U.S. Fish and Wildlife Service
Office of Subsistence Management
Fisheries Resource Monitoring Program

Development and application of microsatellites to population structure and mixed-stock analyses of Dolly Varden from the Togiak River drainage

Final Report for Study 00-011

Penny Crane[1], Mark Lisac[2], Bill Spearman[1], Eric Kretschmer[1], Cara Lewis[1], Steve Miller[1], and John Wenburg[1]

[1]Conservation Genetics Laboratory
U.S. Fish and Wildlife Service
1011 East Tudor Road, Anchorage, AK 99503

[2]Togiak National Wildlife Refuge
U.S. Fish and Wildlife Service
P.O. Box 270, Dillingham, AK 99576

January 2003

Final Report Summary Page

Title: Development and application of microsatellites to population structure and mixed-stock analyses of Dolly Varden from the Togiak River drainage

Study Number: 00-011

Investigator(s)/Affiliation(s): Conservation Genetics Laboratory, U.S. Fish and Wildlife Service; Mark Lisac/U.S. Fish and Wildlife Service, Togiak National Wildlife Refuge

Geographic Area: Bristol Bay (Region 4)

Information Type: Stock Status and Trends

Issue(s) Addressed: Lack of stock structure information to support federal subsistence fishery management of Dolly Varden in the Togiak River drainage.

Study Cost: $34,600

Study Duration: June 2000 to January 2003

Abstract: Dolly Varden *Salvelinus malma* in the Togiak River support a large subsistence fishery for communities in Bristol Bay. Population assessment and management for anadromous Dolly Varden are challenging because populations overwinter and are harvested in mixtures. We used genetic data to test whether population subdivision exists among spawning aggregates of Dolly Varden in three tributaries of the Togiak River drainage and if genetic methods could be used to estimate the stock composition of Dolly Varden sampled from subsistence catches and overwintering aggregates in the Togiak River. Tissue samples were collected from Dolly Varden in prespawning condition from three tributaries to the Togiak River in 1998 and 2000: Trail Creek (N=116), Kashaiak River (N=51), and Ongivinuck River (N=119). Young-of-the-year Dolly Varden from Cobblestone River (N=298), north of Norton Sound, and Kivalina River (N=200), in Kotzebue Sound, were used for comparison at a larger spatial scale. Seven species-specific microsatellite loci were developed specific to Dolly Varden of which six were polymorphic. The number of alleles observed at variable loci ranged from 3 to 38 and mean expected heterozygosity ranged from 0.521 to 0.941. F_{ST} for the Togiak River populations was 0.009 and for all populations was 0.046; both values were significantly greater than zero. Genetic differences were detected among all possible pairwise comparisons of the five collections. Multidimensional scaling analysis demonstrated large spatial differences between the three Togiak River drainage collections and those from the Cobblestone and Kivalina Rivers. A simulation analysis was used to test whether these genetic data could be used in mixed-stock analysis. Mean contribution estimates for artificial mixtures composed 100% from a given population were 81% (Kashaiak River), 93% (Ongivinuck River), 97% (Trail Creek), 99% (Cobblestone River), and 99% (Kivalina River). The levels of population subdivision observed

in this study will likely permit estimation of population contributions to subsistence catches and overwintering mixtures in the Togiak River drainage. However, larger sample sizes and greater representation of Togiak River tributaries and rivers in western Alaska will be needed. Future management actions should take into account the fine spatial scale of population structure for Dolly Varden in the Togiak River drainage in order to maintain genetic diversity and productivity.

Key Words: federal subsistence fisheries, genetic diversity, DNA microsatellites, Dolly Varden, *Salvelinus malma*, Bristol Bay, Togiak River, Togiak National Wildlife Refuge

Project Data: Description – Data for this study consist of biological collections (fin tissue and DNA samples) and information (date, location, and method of capture; gender and maturity index of fish) from Dolly Varden. Format – Fin tissue samples stored in 90% ethanol. Sampling and genetic data are stored in a Microsoft Access database. Custodian(s) – U.S. Fish and Wildlife Service, Conservation Genetics Laboratory, 1011 East Tudor Road, Anchorage, Alaska 99503. Availability – Access to biological samples and data is available upon request to the custodian(s).

Report Availability: Please contact either the author(s) or Alaska Resources Library and Information Services to obtain a copy of this report.

Citation: P. Crane, M. Lisac, B. Spearman, E. Kretschmer, C. Lewis, S. Miller, and J. Wenburg. 2003. Development and application of microsatellites to population structure and mixed-stock analyses of Dolly Varden from the Togiak River drainage. U.S. Fish and Wildlife Service, Office of Subsistence Management, Fisheries Resource Monitoring Program, Final Report for Study 00-011, Anchorage, Alaska.

INTRODUCTION

Dolly Varden *Salvelinus malma* support important subsistence fisheries in western Alaska, with one of the more heavily utilized fisheries occurring in the Togiak River, a lacustrine system draining into Bristol Bay in southwest Alaska. During a 12-month period in 1994-1995, residents harvested 10,462 Dolly Varden in the Togiak River for subsistence use (BBNA and ADFG1996; ADFG 2001). Though an important subsistence resource, population assessment and management for Dolly Varden are difficult because of its complex life history. In this study, we tested whether sufficient genetic differentiation exists among Dolly Varden spawning in tributaries of the Togiak River drainage to identify population contributions to mixtures sampled from subsistence harvests and overwintering aggregates.

In North America, two forms of Dolly Varden are generally recognized based on genetic (Phillips et al. 1999), morphological, meristic, and behavioral traits (Morrow 1980; Armstrong and Morrow 1980). The northern form, *S. m. malma*, is distributed from the Mackenzie River to the north side of the Alaska Peninsula, while the southern form, *S. m. lordi*, is distributed from the south side of the Alaska Peninsula to Puget Sound. Throughout their range, Dolly Varden exhibit both anadromous and resident life history types. Anadromous life history types rear for up to five years in freshwater before beginning a cycle of entering marine waters to feed during the summer and returning to freshwater to spawn and overwinter during the fall. Most anadromous Dolly Varden return to their natal systems to spawn. Overwintering may occur in natal or non-natal systems. Resident life history types complete their life cycle in freshwater streams, springs, or lakes (Armstrong and Morrow 1980).

Migration patterns have been studied most extensively in anadromous southern form Dolly Varden (Armstrong 1974; Armstrong and Morrow 1980; Bernard et al. 1995; Hepler et al.

1996). A "migration model" for southern form Dolly Varden developed by Armstrong (1974) and tested by Bernard et al. (1995) suggests that adults return to their natal streams each year to spawn, but adults and immature fish return to their natal system to overwinter only if it contains a lake. Dolly Varden from natal streams without lakes search for a system containing a lake for overwintering after their first seaward migration, and then show fidelity to that system for overwintering in subsequent years. Northern form Dolly Varden also return to natal streams to spawn, but may not spawn every year (Armstrong and Morrow 1980; DeCicco 1997). Spawners may not undergo a marine migration in the months prior to spawning. Unlike the southern form, northern form Dolly Varden are not dependent on systems with lakes for overwintering and may undertake more extensive migrations between overwintering and spawning areas. For example, non-spawning Dolly Varden tagged in the Wulik River north of Kotzebue Sound have been recovered not only in other rivers of Kotzebue Sound, but also in Norton Sound, St. Lawrence Island, and several locations in the Russian Far East (DeCicco 1997). Tagging data clearly show that overwintering populations in northwestern Alaska contain individuals from a wide variety of geographic areas, although the degree of mixing is not known (DeCicco 1997).

The migratory behavior and life history of northern form Dolly Varden in the Togiak River drainage have been described using data from radio telemetry, tagging, and otolith microchemistry (Lisac and Nelle 2000; Reynolds 2000). The Dolly Varden studied were anadromous and matured between ages 2-8, primarily at age 4. They first entered the ocean or estuarine environment at age 0-3, primarily at age 2. Dolly Varden returned to the Togiak River from saltwater from late July to mid-August. Pre-spawning Dolly Varden returned earlier than immature and most non-spawning Dolly Varden. Dolly Varden entered tributary streams throughout August, and spawned in tributaries from September through mid-October. Dolly

Varden overwintered in mixed aggregates in the mainstem of the Togiak River and Togiak Lake. Evidence from radio telemetry and tagging studies suggests that Dolly Varden in the Togiak River return to tributaries used the previous year for spawning and that at least some Dolly Varden return to the Togiak River to overwinter. However, the degree of population subdivision among Dolly Varden spawning in the Togiak River drainage and the extent that non-Togiak River Dolly Varden use the Togiak River for overwintering are unknown.

Residents of Bristol Bay communities typically harvest Dolly Varden in the lower Togiak River during outmigration in the spring and immigration in the fall. Subsistence harvests are also taken from lakes in the winter and incidentally during commercial and subsistence fisheries for Pacific salmon in the summer. Therefore, harvests generally occur at times when Dolly Varden populations are mixed. We developed genetic markers as a method to test for population subdivision among Dolly Varden spawning aggregations in the Togiak River drainage and to estimate the population composition of overwintering aggregations and subsistence harvests.

Allozyme data have been used to quantify reproductive isolation among Dolly Varden populations in Arctic Alaska and Russia (Pustovoit 1991; Everret et al. 1997) and to estimate the origin of anadromous Dolly Varden sampled in the Beaufort Sea (Krueger et al. 1999). In this study, we used microsatellite markers, a class of highly polymorphic nuclear DNA markers characterized by repeating sequences 1-6 base pairs in length. Microsatellites and allozymes generally provide concordant estimates of population subdivision (Scribner et al. 1998; Olsen et al. 2000; Allendorf and Seeb 2000). However, more microsatellite loci can be resolved in samples collected using nonlethal techniques than allozyme loci, and microsatellite loci are often more polymorphic, potentially providing enough statistical power to estimate the origin of individual fish (Olsen et al. 2000; Banks et al. 2000). Though microsatellites have been used to

estimate interspecific (Bernatchez et al. 1997; Brunner et al. 1998; Spruell et al. 1999; Castric et al. 2001) and intraspecific (Leder 2001; Taylor et al. 1999) relationships in *Salvelinus*, only a handful of microsatellite loci have been developed specifically for *Salvelinus* species (Angers et al. 1995; E. B. Taylor, University of British Columbia, unpublished) and none for Dolly Varden. Application of loci developed for other species can be problematic (Brunner et al. 1998).

OBJECTIVES

Our objectives for this study were to:

1) Develop 8-10 genetic markers specific to Dolly Varden and suitable for assessing population structure in conjunction with study 00-001, *Genetic diversity of Dolly Varden populations in Norton and Kotzebue Sounds, and of Arctic char populations in the Noatak National Preserve.* This is expected to include one mitochondrial DNA marker plus 7-9 microsatellites.

2) Collect tissue samples for genetic work from three tributaries of the Togiak River.

3) Characterize population structure using the genetic markers from Objective 1.

4) Construct genetic baselines and test performance with computer simulations. Assess performance within Togiak River and northwestern Alaska as well as between these two regions.

5) Report the potential for using the genetic markers developed for Dolly Varden population structure assessments, and make recommendations for further baseline development and mixed-stock analyses.

METHODS

Study Area

The Togiak River drainage encompasses 5,178 km^2 in the Togiak National Wildlife Refuge in southwest Alaska (Figure 1). The Togiak River is 93km in length from the outlet of Togiak Lake to Togiak Bay, and ranges in width from 80 to over 120 m. Togiak Lake is approximately 22 km long and is fed by Izavieknik River, and Trail, Sunday, Bruin, Jondik, Truman, and Ougamautamuk Creeks. Six major tributaries enter the Togiak River downstream of Togiak Lake: Gechiak Creek, Pungokepuk Creek, Kashaiak (Nayorurun) River, Kemuk River, Ongivinuck River, and Kipnuktuli Creek. Nine headwater lakes are associated with these tributaries.

Field Collection

Dolly Varden fin tissue was collected from three known spawning aggregates in two Togiak River tributaries, Kashaiak and Ongivinuck Rivers, and one Togiak Lake tributary, Trail Creek (Figure 1; Lisac and Nelle 2000). Collections for the Kashaiak and Ongivinuck Rivers were made from 25 to 27 September 2000 and for Trail Creek from 28 August to 1 September and 25 to 27 September 2000. Dolly Varden were captured using seines, gill nets, or hook and line. Only Dolly Varden in prespawning, spawning or post- spawning condition were sampled for genetic analysis. Fin clips were stored in 90% ethanol. Gender, maturity index, and location and date of sampling were recorded for each fish. Fin clips collected from Dolly Varden in these tributaries in 1998 were also included to increase sample sizes. These samples were taken either from radio-tagged Dolly Varden determined to be prespawners and tracked to these tributaries during the spawning season or mature fish captured in these tributaries in September.

Two Dolly Varden collections from FIS Study 01-136 were included for genetic comparison on a larger spatial scale. Young-of-the-year Dolly Varden were collected from

Cobblestone River north of Norton Sound in July 2001 (N=298) and Kivalina River in Kotzebue Sound in July 2000 (N=200) using baited minnow traps or small dip nets. Young-of the year were collected because of the difficulty of collecting adequate numbers of adults.

Laboratory Analysis

Genetic Identification Services, Inc. (Chatsworth, California, USA) was contracted to construct microsatellite libraries from total genomic DNA isolated from Dolly Varden. Four libraries were enriched for the following repeat motifs: CA, ATG, CATC, and TAGA. The enrichment procedure for the ATG library failed. From the three remaining libraries, 112 clones were sequenced to search for microsatellite loci. Thirty-four primers for regions flanking microsatellite repeats were designed using Oligo 5.1 Primer Analysis Software (Molecular Biology Insights, Cascade, CO). Initial screens indicated seven loci (*Sma-3*, *Sma-5*, *Sma-10*, *Sma-17*, *Sma-21*, *Sma-22*, and *Sma-24*; Table 1) were non-duplicated and amplified in the correct size range with little or no stutter. Furthermore, no evidence of null alleles or linkage was detected among these loci; therefore, they were used in the population-level analysis.

Total genomic DNA was isolated from fin tissues using a quick lysis procedure. PCR amplification of microsatellite loci were carried out in 10ul reaction volumes (approximately 100ng DNA, 1.5 mM $MgCl_2$, 8mM dNTPs, 0.4uM unlabeled/labeled forward primer, and 0.4uM reverse primer) using an MJResearch thermocycler. Cycling conditions were 2 min 92^{o}C; 30 cycles of 15 sec 92^{o}C, 15 sec T_a (Table 1), and 30 sec 72^{o}C; with a final extension for 10 min 72^{o}. Microsatellites were separated on 64-well denaturing polyacrylamide gels with Li-Cor 50-350bp size standards loaded every 14 lanes and at least one positive control (individual of known

genotype). Genotypes were scored using a Li-Cor scanner and the computer program Saga GT ver 2.0 (Lincoln, NE).

Though assay of variation in the mitochondrial genome was originally proposed, we focused on development of microsatellite loci instead. The mitochondrial genome is clonally (maternally) inherited making it a valuable marker in the recovery of maternal lineages, the analysis of hybrid zones, and the analysis of gender-specific dispersal patterns (Avise 2000). However, all mitochondrial DNA variation must be treated as a single locus, reducing the power of this marker type in mixed-stock analysis (Smouse et al. 1994).

Diversity Within Populations

Numbers of alleles observed per locus and observed and expected heterozygosities were calculated to describe within-population diversity. Allele frequencies from samples collected in tributaries in different years were compared using the exact test for sample differentiation in GENEPOP ver 3.3 (Raymond and Rousset 1995) and were combined if no significant differences existed. Genotype frequencies for each locus in each tributary sample were tested for random mating using the exact test for conformation to Hardy-Weinberg equilibrium in GENEPOP ver 3.3 (Raymond and Rousset 1995). Significance of the *p*-values was evaluated by adjusting the table-wide $\alpha = 0.05$ for 30 multiple tests using the sequential Bonferroni technique (Rice 1989).

Diversity Among Populations

Differences in allele frequencies among populations were tested using the exact test for sample differentiation in GENEPOP ver 3.3. F_{ST} was computed to quantify the amount of

population subdivision following the method of Weir and Cockheram (1984) using FSTAT 2.9.3 (Goudet 2001). A randomization-based test was used to determine if F_{ST} was greater than zero with the null distribution created through 1000 permutations of multi-locus genotypes among populations.

The Cavalli-Sforza and Edwards chord distance (Cavalli-Sforza and Edwards 1967; formula 9.16, Nei 1987) was calculated between all pairwise combinations of populations using S-Plus 6.0 (Insightful, Inc.; Seattle, WA). The distance measures were used in a metric multidimensional scaling analysis in S-Plus to infer population relationships.

Mixture Analysis

Population contributions to simulated mixtures were estimated using conditional maximum likelihood in SPAM ver3.65 (Debevec et al. 2000) to test whether populations could be identified in mixtures. For each population under study, 1000 artificial mixtures of 400 genotypes were randomly constructed using Hardy-Weinberg expectations from the baseline allele frequencies (Seeb and Crane 1999a). The mean contribution estimate for the population under study should therefore approximate 100% if the population is genetically distinct. Mean contribution estimates and 90% symmetric confidence intervals were calculated from 1000 resamples of the mixture and baseline to account for sampling error in mixture samples and baseline allele frequencies. We pooled alleles in the baseline to decrease the effect of sampling zeros (i.e., alleles present at low frequencies in a population that are not present in the population sample, Paetkau et al. 1995; Cornuet et al. 1999) and rare alleles. Alleles were pooled in the following manner: for each locus, allele distributions for all pairwise combinations of alleles were tested for homogeneity using a chi-square statistic and a *p*-value calculated using the

randomization method of Roff and Bentzen (1989). Allele pairs with the largest *p*-value greater than 0.1000 were pooled, and the procedure repeated until the *p*-values generated were less than 0.1000. In this manner, the dimension of the baseline was reduced without loss of the information content of the full baseline (J. Bromaghin, USFWS, Anchorage, AK, personal communication).

RESULTS

Field Collections

Tissue samples were collected from a total of 217 adult Dolly Varden from the Togiak River drainage in 2000; Kashaiak River (N = 23), Ongivinuck River (N = 89), and Trail Creek (N = 105). Tissue samples from 69 Dolly Varden collected in 1998 were included to increase samples sizes. Final sample sizes for the three tributaries were: Kashaiak River, 51; Ongivinuck River, 119; and Trail Creek, 116.

Diversity Within Populations

No allele frequency heterogeneity was detected between years in collections made for Kashaiak River (P=0.3862), Ongivinuck River (P=0.2936), and Trail Creek (P=0.2367). Therefore, the 1998 and 2000 collections were pooled for these samples for subsequent analyses.

All loci were polymorphic except *Sma-5* (Table 1 and Table 2). However, we retained *Sma-5* in the laboratory analysis because it was polymorphic in two southern form Dolly Varden populations surveyed during the initial phases of marker development. Number of alleles observed at each variable locus ranged from 3 to 38 (Table 1) and mean expected heterozygosity ranged from 0.521 to 0.941.

Six tests of conformation to Hardy-Weinberg expectation had *p*-values < 0.05 (Table 2). When adjusted for 30 simultaneous tests, only *Sma-21* in Ongivinuck River and *Sma-24* in Kivalina River did not conform to Hardy-Weinberg expectations.

Diversity Among Populations

Allele frequencies for all pairwise combinations of collections were significantly different, even after adjustment for simultaneous tests. Therefore, all five collections are considered to represent distinct populations. The loci with the largest frequency differences among Togiak River tributaries were *Sma-17* and *Sma-22*. The loci with the largest frequencies differences among drainages were *Sma-10* and *Sma-21* (Tables 2, 3). The F_{ST} estimate for the Togiak River drainage was small (0.009), but significant (P<0.001). The F_{ST} for all populations was 0.046 (P<0.001; Table 3).

Multidimensional scaling analysis showed large differences between Cobblestone River and Kivalina River and populations in the Togiak River drainage (Figure 2). Trail Creek was spatially distinct from other populations sampled from the Togiak River drainage.

Mixture Analysis

Analysis of simulated mixtures suggests that genetic analyses could be used for identifying the origin of Dolly Varden sampled from population mixtures. Mean estimates of simulated mixtures from Ongivinuck River, Trail Creek, Kivalina River, and Cobblestone River were all greater than 90%, suggesting that mixture estimates for these populations in mixed-fishery applications would be robust (Table 4). The estimate for Kashaiak River was 81%, with 15% being misallocated to the Ongivinuck River.

DISCUSSION

Dolly Varden Population Structure

Identification and conservation of local reproductive units, formed and maintained through natal homing (McQuinn 1997), is essential to the long-term sustainability of a resource (NRC 1996). Lisac and Nelle (2000) tagged prespawning Dolly Varden from five tributaries to the Togiak River in 1998. Of the 26 Dolly Varden that returned to the Togiak River to spawn the following year, most returned to their tributary of capture, indicating fidelity to spawning tributaries. We detected significant population substructuring among all collections, providing further evidence that Dolly Varden home to their natal tributaries to spawn with sufficient frequency to lead to genetic differences among populations. In order to sustain productivity, management actions need to take into account the population structure of Dolly Varden in the Togiak River drainage to maintain genetic variability and possible local adaptations among tributary populations.

Trail Creek, draining into Togiak Lake, was the most divergent population sampled from the Togiak River drainage. The divergence of Trail Creek may be due to its greater geographic isolation from other populations. Altitude and lake size may act as isolating mechanisms for brook trout *S. fontinalis* (Castric et al. 2001) and could be influencing patterns of population subdivision for Dolly Varden in the Togiak River drainage.

Of the six loci and five populations surveyed, the genotypic frequencies for *Sma-21* in Ongivinuck River and *Sma-24* in Kivalina River did not conform to Hardy-Weinberg expectations. These two deviations are only slightly higher than the 1.5 (0.05%) expected by

chance alone. It is unlikely overwintering adults were sampled in the Ongivinuck River. All adults sampled were checked for prespawning condition and Lisac and Nelle (2000) detected few non-spawning (overwintering) Dolly Varden in tributaries to the Togiak River. Lack of conformation to Hardy-Weinberg in the Ongivinuck River samples could be due to chance, the presence of multiple spawning populations within this tributary, "nosing in" of spawners destined to spawn in other tributaries, or non-random sampling. Lack of Hardy-Weinberg conformation of the Kivalina River samples may be due in part to the sampling of related individuals from family groups or because young-of-the-year from multiple populations were sampled.

Comparison to other *Salvelinus* studies

Expected heterozygosities (0.521-0.941, excluding *Sma-5*) were much larger than those observed using allozyme data for Dolly Varden sampled from rivers draining into the Beaufort Sea (0.016-0.052; Everett et al. 1997) and northeast Russia (average 0.01; Pustovoit 1991). This is consistent with the higher mutation rates documented for microsatellite loci (Jarne and Lagoda 1996). The heterozygosities observed in this study were similar to heterozygosities for microsatellite loci observed for anadromous arctic char *S. alpinus* in Labrador, Canada that have a similar life history pattern to anadromous Dolly Varden in Alaska (H_e=0.74-0.83; Bernatchez et al. 1997).

The estimate of F_{ST} (0.046) in this study, though significant, was unexpectedly small given that significant allele frequency differences were detected among all pairs of populations. This F_{ST} value was smaller than estimates for anadromous char from other studies (0.09, Pustovoit 1991; 0.09, Everett et al. 1997; 0.06, Bernatchez et al. 1997). Similarly, levels of

within- and among-population variability in Pacific salmon in western Alaska are often lower than for other geographic regions (Seeb and Crane 1999b; Gharrett et al. 1987). Low levels of population differentiation in western Alaska Dolly Varden may be an effect of recent population expansion in this region. Though glaciers did not cover the majority of western Alaska during the Pleistocene, spawning and overwintering habitat for river systems draining through the Bering land bridge may have been restricted because of periodic sea level and/or climatic changes (Pielou 1991). In addition, F_{ST} estimates using highly polymorphic loci may be downwardly biased because of high mutation rates (Hedrick 1999; Olsen et al. in press).

Mixed-Stock Analysis

Armstrong (1984) suggested that management programs for Dolly Varden should include identification and estimation of harvest rates on Dolly Varden stocks in overwintering areas. The levels of genetic differentiation we detected in this study indicate that genetic analysis may be an efficient method to estimate population contributions to mixed-stock samples of overwintering Dolly Varden. Questions that can be addressed using mixed-stock analysis relevant to fishery management include: 1) the number and relative abundance of non-local populations overwintering in the Togiak River drainage; 2) differences in mixture composition of overwintering aggregates between Togiak River mainstem and Togiak Lake; and 3) temporal stability of mixture composition across years. However, some assumptions must first be met to obtain accurate estimates of population contributions to overwintering mixtures.

An assumption of the conditional maximum likelihood estimator of mixture proportions used in this study is that the allele frequencies for the baseline populations are known without error. The microsatellite loci examined in this study were highly polymorphic, and larger sample

sizes will be needed to adequately meet this assumption. We reduced the effect of sampling error by pooling alleles and accounted for sampling error in the simulation study by resampling the baseline allele frequencies. However, we recommend increasing sample sizes of populations prior to mixture analysis to improve estimate accuracy and precision (Wood et al. 1987).

Another assumption in mixture analysis is that all populations that contribute to a mixture are represented in the baseline (Pella and Milner 1987). Tagging information and genetic data from the Togiak River drainage indicate that at a minimum the baseline should include information from the five major spawning tributaries, Gechiak River, Kashaiak River, Kemuk River, Ongivinuck River, and Trail Creek. We also suggest more extensive sampling throughout the Ongivinuck River and its tributaries to determine if multiple spawning aggregates occur within this tributary.

Tagging information for northern form Dolly Varden from northwestern Alaska suggests that Dolly Varden collections from a wide geographic area should also be included in the baseline; tagged individuals have been recaptured 1,690 km from their marking site (DeCicco 1997). However, the Togiak River is at the southern extreme of the range for northern form Dolly Varden, and migration habits may not be consistent throughout the range (Armstrong and Morrow 1980). Annual spawning, earlier age at sexual maturity, and reliance on lacustrine systems for overwintering may, in part, limit the distances migrated by southern form Dolly Varden. The median migration distance for studies of southern form Dolly Varden spawning yearly is less than 60 km with the longest documented migration of 250 km (Bernard et al. 1995). Dolly Varden in the Togiak drainage appear to spawn in consecutive years; of 26 prespawning Dolly Varden radio tagged in 1998, 23 were relocated in the same spawning tributaries the following year (Lisac and Nelle 2000). Dolly Varden in the Togiak River

drainage mature primarily at four years of age (Lisac and Nelle 2000; Reynolds 2000), while the northern form in northwestern Alaska does not mature until ages 7-9 (Armstrong and Morrow 1980). Given these similarities with the southern form, Dolly Varden from the Togiak River drainage may not undergo as extensive migrations as northern form Dolly Varden from northwestern Alaska. At a minimum, we recommend including representatives from major spawning aggregates in Bristol and Kuskokwim Bays in a baseline for analysis of mixtures in Togiak River drainage.

CONCLUSIONS

1. We developed seven microsatellite loci for use in assessing genetic relationships among Dolly Varden.

3. Using these loci, we detected significant population subdivision among tributary samples from Kashaiak River, Ongivinuck River, and Trail Creek in the Togiak River drainage.

4. Togiak River populations were also genetically distinct from the young-of-the-year collections from the Cobblestone River north of Norton Sound and the Kivalina River north of Kotzebue Sound.

5. The levels of population subdivision observed in this study suggest that genetic data may be used to estimate population contributions to overwintering mixtures once an adequate baseline is developed.

RECOMMENDATIONS

Based on the results of this study, we recommend:

1. Knowledge of genetic differences among tributary populations should be incorporated into management programs to provide for the longterm sustainability of Dolly Varden in the Togiak River drainage.

2. Additional microsatellite loci should be developed from archived Dolly Varden libraries to increase the power to detect population differences and decrease the variance of mixed-stock estimates.

3. A minimum baseline for mixed-stock analysis of mixtures of Dolly Varden sampled from the Togiak River should include major spawning populations in the Togiak River and other drainages in Bristol Bay and Kuskokwim Bay.

4. Mixture estimates could be used to obtain the following information to refine management of Dolly Varden in the Togiak River system: 1) the number and relative abundance of non-natal Dolly Varden overwintering in the Togiak River system; 2) spatial differences in stock mixtures between Togiak River and Togiak Lake; 3) temporal stability of stock mixtures over years; and 4) stock mixtures in harvests.

ACKNOWLEDGEMENTS

We thank R. D. Nelle, Aaron Poetter, Andy Aderman, Kyle Larson, Schuyler Grove, and Scotty Gibbons for their effort collecting samples in the field, and Lynsey Luiten and Cara Krumenaur for assistance with laboratory analysis. We appreciate the reviews by Steve Fried and Jeff Bromaghin; their comments greatly improved the manuscript. We also thank Jeff Bromaghin for help with statistical analysis. The U.S. Fish and Wildlife Service, Office of Subsistence Management, provided funding for this project through the Fisheries Resource Monitoring Program.

LITERATURE CITED

ADFG (Alaska Department of Fish and Game). 2001. Alaska subsistence fisheries: 1999 annual report. Division of Subsistence, Alaska Department of Fish and Game, Juneau.

Allendorf, F. S., and L. W. Seeb. 2000. Concordance of genetic divergence among sockeye salmon populations at allozyme, nuclear DNA, and mitochondrial DNA markers. Evolution 54:640-651.

Angers, B., L. Bernatchez, A. Angers, and L. Desgroseillers. 1995. Specific microsatellite loci for brook char reveal strong population subdivision on a microgeographic scale. Journal of Fish Biology 47 (supplement A): 177-185.

Armstrong, R. H. 1984. Migration of anadromous Dolly Varden charr in Southeastern Alaska-a manager's nightmare. Pages 559-570 in Johnson, L. and B. L. Burns (editors). Proceedings of the International Symposium on Arctic Charr. University of Manitoba Press, Winnipeg.

Armstrong, R. H. 1974. Migration of anadromous Dolly Varden (*Salvelinus malma*) in southeastern Alaska. Journal of the Fisheries Resource Board of Canada 31:435-444.

Armstrong, R. H., and J. E. Morrow. 1980. The Dolly Varden charr, *Salvelinus malma*. Pages 99-140 in Balon, E. (editor). Charrs: Salmonid fishes of the genus Salvelinus, Vol. 1. The Hague, Netherlands, Dr. W. Junk.

Avise, J. C. 2000. Phylogeography: the history and formation of species. Harvard University Press, Cambridge, MA.

BBNA (Bristol Bay Native Association) and ADFG (Alaska Department of Fish and Game). 1996. The harvest and use of freshwater fish in Togiak and Manokotak, 1994-95. Final Report by Natural Resources Department, Bristol Bay Native Association and Division of Subsistence, Alaska Department of Fish and Game, Dillingham.

Banks, M.A., V. K. Rashbrook, M. J. Calavetta, C. A. Dean, and D. Hedgecock. 2000. Analysis of microsatellite DNA resolves genetic structure and diversity of chinook salmon (*Oncorhynchus tshawytscha*) in California's Central Valley. Canadian Journal of Fisheries and Aquatic Sciences 57: 917-925.

Bernard, D. R., K. R. Hepler, J. D. Jones, M. E. Whalen, and D. N. McBride. 1995. Some tests of the "migration hypothesis" for anadromous Dolly Varden (southern form). Transactions of the American Fisheries Society 124:297-307.

Bernatchez, L., J. B. Dempson, and S. Martin 1997. Microsatellite gene diversity analysis in anadromous arctic char, *Salvelinus alpinus*, from Labrador. Canadian Journal of Fisheries and Aquatic Sciences 55: 1264-1272.

Brunner, P. C., M. R. Douglas, and L. Bernatchez. 1998. Microsatellite and mitochondrial DNA assessment of population structure and stocking effects in arctic charr *Salvelinus alpinus* (Teleostei: Salmonidae) from central Alpine lakes. Molecular Ecology 7: 209-223.

Castric, V., F. Bonney, and L. Bernatchez. 2001. Landscape structure and hierarchical genetic diversity in the brook charr, *Salvelinus fontinalis*. Evolution 55: 1016-1028.

Cavalli-Sforza, L. L., and A. W. F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. Evolution 21: 550-570.

Cornuet, J.M., S. Piry, G. Luikart, A. Estoup, and M. Solignac. 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics 153: 1989-2000.

Debevec, E. M., R. B. Gates, M. Masuda, J. Pella, J. Reynolds, and L. W. Seeb. 2000. SPAM (Version 3.2): Statistics program for analyzing mixtures. Journal of Heredity 91: 509-511.

DeCicco, A. L. 1997. Movements of postsmolt anadromous Dolly Varden in northwestern Alaska. American Fisheries Society Symposium 19:175-183.

Everett, R. J., R. L. Wilmot, and C. C. Krueger. 1997. Population genetic structure of Dolly Varden from Beaufort Sea drainages of northern Alaska and Canada. American Fisheries Society Symposium 19: 240-249.

Gharrett, A. J., S. M. Shirley, and G. R. Tromble. 1987. Genetic relationships among populations of Alaskan chinook salmon (*Oncorhynchus tshawytscha*). Canadian Journal of Fisheries and Aquatic Sciences 44: 1987.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.1). Available from http://www.unil.ch/izea/softwares/fstat.html.

Hedrick, P. 1999. Perspectives: highly variable loci and their interpretation in evolution and conservation. Evolution 53: 313-318.

Hepler, K. R., P. A. Hansen, and D. R. Bernard. 1996. Impact of oil spilled from the Exxon Valdez on survival and growth of Dolly Varden and cutthroat trout in Prince William Sound. American Fisheries Society Symposium 18: 645-658.

Jarne, P., and J. L. Lagoda. 1996. Microsatellites, from molecules to populations and back. Trends in Ecology and Evolution 11: 424-429.

Krueger, C. C., R. L. Wilmot, and R. J. Everett. 1999. Stock origins of Dolly Varden collected from Beaufort coastal sites of Arctic Alaska and Canada. Transactions of the American Fisheries Society 128:49-57.

Leder, E. H. 2001 . Phylogenetic analysis of the arctic char-dolly varden species complex. PhD dissertation, University of Wisconsin, Milwaukee.

Lisac, M. J. and R. D. Nelle. 2000. Migratory behavior and seasonal distribution of Dolly Varden *Salvelinus malma* in the Togiak River watershed, Togiak National Wildlife Refuge. Final Report. U.S. Fish and Wildlife Service. Dillingham, Alaska.

McQuinn, I. H. 1997. Metapopulations and the Atlantic herring. Reviews in Fish Biology and Fisheries 7:297-329.

Morrow, J. E. 1980. Analysis of the Dolly Varden charr, *Salvelinus malma*, of northwestern North America and northeastern Siberia. Pages 323-338 in E. K. Balon (editor). Charrs: salmonid fishes of the genus *Salvelinus*. Dr. W. Junk, the Hague, Netherlands.

NRC (National Research Council). 1996. Upstream: salmon and society in the Pacific Northwest. National Academy Press, WA.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Olsen, J. B., P. Bentzen, M. A. Banks, J. B. Shaklee, and S. Young. 2000. Microsatellites reveal population identity of individual pink salmon to allow supportive breeding of a population at risk of extinction. Transactions of the American Fisheries Society 129: 232-242.

Olsen, J. B., C. Habicht, and J. E. Seeb. In press. Moderate and highly polymorphic microsatellites provide discordant estimates of population differentiation in sockeye salmon (*Oncorhynchus nerka*). Environmental Biology of Fishes.

Paetkau, D., W. Calvert, I. Stirling, and C. Strobeck. 1995. Microsatellite analysis of population structure in Canadian polar bears. Molecular Ecology 4:347-354.

Pella, J. J., and G. B. Milner. 1987. Use of genetic marks in stock composition analysis. Pages 247-276 in Ryman, N. and F. Utter (editors). Population genetics and fishery management. University of Washington, Seattle.

Phillips, R. B., L. I. Gudex, K. M. Westrich, and A. L. DeCicco. 1999. Combined phylogenetic analysis of ribosomal ITS1 sequences and new chromosome data supports three subgroups of Dolly Varden char (*Salvelinus malma*). Canadian Journal of Fisheries and Aquatic Sciences 56:1504-1511.

Pielou, E. C. 1991. After the Ice Age: the return of life to glaciated North America. University of Chicago Press, Chicago, IL.

Pustovoit, S.P. 1991. Genetic variation and interpopulational differences in Dolly Varden charr (*Salvelinus malma* W.). Genetika 27: 106-112.

Raymond, M. and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86: 248-249.

Reynolds, J. B. 2000. Life history analysis of Togiak River char through otolith microchemistry. Final Report. Unit Cooperative Agreement 1434-HG-97-RU-01582. Research Work Order 91. University of Alaska, Alaska Cooperative Fish and Wildlife Research Unit, Fairbanks, Alaska.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43: 223-225.

Roff, D. A. and P. Bentzen. 1989. The statistical analysis of mitochondrial DNA: Chi-square and the problem with small samples. Molecular Biology and Evolution 6:539-545.

Scribner, K. T., P. A. Crane, W. J. Spearman, and L. W. Seeb. 1998. DNA and allozyme markers provide concordant estimates of population differentiation: analyses of US and Canadian populations of Yukon River fall-run chum salmon (*Oncorhynchus keta*). Canadian Journal of Fisheries and Aquatic Sciences 55: 1748-1758.

Seeb, L. W**.** and P. A. Crane. 1999a. Allozymes and mitochondrial DNA discriminate Asian and North American populations of chum salmon in mixed-stock fisheries along the south coast of the Alaska Peninsula. Transactions American Fisheries Society 128: 88-103.

Seeb, L. W., and P. A. Crane. 1999b. High genetic heterogeneity in chum salmon in western Alaska, the contact zone between northern and southern lineages. Transactions of the American Fisheries Society 128: 58-87.

Smouse, P. E., C. J. Kobak, and S. Xu. 1994. Some thoughts on information content in allozymes and DNA markers in genetic stock identification. Pages 121-126 in Park, L. K., P. Moran, and R. S. Waples (editors). Application of DNA technology to the management of Pacific salmon: Proceedings of the workshop. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-NWFSC-17, 178 p.

Spruell, P., B. E. Rieman, K. L. Knudsen, F. M. Utter, and F. W. Allendorf. 1999. Genetic population structure within streams: microsatellite analysis of bull trout populations. Ecology of Freshwater Fish 8: 114-121.

Taylor, E. B., S. Pollard, and D. Louie. 1999. Mitochondrial DNA variation in bull trout (*Salvelinus confluentus*) from northwestern North America: implications for zoogeography and conservation. Molecular Ecology 8: 115-1170.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38: 1358-1370.

Wood, C. C., S. McKinnell, T. J. Mulligan, and D. A. Fournier. 1987. Stock identification with the maximum-likelihood mixture model: sensitivity analysis and application to complex problems. Canadian Journal of Fisheries and Aquatic Sciences 44:866-881.

Table 1. Characterization of seven microsatellite loci in five populations; T_a, annealing temperature; H_o, observed heterozygosity; H_e, expected heterozygosity.

Locus	Primer sequence (5'-3')	Repeat Sequence	T_a (^oC)	Size range (bp)	No. of Alleles	H_o	H_e
Sma-3	F: Tgg CTC AAA TTA AgA TCC TAC R:AgC CAT TAT gCA TTA CTT gTT C	$(CA)_3CG(CA)_{11}$	58	120-124	3	0.478	0.521
Sma-5	F: AgA TgT gTg ATA AAC TCA gCC TC R: AgT TgT TTA AAT Agg gCg gAT Ag	$(CA)_7C(CA)_7$	55	90	1	0	0
Sma-10	F: AAA ATg TCT CCC CTC CCT CTC R: TCC CTA ACA TAA CAA gTT TTC ATC CT	$(TCCA)_{16}$	55	140-252	26	0.798	0.839
Sma-17	F: AAg gAT ggT gAg gAC AAT ACA R: ACC TTg AgA AAT CTA TAT gTg gTC TA	$(CA)_{29}$	56	99-141	19	0.759	0.782
Sma-21	F: ggC TgT TCA CCA CAT AgA gTA AT R: TTA AgA Tgg gAT gCA TAT TCA gT	$(TC)_4TTTC(TC)_{21}$	56	105-149	15	0.666	0.674
Sma-22	F: CCC AAT gCA gAT AAg ACC TT R: TCT ATA ggC TTA TTT gAA Tgg AAT	$(TAGA)_{19}$	55	152-264	27	0.905	0.909
Sma-24	F: CAT TgA TCA AgA AgC CAg TgC R: TgT ATT Tgg CCA ATA TAA CAC AgC	$(TATC)_{33}$	56	158-310	38	0.882	0.941

Table 2. Allele frequency estimates, observed heterozygosity (H_o), expected heterozygosity (H_e), and probability of conformation of genotype frequencies to Hardy-Weinberg expectations (P) for seven microsatellite loci from five populations. Significant deviations from Hardy-Weinberg expectations, after correction for multiple tests with the sequential Bonferroni procedure, are indicated with an asterisk (*).

Locus	Kashaiak River	Ongivinuck River	Trail Creek	Cobblestone River	Kivalina River
Sma-3					
N	48	103	99	292	168
120	0.448	0.437	0.515	0.483	0.354
122	0.531	0.534	0.475	0.479	0.604
124	0.021	0.029	0.01	0.038	0.042
H_o	0.458	0.447	0.424	0.545	0.518
H_e	0.523	0.526	0.512	0.536	0.509
P(HW)	0.6961	0.2658	0.0593	0.7895	0.2756
Sma-5					
N	50	102	113	281	173
90	1.000	1.000	1.000	1.000	1.000
Sma-10					
N	50	100	114	290	166
140	0.000	0.000	0.000	0.005	0.000
144	0.000	0.000	0.004	0.002	0.000
148	0.020	0.010	0.092	0.005	0.000
152	0.060	0.055	0.066	0.036	0.030
156	0.260	0.215	0.145	0.012	0.000
160	0.150	0.250	0.154	0.047	0.048
164	0.030	0.055	0.070	0.019	0.006
168	0.080	0.100	0.096	0.083	0.060
172	0.160	0.095	0.088	0.417	0.223
176	0.050	0.055	0.026	0.162	0.084
180	0.040	0.050	0.096	0.019	0.057
184	0.070	0.040	0.057	0.002	0.003
188	0.010	0.025	0.022	0.029	0.012
192	0.000	0.005	0.000	0.062	0.401
196	0.010	0.025	0.000	0.052	0.015
200	0.000	0.005	0.000	0.007	0.018
204	0.010	0.000	0.000	0.002	0.006
208	0.010	0.000	0.000	0.000	0.012
212	0.010	0.015	0.048	0.002	0.000
224	0.010	0.000	0.000	0.000	0.003
228	0.000	0.000	0.009	0.000	0.012
232	0.020	0.000	0.000	0.000	0.000
236	0.000	0.000	0.004	0.002	0.009
240	0.000	0.000	0.013	0.003	0.000
244	0.000	0.000	0.004	0.033	0.000
252	0.000	0.000	0.004	0.000	0.000

Table 2. Continued.

Locus	Kashaiak River	Ongivinuck River	Trail Creek	Cobblestone River	Kivalina River
Sma-10					
H_o	0.780	0.800	0.842	0.766	0.801
H_e	0.873	0.862	0.909	0.781	0.774
P(HW)	0.0197	0.0562	0.0763	0.3725	0.2681
Sma-17					
N	49	116	112	284	154
99	0.000	0.000	0.000	0.000	0.013
101	0.010	0.022	0.000	0.000	0.000
105	0.020	0.034	0.080	0.011	0.000
107	0.000	0.000	0.000	0.021	0.000
109	0.041	0.043	0.018	0.000	0.000
111	0.041	0.065	0.085	0.014	0.032
113	0.031	0.004	0.018	0.002	0.055
115	0.092	0.108	0.143	0.065	0.049
117	0.061	0.004	0.022	0.185	0.026
119	0.020	0.043	0.107	0.019	0.000
121	0.031	0.047	0.067	0.118	0.065
123	0.000	0.013	0.018	0.004	0.000
125	0.082	0.043	0.058	0.007	0.097
127	0.408	0.440	0.214	0.440	0.516
129	0.112	0.099	0.121	0.109	0.088
131	0.051	0.026	0.040	0.005	0.045
133	0.000	0.000	0.000	0.000	0.013
135	0.000	0.000	0.004	0.000	0.000
141	0.000	0.009	0.004	0.000	0.000
H_o	0.694	0.767	0.911	0.729	0.695
H_e	0.803	0.774	0.887	0.742	0.705
P(HW)	0.0082	0.529	0.2628	0.1867	0.0269

Table 2. Continued.

Locus	Kashaiak River	Ongivinuck River	Trail Creek	Cobblestone River	Kivalina River
Sma-21					
N	50	113	112	278	169
105	0.000	0.000	0.009	0.000	0.000
115	0.130	0.124	0.085	0.223	0.388
117	0.170	0.146	0.161	0.011	0.000
123	0.040	0.000	0.000	0.000	0.000
125	0.000	0.000	0.000	0.007	0.000
127	0.520	0.602	0.607	0.369	0.275
131	0.050	0.044	0.031	0.022	0.009
133	0.060	0.066	0.045	0.122	0.160
135	0.010	0.000	0.000	0.212	0.080
137	0.000	0.000	0.000	0.007	0.068
139	0.000	0.000	0.000	0.025	0.003
141	0.000	0.004	0.036	0.002	0.000
143	0.000	0.000	0.022	0.000	0.000
145	0.020	0.009	0.000	0.000	0.018
149	0.000	0.004	0.004	0.000	0.000
H_o	0.66	0.549	0.652	0.741	0.728
H_e	0.683	0.598	0.596	0.754	0.739
P(HW)	0.2936	0.0003*	0.0578	0.098	0.0602

Table 2. Continued.

Locus	Kashaiak River	Ongivinuck River	Trail Creek	Cobblestone River	Kivalina River
Sma-22					
N	51	118	109	289	160
152	0.000	0.000	0.000	0.000	0.006
160	0.000	0.004	0.005	0.002	0.016
164	0.000	0.000	0.023	0.055	0.000
168	0.010	0.000	0.023	0.019	0.000
172	0.020	0.025	0.000	0.005	0.000
176	0.020	0.034	0.069	0.003	0.025
180	0.010	0.055	0.032	0.014	0.075
184	0.069	0.081	0.050	0.035	0.188
188	0.059	0.097	0.055	0.069	0.047
192	0.196	0.123	0.046	0.057	0.031
196	0.108	0.097	0.078	0.121	0.097
200	0.098	0.102	0.101	0.048	0.125
204	0.039	0.097	0.110	0.187	0.144
208	0.078	0.034	0.161	0.116	0.022
212	0.088	0.072	0.060	0.040	0.181
216	0.078	0.030	0.032	0.017	0.022
220	0.010	0.034	0.028	0.145	0.016
224	0.039	0.051	0.032	0.010	0.003
228	0.049	0.047	0.023	0.017	0.003
232	0.029	0.004	0.023	0.019	0.000
236	0.000	0.004	0.005	0.005	0.000
240	0.000	0.000	0.014	0.014	0.000
244	0.000	0.000	0.014	0.000	0.000
248	0.000	0.004	0.005	0.000	0.000
252	0.000	0.000	0.009	0.000	0.000
256	0.000	0.004	0.000	0.000	0.000
264	0.000	0.000	0.005	0.000	0.000
H_o	0.882	0.966	0.908	0.9	0.869
H_e	0.914	0.925	0.928	0.899	0.878
P(HW)	0.4228	0.8012	0.065	0.0695	0.4116

Table 2. Continued.

Locus		Kashaiak River	Ongivinuck River	Trail Creek	Cobblestone River	Kivalina River
	Sma-24					
	N	51	117	109	291	154
	158	0.000	0.000	0.000	0.002	0.003
	162	0.000	0.000	0.000	0.000	0.010
	170	0.010	0.000	0.000	0.000	0.000
	174	0.010	0.000	0.000	0.010	0.019
	178	0.000	0.000	0.023	0.022	0.000
	182	0.000	0.000	0.005	0.007	0.000
	186	0.010	0.000	0.000	0.033	0.010
	190	0.010	0.034	0.005	0.002	0.019
	194	0.020	0.030	0.009	0.005	0.016
	198	0.029	0.026	0.000	0.033	0.000
	202	0.069	0.009	0.018	0.007	0.065
	206	0.010	0.017	0.014	0.000	0.029
	210	0.039	0.021	0.037	0.007	0.006
	214	0.059	0.021	0.060	0.019	0.019
	218	0.029	0.026	0.046	0.103	0.019
	222	0.088	0.051	0.087	0.017	0.000
	226	0.049	0.038	0.055	0.031	0.026
	230	0.020	0.068	0.073	0.062	0.013
	234	0.049	0.038	0.032	0.007	0.023
	238	0.039	0.068	0.009	0.082	0.104
	242	0.020	0.060	0.023	0.026	0.071
	246	0.039	0.051	0.083	0.021	0.036
	250	0.059	0.111	0.064	0.050	0.117
	254	0.069	0.077	0.060	0.141	0.084
	258	0.049	0.038	0.060	0.024	0.094
	262	0.049	0.030	0.060	0.165	0.016
	266	0.069	0.038	0.032	0.024	0.149
	270	0.029	0.073	0.023	0.009	0.026
	274	0.029	0.009	0.037	0.000	0.019
	278	0.010	0.013	0.023	0.077	0.003
	282	0.010	0.000	0.018	0.003	0.000
	286	0.020	0.026	0.009	0.005	0.000
	290	0.000	0.004	0.014	0.003	0.000
	294	0.010	0.013	0.000	0.000	0.000
	298	0.000	0.000	0.014	0.000	0.000
	302	0.000	0.004	0.005	0.000	0.000
	306	0.000	0.000	0.005	0.003	0.000
	310	0.000	0.004	0.000	0.000	0.000
	H_o	0.922	0.906	0.945	0.897	0.74
	H_e	0.961	0.95	0.953	0.918	0.925
	P(HW)	0.2303	0.045	0.1585	0.1073	<0.0001*

Table 3. Estimates of F_{ST} for six microsatellite loci.

Locus	Togiak River populations (N=3)	All populations (N=5)
Sma-3	0.000	0.012
Sma-10	0.009	0.095
Sma-17	0.023	0.035
Sma-21	0.000	0.078
Sma-22	0.011	0.027
Sma-24	0.004	0.022
overall	0.009	0.046

Table 4. Contribution estimates to simulated mixtures of Dolly Varden where each mixture is composed only of genotypes from the population tributary under study. Mean estimates and 90% symmetric confidence intervals are from 1000 resamples of the mixture and the baseline.

	Mixture														
	Kashaiak			Ongivinuck			Trail			Cobblestone			Kivalina		
		90% CI			90% CI			90% CI			90% CI			90% CI	
Origin	Mean Est.	Low	High	Mean Est.	Low	High	Mean Est.	Low	High	Mean Est.	Low	High	Mean Est.	Low	High
Kashaiak	0.805	0.674	0.935	0.036	0.000	0.094	0.005	0.000	0.026	0.001	0.000	0.007	0.001	0.000	0.006
Ongivinuck	0.149	0.051	0.251	0.929	0.858	0.988	0.019	0.000	0.059	0.001	0.000	0.005	0.002	0.000	0.011
Trail	0.024	0.000	0.071	0.024	0.000	0.062	0.971	0.921	1.000	0.002	0.000	0.008	0.000	0.000	0.000
Cobblestone	0.010	0.000	0.029	0.006	0.000	0.020	0.004	0.000	0.017	0.991	0.976	1.000	0.005	0.000	0.020
Kivalina	0.004	0.000	0.018	0.003	0.000	0.011	0.000	0.000	0.000	0.004	0.000	0.013	0.991	0.969	1.000

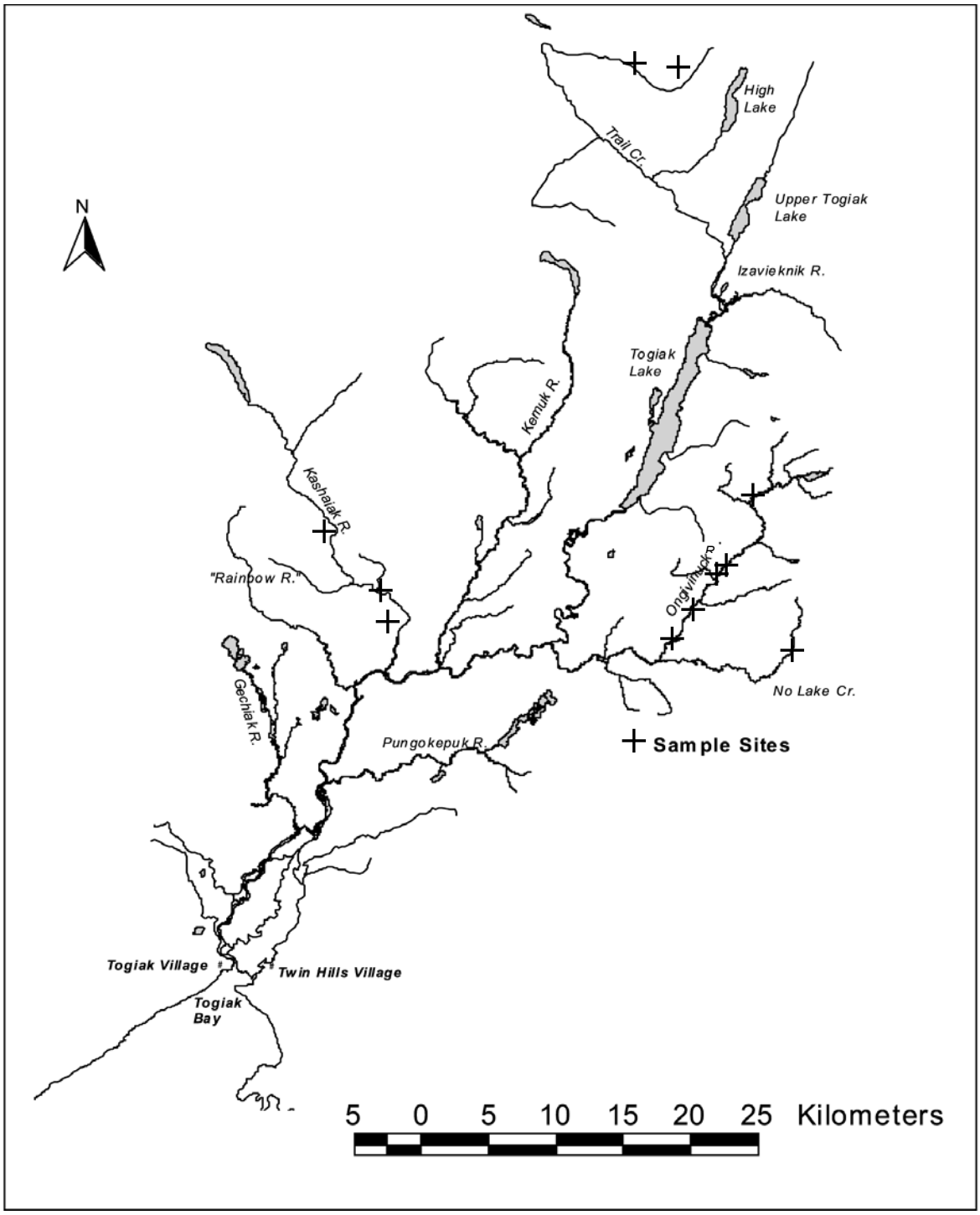

Figure 1. Major tributaries to the Togiak River and approximate sampling areas for Dolly Varden collected in the Kashaiak River, Ongivinuck River, and Trail Creek in 2000.

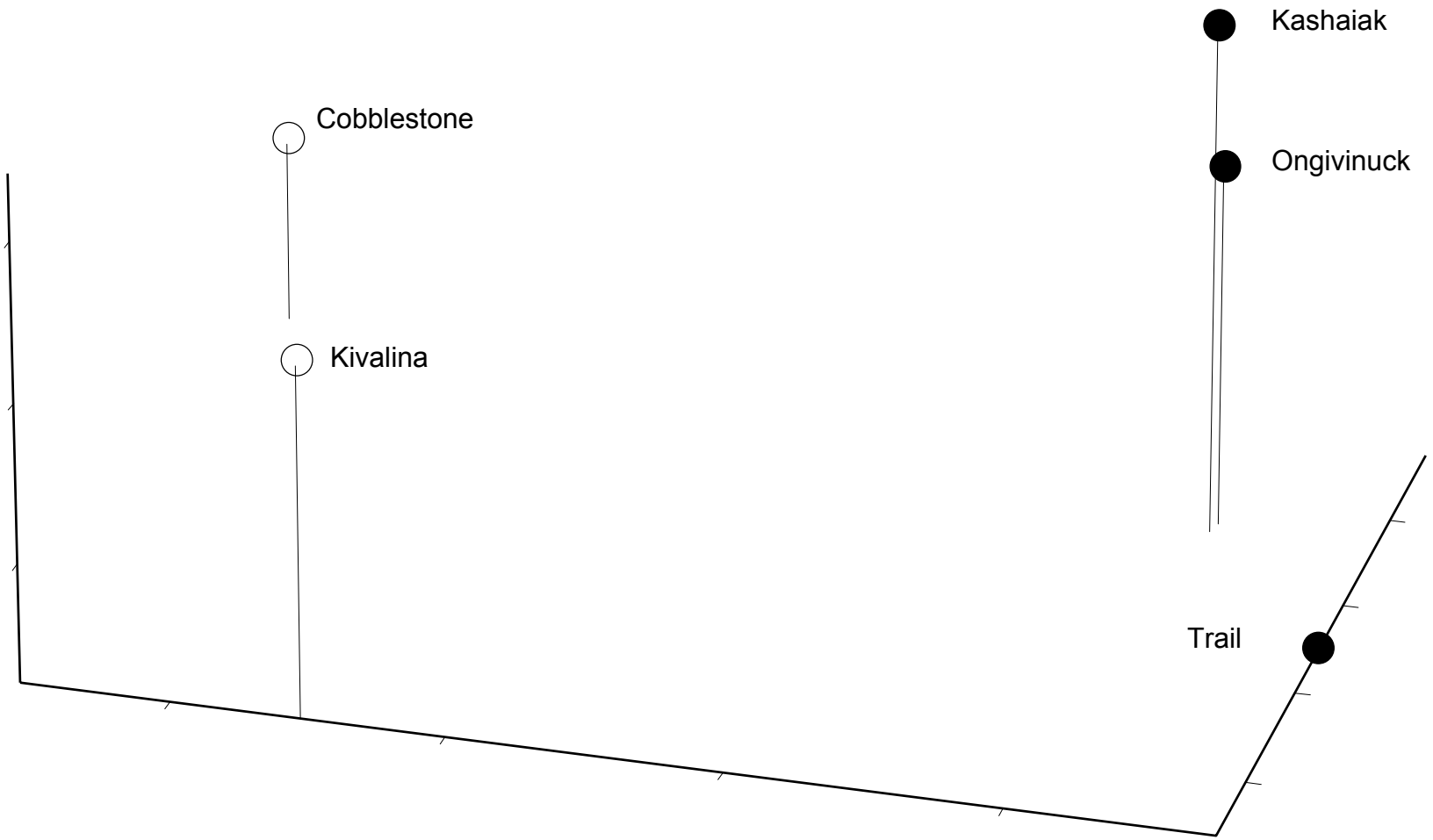

Figure 2. Multidimensional scaling of anadromous Dolly Varden from the Togiak River drainage (solid circles), Norton and Kotzebue Sounds (open circles).