Genetic relationships of rainbow trout *Oncorhynchus mykiss* on Togiak National Wildlife Refuge, Alaska

2012 Progress Report

Patrick Walsh, Jeffrey Olsen, John Wenburg, Jason Dye, and Craig Schwanke

Togiak National Wildlife Refuge
Dillingham, Alaska;
Conservation Genetics Laboratory
Anchorage, Alaska;
Alaska Department of Fish and Game
Dillingham, Alaska
April 2013
Citation:


Keywords:

*Oncorhynchus mykiss*, Togiak National Wildlife Refuge, southwest Alaska

Disclaimer: The use of trade names of commercial products in this report does not constitute endorsement or recommendation for use by the federal government.
Genetic relationships of rainbow trout *Oncorhynchus mykiss* on Togiak National Wildlife Refuge, Alaska

2012 Progress Report

Patrick Walsh, Jeffrey Olsen, John Wenburg, Jason Dye, and Craig Schwanke

**Abstract**

We completed the fourth and final year of field work in a project to inventory populations of rainbow trout *Oncorhynchus mykiss* in the vicinity of the Togiak National Wildlife Refuge, southwestern Alaska. We have inventoried and identified all available archived genetic tissue samples collected during other studies since 1986. We identified river systems in which additional sampling is necessary. We collected 1,207 tissue samples from rainbow trout occurring in 19 streams or rivers located in five watersheds during the summers of 2009 -- 2012.

**Keywords:** *Oncorhynchus mykiss*, Togiak National Wildlife Refuge, southwestern Alaska

**Introduction**

This project is focused on understanding the genetic relationships of rainbow trout *Oncorhynchus mykiss* populations occurring in the vicinity of Togiak National Wildlife Refuge. Conservation of biological diversity is a primary, congressionally-mandated purpose of Togiak Refuge, and populations are arguably the most important organizational unit of biodiversity because they maintain and accumulate genetically based adaptations that enhance species survival (Krueger et al. 1999). Management at the population level is particularly relevant for non-anadromous fish, given their limited dispersal capabilities and restricted ranges.

---

1 **Authors:** Patrick Walsh, U. S. Fish and Wildlife Service, Togiak National Wildlife Refuge, P.O. Box 270, Dillingham, AK 99576. (907) 842-1063, Patrick_Walsh@fws.gov. Jeffrey Olsen, Conservation Genetics Laboratory, U.S. Fish and Wildlife Service, 1011 E. Tudor Rd., Anchorage, AK 99503. (907) 786-3858. John Wenburg, Conservation Genetics Laboratory, U.S. Fish and Wildlife Service, 1011 E. Tudor Rd., Anchorage, AK 99503. (907) 786-3858. Jason Dye, Alaska Department of Fish and Game, Division of Sport Fish, P.O. Box 1030, Dillingham, AK 99576. (907) 842-2427, jason.dye@alaska.gov. Craig Schwanke, Alaska Department of Fish and Game, Division of Sport Fish, P.O. Box 1030, Dillingham, AK 99576. (907) 842-2427, craig.schwanke@alaska.gov.
To address this issue, there has been a single limited study which examined genetic relationships between six southwest Alaska rainbow trout populations, four of which occurred in three Togiak Refuge watersheds (Krueger et al. 1999). The study was based on mitochondrial DNA, and found unexpected results, such as closer genetic relationships between rainbow trout in the Arolik and Gechiak Rivers (which are in different watersheds) than the Gechiak and Pungokepuk Rivers, (which are in the same watershed). The study recommended that it be considered a starting point in the definition of rainbow trout populations refuge-wide, and that populations serve as the conservation unit of interest to management. More advanced genetic methods are now available than those that existed previously, and so a more comprehensive study will support or refute the previous findings, as well as more thoroughly identify the number and distribution of distinct populations. Knowledge of these metapopulation relationships will aid management in determining appropriate harvest strategies, and will serve as a basis for determining appropriate geographic ranges for future studies, such as abundance estimates.

Objectives
1. Determine the relationships and degree of genetic exchange of rainbow trout within and among the watersheds of Togiak National Wildlife Refuge.
2. Inventory the populations of rainbow trout occurring on Togiak Refuge.

Justification
Rainbow trout are found throughout Togiak National Wildlife Refuge and are an important sport and subsistence resource. Anglers reported catching an average of 33,566 rainbow trout annually 2003-2007 in four local river drainages (Togiak, Arolik, Kanektok, and Wood; Jennings et al. 2006, 2007, and in prep.). The Refuge Fisheries Management Plan (U.S. Fish and Wildlife Service 1990) identified rainbow trout as an ecological indicator species. This project relates directly to two of the purposes for which Togiak Refuge was created: conserving fish and wildlife populations and habitats in their natural diversity; and, providing the opportunity for continued subsistence uses by local residents. Further, title VIII of ANILCA (section 812 / 16 USC 3122) states that "the Secretary, in cooperation with the State and other appropriate Federal agencies, shall undertake research on fish and wildlife and subsistence uses on the public lands; seek data from, consult with and make use of, the special knowledge of local residents engaged in subsistence uses; and make the results of such research available to the State, the local and regional councils established by the Secretary or State pursuant to section 805, and other appropriate persons and organizations". The 2002 Togiak Refuge Biological Program Review gave medium priority to defining populations of Togiak Refuge rainbow trout (Heglund and Taylor 2005). This project is expected to take four years of field work beginning in the spring of 2009, followed by a year of data analysis and reporting.
Study area and Background

Overview
The study area includes Togiak Refuge, plus the adjacent areas to a distance of approximately 100 km beyond the refuge borders. This surrounding area includes the adjacent large watersheds of which small portions are included within refuge borders (Fig. 1).

Togiak Refuge is a ~1.7 million ha federal conservation unit located at the confluence of the Bristol and Kuskokwim Bays of the Bering Sea. It extends inland for approximately 100 km creating a range of climate characteristics from subarctic maritime near the coastal areas to subarctic continental toward the interior. Temperatures range from average daily low and high of -11.9 and -5.8°C in December, the coldest month, to 9.1 and 16.1°C in July, the warmest month at Dillingham, the nearest weather station (climate data averaged 1971-2000, National Oceanic and Atmospheric Administration 2008). Annual precipitation averages 64.5 cm and snowfall averages 210.6 cm.

The Togiak Refuge includes all or portions of 35 major rivers, 25 major lakes, and hundreds of smaller lakes, ponds, and streams (USFWS 1990). The Ahklun Mountains occupy the central portion of the refuge while the Nushagak and Kanektok River lowlands occur to the northwest and southeast. The modern-day landscape, and thus the distribution of rainbow trout within this landscape, is strongly influenced by glacial activity in the late Pleistocene (Walsh et al. 2006).

Figure 1. Study area topography.
Rainbow trout distribution:
Fish species inventories have been performed throughout Togiak Refuge for the past two decades to understand species distribution. The refuge includes all or most of 12 large watersheds, and rainbow trout occur in all but 5 of them (Fig. 2). Additionally, the Refuge includes small portions of an additional five large watersheds (Eek, Kwethluk, Nushagak, Wood, and Snake) occurring primarily to the north and east of the Refuge. Rainbow trout have been documented in all of these except Eek watershed. The Indian River watershed, located outside the Togiak Refuge boundary on the west, has also been reported to include rainbow trout.

Figure 2. Major watersheds of the Togiak National Wildlife Refuge. Rainbow trout populations have been documented in watersheds colored green.

The known or suspected rainbow trout populations in the Togiak Refuge vicinity include:

1. **Togiak River watershed.** Draining approximately 4,594 km², this is the largest watershed on Togiak Refuge, and is almost wholly enclosed within the refuge boundary. Rainbow trout are well distributed in this watershed, and have been documented in the mainstem Togiak River, as well as in the following second-order streams: Pungokepuk, Gechiak, and Kashiaak Creeks, and Ongivinuck and Kemuk Rivers (Lisac and MacDonald 1996, MacDonald 1997, Nelle and Lisac 2001). Of these various locations, springtime collections have occurred only in
Pungokepuk and Gechiak Creeks, suggesting that spawning occurs in these locations. Krueger et al. (1999) found genetically distinct groups in these two rivers, suggesting separate populations. Nelle and Lisac (2001) described the seasonal movements of rainbow trout in these rivers, and found little mixing of individuals from the two rivers, corroborating this finding. Rainbow trout have not been documented to occur in the Togiak drainage upstream of the confluence of the main Togiak and the Ongivinuck River (Lisac and MacDonald 1996).

2. **Goodnews River watershed**. At 2,732 km\(^2\) in area, the Goodnews River watershed is the second largest on Togiak Refuge, although about a third of this watershed occurs outside of the refuge. Rainbow trout have been documented in all four of the principal branches of this river system: North Fork, Middle Fork, and South Fork Goodnews River and Kukaktlik River (Alt 1986, Irving and Faustini 1994, Faustini 1996, Nelle 2002a).

3. **Kanektok River watershed**. The Kanektok River watershed is approximately 2,292 km\(^2\) in area and almost wholly enclosed within the refuge boundary. As with the Goodnews River watershed, rainbow trout appear to be widely distributed here as well (Wagner 1991, Adams 1996, Larson 2008). Adams (1996) investigated movements, and found that in spring all radioed rainbow trout were located in the mainstem river, suggesting that tributaries may not be important spawning areas.

4. **Igushik watershed**. This watershed is 2,066 km\(^2\) in area. It contains two large lakes (Amanka and Ualik) and four large second order streams (Tuklung and Ongoke Rivers, and Francis and Longarm Creeks). Rainbow trout are present, but apparently not abundant in this watershed. Rainbow trout have been documented on two occasions in this watershed. In 1997, a single individual was captured in the Ongoke River during a 4-day survey (MacDonald and Lisac 1998), and in 2004, five individuals were captured in the lower Ongoke River during a 10-day survey of Amanka Lake and associated streams (Gwinn 2005a). No rainbow trout were encountered during a 3-day survey in the Ongoke River in 1996 (MacDonald 1997).

5. **Arolik River watershed**. This watershed is 1,381 km\(^2\) in area and is located between the Kanektok and Goodnews River watersheds. Rainbow trout have been well documented in the mainstem and East Fork Arolik Rivers (Lisac and MacDonald 1995, MacDonald 1997, MacDonald and Lisac 1998).

6. **Ungalikthluk River watershed**. This watershed is 585 km\(^2\) in area and includes one major second-order stream, the Negukthlik River. Rainbow trout have been well documented in both rivers (Lisac 1996, Gwinn 2005b, 2005c). Based on radio telemetry locations, Lisac (1996) hypothesized that this system is composed of a single population of rainbow trout, and that spawning occurs in the Negukthlik River.

7. **Osviak River watershed**. This small watershed (514 km\(^2\)) is located between four watersheds where rainbow trout have not been documented: the Quigmy and Matogak River watersheds to the east and the Slug and Kinegnak River watersheds to the west. However, rainbow trout have been well documented in the Osviak (MacDonald 1997, MacDonald and Lisac 1998, Nelle 2002b).

8. **Nushagak River watershed**. At 31,477 km\(^2\) this is largest watershed in the vicinity of Togiak Refuge, although all but a small fraction occurs outside of the Refuge.
boundary. This watershed includes over 20 major tributary streams. Rainbow trout are well documented throughout this watershed with the largest numbers occurring in the mainstem Nushagak River upstream of its confluence with Harris Creek and in second-order tributaries including the Kokwok, Mulchatna, Nuyakuk, and King Salmon rivers (Schwanke unpublished, Schwanke 2007).

9. Kwethluk River watershed. This watershed is 3,391 km² in area and is largely located outside of the Togiak Refuge boundary to the north. Rainbow trout occur in this watershed (D. Gillikin, personal communication, Yukon Delta National Wildlife Refuge).

10. Snake River watershed. This watershed is 1,168 km² in area, approximately half of which is located within Togiak Refuge. The watershed includes one large lake (Nunavugaluk Lake) and a single large second-order stream, the Weary River. Local residents report that rainbow trout currently occur in the upper Snake River and Nunavugaluk Lake, and that their occurrence is relatively recent (A. Aderman, personal communication, Togiak National Wildlife Refuge). Local lore holds that this population is the result of an intentional release of rainbow trout that originated from Ice Creek, a tributary to Lake Aleknagik of the Wood River watershed. Thus, this forms a testable hypothesis.

11. Wood River watershed. This watershed is 3,578 km² in area, and outside the Togiak Refuge boundary except for small portions of mountainous, headwater streams. The watershed includes six large lakes (Aleknagik, Nerka, Little Togiak Beverly, Kulik, and Grant), six interconnecting rivers, and numerous streams draining into the lakes. Rainbow trout have been documented throughout this watershed with the exception of Grant Lake due to an impassable waterfall (Dunaway 1998, Dye 2008, Minard 1989, Rogan and Jaenicke 1997).

12. Eek River watershed. This watershed is 3,478 km² in area, and outside the Togiak Refuge boundary except for small portions of mountainous, headwater streams. Rainbow trout have not been documented here, although local lore states that they are present (D. Gillikin, personal communication, Yukon Delta National Wildlife Refuge).

13. Indian River watershed. At 159 km² this watershed is the smallest in the study area reported to support rainbow trout. The watershed is located outside of the Togiak Refuge boundary on Kuskokwim Bay. Seppi (1995) reported catching three *Oncorhynchus mykiss* in mid-July 1994. No other surveys have occurred in this watershed.

**Existing genetic collections:**

Tissue samples for genetic analysis have been collected and archived in the U.S. Fish and Wildlife Service Conservation Genetics Laboratory, Anchorage, Alaska, for some of the populations of Togiak Refuge (Table 1, Figs. 3-6).
Table 1. Archived genetic tissues available for this study.

<table>
<thead>
<tr>
<th>Watershed</th>
<th>River</th>
<th>Samples</th>
<th>N collections</th>
<th>Years of collections</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Togiak</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Togiak River</td>
<td>3</td>
<td>2</td>
<td>1986, 1987</td>
</tr>
<tr>
<td><strong>Kanektok</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kanektok River</td>
<td>10</td>
<td>3</td>
<td>1988, 2000</td>
</tr>
<tr>
<td><strong>Arolik</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ungalikthluk</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ungalikthluk River</td>
<td>17</td>
<td>2</td>
<td>1990, 2003</td>
</tr>
<tr>
<td><strong>Osviak</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Osviak River</td>
<td>29</td>
<td>2</td>
<td>1996, 2002</td>
</tr>
<tr>
<td><strong>Kwethluk</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kwethluk River</td>
<td>471</td>
<td>1</td>
<td>1998</td>
</tr>
<tr>
<td><strong>Wood</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Agulowak River</td>
<td>50</td>
<td>2</td>
<td>2008, 2009</td>
</tr>
<tr>
<td></td>
<td>Agulukpak River</td>
<td>29</td>
<td>2</td>
<td>2009</td>
</tr>
<tr>
<td></td>
<td>Lynx Creek</td>
<td>50</td>
<td>2</td>
<td>2008, 2009</td>
</tr>
<tr>
<td></td>
<td>Moose Creek</td>
<td>50</td>
<td>2</td>
<td>2008, 2009</td>
</tr>
</tbody>
</table>

- Fig. 3. Locations of individual rainbow trout sampled for genetic material on Gechiak (1-26 June 2000), Pungokepuk Creek (22 May – 26 July 2000), and Ongivinuk Rivers (22 – 23 June 2007,)
Fig. 4. Locations of individual rainbow trout sampled for genetic material on Negukthluk and Ungalikthluk Rivers, 29 May – 5 August 2003.

Fig. 5. Locations of individual rainbow trout sampled for genetic material on Osviak River, 29 May – 19 August 2002.
Methods

Genetic material collection:
Tissue samples were collected from rainbow trout from all known or expected Togiak Refuge populations, including those in the Goodnews, Kanektok, Arolik, Togiak, Ungalikthluk, Osviak and Igushik watersheds (Table 1). Additionally, samples were collected from one or more populations from watersheds on the periphery of Togiak Refuge, including Kwethluk, Nushagak, Wood, and Snake (Table 2). When possible, genetic material will be used from archived samples collected during fish surveys occurring 1986–2007 (Table 1). When archived genetic collections were insufficient or lacking, new collections were made during the period of 2009–2012. Fish were captured by angling and seine netting and genetic material was collected by clipping a ~5mm portion of the left pectoral fin from approximately 50 fish per suspected population and preserving the samples in 90% ethyl alcohol or by drying samples in dessicant vials. Fish were released alive.

Captured rainbow trout were measured to the nearest millimeter fork length, categorized as sexually immature or mature using the criteria of Schwanke and Hubert (2003), and the sex of sexually mature fish was determined. Sexually immature fish lack secondary sexual features, whereas sexually mature fish are those fish believed to be capable of spawning or have already spawned during the spring of capture. Sexually mature males are generally dark colored, have a well-developed kype, often exude milt or clear fluid, and often demonstrate visible physical trauma from the rigors of spawning (Schwanke and Hubert 2003). Sexually mature females are more silver, lack a kype, have an extended abdomen with an ovipositor protruding from the vent, often have flaccid...
stomachs, and sometimes exude eggs or clear fluid from the vent when pressure is applied. Post-spawn fish of either gender often appear emaciated. When sample sizes permit, genetic analysis will be restricted to specimens exhibiting spawning physiology.

In order to plan collections, general population assumptions were necessary. However, all a-priori population assumptions are considered tentative, and will be tested when analyzing the genetic data. For example, we assumed that all collections represent genetically discrete spawning populations. We also assumed that geographically small or hydrologically simple watersheds will include single populations of rainbow trout. With increasing size and complexity of watersheds, the likelihood of multiple populations and greater genetic diversity increases. Therefore, we then initiated collections based on the following assumptions:

1. For geographically small watersheds or those with few large tributaries to the primary watershed river, we assumed a single population. These watersheds included Osviak, Indian, Igushik, and Snake.
2. For systems where previous radio telemetry studies demonstrated discrete aggregations during spring (May and June), we assumed aggregations were independent populations. These include Togiak, Kanektok, Ungalikthluk and Wood.
3. For geographically large and complex systems where no previous information on spawning locations or seasonal movements existed, collection trips were timed to occur as closely as logistically possible to the presumed spawning period (mid-April through June, Morrow 1980).

In order to direct collections to individual populations most efficiently, we first obtained local ecological knowledge on rainbow trout spawning locations by interviewing study area residents and knowledgeable anglers. In addition, if genetic analysis suggests a single sample is an admixture of multiple populations, additional sampling will be considered.

**Bias**

A number of steps will be used to minimize bias and error associated with genotyping. For example, we will use a standardized suite of 16 microsatellite loci for *Oncorhynchus mykiss* (Stephenson et al. 2008) that have been rigorously screened across multiple laboratories to characterize alleles and minimize the probability of scoring error. An internal size standard (Liz) will be run during electrophoresis for instrument calibration on each plate of individuals. Individual genotypes determined by the computer will also be checked manually and independently by two laboratory personnel. Afterwards, disagreements will either be reconciled when viewing electropherograms or the samples in question will be re-run. In addition to these re-runs, an entire column from each plate of individuals will be re-run as a quality check to ensure that genotypes correctly align with their original runs. Finally, the program Microchecker (Oostrhout et al. 2004) will be used as a final check of genotypes to identify any scoring error and aberrant genotypes.
To minimize bias in collecting tissue samples from mixed populations rather than from discrete populations, collection will be timed to occur during or immediately following the spawning season.

**Genetic analysis:**

Total genomic DNA will be isolated from approximately 10-20mg of fin tissue using the Qiagen 96-well DNeasy® procedure (Qiagen, Inc.). Isolated DNA will be quantified using a 96-well Modulus™ Microplate Reader (Turner BioSystems, Inc.) and diluted to 30ng/µl for use in PCR. Sixteen microsatellite loci (Stephenson et al. 2008) will be used to genotype each sample:

- Ogo4
- Oke4
- Oki23
- Omy1001
- Omy1011
- Omy7
- Oneu14
- Ots100
- Ots3m
- Ots4
- Ssa289
- Ssa407
- Ssa408
- Omm1036
- Omm1046
- One102

PCR reactions will be conducted in 6 µl volumes containing equal parts of 10x primer mix and 2x Qiagen Multiplex PCR Master Mix (Qiagen Inc.) PCR will be completed on an MJResearch™ DNA Engine Tetrad™ PCT-225. The amplification profile will be one cycle of 15 min @ 95°C, 28 cycles of 30 sec @ 94°C, 1 min 30 sec @ 51 or 57°C (depending on the multiplex) and a final extension for 30 min @ 60°C. PCR products will be visualized by denaturing polyacrylamide gel electrophoresis on an ABI 3730xl automated capillary sequencer (Applied Biosystems, Inc). Fragment size calling will be conducted using GeneMapper 4.0 software (Applied Biosystems, Inc.).

Estimates of allele frequency, allelic richness ($A_r$), and observed and expected heterozygosity ($H_o$, $H_e$) will be computed for each locus and putative population using the computer program FSTAT version 2.9.3 (Goudet 2001). Randomization tests will be used to test for conformity to Hardy-Weinberg equilibrium (HWE) for each locus and population combination and to test for genotypic disequilibrium among locus pairs over all populations. A permutation test in FSTAT will be used to test if $A_r$ and $H_e$ differ among putative populations from small versus large drainages.

A $G$-test of genotypic frequency homogeneity will be used to test for significant genetic divergence over all populations and among each putative population using FSTAT. In addition, we will use the $G$-test to evaluate temporal stability of gene frequencies within populations for those locations in which we have multi-year samples. The level of population divergence will be estimated using the index $F_{ST}$ (Wright 1943), which will be computed over all populations and for each population pair, over all loci, according to Weir and Cockerham (1984). Principle component analysis (PCA) will used to graphically assess the genetic population structure in order to identify population groups and outlier populations. The computer program PCAGEN version 1.2 (http://www2.unil.ch/popgen/softwares/pcagen.htm) will be used to estimate the proportion of variation among populations along axes in multidimensional space. The statistical significance of individual axes will be determined by performing 1,000 randomizations of genotypes using the program’s randomization function.

To complement the PCA we will infer hierarchical population structure using the program SAMOVA (Dupanloup et al. 2002). SAMOVA uses Arelquin (Schneider et al. 2000) code and spatial data to perform an Analysis of Molecular Variance (AMOVA) and group populations with the constraint that populations within a group must be
geographically adjacent and genetically homogenous. The approach is similar in principle to Structure 2.1 (Pritchard et al. 2000) but populations rather than individuals are grouped. Thus, the resulting hierarchical structure maximizes differences between groups ($F_{CT}$) while minimizing differences within groups ($F_{SC}$). The program requires spatial data for each population; however, geographic coordinates (latitude and longitude) are generally inappropriate for dendritic systems like river drainages. Therefore, as suggested by Manni et al. (2004) we will use the distance matrix of pairwise waterway distances to conduct multi-dimensional scaling and use the MDS dimension 1 and 2 coordinates as surrogates for latitude and longitude.

It is presently believed that rainbow trout in this area rarely if ever leave freshwater. Thus, two approaches will be used to infer patterns of migration among populations and to assess the extent to which saltwater is a barrier to gene flow. First, the program IBD version 1.52 (Bohonak 2002) will be used to test for isolation-by-distance and infer the extent to which saltwater inhibits gene flow. Because the route between many population pairs includes saltwater, evidence of isolation-by-distance would be consistent with ongoing saltwater migration. On the other hand, lack of isolation-by-distance, while not excluding saltwater migration, may indicate these populations are largely restricted to freshwater. Thus, we will also test if genetic divergence ($F_{ST}$) is associated with the presence or absence of a saltwater connection. Mantel tests will be used to assess the influence of each factor (waterway distance and saltwater) separately and partial Mantel tests will be used to assess the influence of each factor while controlling for the influence of the other factor. Estimates of the waterway distance between all population pairs will be determined using the National Hydrologic Dataset in ArcGIS™ version 9.2 (ESRI). Second, we will use the program Structure 2.1 (Pritchard et al. 2000) to infer population structure from individual genotypes. This program uses a Bayesian approach to cluster individuals probabilistically, assuming Hardy-Weinberg equilibrium and linkage equilibrium at each locus in each cluster. The analysis will be performed without a-priori population designations in order to assess the number and makeup of groups that maximizes the log likelihood of K (the number of clusters or populations). Individuals belonging to the same cluster but from drainages separated by saltwater would be consistent with saltwater migration. Such a result may indicate gene flow between two closely related populations or transient migratory behavior (individuals sampled while away from their home drainage – possibly to feed). Results from Structure 2.1 will also be used to test our assumption that each collection represents a single panmictic spawning population.

Results

Fish Sampling

South Fork Goodnews River
During the period 8 June-10 June 2009, two anglers floated the South Fork Goodnews River by inflatable canoe, and collected 17 genetic samples. Sampling began at a floatplane accessible pond at N59.15126 W161.25773 and terminated at the confluence of the South Fork and Middle Fork Goodnews River. Fish were caught throughout the
entire stretch of the sampled portion of the river, rather than being located in discrete concentrations (Fig. 7). Fork length ranged from 383 to 581 mm with a mean of 469.9 mm (SD 12.5). No fish injuries or mortalities were recorded. Of the 17 fish sampled, seven were recorded as mature (Table 1).

![Rainbow trout capture locations](image)

**Fig. 7.** Locations of individual rainbow trout sampled for genetic material on Goodnews River and its main tributaries, 2009—2011.

**Table 1.** Sample sizes and lengths (mm) of rainbow trout by maturity class sampled during summers 2008-12 at Togiak National Wildlife Refuge.

<table>
<thead>
<tr>
<th>River</th>
<th>Postspawner n</th>
<th>Postspawner average</th>
<th>Mature n</th>
<th>Mature average</th>
<th>Immature n</th>
<th>Immature average</th>
<th>Undetermined n</th>
<th>Undetermined average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Middle Fork</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goodnews River</td>
<td>13</td>
<td>519.4</td>
<td>27</td>
<td>474.1</td>
<td>4</td>
<td>339.5</td>
<td>35</td>
<td>439.9</td>
</tr>
<tr>
<td>Kukaktlik River</td>
<td>8</td>
<td>509.2</td>
<td>6</td>
<td>457.0</td>
<td>2</td>
<td>297.5</td>
<td>7</td>
<td>421.4</td>
</tr>
<tr>
<td>South Fork</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goodnews River (mainstem)</td>
<td>7</td>
<td>471.0</td>
<td>49</td>
<td>462.7</td>
<td>19</td>
<td>368.4</td>
<td>10</td>
<td>373.3</td>
</tr>
<tr>
<td>Muklung River</td>
<td>10</td>
<td>509.9</td>
<td></td>
<td>2</td>
<td></td>
<td>284.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ongoke River</td>
<td>8</td>
<td>597.6</td>
<td></td>
<td></td>
<td>4</td>
<td>522.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Igushik River</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kanektok River</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ice Creek</td>
<td>19</td>
<td>437.3</td>
<td></td>
<td>280.75</td>
<td>7</td>
<td>381.7143</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pike Creek</td>
<td>12</td>
<td>462.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fenn Creek</td>
<td>6</td>
<td>446.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Snake River</td>
<td>9</td>
<td>458.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eagle Creek</td>
<td></td>
<td></td>
<td>19</td>
<td>320.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osviak River</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>112</td>
<td>531.6</td>
</tr>
</tbody>
</table>
Kukaktlik and Middle Fork Goodnews River
During the period 15 June-21 June 2009, three anglers sampled the Kukaktlik and Middle Fork Goodnews Rivers. Sampling began at Kukaktlik Lake and proceeded downstream by raft to the confluence of the Middle Fork and North Fork Goodnews Rivers. A total of 23 samples were collected in the Kukaktlik River, followed by an additional 79 samples in the Middle Fork Goodnews River (Fig. 7). Fork lengths ranged from 250 to 625 mm, with postspawners having greatest length of all maturity classes (Table 1). A total of two mortalities and two injuries were recorded. The first rainbow trout was encountered ~3.5 km downstream from Kukaktlik Lake. From this point, rainbow trout occurred throughout the remainder of the sampled area.

Goodnews River (mainstem)

During the period 14-18 June 2011, three anglers sampled the mainstem Goodnews River (Fig. 7), catching 85 rainbow trout. With the exception of five fish caught within 5 km of Goodnews Lake, no rainbow trout were caught in the upper third of the river. A total of two mortalities were recorded. Rainbow trout fork lengths ranged from 285 to 610 mm, and averaged 432 mm.

Ongoke and Igushik Rivers
During the period 29 June-2 July, two anglers motorboated the Ongoke River from Amanka Lake to approximately N59.194 W159.351 and collected 12 rainbow trout tissue samples (Fig. 8). Fork lengths ranged from 500 – 633 mm and averaged 572.6. Of the 12 fish, 8 were classified as postspawners (Table 1). Non-target species caught on the Ongoke River included ~110 grayling, ~19 Dolly Varden and ~5 char.
Rainbow trout capture locations:
- Agulowak River
- Eagle Creek
- Fenno Creek
- Hidden Creek
- Hope Creek
- Ice Creek
- Igushik River
- Lynx Creek
- Moose Creek
- Muklung River
- Ongoke River
- Pike Creek
- Snake River
- Togiak Refuge boundary

Fig. 8. Locations of rainbow trout sample locations in Igushik, Snake, and Wood River watersheds during summer 2008 through 2012.

On completion of the Ongoke River survey, samplers boated across Amanka Lake to the outlet at the Igushik River and collected another three samples. An ADFG biological technical stationed at a field camp at this location collected 21 additional samples from 26 June – 22 July 2009. Igushik River rainbow trout ranged from 270 – 610 mm and averaged 426.6 mm.

Snake River and Eagle Creek
2009
Two to three anglers sampled the Snake River near the Snake Lake outlet on 17 June, 5 August, and 19 August 2009, collecting a total of 30 samples (Fig. 8). Eagle Creek, a small creek feeding Snake Lake located ~5 km from the Snake River sample site, was surveyed on 11 July and 8 August 2009, and produced 19 tissue samples. Fork length ranged from 140 to 490 mm at Eagle Creek, and averaged 320.8. Fork length ranged from 340 to 510 mm at the Snake River, and averaged 424.2.

2010
On 8 July 2010, ADFG anglers collected 8 rainbow trout tissue samples from the outlet of Snake Lake. Lengths ranged from 404—539 mm and averaged 449. Three of the fish were classified as postspawners while the remainder was classified as undetermined.

Wood River watershed
2008-2009
In addition to the archived samples reported in Table 1, ADFG located an additional 50 archived samples each from three streams in the Wood River watershed. On 25 April and 1 May 2008, ADFG anglers collected 50 rainbow trout tissue samples in the Aguluwok River, between Lakes Nerka and Aleknagik in the Wood River watershed (Fig. 8, Table 1). In May of 2008 and 2009, ADFG biologists collected 50 rainbow trout samples at a weir at Moose Creek, Lake Beverly, and an additional 50 samples were caught by angling at Lynx Creek, Lake Nerka. All fish were identified as spawners.

On 18 June 2009, two anglers collected 6 rainbow trout tissue samples in Fenno Creek, a stream feeding Lake Nerka (Fig. 8, Table 1). On the following day, nine samples were collected from Pike Creek, also feeding Lake Nerka. On 24 June and 12 September 2009, five samples were collected from Ice Creek, which flows into Lake Aleknagik. Thirteen samples were collected from the Muklung River on six days between 25 July and 22 August 2009. In all Wood River system sites, fork lengths ranged from 184 – 580 mm, and averaged 451. All but three fish from the Muklung River were classified as postspawners.

2010
On 11 June 2010, a single rainbow trout tissue sample was collected at Ice Creek. On 12 June, a single sample was collected from Hope Creek, which flows into Lake Beverly (Fig. 8, Table 1). On 13 June, ADFG anglers collected 3 samples from Pike Creek. In all 2010 Wood River system sites, fork lengths ranged from 428—511 mm, and averaged 449. All fish were classified as post spawners.

2012
On four occasions (6, 20, 26 June, and 13 July) two to three anglers sampled Ice Creek at Lake Aleknagik (Fig. 8, Table 1). A total of 24 samples were collected. Fork lengths ranged from 267—502 and averaged 396.3 mm. Thirteen individuals were identified as
postspawners. Non-target species caught or seen included Arctic char, round whitefish (*Prosopium cylindraceum*), and sockeye salmon (*Oncorhynchus nerka*).

On 13-14 June 2012, anglers sampled Hidden and Lynx Creeks on Lake Nerka (Fig. 8, Table 1). A total of 10 samples were collected at Hidden Creek, and fork lengths ranged from 310-582 mm and averaged 458 mm. A total of 53 samples were collected at Lynx Creek, and fork lengths ranged from 325-611 mm, averaging 430.6 mm.

Kanektok River

The Kanektok River was sampled by a crew of 8 anglers from 3-12 August 2009, 6 of whom originated at Kagati Lake and two of whom originated at Kanuktik Lake (Fig. 9). Rainbow trout were distributed throughout the entire length of the Kanektok River and were more heavily concentrated in the lower half of the Kanuktik River. A total of 523 tissue samples were collected. Fork lengths ranged from 135 – 680 mm and averaged 433.7. Radio transmitters were placed in 200 of the sampled fish, and were followed for two years as part of a habitat use investigation (Schwanke 2009). The radio telemetry data will be examined to determine whether there is evidence of multiple populations in the Kanektok system. If so, we will determine whether sample sizes from putative populations are sufficient, or whether additional sampling is necessary.

![Map of Kanektok River](image)

Fig. 9. Locations of individual rainbow trout sampled for genetic material on Kanektok River, 3-12 August 2009.

Osviak River

From 19—23 July 2012, three anglers sampled the Osviak River. They were inserted by helicopter in the vicinity of N59.0383 W161.1245 and floated via inflatable boat to Osviak lagoon. A total of 59 rainbow trout samples were taken, although four were
apparently captured and sampled previously during this same survey. Lengths ranged from 240-730 mm, averaging 528.6 (Fig. 10, Table 1).

Two of the three anglers returned to the Osviak River on 31 July 2012, and repeated the sampling effort in the same area of the river until 7 August 2012. A total of 53 rainbow trout were captured and sampled, and lengths ranged from 320-690 mm, averaging 532.2. A total of 12 fish were recaptured from the initial sampling trip. Using a 2-sample closed-population capture-recapture model (modified Petersen estimate, Chapman 1951), population size, with 95% confidence, was estimated as 248.2 +/- 100.7.

**Indian River**

The Indian River was surveyed by a crew of two anglers from 23-26 July 2010. The crew was dropped off by helicopter approximately 8 km from the river outlet, then floated to the outlet. At the outlet, the crew rowed across the bay to the outlet of Carter Creek, then rowed up Carter Creek approximately 0.5 km. No rainbow trout were caught or seen. Non-target fish caught included 30 Dolly Varden, 8 chum salmon, 2 pink salmon, and 2 starry flounder (*Platichthys stellatus*). The Indian River appeared to be suitable summer habitat for rainbow trout, but suitable winter habitat (lakes or deep river pools) appeared missing. It is possible that the rainbow trout which Seppi (1995) reported observing in the Indian River in July 1994 were part of a migratory group that moves back and forth from the Arolik or Goodnews River through salt water. Thus, it would be prudent to sample this river on another occasion.

![Fig. 10. Portion of Indian River and Carter Creek surveyed for rainbow trout 23-26 July 2010.](image-url)
Future Work
Genetic tissue collections have been completed, and analysis of data will proceed through 2013, with a final report completed in spring 2014.

Acknowledgements
This project was funded by the Togiak National Wildlife Refuge, the U.S. Fish and Wildlife Service Alaska Region Conservation Genetics Lab, and Alaska Department of Fish and Game Sport Fish Office in Dillingham. We thank A. Aderman, M. Albert, G.J. Ballentine, T. Bird, J. Boersma, W. Church, J. Chythlook, M. Evenson, J. Everett, A. Garry, K. Gates, M. Lisac, A. Miller, R. Sanders, C. Schwanke, T. Underwood, J. Walsh, and C. Waters for assisting in sampling. We thank the Alaska Department of Game Commercial Fish Office in Dillingham, which provided logistical support.

References

Alt, K.T. 1986. Western Alaska rainbow trout studies. Federal aid in fish restoration, F-10-1. Alaska Department of Fish and Game, Division of Sport Fish, Volume 27, T-6-1, Juneau, Alaska.


