N = 1,066) Dolly Varden sampled from 15 spawning and rearing locations in 7 drainages in southwestern Alaska to 1) evaluate genetic population structure using 11 microsatellite loci and 2) determine if genetic data could be used in mixed-stock analysis (MSA) for southwestern Dolly Varden. Population structure was shallow, $F_{ST} = 0.018$. Populations in a neighbor-joining tree grouped largely according to drainage. Maka and Kashioli creeks in the Kuskokwim River drainage, Trail Creek in the Togiak River drainage, and two Nushagak River tributaries were the most divergent populations. Evaluation of the baseline for MSA showed that drainage-specific estimates of stock composition may be possible. Mean estimates from simulated mixtures composed 100% from each of the 7 drainages under study ranged from 86% to 95% using the program SPAM, and from 79% to 94% using the program Oncor. Estimates from proof tests (where genotypes are sampled without replacement from the baseline data, and deleted from the baseline for MSA) conducted for the Togiak River for stock proportions ranging from 0% to 100%, in 20% increments, were close to the expected values. Mixed-stock analysis may be a useful method to estimate the contributions of Dolly Varden originating from different drainages to in-river samples collected from weir sites or subsistence catches.

Introduction

Dolly Varden *Salvelinus malma malma* contribute to subsistence and sport fisheries in Bristol and Kuskokwim bays in southwestern Alaska, especially within the Togiak National Wildlife Refuge (TNWR; Figure 1). Togiak River communities harvest approximately 10,000 Dolly Varden yearly for subsistence use (Bristol Bay Native Association [BBNA] and Alaska Department of Fish and Game [ADF&G] 1996; ADF&G 2001) and many thousands are harvested in the village of Quinhagak on the Kanektok River (Willard Church, Natural Resources Director, Native Village of Kwinhagak, personal communication). This level of harvest is similar to numbers of Pacific salmon *Oncorhynchus* spp. harvested for subsistence use and accounts for approximately a quarter of the per capita weight of fish harvested (Wolfe et al. 1984). Dolly Varden are heavily relied upon as subsistence food in years of poor salmon returns, in years when salmon fishery restrictions occur, and in winter months as a source of fresh fish

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Authors: Penelope A. Crane, Cara J. Lewis, and John K. Wenburg are geneticists and Mark J. Lisac is a fisheries biologist with the U.S. Fish and Wildlife Service. The authors can be contacted at the Conservation Genetics Laboratory, U.S. Fish and Wildlife Service, 1011 E. Tudor Rd., Anchorage, AK 99503; Togiak National Wildlife Refuge, P. O. Box 270, Dillingham, AK 99576; or Penelope_Crane@fws.gov, Mark_Lisac@fws.gov, and John_Wenburg@fws.gov.

(BBNA and ADF&G 1996; Chythlook 2012). In addition to their value as a local subsistence food source, Dolly Varden also support important sport fisheries in the region. The 10-year (2002-2011) average annual sport fish harvest from southwestern Alaska streams is approximately 4,000 fish (Chythlook 2012; http://www.adfg.alaska.gov/sf/sportfishingsurvey/). The numbers of Dolly Varden incidentally harvested in directed commercial salmon fisheries are not monitored.

In the 1980s residents and sport fishers voiced concerns about declining Dolly Varden size and abundance (USFWS 1990, 1991). At the time there were no data available to assess the legitimacy or severity of these concerns, and there was no reliable documentation of the life history of Dolly Varden in southwestern Alaska. In 1997, TNWR initiated research to accumulate basic life history and abundance information. Results from this effort show that Dolly Varden are highly migratory in southwestern Alaska, and that subsistence fisheries within a river likely target fish originating from multiple drainages.

Dolly Varden are iteroparous and home to natal streams to spawn in late August to October (Lisac and Nelle 2000; Lisac 2004, 2006, 2009; Lisac and Bromaghin 2007). Juveniles rear in fresh water for 0 to 3 years and then begin an annual pattern of migrating to marine waters for feeding in summer and returning to fresh water for overwintering (Reynolds 2000). Otolith microchemical analysis and radiotelemetry studies conducted in the Togiak, Goodnews, and Kanektok rivers indicate that Dolly Varden in this region emigrate to the ocean during May and June shortly after ice out and spend approximately 30 to 90 days at sea (Lisac and Nelle 2000; Lisac 2004, 2006, 2009). Fish return to fresh water beginning in July continuing into September, with mature fish generally entering the rivers earlier in the run (Lisac and Nelle 2000; Lisac 2004, 2006, 2007a, 2007b, 2008a, 2008b, 2010). Fish overwinter in main river channels and occasionally in lakes. Tagging studies have documented movements of Dolly Varden among rivers within TNWR and rivers in western Alaska. For example, fish tagged in the Goodnews River have been recovered in the Aniak River (Kuskokwim River drainage), the Kanektok River in Kuskokwim Bay, and the Kulukak River in Bristol Bay (Lisac 2009). Dolly Varden tagged in TNWR rivers have been recovered as far east as Egegik (Lisac and Nelle 2000) and as far north as Norton Sound (M. J. Lisac, unpublished).

In order to monitor the status of Dolly Varden it is desirable to enumerate the annual abundance of fish returning to spawn, overwinter, or both, in various rivers. Methods to accomplish this have included using aerial surveys (e.g, Viavant 2009; Scanlon 2011), mark-recapture experiments (Lisac and Bromaghin 2007), sonar (Osborne and Melegari 2008) and weirs (Whalen 1993; Larson 1997; Lisac 2004, 2006, 2007a, 2007b, 2008a, 2008b, 2010, 2011).

Dolly Varden abundance has been assessed annually using weir counts in the Kanektok River (Figure 1) since 2002 (Lisac 2011) and in the Middle Fork (MF) Goodnews River (Figure 1) since 1996 (Lisac 2010) by TNWR and ADF&G as part of a long-term monitoring program for the species. Weir counts for Dolly Varden range from 8,140 in 2008 to 43,292 in 2010 in the Kanektok River (Lisac 2011), and from 1,421 in 2008 to 6,616 in 2000 in the MF Goodnews River (Lisac 2010). Because of the migratory nature of the species, weir counts include both local fish (mature and immature Dolly Varden originating from the drainage under study) and non-local fish (mature and immature Dolly Varden originating from other drainages). Without information on the origin of Dolly Varden passing through the weirs, it is difficult to determine if variation observed in annual weir counts is due to actual changes in the local population or changes in the proportion of non-local fish using these systems for overwintering.

Attempts have been made to estimate of the number of mature prespawning fish in the run to aid in evaluating trends in Dolly Varden returns. For example, Larson (1997) systematically sacrificed fish throughout the run in the Anchor River and used gonad development criteria to determine the proportion of spawners. He estimated that the annual runs consisted of 43% to 56% spawners. Lisac (2007a, 2007b, 2008a, 2008b, 2010, 2011) attempted to use nonlethal sampling to estimate the proportion of mature, prespawning Dolly Varden passing the Kanektok and MF Goodnews River weirs to interpret weir counts. Prespawning fish were identified using external characteristics in coloration and head morphology, and confirmed with random dissection. Proportions of prespawning fish varied from 38% to 83% at the MF Goodnews weir, and from 55% to 100% at the Kanektok weir. However, estimation of the proportion of prespawners using external characteristics relies on the experience of staff to recognize fish in prespawning condition and for prespawning fish to exhibit changes in external characteristics at the time of sampling. This method is more reliable if the fish are encountered after spending a sufficient time in fresh water or are nearing the spawning season. In the MF Goodnews River, the weir is only 18 river kilometers (rkm) upstream of the tidal influence of Goodnews Bay and Dolly Varden are not in the river long enough to consistently develop the external characteristic of prespawning char. This is especially true for female Dolly Varden that have more subtle spawning characteristic development and color changes compared to males.

Mixed-stock analysis (MSA) has become commonplace in fisheries management to apportion the contributions of individual populations, or regional groups of populations ("stocks"), to mixtures sampled from marine or in-river fisheries. In MSA, the contributions of stocks of fish to a mixture are estimated using naturally occurring genetic markers (reviewed in Pella and Milner 1987). Two data sets are needed for the analysis, the genetic profile of all stocks of fish potentially contributing to the mixture ("baseline"), and the genotypes of fish sampled from the mixture. This methodology has been applied to estimate the marine migration patterns of Dolly Varden in the Beaufort Sea (Krueger et al. 1999) and to estimate the composition of Dolly Varden in overwintering areas in northwestern Alaska and the North Slope (Crane et al. 2005; Crane et al. 2010).

We collected genetic data from Dolly Varden sampled from 15 locations in 7 drainages in southwestern Alaska. Our objectives were to evaluate the population structure of Dolly Varden within the region and to determine if the genetic variation observed among drainages is sufficient to use MSA to identify the drainage of origin of Dolly Varden sampled from annual run and harvest mixtures in the Togiak, Goodnews, and Kanektok rivers. These three systems represent the majority of the production and harvest of Dolly Varden in TNWR. Our findings show 1) shallow but significant population structure of Dolly Varden within southwestern Alaska, 2) regional structuring of populations across western Alaska, and 3) the genetic variation observed in Dolly Varden in southwestern Alaska should permit future applications using MSA to aid in drainage-specific enumeration of Dolly Varden.

Methods

Sample collection

Dolly Varden adults and juveniles were collected from spawning and juvenile rearing areas in the Kuskokwim, Kanektok, Arolik, Goodnews, Quigmy, Matogak, Togiak, Ungalikthluk, Igushik, and Nushagak rivers from 2006 to 2008. Sample sites were accessed by helicopter or raft. Sample goals were 100 adults or 200 juveniles per site. Adults were captured with hook-and-line while rafting a long reach of the tributary stream, or by seine if concentrations of adults were observed during helicopter surveys conducted in the fall (August to October). Only mature fish in ripe or post-spawning condition were sampled. Juveniles were captured using baited minnow traps and electrofishing. Minnow traps were loaded with rocks and set in fast moving water overnight. In order to minimize the sampling of related individuals in juvenile collections, multiple sites were sampled within a tributary, or sites were sampled in multiple years. A fin clip was taken from each adult or juvenile fish captured and preserved in 95% ethanol.

Laboratory analysis

Total genomic DNA was isolated from approximately 10–20 mg of fin tissue from each fish using the Qiagen 96-well DNeasy® procedure. Genomic DNA was brought to a final concentration of 30 ng/µl using the Packard Fluorocount™ Microplate Fluorometer and I-Smart™ Curve-Fitting and Data Analysis Software (Version 2.0). Variation was assayed at 12 microsatellite loci listed in Table 1 using the following conditions. Polymerase chain reaction amplifications were carried out in 10 ul reaction volumes (approximately 30–50 ng DNA, 1.5–2.5 mM $MgCl_2$, 0.8–1 mM dNTPs, 0.1–0.6 µM labeled forward primer, 0.1–0.6 µM reverse primer, and 0.025–0.05 U/µl *Taq* polymerase) using an MJResearch thermocycler. Cycling conditions were 1 cycle of 2 min at 92^o; 30 cycles of 15 sec at 92^o, 15 sec at 55^o-60^o, and 30 sec at 72^o; with a final extension for 10 min at 72^o. The PCR products were size fractioned using an Applied Biosystems 3730 Genetic Analyzer utilizing a polymer denaturing capillary system. Size scoring was completed using GeneMapper® Software ver 4.1. Applied Biosystems GeneScan™-600 LIZ® size standard, 20-600 bases, were loaded as internal size standards. Two researchers scored alleles independently. Samples with score discrepancies between researchers were re-amplified at the loci in question and rescored. Lastly, PCR amplifications were repeated for 8% of the samples, size fractioned and re-scored to check and correct for laboratory errors.

Statistical analysis

Species confirmation—Taylor et al. (2008) reported that the microsatellite locus, *Sfo18*, exhibits diagnostic alleles for Dolly Varden (152 base-pair [bp] allele) and Arctic char *S. alpinus* (158, 160, and 162 bp alleles). Arctic char sampled from southwestern Alaska (Goodnews River, Kukaktlim Lake, Gechiak Lake, Lake Aleknagik), southcentral Alaska (Cooper Lake), and Ireland (Muckross Lake) exhibit 103 and 105 bp alleles at the locus *Sma21* whereas Dolly Varden sampled to date in Alaska exhibit alleles ranging in size from 109 to 155 bp (P. A. Crane, unpublished). Fish expressing only Arctic char alleles at both *Sfo18* and *Sma21* were considered Arctic char and were deleted from subsequent analysis.

Within- and among population variation—Individuals sampled at the same location (Table 2) in multiple years were pooled into a single collection for that location. For each collection, conformation of genotypic frequencies to Hardy-Weinberg expectation was evaluated by testing for a deficit of heterozygotes in FSTAT version 2.9.3 (Goudet 2001). Significance was assessed through randomization tests, permuting alleles among individuals within samples. Significance of the *p*-values for simultaneous tests was evaluated by adjusting the table-wide $\alpha = 0.05$ using the sequential Bonferroni method (Rice 1989). Adjustments were done for 15 simultaneous tests for each locus to evaluate whether deviations were locus-specific and for 12 simultaneous tests for each population to evaluate whether deviations were significant within population.

Genotypic disequilibrium was tested for all pairwise combinations of loci using log-likelihood ratio G-statistics in FSTAT. *P*-values for each locus pair were obtained using randomization tests, permuting genotypes among individuals. A locus-pair was considered linked if *P*-values were less than 0.05 in greater than 50% of the collections and one of the loci was deleted from remaining analyses following McGlauflin et al. (2011). Expected and observed heterozygosity and allelic richness, the number of alleles adjusted for variation in sample size (El Mousadik and Petit 1996), were calculated in FSTAT to describe within-sample diversity. Population differentiation between all pairwise combinations of populations was tested using log-likelihood G-statistics in FSTAT. *P*-values for each locus pair were obtained using randomization tests, permuting genotypes among collections.

Cavalli-Sforza and Edwards chord distances (Cavalli-Sforza and Edwards 1967) were calculated between all population pairs in Phylip version 3.68 (Felsenstein 2004) and grouped in a neighbor-joining tree in NTSYS version 2.10p to visualize spatial genetic relationships. To evaluate population structure at a larger spatial scale, allele frequencies from this study were combined with allele frequency data for seven microsatellite loci (*Sma10, 17, 21, 22, 24, 3*, and *5*) from 14 populations from coastal streams in Norton Sound and the Imuruk Basin (Crane et al. 2010); 9 populations from the Kobuk, Noatak, Wulik, and Kivalina rivers (Crane et al. 2010); and 10 populations from streams draining the Alaska North Slope (Crane et al. 2005). Genotypes for these populations were obtained using the Li-Cor sequencing platform (Crane et al. 2010). To ensure consistency of allele sizes between the Li-Cor and Applied Biosystems platforms, all samples from the Kashaiak River, Ongivinuck River, and Trail Creek sampled in 1998 and 2000 (Crane et al. 2003) were run on both platforms. Some alleles at the *Sma17* locus for individuals genotyped on the Li-Cor platform were pooled for comparability to alleles resolved on the AB platform (Appendix 2). Cavalli-Sforza and Edwards chord distance (Cavalli-Sforza and Edwards 1967) was calculated between all population pairs in the larger data set using Phylip, and multidimensional scaling was used to plot populations in two-dimensional space using NTSYS.

The proportion of variation due to an among-population component (F_{ST}) was estimated using FSTAT according to the method of Weir and Cockerham (1984). The genetic distance $F_{ST}/1\text{-}F_{ST}$ (Rousset 1997) was plotted against waterway distance (km) between population pairs and least-square and locally weighted (loess) regression lines were drawn in SPlus 6.1 (Insightful Corporation, Seattle). Mantel tests were performed in the program IBD version 1.52 (Bohonak 2002) to determine if genetic distance ($F_{ST}/1\text{-}F_{ST}$) between population pairs increases with the geographic distance separating them, as expected under isolation by distance (reviewed in Hutchinson and Templeton 1999). Waterway distances between collections were obtained using Google Earth (http://earth.google.com/download-earth.htm).

Baseline Evaluation—Maximum likelihood estimation of simulated mixtures and Bayesian estimation of the stock composition of known mixtures were used to evaluate baseline performance for MSA. Prior to these analyses, alleles in the baseline populations were binned using the program OptiBin (Bromaghin 2006) to reduce the effects of sampling error and rare alleles. For each locus, exact tests of homogeneity were used to test if allele pairs were similarly distributed among populations, with Monte Carlo simulation to estimate significance, to determine the binning strategy. Log-likelihood ratios were used as the test statistic, and the binning procedure was executed until $P < 0.10$.

Baseline performance was evaluated for seven drainages: Kuskokwim, Kanektok, Goodnews, Matogak, Togiak, Ungalikthluk, and Nushagak. Artificial mixtures of 400 genotypes from a single drainage ("100% simulations", e.g., Seeb et al. 2000), with each population within the drainage contributing equally, were randomly constructed using Hardy-Weinberg expectations from the baseline population allele frequencies and composition by drainage was estimated using conditional maximum likelihood. Bayesian estimation of baseline allele proportions (Rannala and Mountain 1997) was used to further reduce the effects of sampling error in the baseline allele frequencies. The baseline and artificial mixtures were parametrically bootstrapped 1,000 times to compute a mean contribution estimates. The mean contribution estimate for a drainage is expected to be 100%; mean estimates approximating 90% are generally considered acceptable for mixture analysis (Seeb et al. 2000). Simulations were conducted using two programs, SPAM version 3.7b (Debevec et al. 2000) and Oncor (http://www.montana.edu/kalinowski/Software/ONCOR.htm; Anderson et al. 2008) which incorporates a "leave-one-out" procedure, where the gene copies in the simulated mixture genotype are deleted from the population from which it was drawn.

A second set of simulations was performed for the Kanektok, Goodnews, and Togiak drainages. For these simulations, the mixture proportions for the drainage under study were set from 0% to 100% in 20% increments, with the remaining proportion created from equal contributions from the remaining drainages. Lastly, we repeated the analysis described above varying mixture proportions for the Togiak River from 0% to 100% in 20% increments, but instead of simulating mixture samples, we drew genotypes from the baseline to create the mixture ("proof tests"; Templin et al. 2011). Mixtures were created by randomly sampling $N = 300$ genotypes from the baseline without replacement, deleting mixture genotypes from the baseline, and re-computing baseline allele frequencies. For this analysis, mixture samples were only drawn from populations with sample sizes exceeding 100 fish, so that baseline sample sizes would remain large (i.e., Kashaiak River and the Nushagak River sites were not used as a source for mixture individuals).

The stock composition of each mixture was estimated using Bayesian mixture modeling as implemented in the program Bayes (ftp://www.abl.afs.noaa.gov/sida/mixture-analysis/bayes; Pella and Masuda 2001). Initial starting proportions for three chains of 20,000 iterations each were 95% from a single population and 5% distributed evenly among the remaining populations. Values for flat genetic prior parameters were determined as described in Pella and Masuda (2001). The Raftery and Lewis (1996) diagnostic was used to verify that the number of iterations was sufficient. Convergence among chains was determined using Gelman and Rubin (1992) shrink factors. Shrink factors that had a value of less than 1.2 were used to indicate convergence. Mean stock composition estimates were generated after a burn-in discard of the first half of the samples. For each of the six mixture proportions, three proof tests were conducted, for a total of 18 tests. Mean stock contribution estimates and 95% credibility intervals were computed by averaging across the three proof tests per proportion.

Results

Sample collection

In 2006 and 2007, Dolly Varden collections exceeding $N = 50$ were obtained from the Kuskokwim (3 sites), Kanektok, Goodnews (2 sites), Matogak, Togiak (2 sites), Ungalikthluk, and Nushagak (2 sites) rivers (Table 2, Figure 2). Samples were combined with archive samples collected in southwestern Alaska from 1996 to 2003 (Table 2), and analyzed for this study. Additional collections were made from the Arolik, Kanektok, Goodnews, Quigmy, Igushik (Ongoke), and Togiak rivers in 2006 and 2008 (Appendix 1) that are not included in this analysis. These additional samples are archived for future analysis at the Conservation Genetics Laboratory, U.S. Fish and Wildlife Service.

Species confirmation

Three fish exhibited two Arctic char alleles at both *Sfo18* and *Sma21*, one fish each from Kemuk River (*Sfo18* 162/162; *Sma21* 105/105), Trail Creek (*Sfo18* 158/158; *Sma21* 105/105), and Ungalikthluk River (*Sfo18* 158/162; *Sma21* 105/105). These fish were deleted from subsequent analyses. One other fish from the Kokwok River showed an Arctic char allele at *Sfo18* (152/158); this fish was retained for subsequent analyses.

Within- and among population variation

Eighteen of 163 tests for heterozygote deficiency were different, $P < 0.05$ (Appendix 2). One population (Salmon River) had more than one locus not conforming to Hardy-Weinberg expectation after adjusting for 12 multiple tests within each population. One locus, *Sma10*, had a significant number of deviations from Hardy-Weinberg expectation after adjusting for 15 multiple tests for each population, suggesting the presence of a non-amplifying (null) allele. This locus was retained for remaining analysis because the presence of a null allele does not significantly bias estimates of population differentiation given the level of among population variation observed in this study (see below; Chapuis and Estoup 2007). Tests for genotypic disequilibrium were significant in all collections for one locus pair, *Smm22* and *Smm47*. We deleted *Smm47* from all analyses evaluating: 1) genetic variation among populations; 2) allele richness, observed heterozygosity, and expected heterozygosity averaged over loci within collections; and 3) mixed-stock analysis.

The number of alleles observed at the 12 loci assayed in southwestern Dolly Varden ranged from 3 (*Sfo18*) to 43 (*Sma24*) (Appendix 2). Allele richness ranged from 7.59 (Kashioli Creek) to 10.52 in MF Goodnews River. Expected heterozygosity ranged from 0.60 (Kashioli Creek) to 0.66 (South Fork [SF] Goodnews River), and observed heterozygosity ranged from 0.59 (Kashioli Creek) to 0.65 (MF Goodnews River).

Allele frequency differences were detected between all comparisons after adjusting for 105 simultaneous tests ($P < 0.016$). Pairwise F_{ST} ranged from 0.001 between Kashaiak and Kemuk Rivers and 0.074 between Kashioli Creek and Klutapuk River (Table 3). Overall F_{ST} was 0.018.

Maka and Kashioli creeks in the Kuskokwim River drainage were the most divergent populations in the neighbor-joining tree (Figure 3). Populations within drainages tend to group together, with two exceptions. Gechiak, Ongivinuck, Kashaiak, and Kemuk rivers in the Togiak River drainage clustered together, but Trail Creek from the upper Togiak River drainage clustered with Klutapuk River in the Nushagak River drainage. Additionally, Kokwok and Klutapuk rivers in the Nushagak drainage were divergent from each other.

Dolly Varden populations from southwestern Alaska were clearly distinct from Norton Sound, Kotzebue Sound, and North Slope populations in the multidimensional scaling analysis (Figure 4). In this analysis, populations from southwestern Alaska grouped tightly compared to populations in the other regions, with the exception of Maka, Kashioli, and Trail creeks.

The scatter plot of genetic ($F_{ST}/1\text{-}F_{ST}$) versus geographic distance, along with least-square and loess fitted lines, is shown in Figure 5. The Mantel test indicated that genetic and geographic distance was positively correlated (P = 0.002). The loess line indicated that the relationship between geographic and genetic distance is not the same over all spatial scales; the line is steeper for population pairs separated by approximately 1,000 km or greater (i.e., population pairs including Maka or Kashioli creeks). A Mantel test repeated without these two populations showed that populations distributed from the Salmon River (Aniak River/Kuskokwim River drainage) to the Nushagak River do not conform to an isolation by distance model (P = 0.075).

Baseline evaluation

Alleles in the baseline populations were binned using the program OptiBin (Bromaghin 2006) to reduce the effects of sampling error and rare alleles at the following loci; 1 allele was binned at *Ogo1A*, 1 allele at *Sfo18*, 11 alleles at *Smm10*, 2 alleles at *Smm17*, 8 alleles at *Smm21*, 14 alleles at *Smm22*, 3 alleles at *Smm3*, 1 allele at *Smm5*, 21 alleles at *Smm24*, 10 alleles at *Smm41*, and 14 alleles at *Smm44*. Mean mixture estimates for 100% simulations conducted using SPAM version 3.7b for the seven drainages (Kuskokwim, Kanektok, Goodnews, Matogak, Togiak, Ungalikthluk, and Nushagak) varied from 0.86 for Goodnews to 0.95 for Kuskokwim (Table 4). Mean estimates for 100% simulations conducted in Oncor ranged from 0.79 for Goodnews to 0.94 for Kuskokwim.

Mean mixture estimates for contributions of Kanektok, Goodnews, and Togiak in proportions ranging 0% to 100% in 20% increments are shown in Figure 6. Mean estimates were within 10% of expected values for mixture proportions ranging from 0% to 60% for the Kanektok using SPAM and Oncor (Figure 6). For Goodnews, mean estimates were within 10% of expected values for mixture proportions ranging from 0% to 60% in SPAM and 0% to 40% in Oncor (Figure 6). For Togiak, mean estimates were within 10% of the expected value for all mixture proportions in the analysis using SPAM, and for all mixture proportions except 100% in the analysis using Oncor (Figure 6). The mean estimates for the proof tests for Togiak were within 10% of expected values for all mixture proportions (Figure 6). The mean estimate for the 100% proof test was 97%, exceeding the upper value of the 95% confidence interval of the simulation estimates.

Discussion

Identification of Arctic char

Three fish sampled from the Kemuk River and Trail Creek (Togiak River drainage) and the Ungalikthluk River were identified as suspect Arctic char using diagnostic alleles at the microsatellite loci *Sfo18* (Taylor et al. 2008) and *Smm21*. Though Arctic char in southwestern

Alaska are generally considered non-migratory, lake-dwelling residents (Mecklenburg et al. 2002), there have been significant numbers of Arctic char observed in Trail Creek and Izavieknik River in the Togiak River drainage (Lisac and MacDonald 1996; MacDonald and Lisac 1998; Lisac and Nelle 2000). Scanlon (2000) found strontium isotope signals in otoliths from Arctic char sampled from Becharof Lake draining into Bristol Bay that were consistent with four movement patterns; lake resident, full anadromy, migration between drainages, and migration within a drainage. An analysis of 13 Arctic char otoliths randomly selected from Upper Togiak Lake and Trail Creek found no strontium signature that indicated any migration into marine or estuarine waters (Reynolds 2000). The occurrence of Arctic char in the Kemuk River and Trail Creek is not surprising because they are documented in the lakes connected to both tributaries; however these fish were sampled greater than 30 km from the closest lake. The identification of an Arctic char in the Ungalikthluk River is surprising. Arctic char have not been described in this watershed, which contains no large, deep lakes. Thus, the presence of Arctic char could indicate previously unsuspected spawning habitat or movement of Arctic char between drainages.

Taylor et al. (2008) did not find evidence of contemporary hybridization between Dolly Varden and Arctic char. They did not detect any genotypes indicative of F_1 hybrids (*Sfo18* genotypes with a Dolly Varden 152 and an Arctic char 158, 160, or 162 allele) in their study areas in Bristol Bay. In our study, one fish from Kokwok River in the Nushagak River drainage had a putative hybrid (152/158) genotype at *Sfo18*. However, this fish was unlikely an F_1 hybrid because it was not heterozygous for Arctic char and Dolly Varden alleles at a second microsatellite locus with nearly non-overlapping allele frequency distributions.

Arctic char and Dolly Varden are managed as one species in sport fisheries because of their morphological similarity (e.g., Chythlook 2012). Subsistence surveys for freshwater fish generally do not distinguish between the species (Fall et al. 1996). Arctic char are likely harvested in subsistence fisheries by Bristol Bay residents, particularly those that fish through the ice in lakes in the winter (Fall et al. 1996). The microsatellite markers presented here and in Taylor et al. (2008) could be used to estimate the proportion of Arctic char in subsistence catches. Similarly, the genetic markers could also be used to determine if Arctic char are present in bycatch of *Salvelinus* in Bristol Bay salmon fisheries as another method to indicate anadromy in Arctic char.

Population structure

Among regions—Dolly Varden sampled in spawning and rearing areas in rivers in southwestern Alaska were distinct from those sampled from Norton Sound, Kotzebue Sound, and the Alaska North Slope (Figure 4). Though Dolly Varden are clearly capable of long distance movements in southwestern Alaska (e.g., Lisac 2009), two factors may limit gene flow between populations in southwestern Alaska and those further north. The Yukon-Kuskokwim Delta (Figure 4) presents somewhat of a geographic barrier to gene flow due to a lack of spawning and rearing habitat over a large area (>600 km) of coastline. Dolly Varden are widely distributed in coastal Alaska streams (Mecklenburg et al. 2002), but their presence is associated with rivers draining higher elevations areas exceeding 100 m (Kirsch et al. 2011; Buckwalter et al. 2010; USFWS 1987). Most of the Yukon-Kuskokwim Delta is less than 30 m in elevation. In addition, the strong regional structure seen in the multidimensional scaling may be influenced by variation in temperature regime corresponding to the polar (North Slope), boreal (Kotzebue Sound and Norton Sound), and taiga (southwestern Alaska) ecological regions described for Alaska (Gallant et al. 1995). Temperature regime as measured by the number of degree days over 5^{o}C is significantly correlated with population differentiation in Atlantic salmon *Salmo salar*, suggesting that local adaptation to regional temperature can limit gene flow (Dionne et al. 2008).

Within southwestern Alaska—Dolly Varden populations in southwestern Alaska showed shallow, but significant population structure. The overall F_{ST} estimate was low but did not contain zero in its 95% confidence interval (0.018, 95% CI 0.012-0.025). Further, significant allele frequency heterogeneity was detected between all population pairs. Populations in the region were generally structured by drainage in the neighbor-joining tree (Figure 3). Populations in the Kuskowkim River drainage (Maka and Kashioli creeks, Salmon River) grouped together, as did the two populations from the Goodnews River drainage, and four of the five populations from the Togiak River drainage (Gechiak, Ongivinuck, Kashaiak, and Kemuk rivers). One exception to the structuring of populations by drainage was Trail Creek in the upper Togiak River drainage, which clustered with Klutapuk Creek in the Nushagak River drainage. Stream capture has been used to explain the clustering of populations from headwater areas of different drainages (Poissant et al. 2005) or instances of weak geographic structuring (Garvin et al. 2013). Stream capture between the upper Togiak and upper Nushagak rivers drainages is a possible cause of the genetic clustering of Trail Creek and the Klutapuk River.

The most divergent populations within the region were Maka and Kashioli creeks in the Kuskokwim River drainage, Trail Creek in the Togiak River drainage, and Kokwok and Klutapuk rivers in the Nushagak River drainage (Figure 3 and Figure 4). Geographical variables shown to limit dispersal and gene flow include stream gradient (Boizard et al. 2009), altitude differential (Castric et al. 2001), and freshwater migration length (Olsen et al. 2011). With the exception of Kokwok River, these divergent populations were the most distant from the river mouth and at the highest elevation within the sampled drainage. Elevation may be the underlying reason for the population divergence of Kokwok Creek. This tributary is low in the Nushagak River drainage, but originates in the Muklung and Okstukuk Hills which are the few areas of elevation in the lower drainage (Mike Wiedmer, Alaska Department of Fish and Game, Habitat Division [retired], personal communication).

Though Mantel tests revealed a significant relationship between pairwise genetic and geographic distance, the loess regression line had a steeper slope for population pairs separated by greater than ~1,000 km (Figure 5). This includes all pairwise comparisons with Maka or Kashioli creeks in the Kuskokwim River drainage. In a separate analysis deleting these two populations, geographic and genetic distance was not significantly correlated. Conformation of isolation by distance is used to infer that populations are in migration-drift equilibrium, i.e., genetic diversity introduced through gene flow is balanced by a loss of variability due to genetic drift (Hutchison and Templeton 1999). With this set of populations, it is difficult to make an inference regarding migration-drift equilibrium. Power to detect an isolation by distance signal is limited in small geographic areas (e.g., Castric and Bernatchez 2004) or in analyses involving too few populations (e.g., Jenkins et al. 2010). However, a false-positive signal of isolation by distance can occur when a few highly divergent populations are included in the analysis (Koizumi et al. 2006). In either case, it appears that dispersal is more limited for the two Kuskokwim populations either because of the geographical factors discussed above or perhaps variation in life history strategy.

Alt (1977) reported that Dolly Varden spawning in the Kuskokwim River are likely non-anadromous based on observations from local residents of a lack of large-scale seasonal migrations of Dolly Varden such as are observed in the Kanektok and Goodnews rivers. However, a Dolly Varden captured by a sport angler in the Aniak River tributary of the Kuskokwim River was originally tagged in the Goodnews River (Lisac 2009). Although the natal origin of this fish is unknown, it does suggest the possibility of anadromy in Aniak River Dolly Varden. In an analysis of six otoliths collected from Dolly Varden in the Holitna River further upstream in the Kuskokwim River drainage, only one had a strontium signature that indicated marine or estuarine migration (USFWS, unpublished data). The benefit in growth obtained by anadromy may be offset by the cost of long freshwater migration distances. To determine if the proportion of anadromous adults in tributary populations of Dolly Varden in the Kuskokwim drainage decreases with increasing distance from the Kuskokwim River mouth would require a more in-depth otolith microchemistry or radiotelemetry study.

Evaluation for mixed-stock analysis

We evaluated the baseline for its ability to assign Dolly Varden to drainage using both mixtures of simulated and real-fish genotypes. The results of the SPAM and Oncor simulation analysis were similar. The mean stock contribution estimates using Oncor were lower, but not substantially different than the SPAM estimates. Estimates derived using SPAM exceeded 86% and fell within the 95% confidence intervals of the point estimates derived using Oncor for all drainages except the Nushagak (Table 4, Figure 6). In 100% simulations, mean contribution estimates approximating 90% are generally considered robust for mixture analysis using conditional maximum likelihood (Seeb et al. 2000). Perfect allocation is rare because of the propensity of this method to overestimate the contributions of stocks (populations or aggregates of populations) that are absent in the mixture or present in low numbers (Pella and Milner 1987).

Recently, baselines are being evaluated for mixed-stock analysis using proof tests, where genotypes are sampled without replacement from the baseline for the mixture, and the stock composition is estimated using Bayesian methods with a reduced baseline (e.g., Templin et al. 2011). This avoids a major problem of simulation analyses where mixture samples are not truly independent of the baseline because the mixture genotypes are created from the baseline allele frequencies assuming Hardy-Weinberg proportions (Koljonen et al. 2005). In addition, proof tests can take advantage of the greater power of Bayesian methods in mixed-stock analysis; these methods have routinely been shown to more accurately estimate the stock composition of known mixture samples than conditional maximum likelihood (Koljonen et al. 2005; Griffiths et al. 2010; Habicht et al. 2012).

We could only evaluate the Togiak River using proof tests. With proof tests, baseline sample sizes need to be sufficiently large so that the baseline remains large (>200 individuals; Templin et al. 2011) after mixtures samples are drawn and deleted from the baseline. We created mixtures ranging from 0% to 100% in increments of 20% to investigate a variety of mixture scenarios. Mean estimates for the proof tests estimated in Bayes were close to the expected proportions for all mixture scenarios, especially for the 80% and 100% mixture proportions (Figure 6). This is not unexpected because mixtures created 100% from a given stock provide the optimal scenario for analysis using Bayes, where the information contained in the mixture to update the baseline allele frequency estimates, is maximal.

Potential application using mixed-stock analysis

The underlying goal of this project was to develop a genetic baseline for use in MSA of Dolly Varden in southwestern Alaska. We foresee two MSA applications pertinent to TNWR: stock apportionment in weir samples to aid in evaluating trends in counts of Dolly Varden runs in the Kanektok and Goodnews rivers and stock apportionment of subsistence harvests in the Togiak River.

Weirs—Weir counts of Dolly Varden monitored in rivers in Alaska vary annually and are composed of two parts: fish returning to spawn in their natal stream and fish returning to overwinter in fresh water that consist of mixed stocks. The proportion of mature, pre-spawning Dolly Varden passing through weirs has been estimated as a method to monitor spawning population size, but the most accurate way to determine pre-spawning condition is to systematically sacrifice a portion of fish passing through the weir. The 100% simulations conducted in this study appear promising for mixed-stock analysis to estimate the proportion of fish natal to that drainage as an alternate way to interpret fluctuations in weir counts without necessitating the sacrifice of fish. Proof tests could not be conducted for the Kanektok River and Goodnews River because baseline sample sizes were less than 400 fish. In the future, archive samples from the Kanektok and Goodnews River (Appendix 1) could be analyzed and used to conduct proof tests for these drainages to verify that drainage specific estimates will be possible, or if stock composition estimates for these drainages will need to be aggregated.

Togiak River—Currently there are no enumeration projects for Dolly Varden returning to the Togiak River. The Togiak River supports one of the largest subsistence fisheries for Dolly Varden in southwestern Alaska (BBNA and ADF&G 1996). If samples were collected annually at a time when Dolly Varden are fully mixed (i.e., when both mature and immature fish have migrated into overwintering areas in the main river), the consistency of the Togiak River contribution to the subsistence catch could be monitored as a proxy for the stability of the Togiak River population.

Conclusions

1. Dolly Varden sampled from 15 locations in seven drainages in southwestern Alaska showed significant allele frequency heterogeneity.

2. Populations in a neighbor-joining tree grouped mostly according to drainage. Dolly Varden from Maka and Kashioli creeks in the Kuskokwim River, Trail Creek in the Togiak River, and Kokwok and Klutapuk rivers in the Nushagak River were the most divergent among the populations sampled.

3. Populations in western Alaska showed strong regional structure; southwestern Alaska populations were distinct from Dolly Varden sampled from Norton Sound, Kotzebue Sound, and the North Slope.

4. Analysis of simulated mixtures and proof tests suggest that drainage-specific estimates of stock composition should be possible for mixtures of southwestern Alaska Dolly Varden.

Recommendations

1. We recommend analysis of N = 200 baseline samples (Appendix 1) from the Kanektok and Goodnews rivers to use as mixtures of known origin as a final test of the baseline before application of mixed-stock analysis on weir samples before MSA application.

Acknowledgements

We thank Ralph Anderson, Robbin Lavine and the Bristol Bay Native Association for providing funding for and establishing a laboratory internship for this project, and Laura Sorenson, intern, for assisting with the laboratory analysis. Mike Wiedmer, Alaska Department of Fish and Game (retired), provided samples from the Kokwok River. Andy Aderman, M. James LaWonn, Troy Jaecks, Jack Savo, Michael Winfree, Dan Gwinn, Pat Walsh, Seth Beaudreault and Ora Schlei assisted with field collections. We appreciate the thorough reviews provided by Trent Liebich, Pat Walsh, Chris Habicht and Mike Buntjer. The U.S. Fish and Wildlife Service, Office of Subsistence Management provided funding for this project through the Fisheries Resource Monitoring Program, under agreement number FIS 06-701.

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Table 1. Microsatellite loci and primer references for loci surveyed for genetic variation in Dolly Varden *Salvelinus malma* in or near the Togiak National Wildlife Refuge, Alaska, 1996-2007.

Locus	Primer sequences (5'-3')	Primer Reference
Ogo1A	F: gAT CTg ggC CTA Agg gAA AC R: ACT AgC ggT Tgg AgA ACC C	Olsen et al. 1998
Sfo18	F: Tgg TgT ATC CTg CTC CTg R: Tgg AAT gTg TgT CTg TTT TCT	Angers et al. 1995
Sma10	F: AAA ATg TCT CCC CTC CCT CTC R: TCC CTA ACA TAA CAA gTT TTC ATC CT	Crane et al. 2004
Sma17	F: AAg gAT ggT gAg gAC AAT ACA R: ACC TTg AgA AAT CTA TAT gTg gTC TA	Crane et al. 2004
Sma21	F: ggC TgT TCA CCA CAT AgA gTA AT R: TTA AgA Tgg gAT gCA TAT TCA gT	Crane et al. 2004
Smm22	F: CCC AAT gCA gAT AAg ACC TT R: TCT ATA ggC TTA TTT gAA Tgg AAT	Crane et al. 2004
Sma3	F: Tgg CTC AAA TTA AgA TCC TAC R: AgC CAT TAT gCA TTA CTT gTT C	Crane et al. 2004
Sma5	F: AgA TgT gTg ATA AAC TCA gCC TC R: AgT TgT TTA AAT Agg gCg gAT Ag	Crane et al. 2004
Sma24	F: CAT TgA TCA AgA AgC Cag TgC R: TgT ATT Tgg CCA ATA TAA CAC AgC	Crane et al. 2004
Smm41	F: gAC AAA Tgg CTC TgA ATT gC R: CCC CAC AAT TAA CAA AgA TAg C	USFWS, Conservation Genetics Laboratory, unpublished
Smm44	F: TCT ggg TCT TTT ATT TCA gC R: gTg AAT gCA CAC ACA TgC	USFWS, Conservation Genetics Laboratory, unpublished
Smm47	F: ATT ggg ATg TTg TCT TTg AC R: ACA gTC AgA CCA ACA ggA Ag	USFWS, Conservation Genetics Laboratory, unpublished

Table 2. Location, year, sample size, and life stage of Dolly Varden *Salvelinus malma* sampled and analyzed for genetic variation from southwestern Alaska, 1996-2007.

Drainage/Location	Year	N	Latitude	Longitude	life stage
Kuskokwim River					
Maka Creek	2007	101	60.5374	-158.3318	adult
Kashioli Creek	2007	101	60.5170	-158.4356	adult
Salmon River	2006	131	60.7937	-159.5436	adult
Kanektok River					
Nukluk Creek	2007	31	59.6770	-160.7523	adult
	2007	200			juvenile
Goodnews River					
Middle Fork	2003	19	59.2949	-160.8569	adult
	2006	100			adult
South Fork	2006	3	59.1716	-161.1005	adult
	2006	133			juvenile
Matogak River	2006	189	59.1837	-160.7668	juvenile
	1996	25			adult
Togiak River					
Gechiak Creek	2006	4	59.3039	-160.2933	adult
	2006	110			juvenile
	1997	27			adult
Kashaiak River	1998	28	59.4618	-160.1280	adult
	2000	23			adult
Kemuk River	2003	38	59.6055	-159.9352	adult
	2006	15			adult
	2006	188			juvenile
Ongivinuck River	1998	30	59.4155	-159.5861	adult
	2000	89			adult
	2003	44			adult
Trail Creek	1998	10	59.9760	-159.6624	adult
	2000	103			adult
	2003	100			adult
Ungalikthluk River	2003	34	59.0850	-159.9506	adult
	2006	10			adult
	2006	141			juvenile
Nushagak River					
Kokwok River	2006	39	59.3373	-158.1189	juvenile[2]
	2007	35			juvenile
Klutapuk River	2007	69	60.3501	-157.4757	juvenile

[1] Coordinates are approximate midpoints of collection locations.

[2] Samples from M. Wiedmer, Alaska Department of Fish and Game (retired)

Table 3. Pairwise F_{ST} for Dolly Varden *Salvelinus malma* populations sampled in southwestern Alaska, 1996-2007.

Collection	Maka	Kashi.	Salm.	Nukl.	MF G.	SF G.	Mato.	Gech.	Kasha.	Ongiv.	Kemuk	Trail	Unga.	Kokw.
Kashioli	0.0054													
Salmon	0.0294	0.0486												
Nukluk	0.0432	0.0637	0.0062											
MF Good.	0.0416	0.0611	0.0108	0.0086										
SF Good.	0.0377	0.0570	0.0094	0.0061	0.0032									
Matogak	0.0355	0.0546	0.0077	0.0083	0.0069	0.0069								
Gechiak	0.0428	0.0640	0.0122	0.0079	0.0053	0.0052	0.0107							
Kashaiak	0.0386	0.0592	0.0097	0.0062	0.0025	0.0067	0.0067	0.0052						
Ongivinuck	0.0446	0.0684	0.0127	0.0103	0.0063	0.0109	0.0089	0.0074	0.0029					
Kemuk	0.0375	0.0567	0.0079	0.0044	0.0037	0.0079	0.0063	0.0065	0.0010	0.0039				
Trail	0.0421	0.0636	0.0113	0.0098	0.0095	0.0115	0.0154	0.0131	0.0119	0.0115	0.0083			
Ungalikthluk	0.0433	0.0633	0.0123	0.0076	0.0110	0.0108	0.0078	0.0142	0.0110	0.0133	0.0100	0.0141		
Kokwok	0.0459	0.0668	0.0225	0.0219	0.0269	0.0267	0.0284	0.0277	0.0255	0.0252	0.0219	0.0232	0.0245	
Klutapuk	0.0531	0.0744	0.0166	0.0072	0.0178	0.0148	0.0207	0.0178	0.0181	0.0174	0.0127	0.0089	0.0149	0.0262

Table 4. Mean stock contribution estimates and 95% confidence intervals for 1,000 simulated mixtures (*N* = 400) of Dolly Varden *Salvelinus malma* sampled from rivers in or near the Togiak National Wildlife Refuge, Alaska. Simulations were conducted using SPAM version 3.7b (A.) and Oncor (B.). Mixtures were created from a single river with all populations sampled within the river contributing equally. Expected values (in bold) should be close to 1.0.

A.

	Mean Mixture Estimate (95% Confidence Interval)						
	Kuskokwim	Kanektok	Goodnews	Matogak	Togiak	Ungalikthluk	Nushagak
Kuskokwim	**0.95 (0.91, 0.98)**	0.02 (0,0.05)	0.01 (0, 0.03)	0.01 (0, 0.04)	0.01 (0, 0.03)	0.00 (0, 0.01)	0.00 (0, 0.02)
Kanektok	0.01 (0, 0.04)	**0.89 (0.82, 0.95)**	0.02 (0, 0.07)	0.01 (0, 0.05)	0.04 (0, 0.10)	0.01 (0, 0.03)	0.04 (0, 0.08)
Goodnews	0.01 (0, 0.03)	0.02 (0, 0.07)	**0.86 (0.79, 0.93)**	0.03 (0, 0.07)	0.03 (0, 0.07)	0.01 (0, 0.04)	0.02 (0, 0.06)
Matogak	0.01 (0, 0.03)	0.01 (0, 0.04)	0.03 (0, 0.07)	**0.90 (0.84, 0.96)**	0.01 (0, 0.05)	0.01 (0, 0.04)	0.01 (0, 0.04)
Togiak	0.01 (0, 0.04)	0.05 (0.01, 0.11)	0.07 (0.02, 0.13)	0.04 (0.00, 0.08)	**0.90 (0.84, 0.96)**	0.02 (0, 0.06)	0.04 (0.01, 0.09)
Ungalik.	0.00 (0, 0.01)	0.01 (0, 0.02)	0.00 (0, 0.02)	0.01 (0, 0.03)	0.00 (0, 0.02)	**0.94 (0.90, 0.98)**	0.01 (0, 0.02)
Nushagak	0.00 (0, 0.01)	0.01 (0, 0.03)	0.00 (0, 0.02)	0.00 (0, 0.02)	0.00 (0, 0.02)	0.00 (0, 0.01)	**0.88 (0.82, 0.93)**

B.

	Mean Mixture Estimate (95% Confidence Interval)						
Region	Kuskokwim	Kanektok	Goodnews	Matogak	Togiak	Ungalikthluk	Nushagak
Kuskokwim	**0.94 (0.90, 0.97)**	0.04 (0.00, 0.08)	0.01 (0, 0.03)	0.01 (0, 0.05)	0.01 (0, 0.03)	0.00 (0, 0.01)	0.00 (0, 0.01)
Kanektok	0.02 (0, 0.05)	**0.82 (0.74, 0.89)**	0.04 (0, 0.09)	0.02 (0, 0.06)	0.06 (0, 0.12)	0.01 (0, 0.04)	0.08 (0.02, 0.13)
Goodnews	0.01 (0, 0.03)	0.03 (0, 0.08)	**0.79 (0.72, 0.87)**	0.05 (0.00, 0.10)	0.02 (0, 0.07)	0.01 (0, 0.04)	0.03 (0, 0.08)
Matogak	0.01 (0, 0.04)	0.01 (0, 0.04)	0.04 (0.00, 0.10)	**0.85 (0.78, 0.91)**	0.01 (0, 0.04)	0.01 (0, 0.04)	0.01 (0, 0.04)
Togiak	0.02 (0, 0.04)	0.08 (0.03, 0.15)	0.10 (0.04, 0.16)	0.05 (0.01, 0.11)	**0.89 (0.81, 0.95)**	0.02 (0, 0.05)	0.05 (0.01, 0.10)
Ungalik.	0.00 (0, 0.02)	0.01 (0, 0.03)	0.01 (0, 0.03)	0.01 (0, 0.04)	0.01 (0, 0.02)	**0.94 (0.91, 0.98)**	0.01 (0, 0.03)
Nushagak	0.00 (0, 0.01)	0.01 (0, 0.05)	0.01 (0, 0.03)	0.01 (0, 0.03	0.00 (0, 0.02)	0.00 (0, 0.01)	**0.82 (0.75, 0.87)**

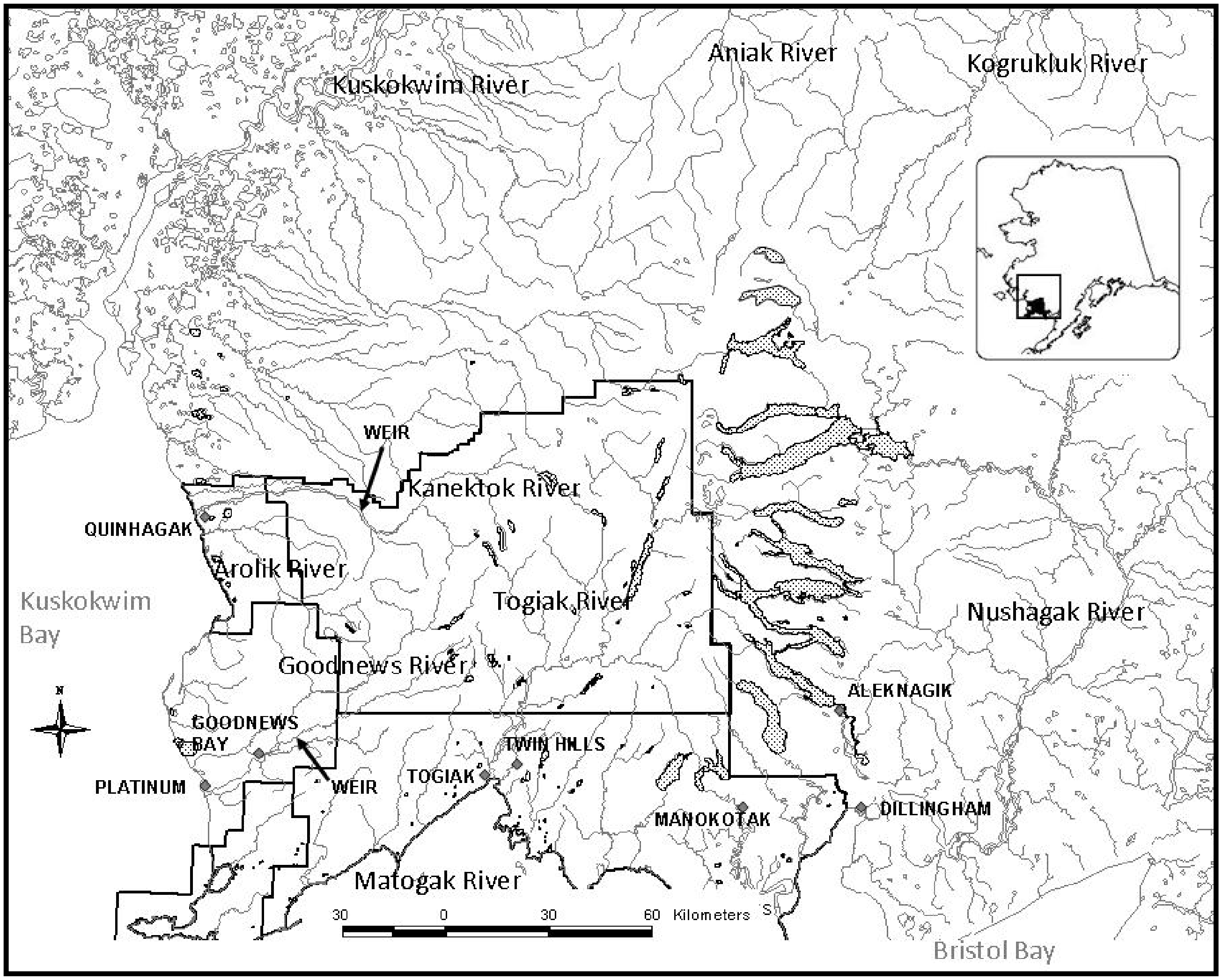

Figure 1. Location of the Togiak National Wildlife Refuge, including major river drainages, communities, and weirs for fish enumeration in southwestern Alaska.

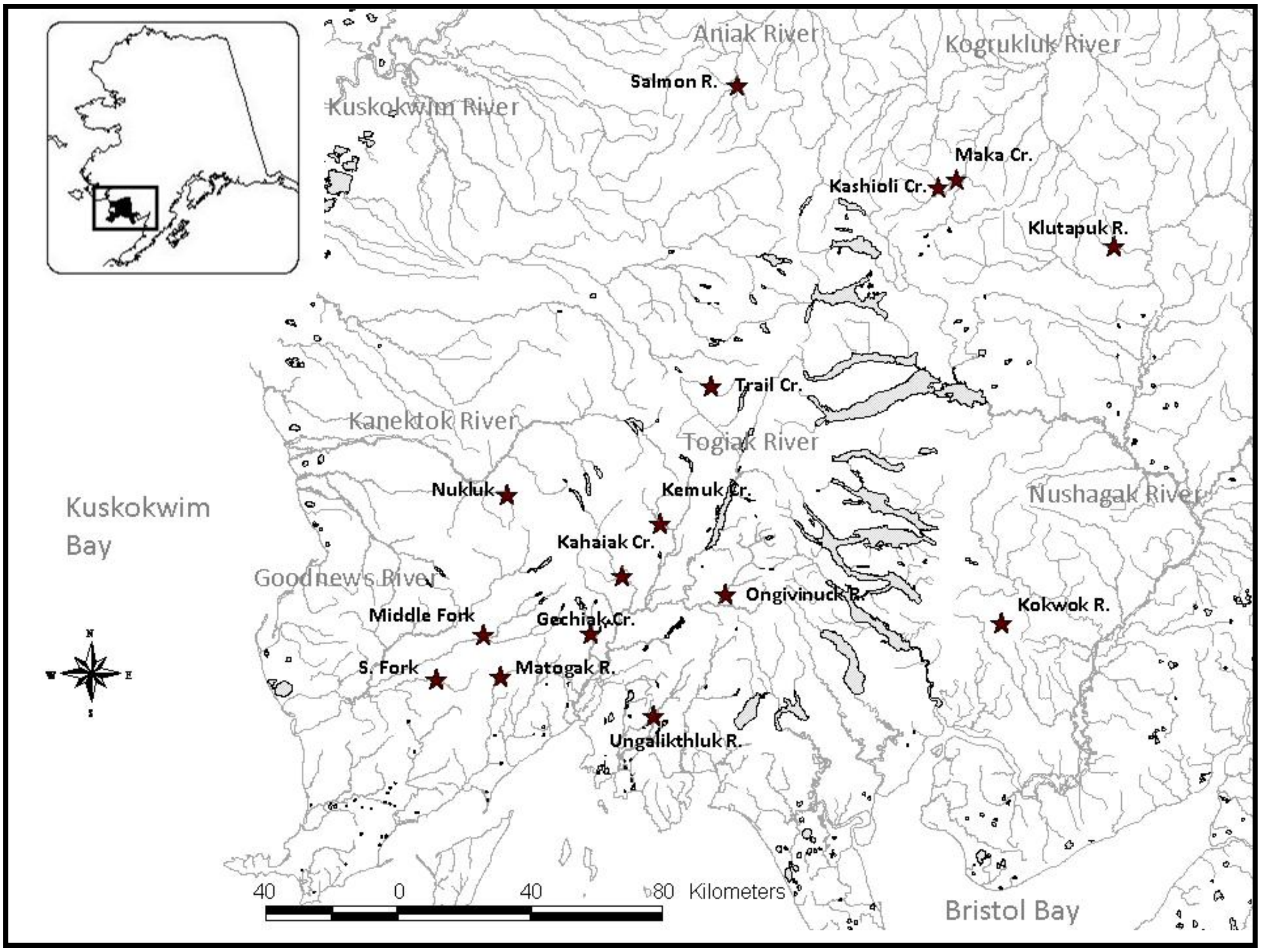

Figure 2. Locations of Dolly Varden *Salvelinus malma* sampled for genetic analysis in or near the Togiak National Wildlife Refuge in southwestern Alaska from 1996-2007.

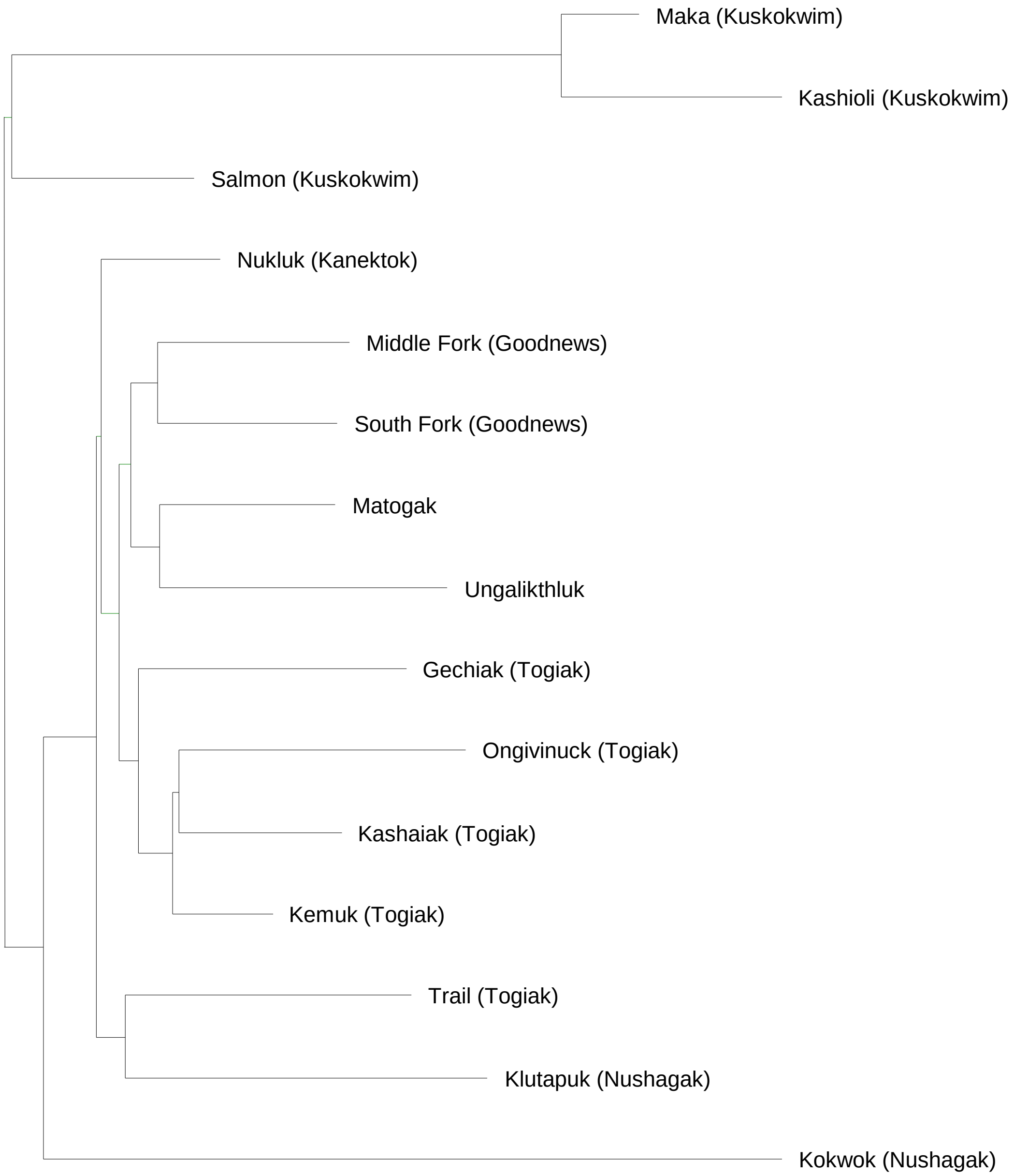

Figure 3. Neighbor-joining tree of pairwise Cavalli-Sforza and Edwards chord distances between populations of Dolly Varden *Salvelinus malma* sampled in rivers in or near the Togiak National Wildlife Refuge, Alaska, from 1996-2007.

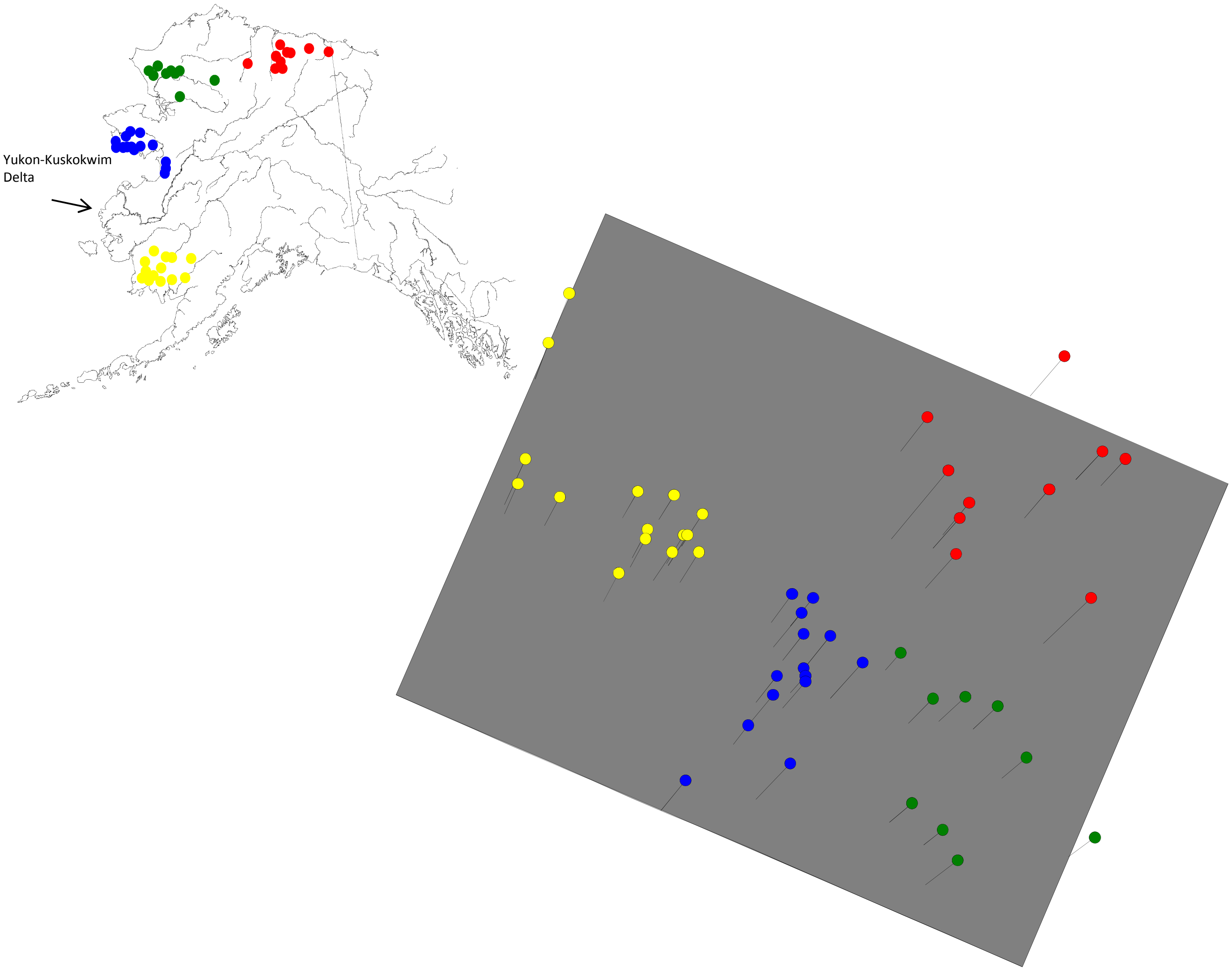

Figure 4. Multidimensional scaling analysis of pairwise Cavalli-Sforza and Edwards chord distances between populations of Dolly Varden *Salvelinus malma* from the Alaska North Slope (red), northwestern Alaska (green), Norton Sound (blue), and southwestern Alaska (yellow). Data for North Slope, northwestern Alaska, and Norton Sound populations are from Crane et al. (2005) and Crane et al. (2010).

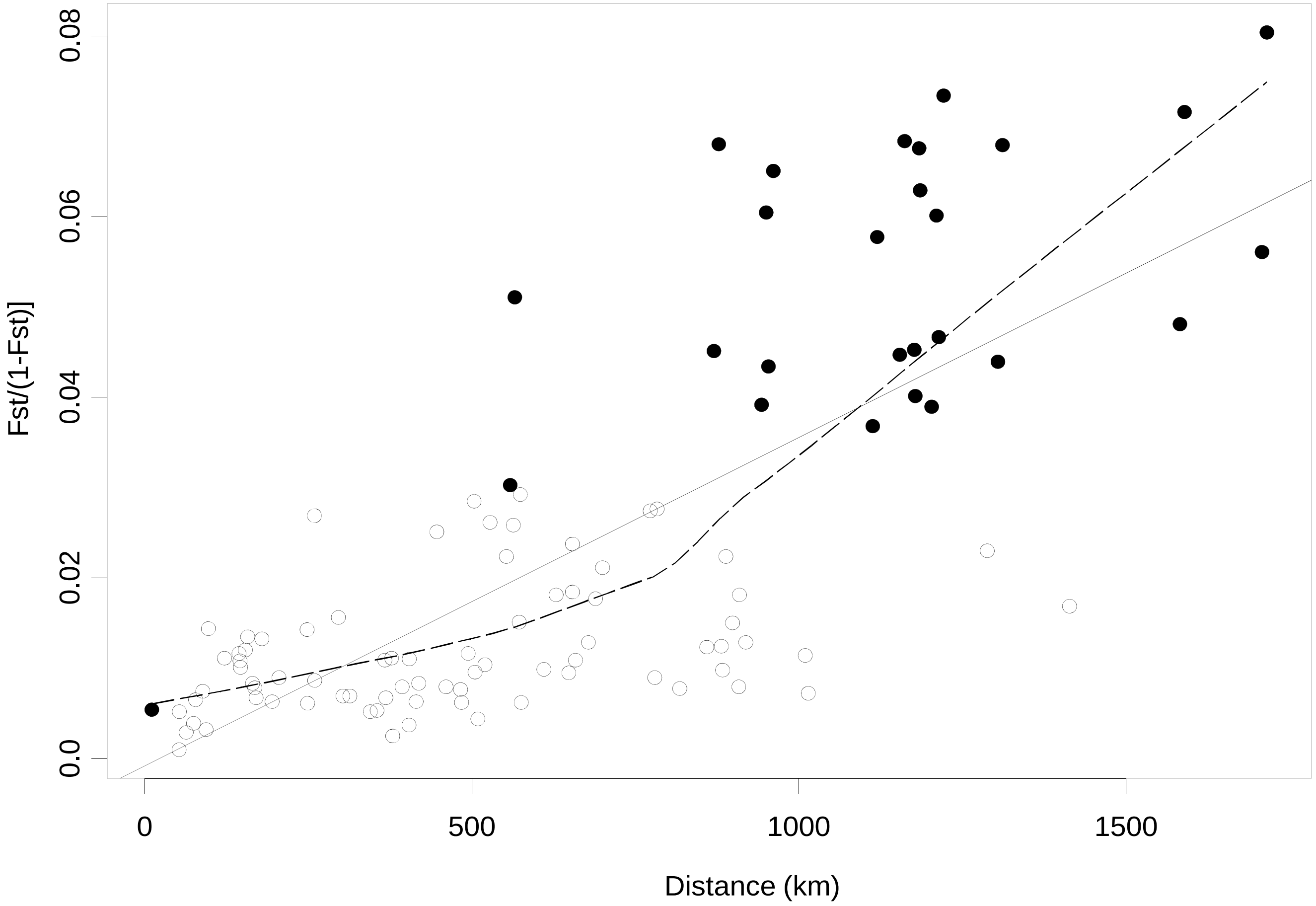

Figure 5. Scatter plots and least square (solid) and locally-weighted (dashed) regression lines of genetic distance versus geographic distance for populations of Dolly Varden *Salvelinus malma* sampled in southwestern Alaska from 1996-2007. Filled circles represent population comparisons with Maka and Kashioli creeks in the Kuskokwim River drainage.

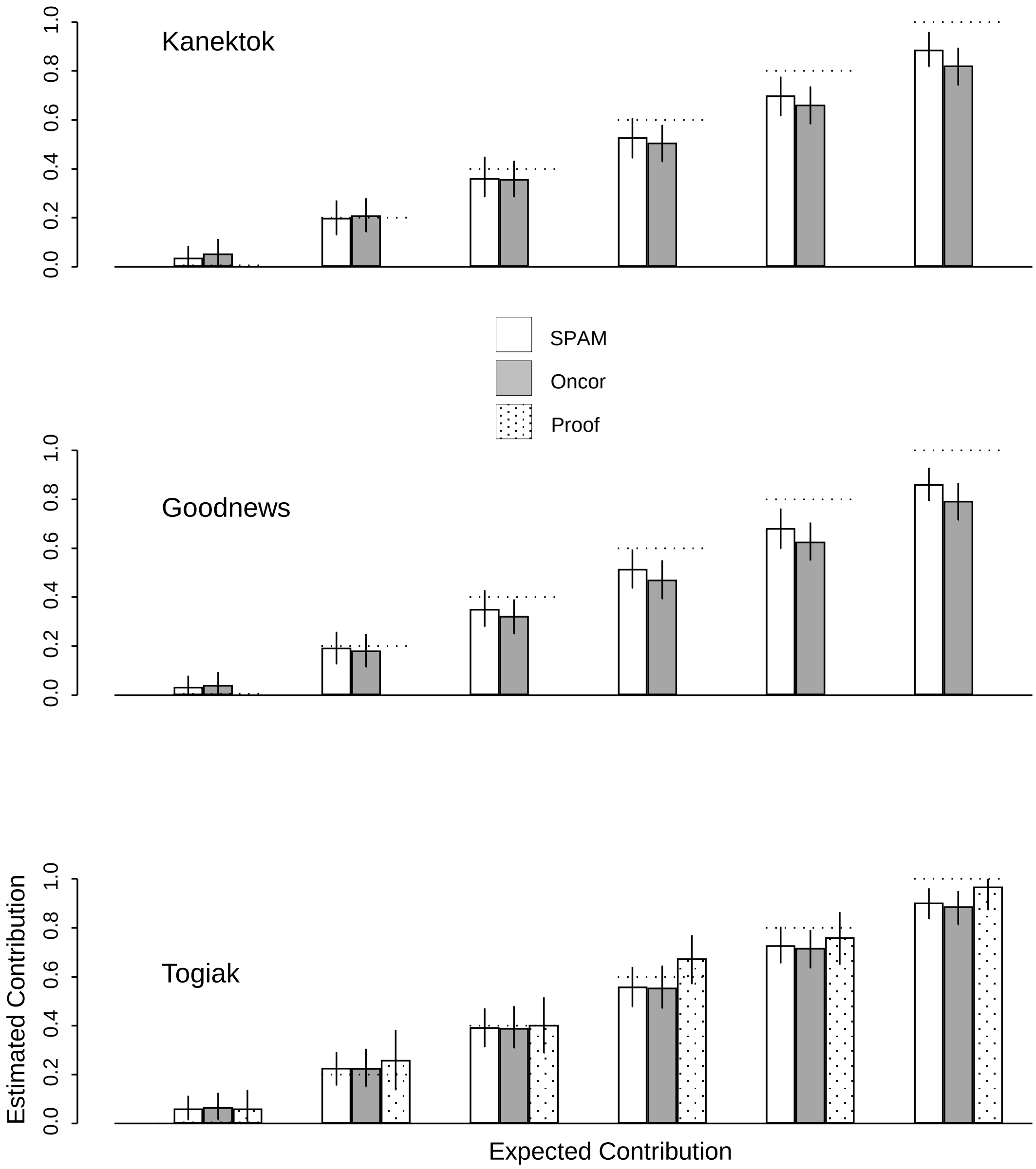

Figure 6. Results of mixed-stock analysis of simulated mixtures for Kanektok, Goodnews, and Togiak river Dolly Varden *Salvelinus malma* using conditional maximum likelihood (SPAM) and conditional maximum likelihood with a "leave-one-out" procedure (Oncor), and "proof tests" for Togiak River. For proof tests, mixture genotypes were taken from the baseline and stock compositions were estimated with a reduced baseline. Mixtures were composed of increasing 20% contributions from each reporting group, with all other populations contributing proportionally. Bars are average contribution estimates (*N* = 1,000 for simulated mixtures and *N* = 3 for proof tests), error bars are 95% confidence intervals, and dashed lines represent expected contributions.

Appendix 1. Location, year, sample size, and life stage of archived Dolly Varden *Salvelinus malma* genetic tissue collections made in southwestern Alaska from 2004-2010.

Location	Year	N	Latitude	Longitude	life stage
Baseline					
Arolik River					
Faro Creek	2008	197	59.4130	-161.4664	juvenile
Keno Creek	2008	193	59.5434	-161.2944	juvenile
South Fork Arolik River	2008	217	59.4195	-161.3497	juvenile
Kanektok River					
Kanuktik Creek	2008	51	59.8302	-160.3931	juvenile
Klak River	2008	99	59.6902	-160.5735	juvenile
Ohnlik Creek	2008	137	59.7891	-160.2954	juvenile
Takshilik Creek	2008	116	59.6169	-161.0331	juvenile
Goodnews River					
Awayak Creek	2008	225	59.5079	-160.6616	juvenile
Canyon Creek	2008	158	59.4556	-161.0187	juvenile
Nimgun Creek	2008	209	59.5903	-160.9548	juvenile
South Branch Middle Fork Goodnews River	2008	184	59.3594	-160.6443	juvenile
North Branch Middle Fork Goodnews River	2008	204	59.4006	-160.6513	juvenile
Quigmy River	2006	175	59.2187	-160.5584	juvenile
Togiak River					
No Lake Creek	2008	70	59.41803	-159.3855	juvenile
Igushik River					
Ongoke River	2008	77	59.31402	-159.43917	juvenile
Mixture					
Kanektok River weir	2004	297	59.76762	-161.06027	adult
	2005	399			adult
	2007	162			adult
	2010	221			adult
Middle Fork Goodnews River weir	2006	300	59.15992	-161.38812	adult
	2007	230			adult
	2010	433			adult

Appendix 2. Sample size (N), allele richness (A_R), expected heterozygosity (H_E), observed heterozygosity (H_O), and p-values for tests for conformation of genotypic frequencies to Hardy-Weinberg expectation (*P-HW*) for Dolly Varden *Salvelinus malma* sampled from rivers in and adjacent to the Togiak National Wildlife Refuge, Alaska, 1996-2007.

	Location														
	Maka	Kashi.	Salmon	Nukluk	MF G.	SF G.	Matog.	Gechi.	Kasha.	Ongiv.	Kemuk	Trail	Ungal.	Kokw.	Kluta.
Ogo1A															
N	100	100	130	231	119	133	203	130	51	162	240	207	177	68	66
136	0.105	0.11	0.165	0.11	0.113	0.117	0.106	0.1	0.088	0.08	0.11	0.109	0.045	0.044	0.114
140	0	0	0	0	0.004	0	0	0	0	0.009	0.008	0.002	0	0	0.008
142	0.085	0.09	0.3	0.394	0.349	0.38	0.256	0.396	0.343	0.256	0.298	0.309	0.347	0.257	0.417
148	0.24	0.16	0.215	0.271	0.218	0.274	0.256	0.231	0.196	0.29	0.229	0.329	0.302	0.368	0.318
160	0	0	0	0	0	0.008	0.01	0.015	0	0.019	0.004	0	0	0	0
166	0.57	0.64	0.319	0.225	0.315	0.222	0.372	0.258	0.373	0.346	0.35	0.251	0.305	0.331	0.144
A_R	3.998	3.999	4	4	4.26	4.412	4.485	4.666	4	5.193	4.667	4.149	3.957	3.976	4.47
H_E	0.602	0.547	0.737	0.71	0.721	0.721	0.721	0.716	0.705	0.726	0.726	0.723	0.695	0.692	0.697
H_O	0.64	0.56	0.75385	0.7013	0.7395	0.66165	0.71429	0.8	0.66667	0.75926	0.6875	0.70048	0.76271	0.79412	0.63636
P-HW	0.8627	0.6836	0.703	0.3877	0.712	0.0676	0.434	0.9924	0.3227	0.8593	0.1002	0.2507	0.9808	0.9778	0.1595
Sfo18															
N	100	100	130	231	119	131	202	131	51	159	239	207	178	70	67
152	1	1	0.988	0.998	0.996	1	0.99	1	1	1	1	1	1	0.993	1
154	0	0	0.012	0.002	0.004	0	0.01	0	0	0	0	0	0	0	0
158	0	0	0	0	0	0	0	0	0	0	0	0	0	0.007	0
A_R	1	1	1.56	1.134	1.261	1	1.488	1	1	1	1	1	1	1.443	1
H_E	0	0	0.023	0.004	0.008	0	0.02	0	0	0	0	0	0	0.014	0
H_O	0	0	0.02308	0.00433	0.0084	0	0.0198	0	0	0	0	0	0	0.01429	0
P-HW	NA	NA	1	1	1	NA	1	NA	NA	NA	NA	NA	NA	1	NA
Smm10															
N	100	99	130	229	119	133	204	131	50	148	238	207	177	70	66
140	0	0	0	0	0	0	0	0	0	0	0.002	0	0	0	0
144	0	0	0	0.004	0	0	0	0	0	0	0	0.002	0	0	0
148	0.015	0.005	0	0.017	0.021	0.03	0.015	0	0.03	0.014	0.023	0.072	0.056	0.007	0.03
152	0.015	0.01	0.046	0.033	0.118	0.09	0.059	0.034	0.07	0.061	0.076	0.092	0.09	0.05	0.053
156	0.315	0.379	0.112	0.142	0.176	0.15	0.086	0.126	0.25	0.186	0.164	0.133	0.136	0.193	0.136
160	0.17	0.111	0.135	0.155	0.13	0.143	0.142	0.29	0.14	0.243	0.147	0.133	0.144	0.171	0.167

Appendix 2. Continued.

	Location														
	Maka	Kashi.	Salmon	Nukluk	MF G.	SF G.	Matog.	Gechi.	Kasha.	Ongiv.	Kemuk	Trail	Ungal.	Kokw.	Kluta.
Smm10															
164	0.05	0.051	0.038	0.066	0.046	0.053	0.071	0.042	0.03	0.068	0.086	0.072	0.054	0.007	0.152
168	0.135	0.126	0.242	0.177	0.088	0.128	0.127	0.099	0.08	0.088	0.111	0.106	0.059	0.15	0.129
172	0.135	0.131	0.219	0.207	0.109	0.113	0.152	0.145	0.16	0.105	0.162	0.085	0.172	0.2	0.091
176	0.05	0.076	0.065	0.057	0.084	0.068	0.103	0.084	0.05	0.084	0.09	0.034	0.056	0.007	0.053
180	0	0	0.05	0.05	0.105	0.113	0.091	0.031	0.04	0.03	0.044	0.072	0.15	0.036	0.083
184	0.005	0	0.023	0.017	0.021	0.015	0.027	0.027	0.06	0.024	0.038	0.072	0.011	0.007	0.023
188	0	0	0.015	0.009	0.021	0.004	0.02	0.034	0.01	0.017	0.004	0.022	0.006	0.021	0.008
192	0	0	0.019	0.009	0.017	0.023	0.025	0	0	0.017	0.004	0	0.008	0.079	0
196	0	0	0.004	0.002	0.004	0.019	0.012	0.015	0.01	0.051	0.013	0	0.034	0	0
200	0	0	0	0.011	0	0.011	0.01	0.008	0	0	0	0	0.014	0	0.015
204	0	0	0.004	0.007	0.004	0.026	0.022	0	0.01	0	0.002	0	0.003	0.064	0.023
208	0	0	0	0.011	0.017	0.004	0	0.015	0.01	0.003	0.008	0	0.006	0	0
212	0	0	0.004	0.002	0.021	0.004	0.012	0.031	0.01	0.01	0.013	0.043	0	0	0.015
216	0	0	0	0.002	0.008	0	0.01	0	0	0	0.004	0.002	0	0	0
224	0	0	0.019	0.002	0	0	0	0	0.01	0	0.002	0	0	0	0
228	0.015	0.005	0.004	0.007	0	0	0	0	0	0	0	0.017	0	0	0
232	0.035	0.076	0	0.009	0.004	0	0.01	0	0.02	0	0	0.007	0	0	0
236	0.015	0.02	0	0	0.004	0	0	0.019	0	0	0	0.01	0	0.007	0.008
240	0.03	0.01	0	0	0	0.008	0.005	0	0.01	0	0.006	0.012	0	0	0.015
244	0	0	0	0.002	0	0	0	0	0	0	0	0.01	0	0	0
248	0.015	0	0	0	0	0	0.002	0	0	0	0	0	0	0	0
252	0	0	0	0	0	0	0	0	0	0	0	0.002	0	0	0
260	0	0	0	0.002	0	0	0	0	0	0	0	0	0	0	0
A_R	11.459	9.439	11.791	13.346	13.995	13.558	14.779	12.898	15.065	12.359	12.683	14.624	12.241	10.974	13.712
H_E	0.831	0.801	0.854	0.871	0.9	0.901	0.905	0.858	0.88	0.871	0.889	0.915	0.889	0.862	0.897
H_O	0.81	0.75758	0.75385	0.83406	0.81513	0.79699	0.89216	0.76336	0.76	0.69595	0.79412	0.86957	0.81356	0.85714	0.90909
P-HW	0.3081	0.1433	0.0005	0.0366	0.0049	0.0005	0.2991	0.0014	0.0086	0.0002	0.0002	0.0044	0.0016	0.5081	0.6903
Smm17															
N	99	100	128	228	119	133	196	129	49	163	238	206	177	66	66
93	0	0	0.008	0.002	0	0.015	0	0	0	0	0.006	0	0	0.008	0.008
99	0	0	0.004	0.02	0.017	0	0	0	0.01	0.028	0.023	0	0.003	0	0.038

Appendix 2. Continued.

	Location														
	Maka	Kashi.	Salmon	Nukluk	MF G.	SF G.	Matog.	Gechi.	Kasha.	Ongiv.	Kemuk	Trail	Ungal.	Kokw.	Kluta.
Smm17															
101	0	0	0.008	0	0	0	0	0	0	0	0	0	0	0	0
103	0.121	0.125	0.109	0.086	0.046	0.12	0.036	0.074	0.02	0.04	0.017	0.083	0.037	0.045	0.061
105	0.02	0.005	0.016	0.013	0.017	0.026	0.015	0	0	0	0	0	0	0.045	0
107	0.025	0.035	0.012	0.004	0.034	0.023	0.01	0.004	0.041	0.043	0.061	0.017	0.02	0.045	0.023
109	0.197	0.25	0.051	0.048	0.059	0.041	0.069	0.043	0.051	0.064	0.061	0.068	0.082	0.182	0.091
111	0.051	0.045	0.043	0.031	0.034	0.056	0.023	0.027	0.051	0.003	0.025	0.024	0.011	0	0.03
114	0.157	0.175	0.105	0.11	0.084	0.079	0.064	0.14	0.133	0.098	0.149	0.146	0.062	0.159	0.197
119	0.096	0.06	0.125	0.081	0.134	0.075	0.107	0.116	0.061	0.098	0.082	0.221	0.136	0.114	0.136
125	0.298	0.28	0.504	0.601	0.571	0.553	0.656	0.597	0.633	0.613	0.571	0.437	0.633	0.402	0.417
130	0.035	0.025	0.016	0.004	0.004	0.011	0.005	0	0	0	0.004	0	0.017	0	0
132	0	0	0	0	0	0	0.003	0	0	0.003	0	0.002	0	0	0
138	0	0	0	0	0	0	0.013	0	0	0.009	0	0.002	0	0	0
A_R	8.541	8.044	8.938	7.796	8.449	8.781	7.86	6.048	7.471	7.583	7.833	6.781	7.052	7.41	8.203
H_E	0.823	0.809	0.705	0.611	0.643	0.664	0.549	0.605	0.578	0.598	0.637	0.729	0.57	0.767	0.761
H_O	0.79798	0.82	0.69531	0.60088	0.58824	0.6391	0.53571	0.58915	0.44898	0.57055	0.60084	0.72816	0.58757	0.74242	0.65152
P-HW	0.291	0.653	0.4215	0.3403	0.0539	0.2222	0.3072	0.3354	0.0053	0.162	0.0574	0.5206	0.7986	0.3502	0.0146
Smm21															
N	100	100	130	231	119	133	203	131	51	163	240	207	177	69	66
115	0.5	0.56	0.262	0.147	0.155	0.248	0.236	0.218	0.127	0.12	0.135	0.092	0.189	0.094	0.083
117	0.095	0.04	0.104	0.126	0.076	0.143	0.133	0.111	0.167	0.156	0.104	0.171	0.107	0.13	0.159
121	0	0	0	0.004	0	0	0	0	0	0	0	0	0	0	0.008
123	0	0	0	0.002	0	0	0.005	0.004	0.039	0	0	0	0	0	0
125	0	0	0	0	0.008	0	0	0	0	0	0	0	0	0	0
127	0.36	0.335	0.515	0.58	0.508	0.432	0.441	0.531	0.52	0.598	0.596	0.618	0.514	0.565	0.705
129	0	0	0.004	0.002	0	0	0	0	0	0.003	0	0	0.003	0.007	0
131	0	0.015	0.015	0.028	0.034	0.041	0.02	0.023	0.049	0.034	0.023	0.024	0.011	0.007	0
133	0.045	0.05	0.081	0.078	0.13	0.086	0.113	0.042	0.069	0.067	0.085	0.039	0.147	0.196	0.045
135	0	0	0	0.013	0.008	0	0.002	0	0.01	0.003	0.006	0	0	0	0
137	0	0	0.015	0.002	0.004	0.008	0.002	0.004	0	0	0.004	0.002	0.003	0	0
139	0	0	0.004	0	0.017	0.004	0	0.019	0	0	0.008	0	0.023	0	0
141	0	0	0	0.002	0.021	0.019	0.037	0.015	0	0.009	0.017	0.027	0	0	0

Appendix 2. Continued.

	Location														
	Maka	Kashi.	Salmon	Nukluk	MF G.	SF G.	Matog.	Gechi.	Kasha.	Ongiv.	Kemuk	Trail	Ungal.	Kokw.	Kluta.
Smm21															
143	0	0	0	0	0	0	0.002	0.015	0	0	0.008	0.024	0	0	0
145	0	0	0	0.015	0.017	0.008	0.002	0.015	0.02	0.006	0.01	0	0.003	0	0
147	0	0	0	0	0	0	0.002	0	0	0	0	0	0	0	0
149	0	0	0	0	0.021	0.011	0.002	0.004	0	0.003	0.002	0.002	0	0	0
A_R	3.967	4.604	5.806	6.852	9.051	7.294	6.857	8.2	7.426	6.283	7.518	6.676	5.853	4.898	4.449
H_E	0.613	0.573	0.651	0.62	0.695	0.724	0.719	0.658	0.684	0.6	0.609	0.578	0.668	0.62	0.474
H_O	0.59	0.57	0.53846	0.60606	0.68067	0.74436	0.71429	0.61069	0.66667	0.57055	0.63333	0.6087	0.69492	0.66667	0.37879
P-HW	0.338	0.5132	0.0016	0.2991	0.3634	0.7613	0.4588	0.0838	0.4387	0.1771	0.8979	0.9197	0.8498	0.8757	0.0197
Smm22															
N	100	99	129	230	118	133	200	131	51	163	240	207	178	70	67
152	0	0	0	0.002	0.008	0	0	0.004	0	0	0.002	0	0.02	0	0
156	0	0	0	0.002	0	0	0.005	0	0	0	0	0	0.02	0	0.007
160	0	0	0	0.004	0	0	0.003	0	0	0.003	0.002	0.002	0.003	0	0
164	0	0	0.008	0.009	0.008	0	0.008	0	0	0	0.002	0.014	0	0	0.007
168	0.005	0.005	0.004	0.007	0	0	0.015	0	0.01	0.003	0.013	0.019	0.003	0.029	0
172	0	0	0.023	0.011	0.017	0.03	0.008	0.008	0.02	0.018	0.002	0.014	0.006	0	0.007
176	0.03	0.005	0.008	0.013	0.03	0.045	0.053	0.008	0.02	0.037	0.029	0.065	0.008	0	0.015
180	0.025	0.005	0.023	0.02	0.03	0.019	0.025	0.069	0.01	0.052	0.063	0.031	0.028	0.021	0.022
184	0.04	0.061	0.031	0.039	0.081	0.045	0.075	0.05	0.069	0.067	0.044	0.041	0.037	0.036	0.052
188	0.135	0.091	0.101	0.089	0.097	0.071	0.06	0.065	0.059	0.101	0.065	0.065	0.065	0.014	0.075
192	0.11	0.111	0.109	0.1	0.093	0.132	0.065	0.134	0.196	0.141	0.09	0.043	0.101	0.1	0.112
196	0.085	0.096	0.101	0.139	0.093	0.105	0.08	0.099	0.108	0.095	0.146	0.077	0.062	0.157	0.06
200	0.1	0.141	0.097	0.104	0.089	0.056	0.09	0.05	0.098	0.098	0.1	0.104	0.093	0.05	0.164
204	0.075	0.126	0.101	0.109	0.072	0.105	0.083	0.084	0.039	0.095	0.081	0.101	0.09	0.129	0.112
208	0.1	0.121	0.112	0.076	0.114	0.083	0.07	0.061	0.078	0.028	0.079	0.133	0.028	0.064	0.097
212	0.1	0.101	0.081	0.078	0.051	0.071	0.065	0.134	0.088	0.074	0.09	0.07	0.121	0.129	0.052
216	0.06	0.045	0.078	0.076	0.055	0.105	0.07	0.111	0.078	0.028	0.046	0.056	0.067	0.193	0.082
220	0.045	0.061	0.047	0.024	0.059	0.038	0.068	0.057	0.01	0.04	0.033	0.029	0.096	0.036	0.03
224	0.06	0.015	0.019	0.033	0.038	0.034	0.055	0.034	0.039	0.043	0.033	0.046	0.07	0	0.06
228	0.02	0.005	0.008	0.024	0.034	0.011	0.06	0.011	0.049	0.04	0.029	0.019	0.045	0.014	0.022
232	0.01	0.01	0.016	0.015	0.021	0.03	0.028	0.023	0.029	0.018	0.033	0.017	0.014	0	0

Appendix 2. Continued.

	Location														
	Maka	Kashi.	Salmon	Nukluk	MF G.	SF G.	Matog.	Gechi.	Kasha.	Ongiv.	Kemuk	Trail	Ungal.	Kokw.	Kluta.
Smm22															
236	0	0	0.019	0.013	0	0.019	0.01	0	0	0.006	0.015	0.007	0.006	0.007	0.015
240	0	0	0.008	0.002	0	0	0.008	0	0	0	0.002	0.012	0.008	0	0
244	0	0	0.004	0.002	0.008	0	0	0	0	0.006	0	0.012	0	0.021	0.007
248	0	0	0	0.002	0	0	0	0	0	0.003	0.002	0.002	0.011	0	0
252	0	0	0.004	0.002	0	0	0	0	0	0	0	0.005	0	0	0
256	0	0	0	0.002	0	0	0	0	0	0.003	0	0.002	0	0	0
260	0	0	0	0.002	0	0	0	0	0	0	0	0	0	0	0
264	0	0	0	0	0	0	0	0	0	0	0	0.01	0	0	0
A_R	14.248	12.408	16.058	16.579	16.428	15.533	17.291	14.252	15.407	16.313	16.234	18.633	17.044	13.272	15.856
H_E	0.918	0.904	0.921	0.919	0.93	0.923	0.938	0.916	0.914	0.924	0.924	0.932	0.928	0.89	0.918
H_O	0.94	0.84848	0.92248	0.9087	0.97458	0.91729	0.935	0.90076	0.88235	0.93865	0.92083	0.9372	0.90449	0.87143	0.92537
P-HW	0.8359	0.0465	0.5613	0.3111	0.9914	0.4343	0.4898	0.3083	0.2634	0.8206	0.4451	0.6593	0.1322	0.3701	0.6507
Smm3															
N	100	100	130	230	118	133	204	131	51	163	240	207	178	70	67
116	0	0	0	0.002	0	0	0	0	0	0	0	0	0	0	0.015
118	0	0	0	0.002	0	0	0	0	0	0.003	0.013	0	0	0	0
120	0.59	0.61	0.546	0.567	0.39	0.474	0.515	0.397	0.441	0.423	0.473	0.495	0.618	0.564	0.634
122	0.405	0.385	0.446	0.402	0.581	0.515	0.461	0.573	0.529	0.555	0.492	0.5	0.357	0.436	0.343
124	0	0	0.004	0.02	0.03	0.011	0.025	0.031	0.029	0.018	0.023	0.005	0.025	0	0.007
128	0.005	0.005	0	0.002	0	0	0	0	0	0	0	0	0	0	0
172	0	0	0.004	0.004	0	0	0	0	0	0	0	0	0	0	0
A_R	2.31	2.31	2.477	3.388	2.885	2.55	2.811	2.889	2.942	2.911	3.351	2.277	2.825	2	3.176
H_E	0.49	0.482	0.504	0.517	0.512	0.512	0.523	0.516	0.53	0.514	0.535	0.506	0.492	0.496	0.484
H_O	0.51	0.44	0.6	0.56522	0.61017	0.50376	0.54412	0.44275	0.4902	0.46012	0.5125	0.44928	0.50562	0.41429	0.41791
P-HW	0.6819	0.2157	0.9894	0.9491	0.9894	0.475	0.7516	0.0468	0.3373	0.0907	0.266	0.0657	0.6938	0.1266	0.1477
Smm5															
N	100	100	130	230	119	133	204	131	50	161	240	207	177	70	67
88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.007
90	0.96	0.99	1	1	1	0.985	0.988	0.962	1	0.988	0.996	1	1	1	0.978
92	0.04	0.01	0	0	0	0.004	0.012	0.038	0	0.012	0.004	0	0	0	0.015

Appendix 2. Continued.

	Location														
	Maka	Kashi.	Salmon	Nukluk	MF G.	SF G.	Matog.	Gechi.	Kasha.	Ongiv.	Kemuk	Trail	Ungal.	Kokw.	Kluta.
Smm5															
106	0	0	0	0	0	0.011	0	0	0	0	0	0	0	0	0
A_R	1.952	1.525	1	1	1	1.784	1.563	1.936	1	1.577	1.242	1	1	1	2.176
H_E	0.077	0.02	0	0	0	0.03	0.024	0.074	0	0.025	0.008	0	0	0	0.045
H_O	0.06	0.02	0	0	0	0.03008	0.02451	0.07634	0	0.02484	0.00833	0	0	0	0.01493
P-HW	0.1384	1	NA	NA	NA	1	1	1	NA	1	1	NA	NA	NA	0.0088
Smm24															
N	99	100	129	230	119	133	199	129	51	161	239	207	175	69	66
162	0.02	0	0	0.004	0	0.011	0.003	0	0	0	0	0	0	0.007	0.023
166	0	0.01	0	0.002	0	0	0	0	0	0	0	0	0	0	0.008
170	0.015	0	0	0	0.004	0	0.01	0	0.01	0.003	0.004	0	0	0	0
174	0	0	0	0.002	0	0.004	0	0	0.01	0	0.006	0	0.006	0	0
178	0.01	0	0.008	0	0.013	0.008	0.008	0	0	0	0.002	0.014	0.011	0	0
182	0	0	0	0.011	0	0.019	0	0.027	0	0	0.008	0.002	0.003	0	0
186	0	0	0.004	0.004	0	0	0.015	0	0.01	0	0	0	0.017	0	0
190	0.03	0.015	0.008	0.009	0.013	0.019	0.008	0.012	0.01	0.034	0.008	0.005	0	0	0.015
194	0	0.005	0.012	0.017	0.017	0.008	0.01	0.008	0.02	0.031	0.044	0.005	0.009	0	0.015
198	0.01	0.01	0.012	0.013	0	0	0.01	0.004	0.029	0.025	0.002	0	0.02	0	0
202	0.005	0.005	0.019	0.011	0	0.015	0.028	0.008	0.069	0.019	0.027	0.014	0.08	0	0.023
206	0.015	0.025	0.031	0.037	0.046	0.011	0.023	0.004	0.01	0.019	0.013	0.007	0.006	0.014	0
210	0.106	0.085	0.058	0.065	0.025	0.026	0.015	0.097	0.039	0.028	0.033	0.029	0.006	0.138	0.045
214	0.03	0.13	0.062	0.039	0.038	0.049	0.04	0.039	0.059	0.034	0.056	0.065	0.02	0.036	0.068
218	0.056	0.06	0.089	0.057	0.055	0.06	0.06	0.027	0.029	0.031	0.067	0.036	0.023	0.007	0.076
222	0.03	0.02	0.093	0.037	0.071	0.064	0.03	0.047	0.088	0.056	0.025	0.106	0.097	0.029	0.03
226	0.015	0.03	0.112	0.048	0.046	0.038	0.033	0.054	0.049	0.043	0.05	0.082	0.06	0.014	0.015
230	0.005	0	0.05	0.05	0.076	0.045	0.053	0.074	0.02	0.068	0.067	0.085	0.089	0.065	0.03
234	0.035	0.01	0.047	0.061	0.025	0.041	0.09	0.047	0.049	0.034	0.038	0.036	0.034	0.058	0.03
238	0.051	0.015	0.012	0.061	0.059	0.049	0.05	0.039	0.039	0.068	0.059	0.01	0.046	0.08	0.045
242	0.03	0.025	0.043	0.046	0.084	0.083	0.05	0.074	0.02	0.047	0.04	0.029	0.071	0.014	0.114
246	0.04	0.095	0.043	0.028	0.08	0.132	0.038	0.066	0.039	0.047	0.046	0.068	0.02	0.072	0.061

Appendix 2. Continued.

	Location														
	Maka	Kashi.	Salmon	Nukluk	MF G.	SF G.	Matog.	Gechi.	Kasha.	Ongiv.	Kemuk	Trail	Ungal.	Kokw.	Kluta.
Smm24															
250	0.035	0.055	0.058	0.061	0.025	0.034	0.05	0.05	0.059	0.109	0.088	0.063	0.063	0.007	0.083
254	0.081	0.04	0.039	0.083	0.059	0.071	0.073	0.085	0.069	0.068	0.056	0.051	0.057	0.123	0.083
258	0.071	0.045	0.05	0.065	0.097	0.045	0.038	0.039	0.049	0.037	0.033	0.056	0.103	0.159	0.061
262	0.136	0.095	0.066	0.026	0.025	0.015	0.068	0.062	0.049	0.028	0.044	0.043	0.037	0.043	0.015
266	0.061	0.17	0.012	0.065	0.008	0.03	0.035	0.062	0.069	0.037	0.044	0.034	0.026	0.101	0.038
270	0.035	0.015	0.023	0.024	0.034	0.011	0.03	0.012	0.029	0.062	0.046	0.017	0.014	0.022	0.045
274	0.04	0.005	0.004	0.017	0.017	0.038	0.018	0.019	0.029	0.012	0.033	0.039	0.017	0	0.015
278	0.01	0.015	0.016	0.02	0.029	0.026	0.015	0.008	0.01	0.009	0.021	0.019	0.009	0.007	0.008
282	0.01	0.015	0.012	0.011	0.004	0.004	0.018	0	0.01	0	0.008	0.027	0.011	0	0.008
286	0	0.005	0.008	0.004	0.013	0.008	0.018	0.008	0.02	0.025	0.004	0.012	0.023	0	0.015
290	0.015	0	0.008	0.004	0.004	0.011	0.033	0	0	0.003	0.004	0.012	0	0	0
294	0	0	0	0.004	0.004	0	0	0.008	0.01	0.012	0.004	0.002	0.006	0	0
298	0	0	0.004	0	0.004	0.008	0.015	0	0	0	0	0.019	0.006	0	0.015
302	0	0	0	0	0.013	0.011	0.008	0	0	0.003	0.002	0.002	0.006	0	0
306	0	0	0	0.007	0.004	0	0	0	0	0	0.006	0.007	0.003	0	0.008
310	0	0	0	0.004	0.004	0	0.005	0	0	0.003	0	0.002	0.003	0	0.008
314	0	0	0	0.002	0	0.008	0.003	0.016	0	0	0	0	0	0	0
318	0	0	0	0	0	0	0.003	0.004	0	0	0	0	0	0	0
322	0	0	0	0	0.004	0	0	0	0	0	0.002	0	0	0	0
326	0	0	0	0	0	0	0	0.004	0	0.003	0.004	0	0	0	0
330	0	0	0	0	0	0	0	0	0	0	0.002	0	0	0	0
A_R	21.482	18.442	21.07	22.98	22.307	23.213	24.802	21.007	24.922	22.582	22.992	21.992	21.851	15.553	22.759
H_E	0.942	0.919	0.944	0.953	0.949	0.948	0.957	0.947	0.961	0.952	0.954	0.947	0.943	0.912	0.95
H_O	0.94949	0.89	0.89922	0.93043	0.93277	0.96241	0.94472	0.93023	0.92157	0.93789	0.94561	0.93237	0.96	0.88406	0.92424
P-HW	0.7065	0.1743	0.028	0.0729	0.2507	0.8412	0.2262	0.2426	0.1414	0.2444	0.297	0.2014	0.8674	0.2491	0.2375
Smm41															
N	100	100	130	230	119	133	200	131	51	163	240	206	178	69	67
211	0	0	0	0.004	0.008	0.004	0.003	0.023	0.02	0.009	0.017	0	0	0	0
223	0	0	0	0.002	0	0	0	0	0	0	0	0	0	0	0
227	0.03	0.015	0.008	0.011	0.008	0.004	0.025	0	0	0.012	0.017	0.002	0.053	0	0.015
231	0.015	0.025	0.038	0.076	0.101	0.105	0.043	0.118	0.059	0.092	0.075	0.085	0.118	0.138	0.075

Appendix 2. Continued.

	Location														
	Maka	Kashi.	Salmon	Nukluk	MF G.	SF G.	Matog.	Gechi.	Kasha.	Ongiv.	Kemuk	Trail	Ungal.	Kokw.	Kluta.
Smm41															
235	0.07	0.03	0.112	0.065	0.046	0.064	0.078	0.073	0.108	0.141	0.1	0.066	0.07	0.051	0.075
239	0.35	0.535	0.196	0.196	0.202	0.195	0.21	0.229	0.176	0.141	0.183	0.209	0.213	0.152	0.239
243	0.085	0.085	0.165	0.096	0.059	0.075	0.098	0.042	0.078	0.113	0.088	0.092	0.053	0.355	0.082
247	0.11	0.055	0.104	0.137	0.109	0.09	0.085	0.141	0.118	0.092	0.098	0.133	0.138	0.094	0.142
251	0.05	0.075	0.038	0.148	0.164	0.192	0.188	0.141	0.157	0.086	0.14	0.066	0.084	0.014	0.149
255	0	0	0.058	0.024	0.067	0.034	0.04	0.034	0.049	0.021	0.058	0.002	0.039	0.051	0.022
259	0.005	0	0.035	0.059	0.034	0.038	0.07	0.05	0.01	0.034	0.067	0.083	0.059	0.007	0.022
263	0.01	0	0.012	0.011	0.017	0.008	0.015	0.008	0	0.015	0.004	0.017	0.014	0.065	0.03
267	0.11	0.045	0.046	0.024	0.05	0.064	0.013	0.011	0.039	0.071	0.027	0.032	0.025	0	0
271	0.065	0.065	0.027	0.057	0.042	0.049	0.045	0.031	0.059	0.089	0.04	0.053	0.017	0.022	0.06
275	0.03	0.05	0.031	0.011	0	0.011	0.01	0	0.039	0.006	0.002	0.01	0.022	0	0.007
279	0	0	0.004	0	0.008	0	0	0.004	0	0.009	0.002	0.019	0.022	0	0
283	0	0	0	0.002	0	0	0.003	0	0	0.006	0.004	0	0.022	0	0.007
287	0.02	0	0	0	0	0	0.008	0	0	0	0	0.019	0	0	0
291	0.03	0	0.004	0	0.008	0	0	0	0	0	0	0	0	0	0
295	0.005	0.005	0.008	0.004	0	0.008	0.005	0.015	0	0.009	0.002	0	0.006	0.014	0.03
299	0	0	0	0	0	0	0.008	0	0	0	0	0	0	0	0.03
303	0	0	0	0	0	0	0	0	0	0.003	0.006	0	0	0	0
307	0	0	0	0.002	0.004	0.008	0	0	0.01	0.009	0	0.002	0	0	0
311	0	0	0	0.004	0	0	0	0	0	0	0	0	0	0	0
315	0	0	0	0.007	0.008	0.004	0	0	0	0.003	0	0	0	0.014	0.007
319	0	0	0.027	0.004	0.004	0.004	0	0.008	0	0	0.002	0	0	0	0
323	0.005	0.015	0.038	0.022	0.025	0.004	0.015	0.011	0.029	0.009	0.033	0.061	0.003	0.007	0
327	0.01	0	0.012	0.004	0.025	0.004	0.015	0.011	0.039	0.018	0.006	0.022	0.003	0.014	0
331	0	0	0.015	0.009	0.004	0.026	0.008	0.019	0	0.003	0.008	0.012	0	0	0
335	0	0	0.019	0.004	0.004	0.011	0.018	0.031	0	0.003	0.01	0.012	0.037	0	0.007
339	0	0	0.004	0.017	0	0	0.003	0	0.01	0.003	0.01	0.002	0	0	0
343	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A_R	13.082	10.32	16.372	15.261	15.487	14.377	15.258	14.568	14.536	15.606	15.079	14.939	14.94	11.498	14.01
H_E	0.835	0.691	0.9	0.895	0.897	0.889	0.888	0.883	0.906	0.91	0.904	0.9	0.897	0.817	0.881

Appendix 2. Continued.

	Location														
	Maka	Kashi.	Salmon	Nukluk	MF G.	SF G.	Matog.	Gechi.	Kasha.	Ongiv.	Kemuk	Trail	Ungal.	Kokw.	Kluta.
Smm41															
H_O	0.83	0.7	0.89231	0.90435	0.89076	0.92481	0.935	0.8626	0.94118	0.90798	0.94167	0.89806	0.8764	0.89855	0.97015
P-HW	0.4944	0.6567	0.415	0.7354	0.466	0.9435	0.9947	0.2611	0.8741	0.4896	0.9891	0.5051	0.2014	0.9882	0.9986
Smm44															
N	99	100	129	231	119	133	196	128	49	161	234	199	166	68	67
222	0	0	0	0.015	0.05	0.004	0.008	0.023	0.031	0.056	0.036	0.02	0.024	0.059	0.015
242	0	0	0	0	0	0	0	0.016	0	0	0	0	0	0	0
258	0	0	0	0.002	0	0	0	0	0	0	0	0	0	0	0
262	0	0	0.008	0.013	0.004	0.008	0.008	0	0.01	0.003	0.004	0.02	0	0	0.015
266	0.051	0.075	0.066	0.061	0.059	0.124	0.105	0.105	0.041	0.115	0.071	0.075	0.127	0.176	0.097
270	0.051	0.045	0.012	0.035	0.063	0.041	0.02	0.078	0.163	0.053	0.062	0.045	0.042	0.088	0.052
274	0.005	0	0.004	0.061	0.046	0.064	0.028	0.027	0.051	0.016	0.06	0.035	0.033	0.022	0.06
278	0	0	0.004	0.035	0.034	0.026	0.008	0.004	0.02	0.04	0.028	0.035	0.015	0.007	0.045
282	0.076	0.14	0.039	0.056	0.063	0.075	0.046	0.043	0.02	0.037	0.021	0.033	0.042	0.015	0.03
286	0.025	0.04	0.116	0.063	0.071	0.056	0.13	0.055	0.082	0.124	0.047	0.101	0.09	0.051	0.127
290	0.056	0.075	0.151	0.084	0.076	0.045	0.102	0.09	0.112	0.109	0.088	0.075	0.096	0.059	0.09
294	0.146	0.1	0.163	0.123	0.092	0.094	0.173	0.086	0.153	0.109	0.13	0.133	0.117	0.088	0.067
298	0.313	0.34	0.128	0.154	0.122	0.15	0.084	0.133	0.112	0.071	0.15	0.209	0.142	0.169	0.09
302	0.141	0.095	0.078	0.065	0.067	0.094	0.069	0.094	0.061	0.075	0.071	0.045	0.018	0	0.09
306	0.02	0.015	0.027	0.048	0.025	0.056	0.048	0.055	0.01	0.047	0.026	0.055	0.036	0.007	0.045
310	0.04	0.025	0.039	0.05	0.021	0.015	0.008	0.035	0.051	0.025	0.015	0.023	0.024	0	0
314	0	0	0.012	0.006	0.004	0.019	0.008	0.004	0.02	0.016	0.03	0.003	0	0	0.022
318	0	0.005	0	0.006	0	0.004	0.005	0.004	0.01	0.003	0.013	0.003	0	0.007	0.007
322	0	0	0.008	0.013	0.017	0.008	0	0.004	0	0.003	0.002	0.028	0.009	0.007	0
326	0	0	0.004	0.006	0.029	0	0.005	0	0	0.003	0.009	0	0.027	0	0.045
330	0	0	0.012	0.002	0.013	0.019	0.01	0.004	0	0.012	0.002	0.005	0.003	0.015	0.022
334	0	0	0.004	0.002	0	0.008	0.005	0.004	0.01	0	0	0	0	0	0.022
338	0	0	0.004	0.009	0.021	0	0.015	0.039	0.01	0.003	0.028	0.003	0.033	0.199	0.015
342	0	0	0.019	0.011	0.021	0.008	0.015	0	0.01	0.019	0.015	0	0.03	0.029	0
346	0	0	0.016	0.015	0.029	0.011	0.015	0.004	0	0.019	0.011	0	0.045	0	0.007
350	0.051	0.02	0.012	0.024	0.021	0.015	0.023	0.031	0.02	0.016	0.038	0.005	0.015	0	0
354	0.025	0.025	0.031	0.013	0.004	0.026	0.018	0.027	0	0.016	0.019	0.01	0.027	0	0.007

Appendix 2. Continued.

	Location														
	Maka	Kashi.	Salmon	Nukluk	MF G.	SF G.	Matog.	Gechi.	Kasha.	Ongiv.	Kemuk	Trail	Ungal.	Kokw.	Kluta.
Smm44															
358	0	0	0.019	0.015	0.021	0.008	0.015	0.016	0	0.009	0.013	0.013	0.003	0	0.007
362	0	0	0.004	0.009	0.008	0.015	0	0.012	0	0.003	0.009	0.028	0	0	0.015
366	0	0	0.008	0.002	0.017	0.004	0.028	0	0	0	0	0	0	0	0.007
370	0	0	0.016	0	0	0	0	0.004	0	0	0	0	0	0	0
374	0	0	0	0.002	0	0	0	0.004	0	0	0.004	0	0	0	0
382	0	0	0	0	0	0.004	0	0	0	0	0	0	0	0	0
A_R	11.669	11.367	17.423	19.232	20.567	18.549	17.846	18.296	17.189	17.896	19.179	16.677	17.617	12.967	19.543
H_E	0.845	0.833	0.907	0.928	0.943	0.925	0.913	0.93	0.914	0.926	0.928	0.906	0.922	0.88	0.937
H_O	0.89899	0.86	0.89922	0.92208	0.95798	0.94737	0.90816	0.89063	0.89796	0.91304	0.94444	0.88442	0.95181	0.88235	0.9403
P-HW	0.969	0.8403	0.4123	0.384	0.8303	0.8931	0.4394	0.0648	0.431	0.3123	0.8794	0.1727	0.9553	0.5771	0.6171
Smm47															
N	100	100	130	230	119	133	200	131	50	162	240	207	177	70	67
301	0	0	0	0.002	0.008	0	0	0.004	0	0	0.002	0	0.02	0	0
305	0	0	0	0.002	0	0	0.005	0	0	0	0	0	0.02	0	0.007
309	0	0	0	0.004	0	0	0.003	0	0	0.003	0.002	0.002	0.003	0	0
313	0	0	0.008	0.009	0.008	0	0.005	0	0	0	0.002	0.014	0	0	0.007
317	0.005	0.005	0.004	0.007	0	0	0.015	0	0.01	0.003	0.013	0.019	0.003	0.029	0
321	0	0	0.023	0.011	0.017	0.026	0.008	0.008	0.02	0.015	0.002	0.012	0.006	0	0.007
325	0.03	0.005	0.008	0.013	0.029	0.045	0.05	0.008	0.02	0.04	0.027	0.065	0.008	0	0.015
329	0.025	0.005	0.023	0.02	0.029	0.019	0.025	0.069	0.01	0.052	0.063	0.031	0.028	0.021	0.022
333	0.04	0.06	0.035	0.039	0.084	0.045	0.075	0.05	0.07	0.065	0.042	0.039	0.048	0.036	0.052
337	0.135	0.09	0.1	0.087	0.097	0.068	0.055	0.065	0.06	0.102	0.063	0.065	0.065	0.014	0.075
341	0.11	0.11	0.108	0.098	0.097	0.128	0.065	0.134	0.2	0.142	0.088	0.043	0.09	0.1	0.112
345	0.085	0.095	0.1	0.139	0.092	0.105	0.08	0.099	0.1	0.093	0.148	0.077	0.059	0.143	0.06
349	0.1	0.14	0.096	0.102	0.088	0.06	0.09	0.05	0.1	0.099	0.1	0.106	0.093	0.05	0.164
353	0.075	0.13	0.1	0.109	0.071	0.105	0.085	0.084	0.04	0.096	0.083	0.101	0.088	0.129	0.112
357	0.1	0.12	0.112	0.076	0.113	0.083	0.073	0.061	0.07	0.028	0.079	0.13	0.028	0.064	0.097
361	0.1	0.1	0.077	0.078	0.05	0.075	0.063	0.134	0.09	0.074	0.09	0.07	0.121	0.143	0.052
365	0.06	0.045	0.077	0.076	0.055	0.105	0.07	0.111	0.08	0.028	0.046	0.056	0.068	0.193	0.082
369	0.045	0.06	0.046	0.024	0.059	0.038	0.068	0.057	0.01	0.04	0.033	0.029	0.096	0.036	0.03
373	0.06	0.015	0.019	0.033	0.038	0.038	0.06	0.034	0.04	0.043	0.033	0.048	0.071	0	0.06

Appendix 2. Continued.

	Location														
	Maka	Kashi.	Salmon	Nukluk	MF G.	SF G.	Matog.	Gechi.	Kasha.	Ongiv.	Kemuk	Trail	Ungal.	Kokw.	Kluta.
Smm47															
377	0.02	0.01	0.008	0.024	0.034	0.011	0.06	0.011	0.05	0.04	0.031	0.022	0.045	0.014	0.022
381	0.01	0.01	0.019	0.015	0.021	0.03	0.028	0.023	0.03	0.019	0.035	0.017	0.014	0	0
385	0	0	0.023	0.017	0	0.019	0.01	0	0	0.006	0.015	0.007	0.006	0.007	0.015
389	0	0	0.008	0.002	0	0	0.01	0	0	0	0.002	0.012	0.008	0	0
393	0	0	0.004	0.002	0.008	0	0	0	0	0.006	0	0.012	0	0.021	0.007
397	0	0	0	0.002	0	0	0	0	0	0.003	0.002	0.002	0.011	0	0
401	0	0	0.004	0.002	0	0	0	0	0	0	0	0.005	0	0	0
405	0	0	0	0.004	0	0	0	0	0	0.003	0	0.002	0	0	0
409	0	0	0	0.002	0	0	0	0	0	0	0	0	0	0	0
413	0	0	0	0	0	0	0	0	0	0	0	0.01	0	0	0
A_R	14.248	12.599	16.191	16.802	16.4	15.521	17.269	14.252	15.475	16.282	16.235	18.603	17.109	13.272	15.856
H_E	0.918	0.905	0.922	0.92	0.929	0.924	0.937	0.916	0.914	0.923	0.924	0.932	0.93	0.891	0.918
H_O	0.94	0.85	0.92308	0.92174	0.97479	0.93233	0.95	0.90076	0.88	0.93827	0.92083	0.94686	0.90395	0.87143	0.92537
P-HW	0.8475	0.0523	0.5553	0.5623	0.9914	0.6935	0.8	0.2986	0.2454	0.8028	0.469	0.8551	0.1137	0.3549	0.6546
Overall (not including *Smm47*)															
A_R	8.519	7.587	9.681	10.143	10.517	10.096	10.458	9.615	10.087	9.937	10.162	9.886	9.580	7.726	9.941
H_E	0.634	0.598	0.650	0.639	0.654	0.658	0.651	0.646	0.643	0.641	0.647	0.649	0.637	0.632	0.640
H_O	0.639	0.588	0.634	0.634	0.654	0.648	0.652	0.624	0.607	0.616	0.635	0.637	0.642	0.639	0.615

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[1] *Smm17* alleles reported here (allele-size listed first) correspond to those reported in Crane et al. (2005) and Crane et al. (2010) (allele-size listed second) as follows: 93=95; 97=99; 99=101; 101=103; 103=105; 105=107; 107=109; 109=111; 111=113; 114=115 and 117; 119=119, 121, and 123; 125=125, 127, 129, and 131; 130=133; 132=135; and 138=141.