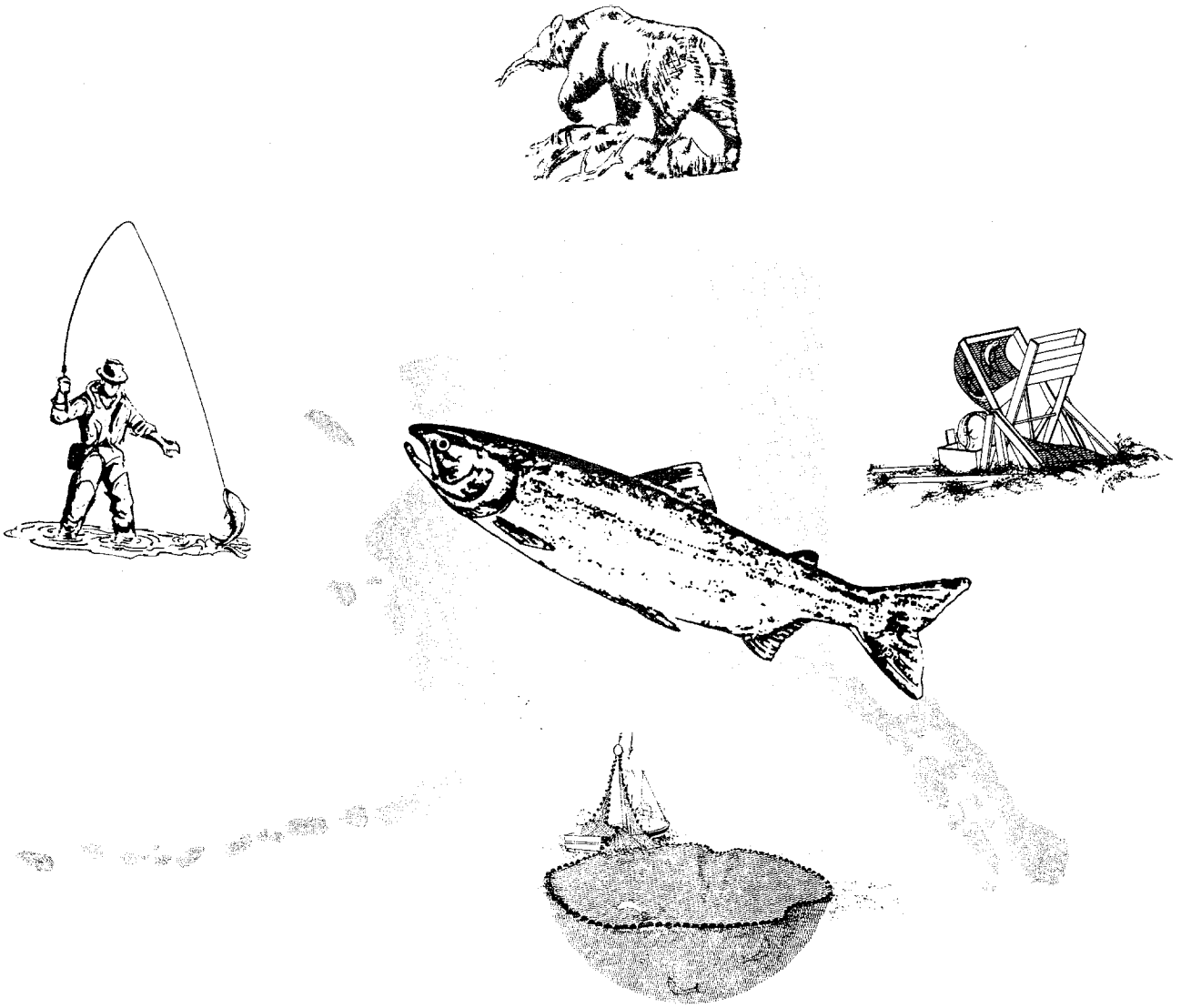
Alaska Fisheries Technical Report Number 51

# Genetic Differentiation of Rainbow Trout (*Oncorhynchus mykiss*) in the Kenai River, Alaska



August 1999

Region 7
U.S. Fish and Wildlife Service • Department of the Interior

Alaska Fisheries Technical Report

Number 51 August 1999

# Genetic Differentiation of Rainbow Trout (*Oncorhynchus mykiss*) in the Kenai River, Alaska

William J. Spearman
Steve J. Miller
Matthew A. Cronin[1]
Douglas E. Palmer[2]
and
Charles C. Krueger

Fish Genetics Laboratory
U.S. Fish and Wildlife Service
1011 East Tudor Road
Anchorage, Alaska 99503

[1]LGL Alaska Research Associates, Inc., 4175 Tudor Centre Drive, Anchorage, Alaska 99508

[2]USFWS Kenai Fishery Resources Office, P.O. Box 1670, Kenai, Alaska 99611

# Table of Contents

Abstract . . . . . 1

Introduction . . . . . 1

Methods . . . . . 2

Results . . . . . 5
- Restriction fragments . . . . . 5
- Genotypes compared . . . . . 5
- Populations compared . . . . . 6

Discussion . . . . . 7

Summary . . . . . 8

Recommendations . . . . . 9

Acknowledgments . . . . . 9

References . . . . . 9

# Tables

Table 1. Mitochondrial DNA genotypes in rainbow trout and steelhead identified with composite restriction fragment patterns. . . . . 4

Table 2. Estimated proportions of shared restriction sites (S) and nucleotide substitutions per site (*p*) between mitochondrial DNA genotypes in rainbow trout and steelhead. . . . . 5

Table 3. Proportions of mitochondrial DNA genotypes in collections of rainbow trout and steelhead from three National Wildlife Refuges (NWR). . . . . 6

Table 4. Pairwise genetic distances (Cavalli–Sforza and Edwards 1967, chord distance) among collections of rainbow trout and steelhead from three National Wildlife Refuges (NWR) based on mitochondrial DNA genotype frequencies. . . . . 7

# Figures

Figure 1. Map of the Kenai River showing collection locations above and below Skilak Lake. . . . . 3

Figure 2. Map showing the geographic distribution of collections. . . . . 3

Figure 3. Dendrogram (UPGMA; Sneath and Sokal 1973) of genetic distances (Cavalli–Sforza and Edwards 1967, chord distance) showing the degree of genetic relatedness among collections of rainbow trout. . . . . 8

# Appendix

Appendix 1. Restriction fragment patterns for mitochondrial DNA *cyt*B and ND5/6 in rainbow trout and steelhead. . . . . 11

## Mission of the Fish Genetics Laboratory

The mission of the Fish Genetics Laboratory is to provide the necessary genetics expertise and support to permit sound stewardship of Alaska fishery resources, including conservation of the natural diversity of wild fish populations and aquatic ecosystems. Responsibilities include providing U.S. Fish and Wildlife Service leadership for conservation of genetic resources, particularly Pacific salmon stocks; development and implementation of genetic stock identification studies to delineate stocks for use in fisheries management and allocation decisions; evaluations of genetic impacts resulting from stock introductions, exploitation, and other activities; monitoring stocks for genetic change; coordination of genetic issues within and outside the Service; and conducting outreach activities that promote the importance of maintaining genetic diversity.


## Nondiscrimination Clause

The U.S. Department of Interior prohibits discrimination in programs on the basis of race, color, national origin, religion, sex, age, or disability. If you believe that you have been discriminated against in any program, activity, or facility operated by the U.S. Fish and Wildlife Service or if you desire further information please write to:

U.S. Department of the Interior
Office for Equal Opportunity
1849 C Street, NW
Washington, D.C. 20240

# Genetic Differentiation of Rainbow Trout (*Oncorhynchus mykiss*) in the Kenai River, Alaska

William J. Spearman, Steve J. Miller, Matthew A. Cronin[1], Douglas E. Palmer[2], and Charles C. Krueger
*Fish Genetics Laboratory, U.S. Fish and Wildlife Service, 1011 East Tudor Road, Anchorage, Alaska 99503*

**Abstract:** Population structure of rainbow trout (*Oncorhynchus mykiss*) in the Kenai River was examined by comparing two collections from the Kenai River mainstem — one collection from above and one from below Skilak Lake. Genetic variation in the Kenai River was also compared to seven other collections from Alaska comprising two anadromous steelhead collections from Kodiak Island and five rainbow trout collections from southwest Alaska. At the molecular level, five mitochondrial DNA (mtDNA) genotypes displayed low DNA sequence divergence ($p$=0.008–0.04). At the population level, significant genetic differences were observed among all nine collections, including presence and absence of genotypes ($P \leq 0.001$; $F_{st}$=0.3). Some of the greatest genetic distances occurred between collections that were geographically close to each other. The two collections from the Kenai River were different as indicated by two genotypes that were in significantly different ($P \leq 0.001$) proportions in each collection. The most common genotype in rainbow trout below Skilak Lake (55%) was the least common above the lake (4%). Genetic results were consistent with movements based on radio telemetry findings and supported the conclusion that at least two populations apparently exist in the Kenai River — one above Skilak Lake and one below. Additional populations may be present in the Kenai River; however, a more thorough study design would be required to fully characterize the population structure. Our results have clear implications for management and suggest the possibility of multiple populations of rainbow trout occurring in other river systems.

## Introduction

Rainbow trout (*Oncorhynchus mykiss*) and steelhead occur in southern Alaska, including the Kodiak archipelago, and support important recreational and subsistence fisheries. The Kenai River, world renowned for its trophy chinook salmon (*O. tshawytscha*) fishery, also hosts a world class rainbow trout fishery. The rainbow trout fishery of the Kenai River is an important element of the economy and culture of the Kenai Peninsula. About 54% of the Kenai River drainage lies within the Kenai National Wildlife Refuge (NWR) and the catch of rainbow trout from the Kenai River alone has comprised 50% of the total catch of rainbow trout from the entire Kenai Peninsula (USFWS 1995). Catches of rainbow trout in the Kenai River have ranged from 8,720 to 62,152 fish annually since 1984 (Nelson 1995; Howe et al. 1996) and the river is one of the most intensively fished in Alaska (Howe et al. 1996).

Rainbow trout are thought to occur as semi–isolated populations when they occupy major river drainages. While relatively little reproductive interchange of resident fish occurs among geographically separate major river drainages (Allendorf 1975; Wishard et al. 1984; Nielsen et al.

[1]LGL Alaska Research Associates, Inc., 4175 Tudor Centre Drive, Anchorage, Alaska 99508
[2]USFWS Kenai Fishery Resources Office, P.O. Box 1670, Kenai, Alaska 99611

1994), little is known about the population structure of rainbow trout within river systems. Genetic differentiation within drainages has been reported for brown trout (*Salmo trutta*; Allendorf et al. 1976), cutthroat trout (*S. clarki clarki*; Campton and Utter 1987), as well as rainbow trout (Northcote et al. 1970; Krueger and May 1987; Currens et al. 1990). For example, Krueger and May (1987) described population structure of rainbow trout in a portion of the Lake Superior watershed. They reported greater genetic distances among populations within the Brule River drainage than among some of the more geographically distant sites. This indicates that forces, such as homing to natal streams to spawn, can exist within drainages to keep populations reproductively isolated and thus maintain genetic differences.

To meet management and conservation objectives, fishery managers need to know whether the rainbow trout in a river represent a single population or multiple, smaller populations. If multiple populations exist, managers should also know the number of populations and their spatial and temporal boundaries. Different populations may have different population dynamics where production, recruitment, and age class structure differ among populations. Such information on population structure has implications for stock conservation, habitat protection, population modeling, environmental assessments, and developing management plans. In general, different populations may require different management regimens.

The Kenai River above Skilak Lake has been managed differently from below the lake since the mid 1980s in response to increasing fishing pressure on rainbow trout (Nelson 1995). Results from a tagging study during that period suggested that the rainbow trout in the upper Kenai River above Skilak Lake could be a population separate from the rainbow trout below Skilak Lake (Lafferty 1989). In recognition of differential fishing pressure in the upper and lower river plus the indication of different populations, the Alaska Board of Fisheries classified the upper Kenai River as a trophy fishery in 1986, while the river from Skilak Lake downstream was managed for sustained yield (Nelson 1995). In 1997, the management status of the upper Kenai River above Skilak Lake was changed to catch–and–release.

Increasing sport fishing pressure in recent years, coupled with a lack of biological information on rainbow trout in the Kenai River, generated concern about potential negative impacts to populations in the middle and upper reaches of the river (USFWS 1995). This concern prompted studies by the Alaska Department of Fish and Game (ADFG) and the U.S. Fish and Wildlife Service (USFWS). ADFG conducted a stock assessment of the rainbow trout population in the upper Kenai River during 1995 (Hayes and Hasbrouck 1996). Concurrent studies were conducted by the USFWS which compared migratory behavior, seasonal distribution, and genetic characteristics of rainbow trout above and below Skilak Lake (Palmer 1998; this study).

The primary objective of this study was to determine if there was evidence of more than one population of rainbow trout in the Kenai River based on genetic variation. In the process of examining Kenai River rainbow trout, we also assessed genetic variation on a broad geographic scale using collections of rainbow trout and steelhead from other locations in Alaska. This provided a relative benchmark of variation by which to interpret our results from the Kenai River. Our objective did not include full characterization of the population structure of rainbow trout in the Kenai River as that would require a more elaborate study design; thus, this study was designed as a preliminary evaluation of population structure in the Kenai River.

## Methods

Clips of fin rays from rainbow trout were collected during May–September 1995 from the Kenai River mainstem above (N=29) and below (N=32) Skilak Lake (Figure 1). For the purposes of this study, we considered these collections to be adequately representative of purported populations above and below Skilak Lake because evidence from a radio telemetry study indicated that fish tagged above the lake did not commonly venture into the mainstem below the lake and vice versa (Palmer 1998). Samples from the Togiak NWR were collected in July 1995 and May–June 1996 (N=51) from three separate drainages: the Arolik, Osviak, and Togiak rivers, where three tributaries of the Togiak River were sampled (Ongivinuk, Pungokepuk, and Gechiak rivers). Samples from the Kodiak NWR were

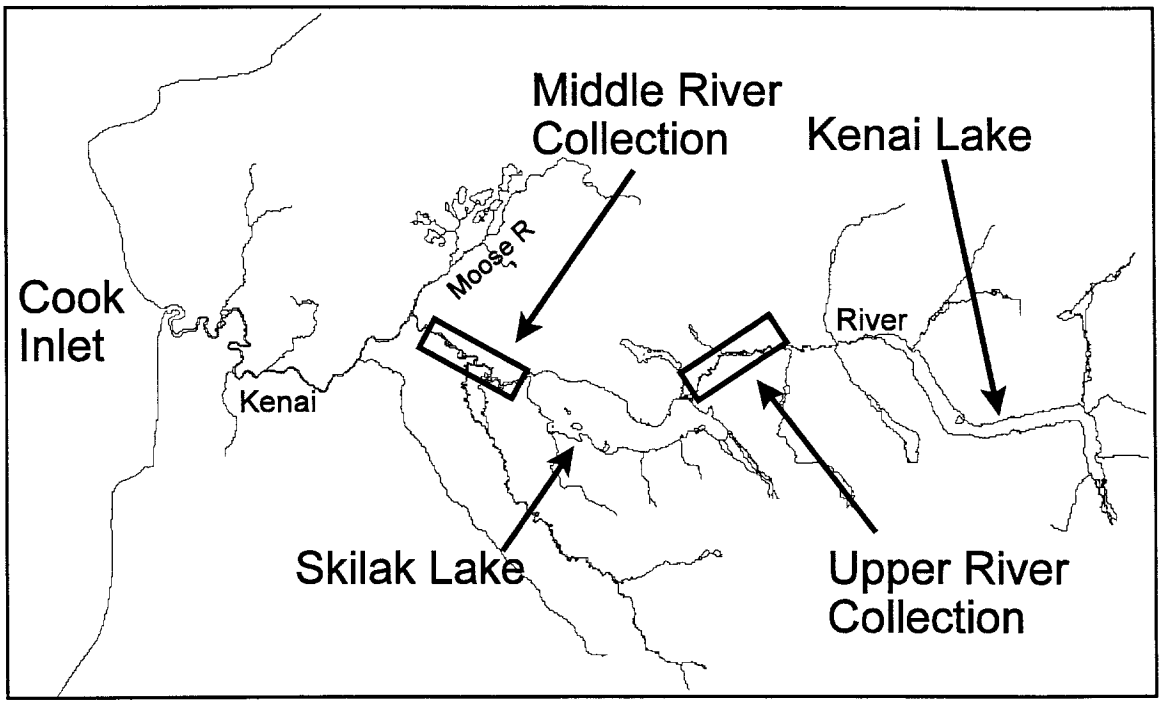



Figure 1. Map of the Kenai River showing collection locations above and below Skilak Lake.

collected in October 1996 (N=20; Figure 2) from two drainages in the southwest portion of Kodiak Island. Fin clip samples were stored in individually numbered vials with 70% ethanol until processed. For the Kenai NWR collections, trout ranged in size from 220 mm to 311 mm fork length (FL; except for one fish where FL=150 mm). Collections from the Kodiak and Togiak NWRs (FL≥360 mm) were used as outgroups

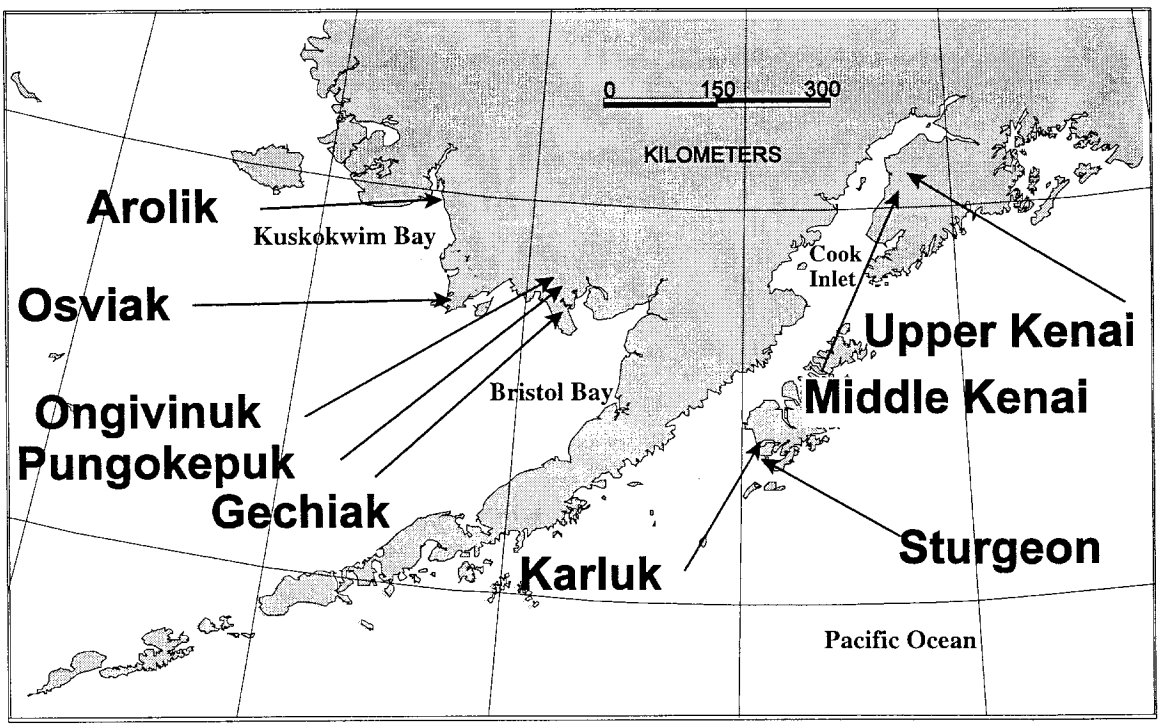



Figure 2. Map showing the geographic distribution of collections.

to permit comparisons of genetic differentiation on varying geographic scales (Figure 2). The Kenai and Togiak collections were from fresh water resident populations, while the Kodiak collections were from sea–run steelhead populations.

Nucleic acids were extracted from about 25 mg of fin tissue incubated in 500 µL of STE buffer (0.1 M NaCl, 10 mM Tris–HCl, 1 mM EDTA, pH 8.0), 50 µL 10% SDS, and 25 µL of proteinase–K (10 mg•mL$^{-1}$) at 65°C for ≥60 min. Buffered phenol:chloroform: isoamyl alcohol (25:24:1; 500 µL) was then added, gently vortexed, and centrifuged for 15 min at 13,800 rcf (relative centrifugal force). Supernate (500 µL) was recovered and transferred to new 1.5 mL tubes and the phenol step repeated. Supernate (500 µL) was again recovered, transferred to a new set of tubes and chloroform:isoamyl alcohol (24:1; 500 µL) was added, vortexed and spun at13,800 rcf for 15 min. Supernate (500 µL) was transferred to new tubes and 15 µL of 5M NaCl and 1 mL of 100% ethanol were added in that order. The tubes were inverted several times and then centrifuged at13,800 rcf for 5 min. The supernate was poured off and the DNA pellet was washed with 70% ethanol, air dried for >60 min and then dissolved in 100 µL TE buffer (10 mM Tris, 1 mM EDTA, pH 7.6). DNA samples were electrophoresed in 0.8% agarose gels cast in TBE buffer (Sambrook et al. 1989), stained with ethidium bromide, and photographed with Polaroid® film on an ultraviolet light table.

Two mitochondrial DNA (mtDNA) segments were amplified using the polymerase chain reaction(PCR) with the following primers:

cytochrome–B (*cyt*B; Bickham et al. 1995);
LGL765 5'-GAAAAACCAYCGTTGTWATTCAACT-3'
LGL766 5'-GTTTAATTAGAATYTYAGCTTTGGG-3'

NADH dehydrogenase 5/6 (ND5/6; Park et al. 1993)
cGlu 5'-CAACGGTGGTTCTTCAAGTC-3'
cLeu3 5'-GGAACCAAAAACTCTTGGTGCAACTCC-3'

Previous assessments of these segments of DNA in chinook, chum (*O. keta*), or sockeye (*O. nerka*) salmon had revealed variation (Cronin et al. 1993; Adams et al. 1994; Bickham et al. 1995; Burger et al. 1997).

Each PCR reaction comprised 0.1–0.5 µg of genomic DNA, 5 µL of 10X buffer (0.1 M Tris–HCl, pH 8.5, 0.025 M $MgCl_2$, 0.5 M KCl, 1 µg•µL$^{-1}$ bovine serum albumin), 5 µL of dNTP mix (2 mM each of dATP, TTP, dCTP and dGTP in 10 mM Tris–HCl, pH 8.0), 1 µL of a 10 µM solution of each of two primers, 2.0 units of *Taq* polymerase, and deionized water added for a final volume of 50 µL. The amplification cycle for *cyt*B consisted of 95°C for 4 min for 1 cycle, then 94°C for 45 s, 50°C for 50 s, and 70°C for 2 min and 30 s, cycled 32 times; and for ND5/6, 95°C for 4 min for one cycle, then 94°C for 1 min, 52°C for 1 min, and 72°C for 2 min, cycled 35 times with a 1 s extension per cycle, followed by a final cycle of 72°C for 5 min. PCR products were electrophoresed on 1.2% agarose gels, stained with ethidium bromide and photographed under ultraviolet light.

Five restriction enzymes (RE) were used to identify different genotypes (Appendix 1). Each RE recognizes a unique sequence of four to six bases and cuts the DNA at that site. For example, if the *cyt*B segment from one fish has two restriction sites and another fish has only one restriction site, those fish have different genotypes. RE digests were electrophoretically separated in 2.5% agarose gels, stained with ethidium bromide, and photographed. Sizes of restriction fragments were estimated by comparison to a 100 basepair (bp) ladder (Amersham Pharmacia Biotech). Restriction fragment patterns were visually identified from gels and photographs.

A composite mtDNA genotype for each fish was defined from the individual genotypes for each DNA–segment/RE combination (i.e, *Dde*I, *Dpn*II, *Msp*I, and *Nci*I for *cytB*, and *Taq*I for ND5/6; Table 1; Lansman et al. 1981; Cronin et al. 1993). The fragment patterns

Table 1. Mitochondrial DNA genotypes in rainbow trout and steelhead identified with composite restriction fragment patterns. Composite genotypes comprise the fragment patterns (from left to right) *Dde*I, *Dpn*II, *Msp*I, and *Nci*I with *cyt*B, and *Taq*I with ND5/6 (see Appendix 1).

| Genotype | Composite Genotype |
|---|---|
| A | ABAAB |
| B | ABBBB |
| C | BBAAB |
| D | AAAAA |
| E | CBAAB |

for each RE and segment were designated A, B, or C, and combined into composite sets of five fragment patterns designated as genotypes. Each genotype was thus defined by at least one fragment pattern that differed from the others.

Differences among genotypes were quantified with estimates of the proportion of shared restriction sites (S), and the nucleotide substitutions per nucleotide site (i.e., DNA sequence divergence; $p$), with equations 2 and 3 of Nei and Miller (1990). These parameters measure the differences among the genotypes in terms of DNA sequence (i.e., How different are the genotypes?). As such, they do not measure population differentiation. In addition, we used only restriction enzymes that showed variation, so $p$ estimates are biased upwards.

To measure population differentiation, the computer program BIOSYS–1 (Swofford and Selander 1989) was used to estimate $F_{st}$, calculate genetic distances (Cavalli–Sforza and Edwards 1967, chord distance), and perform unweighted pair–group method with arithmetic averages (UPGMA; Sneath and Sokal 1973) clustering of the distances to illustrate relationships among the populations. Tests of homogeneity using the log likelihood ratio statistic ($G$; Sokal and Rohlf 1981) were used to assess differentiation of mtDNA frequencies among collections ($H_0$: no differences in genotype frequencies). Frequencies were considered significantly different if $P<0.05$. These analyses help characterize the level of the differentiation of populations based on genotype frequencies.

## Results

### *Restriction fragments*

The *cyt*B segment was ~1350 bp in size; the ND5/6 segment was ~2460 bp in size. Segment sizes were similar to those reported in salmon (Cronin et al.1993; Bickham et al. 1995). Restriction site analysis yielded restriction fragment length polymorphisms from four restriction enzymes (*Dde*I, *Dpn*II, *Msp*I, and *Nci*I) for the *cyt*B segment and one restriction enzyme (*Taq*I) for the ND5/6 segment (Appendix 1). The summed sizes of each of the fragment patterns for each segment/RE combination were similar, and in most cases summed to the size of the amplified DNA segment. However, the sum of the fragments for each *Dde*I genotype was consistently less than 1350 bp by about 350 bp. This difference may have been due to an additional restriction site causing double fragments at the 346 bp fragment position or other positions (Appendix 1); however, indications of this, such as intensity differences of the fragments in the gels, were not observed. Alternatively, small fragments (<50 bp) summing to 350 bp would have been too small to observe in the agarose gels.

### *Genotypes compared*

Five composite genotypes, designated A–E, were observed among the 124 fish analyzed (Table 1). The genotypes displayed high estimates for the proportion of shared sites (S) and low estimates for the nucleotide substitutions per nucleotide site ($p$). Estimates of S ranged from 0.8667 to 0.9677, and of $p$ from 0.0082 to 0.0358 (Table 2). Estimates of S and $p$ were biased downward and upward, respectively, because only restriction enzymes that resulted in variable fragment patterns were used. Inclusion of the invariant enzymes would increase the S estimates and decrease the $p$ estimates. The most divergent genotypes, B and D, occurred in different collections and also together in the Karluk collection.

Table 2. Estimated proportions of shared restriction sites (S) and nucleotide substitutions per site ($p$) between mitochondrial DNA genotypes in rainbow trout and steelhead.

| Genotypes compared | S | $p$ |
|---|---|---|
| A v. B | 0.9375 | 0.0161 |
| A v. C | 0.9677 | 0.0082 |
| A v. D | 0.9286 | 0.0185 |
| A v. E | 0.9677 | 0.0082 |
| B v. C | 0.9091 | 0.0238 |
| B v. D | 0.8667 | 0.0358 |
| B v. E | 0.9091 | 0.0238 |
| C v. D | 0.8966 | 0.0273 |
| C v. E | 0.9375 | 0.0161 |
| D v. E | 0.8966 | 0.0273 |

Table 3. Proportions of mitochondrial DNA genotypes in collections of rainbow trout and steelhead from three National Wildlife Refuges (NWR).

| Population | N | Genotype A | B | C | D | E |
|---|---|---|---|---|---|---|
| Kenai NWR (rainbow trout) | | | | | | |
| Middle Kenai R. | 31 | 0.45 | — | — | 0.55 | — |
| Upper Kenai R. | 28 | 0.96 | — | — | 0.04 | — |
| Kodiak NWR (steelhead) | | | | | | |
| Karluk R. | 10 | 0.40 | 0.50 | 0.10 | — | — |
| Sturgeon R. | 9 | 0.22 | — | 0.11 | 0.33 | 0.33 |
| Togiak NWR (rainbow trout) | | | | | | |
| Osviak R., Bristol Bay | 4 | 1.00 | — | — | — | — |
| Arolik R., Kuskokwim Bay | 10 | 0.90 | — | — | 0.10 | — |
| Gechiak R., Togiak R. | 15 | 1.00 | — | — | — | — |
| Ongivinuk R., Togiak R. | 2 | 1.00 | — | — | — | — |
| Pungokepuk R., Togiak R. | 15 | 0.60 | 0.40 | — | — | — |
| Totals | 124 | 0.69 | 0.09 | 0.02 | 0.18 | 0.02 |

### *Populations compared*

Genetic differences among the collections were observed based on the presence or absence of genotypes in collections and by differences in the frequencies of genotypes (Table 3). The Middle and Upper Kenai collections shared the A and D genotypes, but those genotypes occurred at different frequencies ($G$=22, df=1, $P \leq 0.001$). The most common genotype in the Middle Kenai collection (D at 55%) was the least common in the Upper Kenai collection (4%; Table 3). The genetic distance between these two collections was intermediate to the range of distances among all collections, indicating there is considerable differentiation within the Kenai River (Table 4).

Differentiation was also evident between the Kenai collections and Kodiak collections ($G$=33, df=4, $P \leq 0.001$, pooled by NWR). Whereas the Kenai collections had two genotypes, the Kodiak collections had all five genotypes (Table 3). The two Kodiak collections shared the A and D genotypes as in the Kenai collections, but also had three additional genotypes (B, D, and E) not found in the Kenai collections. The Togiak collections were more similar to the Kenai collections than were the Kodiak collections (Figure 3); however, they were still markedly different ($G$=25, df=2, $P \leq 0.001$, pooled by NWR). The Togiak collections also contained the A and D genotypes, plus the B genotype that also occurred in a Kodiak collection.

The $F_{st}$ estimate for all nine collections was 0.349, indicating that approximately 35% of total variation occurred among the collections and that a high degree of differentiation exists on a broad geographic scale. A test of homogeneity among all nine collections indicated there was differentiation on the broad geographic scale ($G$=115, df=40, $P \leq 0.001$). Sample sizes were small for some collections, so the degree of population differentiation reported here should be considered preliminary.

Assessment of genetic distances showed no clear geographic patterns of differentiation (Figure 3; Table 4). Some geographically close collections were the most genetically divergent (e.g., Middle Kenai versus Upper Kenai collections). Pairwise distances ranged from 0.000 (the populations with only genotype A) to 0.717 (Sturgeon versus Pungokepuk).

Table 4. Pairwise genetic distances (Cavalli–Sforza and Edwards 1967, chord distance) among collections of rainbow trout and steelhead from three National Wildlife Refuges (NWR) based on mitochondrial DNA genotype frequencies.

| | Kenai NWR | | Kodiak NWR | | Togiak NWR | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Population | Middle Kenai | Upper Kenai | Karluk | Sturgeon | Osviak | Arolik | Gechiak | Ongiv-inuk | Pungo-kepuk |
| Kenai NWR | | | | | | | | | |
| Middle Kenai R. | — | | | | | | | | |
| Upper Kenai R. | 0.403 | — | | | | | | | |
| Kodiak NWR | | | | | | | | | |
| Karluk R. | 0.683 | 0.554 | — | | | | | | |
| Sturgeon R. | 0.455 | 0.589 | 0.695 | — | | | | | |
| Togiak NWR | | | | | | | | | |
| Osviak R., Bristol Bay | 0.516 | 0.121 | 0.546 | 0.655 | — | | | | |
| Arolik R., Kuskokwim Bay | 0.322 | 0.084 | 0.569 | 0.548 | 0.204 | — | | | |
| Gechiak R., Togiak R. | 0.516 | 0.121 | 0.546 | 0.655 | 0.000 | 0.204 | — | | |
| Ongivinuk R., Togiak R. | 0.516 | 0.121 | 0.546 | 0.655 | 0.000 | 0.204 | 0.000 | — | |
| Pungokepuk R., Togiak R. | 0.623 | 0.440 | 0.226 | 0.717 | 0.427 | 0.464 | 0.427 | 0.427 | — |

## Discussion

At least two populations of rainbow trout appear to exist in the Kenai River — one above Skilak Lake and one below the lake. If the rainbow trout in the Kenai River represented a single population, we would have expected the Middle and Upper collections to have been genetically similar. The population structure that we observed plus the habitat diversity in the Kenai River system, suggest the possibility of additional populations occurring in the system (e.g., the Moose River drainage) yielding a more complex population structure than what is apparent from this study.

The level of differentiation observed in the Kenai River indicates that gene flow can be restricted (e.g., due to homing to natal streams to spawn) between geographically close populations. Radio telemetry results showed that Middle and Upper Kenai River populations were segregated above and below Skilak Lake for most of the year including the spawning season but used the lake as a common overwintering area (Palmer 1998). Lafferty (1989) reported similar segregation of Kenai River rainbow trout based on tag recoveries. This pattern of habitat use suggests that these populations maintain well defined, but slightly overlapping, home ranges within a single river system. Genetic differentiation within drainages has also been observed in rainbow trout elsewhere (e.g., Krueger and May 1987; Currens et al. 1990) and in brown trout (e.g., Ryman et al. 1979; Hansen and Mensberg 1996). The patterns of mtDNA differentiation reported from our study reflect female mediated dispersal only, whereas analysis of the nuclear DNA would reflect both male and female dispersal; thus yielding a more complete picture of population structure.

The presence of multiple populations of rainbow trout in the Kenai River has clear implications for their management and conservation. Generally, the

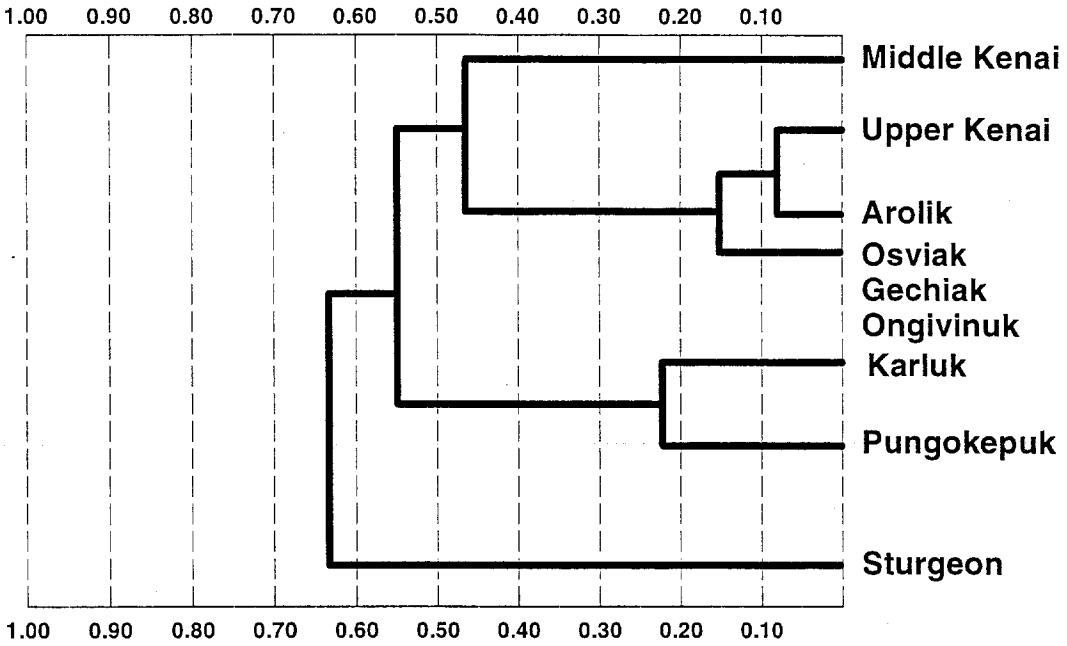



Figure 3. Dendrogram (UPGMA; Sneath and Sokal 1973) of genetic distances (Cavalli–Sforza and Edwards 1967, chord distance) showing the degree of genetic relatedness among collections of rainbow trout. Osviak, Gechiak, and Ongivinuk clustered together with zero genetic distance among them.

ideal unit of management is a population. Individuals in a population share a single life history cycle and experience the same density dependent and independent factors driving the dynamics of that population. This means that different populations may have different rates of production, recruitment, and age class structure that may require different management regimens. Treating multiple populations as a single population under a single management regimen can result in the overharvest of less productive populations. Thus, determining the nature of population structure is an important step in establishing an effective management plan.

Genetic methods provide managers the necessary tools to identify populations and their boundaries so that they can better formulate management plans. This study used genetic methods to conclude that multiple populations of rainbow trout appear to exist in the Kenai River, complementing the results of a movement study (Palmer 1998). These results lend support to past, present, and future management decisions regarding differential management of rainbow trout in the Kenai River, above and below Skilak Lake.

## Summary

1. Considerable mtDNA genetic variation apparently occurs among rainbow trout and steelhead populations in Alaska; although, larger sample sizes will be necessary for more thorough assessments.
2. At the molecular level, mtDNA genotypes displayed relatively low DNA sequence divergence (i.e., the genotypes were similar at the molecular level).
3. At the population level, mtDNA genotype frequencies varied among the nine collections; significant genetic differences occurred among collections on near and distant geographic scales.
4. Rainbow trout in the Middle and Upper Kenai River have different mtDNA genotype frequencies and represent different populations.

## Recommendations

1. Fully characterize population structure of rainbow trout in the Kenai River drainage by using a more thorough and extensive study design with larger sample sizes to determine the number of populations and their boundaries; plus, examine the temporal stability of genotype and allele frequencies in populations.
2. Apply nuclear DNA markers to more thoroughly assess genetic variation.
3. Characterize population structure of rainbow trout in other river systems to enable managers to formulate management strategies based on knowledge of population structure.

## Acknowledgments

This study was a collaborative effort of LGL Alaska Research Associates, Inc., the USFWS Kenai Fishery Resources Office (FRO), and the USFWS Fish Genetics Laboratory. We appreciate the support for field collections from the staff of the USFWS Kenai FRO, Tony Chatto (Kodiak NWR), Mark Lisac (Togiak NWR), and Rob McDonald (Togiak NWR). Laboratory support was provided by Andrea Medeiros and Julee Beasley of the USFWS Fish Genetics Laboratory. Graphics support was furnished by Dave Douglas (USGS–Biological Resources Division, Alaska Biological Science Center) and Julee Beasley (USFWS). The reviews kindly provided by Randy Brown (USFWS Fairbanks FRO), Jim Larson (USFWS King Salmon FRO), Mark Lisac (Togiak NWR), and Jennifer Nielsen (USGS–Biological Resources Division, Alaska Biological Science Center) contributed to the quality of the report.

## References

Adams N., W. Spearman, C. Burger, K. Currens, C. Schreck, and H. Li. 1994. Variation in mitochondrial DNA and allozymes discriminates early and late forms of chinook salmon in the Kenai and Kasilof rivers, Alaska. Canadian Journal of Fisheries and Aquatic Sciences 51 (Supplement 1): 172–181.

Allendorf, F.W. 1975. Genetic variability in a species possessing extensive gene duplication: genetic interpretation of duplicate loci and examination of genetic variation in populations of rainbow trout. Ph.D. thesis. University of Washington, Seattle.

Allendorf, F., N. Ryman, A. Stennek, and G. Ståhl. 1976. Genetic variation in Scandinavian brown trout (*Salmo trutta* L.): evidence of distinct sympatric populations. Hereditas 83: 73–82.

Bickham, J.W., C.C. Wood, and J.C. Patton. 1995. Biogeographic implications of cytochrome b sequences and allozymes in sockeye salmon (*Oncorhynchus nerka*). Journal of Heredity 86: 140–144.

Burger C.V., W.J. Spearman, and M.A. Cronin. 1997. Genetic differentiation of sockeye salmon subpopulations from a geologically young Alaskan lake system. Transactions of the American Fisheries Society 126: 926–938.

Campton, D.E., and F.M. Utter. 1987. Genetic structure of anadromous cutthroat trout (*Salmo clarki clarki*) populations in the Puget Sound area: evidence for restricted gene flow. Canadian Journal of Fisheries and Aquatic Sciences 44: 573–582.

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

Cronin M. W. Spearman, R. Wilmot, J. Patton, and J. Bickham. 1993. Mitochondrial DNA variation in chinook salmon and chum salmon detected by restriction enzyme analysis of polymerase chain reaction (PCR) products. Canadian Journal of Fisheries and Aquatic Sciences. 50: 708–715.

Currens, K.P., C.B. Schreck, and H.W. Li. 1990. Allozyme and morphological divergence of rainbow trout (*Oncorhynchus mykiss*) above and below waterfalls in the Deschutes River, Oregon. Copeia 1990(3): 730–746.

Hansen, M.M., and K.–L.D. Mensberg. 1996. Founder effects and genetic population structure of brown trout (*Salmo trutta*) in a Danish river system. Canadian Journal of Fisheries and Aquatic Sciences 53: 2229–2237.

Hayes, S.R., and J.J. Hasbrouck. 1996. Stock assessment of rainbow trout in the upper Kenai River, Alaska, in 1995. Alaska Department of Fish and Game, Fishery Data Series Number 96-43, Anchorage.

Howe, A.L., G. Fidler, A.E. Bingham, and M.J. Mills.

1996. Harvest, catch, and participation in Alaska sport fisheries during 1995. Alaska Department of Fish and Game, Fishery Data Series Number 96–32, Anchorage.

Krueger, C.C., and B. May. 1987. Genetic comparison of naturalized rainbow trout populations among Lake Superior tributaries: differentiation based on allozyme data. Transactions of the American Fisheries Society 116: 795–806.

Lafferty, R. 1989. Population dynamics of rainbow trout, Kenai River, Alaska. Master's thesis. University of Alaska, Fairbanks.

Lansman, R.A., R.O. Shade, J.F. Shapira, and J.C. Avise. 1981. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. III. Techniques and potential applications. Journal of Molecular Evolution 17: 214–226.

Nei, M., and J. Miller. 1990. A simple method for estimating average number of nucleotide substitutions within and between populations from restriction data. Genetics 125: 873–879.

Nelson, D.C. 1995. Area management report for the recreational fisheries of the Kenai Peninsula, 1994. Alaska Department of Fish and Game, Fishery Management Report Number 95-4, Anchorage.

Nielsen, J.L., C. Carpanzano, M.C. Fountain, and C.A. Gan. 1994. Mitochondrial DNA and nuclear microsatellite diversity in hatchery and wild *Oncorhynchus mykiss* from freshwater habitats in southern California. Transactions of the American Fisheries Society 126: 397–417.

Northcote, T.G., S.N. Williscroft, and H. Tsuyuki. 1970. Meristic and lactate dehydrogenase genotype differences in stream populations of rainbow trout below and above a waterfall. Journal of Fisheries Research Board of Canada 27: 1987–1995.

Palmer, D.E. 1998. Migratory behavior and seasonal distribution of radio–tagged rainbow trout in the Kenai River, Alaska. U.S. Fish and Wildlife Service, Alaska Fisheries Technical Report Number 46, Kenai, Alaska.

Park, L.K., M.A. Brainard, D.A. Dightman, and G.A. Winans. 1993. Low levels of intraspecific variation in the mitochondrial DNA of chum salmon (*Oncorhynchus keta*). Molecular Marine Biology and Biotechnology 2: 362-370.

Ryman, N., F.W. Allendorf, and G. Ståhl. 1979. Reproductive isolation with little genetic divergence in sympatric populations of brown trout (*Salmo trutta*). Genetics 92: 247–262.

Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular cloning, a laboratory manual. 2nd edition. Cold Spring Harbor, NY.

Sneath, P.H.A., and R.R. Sokal. 1973. Numerical Taxonomy. W.H. Freeman, San Francisco.

Sokal, R. R. and R. J. Rohlf. 1981. Biometry. 2nd edition. W. H. Freeman, San Francisco, California.

Swofford, D., and R. Selander. 1989. BIOSYS–1: a computer program for analysis of allelic variation in population genetics and biochemical systematics. Release 1.7. Illinois Natural History Survey.

USFWS. 1995. Fishery Management Plan, Kenai National Wildlife Refuge, FY1996–2000. Kenai Fishery Resources Office, U.S. Fish & Wildlife Service, Alaska.

Wishard, L.N., J.E. Seeb, F.M. Utter, and D. Stefan. 1984. A genetic investigation of suspected redband trout populations. Copeia 1984: 120–132.

Appendix 1. Restriction fragment patterns for mitochondrial DNA *cyt*B and ND5/6 in rainbow trout and steelhead. The numbers in the table are the fragment sizes (in nucleotide basepairs, bp) for each enzyme. Uncut sizes: *cyt*B≅1350 bp; ND5/6≅2460 bp.

| Fragment Size | *cyt*B | | | | | | | | | ND5/6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Dde*I | | | *Dpn*II | | *Msp*I | | *Nci*I | | *Taq*I | |
| | A | B | C | A | B | A | B | A | B | A | B |
| 1200 | — | — | — | — | — | — | — | + | + | — | — |
| 1048 | — | — | — | — | — | + | + | — | — | — | — |
| 852 | — | — | — | — | — | — | — | — | — | + | + |
| 733 | — | — | — | — | — | — | — | — | — | + | — |
| 667 | — | — | — | + | + | — | — | — | — | — | — |
| 600 | — | — | — | — | — | — | — | — | — | — | + |
| 568 | — | — | — | — | — | — | — | — | — | + | + |
| 387 | — | — | — | + | + | — | — | — | — | — | — |
| 346 | + | + | + | — | — | — | — | — | — | — | — |
| 290 | — | — | — | — | — | — | — | — | — | + | + |
| 282 | + | + | — | — | — | — | — | — | — | — | — |
| 262 | + | — | ++ | — | — | — | — | — | — | — | — |
| 215 | — | — | — | + | — | — | — | — | — | — | — |
| 208 | — | + | — | — | — | — | — | — | — | — | — |
| 194 | — | — | — | — | — | — | — | + | — | — | — |
| 182 | — | — | — | — | — | + | — | — | — | — | — |
| 167 | — | — | — | — | + | — | — | — | — | — | — |
| 130 | — | — | — | — | — | — | — | — | — | — | + |
| 122 | + | + | + | — | — | — | — | — | — | — | — |
| 112 | — | — | — | — | — | — | — | — | + | — | — |
| 108 | — | — | — | — | — | — | + | — | — | — | — |
| 96 | — | — | — | — | — | + | + | — | — | — | — |
| 86 | — | — | — | — | — | — | — | — | + | — | — |
| 82 | — | — | — | — | — | — | + | — | — | — | — |
| 80 | — | — | — | + | + | — | — | — | — | — | — |
| 62 | — | — | — | — | + | — | — | — | — | — | — |
| 50 | + | + | + | — | — | — | — | — | — | — | — |
| 20 | — | — | — | — | — | — | — | — | — | — | — |
| Total | 1062 | 1008 | 1042 | 1349 | 1363 | 1326 | 1334 | 1394 | 1398 | 2443 | 2440 |