

FISHERY RESEARCH



NATIVE SPECIES INVESTIGATIONS

Grant # F-73-R-24

July 1, 2001 to June 30, 2002

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Fisheries Research Biologist**

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Annual Performance Report

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Grant # F-73-R-24

Project 2: Native Species Investigations

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TABLE OF CONTENTS

| | <u>Page</u> |
|---|-------------|
| ABSTRACT..... | 1 |
| INTRODUCTION | 2 |
| MANAGEMENT GOAL | 2 |
| OBJECTIVES..... | 3 |
| METHODS | 3 |
| Eagle Fish Genetics Laboratory | 3 |
| Lab Design and Renovation..... | 3 |
| Lab Equipment Research and Purchasing | 8 |
| Microsoft® Access Genetic Database..... | 8 |
| Yellowstone Cutthroat Trout and Rainbow Trout Genetics Proposal | 10 |
| Genetics Work Plan (Current and Proposed Fishery Genetic Projects)..... | 10 |
| RESULTS | 11 |
| Eagle Fish Genetics Laboratory | 11 |
| Microsoft® Access Genetic Database..... | 14 |
| Yellowstone Cutthroat Trout and Rainbow Trout Genetics Proposal | 15 |
| Genetics Work Plan (Current and Proposed Fishery Genetic Projects)..... | 15 |
| ACKNOWLEDGEMENTS | 16 |
| LITERATURE CITED..... | 17 |
| APPENDICES..... | 18 |

LIST OF TABLES

| | |
|---------------------------------|---|
| Table 1. Database Outline. | 9 |
|---------------------------------|---|

LIST OF FIGURES

| | <u>Page</u> |
|--|-------------|
| Figure 1. Old Visitor Center, Eagle, Idaho (front room, facing west) | 4 |
| Figure 2. Old Visitor Center, Eagle, Idaho (back room, facing west). | 5 |
| Figure 3. Example of lab layout outline (right side of page is west end of lab). | 5 |
| Figure 4. Cement wall that housed viewing tanks. | 6 |
| Figure 5. Wooden support beam. | 6 |
| Figure 6. Window lowering. | 7 |
| Figure 7. Southeast wall prior to cement cutting to access outside entrance restrooms (on right)..... | 7 |
| Figure 8. Office construction. | 8 |
| Figure 9. PCR clean room. | 12 |
| Figure 10. Electrophoresis gel room | 12 |
| Figure 11. Main lab including stainless steel workbench (looking east)..... | 13 |
| Figure 12. Main lab (looking northwest). | 13 |
| Figure 13. Main lab (looking east). | 14 |
| Figure 14. Main genetic table in Microsoft® Access. | 15 |

LIST OF APPENDICES

| | |
|---|----|
| Appendix A. Equipment Inventory List | 19 |
| Appendix B. Estimated Cost Analysis | 20 |
| Appendix C. Proposal: "Assessment of genetic population structure and risk of introgression and hybridization to native trout in the Middle and Upper Snake River basins" | 21 |
| Appendix D. Genetics Work Plan. Idaho Department of Fish and Game, Current and Proposed Genetic Projects. | 42 |

ANNUAL PERFORMANCE REPORT

State of: Idaho

Grant No.: F-73-R-24, Fishery Research

Project No.: 2

Title: Native Species Investigations

Contract Period: July 1, 2001 to June 30, 2002

ABSTRACT

In the fall of 2001, the Idaho Department of Fish and Game (IDFG) began construction of a new genetics lab at the Eagle Fish Hatchery. This report describes the construction of this facility and documents several projects completed during the July 1, 2001 to June 30, 2002 contract period. Projects initiated included the development of an inventory database in Microsoft® Access to track samples and record genetic results, the development of a draft genetics work plan of IDFG's current and proposed fishery genetic projects, and the development of a proposal to provide detailed genetic information on Yellowstone cutthroat trout *Oncorhynchus clarki bouvieri* and rainbow trout *Oncorhynchus mykiss gairdneri* populations in Idaho.

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INTRODUCTION

Genetic analyses have increasingly become an invaluable tool in the management and conservation of native fish populations. Genetic information can assist managers in determining population structure and purity (Spruell et al. 1999; Campbell et al. 2002), delineating and prioritizing populations for conservation and management purposes (Allendorf et al. 1997), identifying suitable populations for translocations and reintroductions, identifying suitable populations for broodstock development (King et al. 1995), monitoring genetic diversity and effective population size in hatchery programs (Bartley et al. 1992; Hedrick et al. 2000), assessing the reproductive success of hatchery and wild fish (Leider et al. 1990; Gross and Kapuscinski 1997), and diagnosing disease (Batts et al. 1993; Andree et al. 1998).

A good example of genetic analyses providing useful information for management purposes is recent work completed on the native Yellowstone cutthroat trout *Oncorhynchus clarki bouvieri* population in Henry's Lake, Idaho (Campbell et al. 2002). Idaho Department of Fish and Game (IDFG) hatchery managers were concerned that the inability to distinguish hybrids (produced at the hatchery) from cutthroat trout could result in the accidental introduction of rainbow trout *Oncorhynchus mykiss gairdneri* genes into the hatchery-produced cutthroat trout population. Genetic analyses (species specific DNA markers), however, demonstrated that IDFG staff phenotype-based identifications were highly accurate in distinguishing cutthroat from F₁ hybrids when selecting broodstock (no F₁ hybrids detected among 80 samples identified as "pure"). Rather, the introgression of rainbow trout genes into the Yellowstone cutthroat trout population was probably the result of past rainbow trout introductions and the straying of hatchery-produced F₁ hybrids into tributary streams. Importantly, these results support IDFG's current management goal of implementing a hybrid sterilization program at the Henry's Lake Hatchery.

In 2001, IDFG began construction of a genetics lab to provide an efficient, cost-effective means of generating the detailed genetic information necessary for the proper management and conservation of Idaho's native fish species. This report describes the construction of the new lab at IDFG's Eagle Fish Hatchery. It also describes the development of an inventory database in Microsoft® Access to track samples and record genetic results, the development of a genetics work plan of the department's current and proposed fishery genetic projects, and includes a proposal developed during the July 1, 2001 to June 30, 2002 contract period focused on providing detailed genetic information on Yellowstone cutthroat trout and rainbow trout.

MANAGEMENT GOAL

1. Develop a working, state-of-the-art, fisheries genetics laboratory to provide detailed genetic information necessary for the proper management and conservation of Idaho's native fish species.

OBJECTIVES

1. Develop (plan, design, construct, and operate) a fisheries genetics laboratory for investigating the genetic purity, diversity, and structure of native fish populations and to provide PCR-based technology for confirmation testing of samples for Whirling Disease and Bacterial Kidney Disease.
2. Develop a Microsoft® Access database system to track sample inventory and genetic results.
3. Develop a project proposal for the genetic investigation of native Yellowstone cutthroat trout and native rainbow trout populations.
4. Develop a genetics work plan of IDFG's current and proposed fishery genetic projects to assist the bureau, management, and research staff, and to organize and prioritize genetic samples and projects for analysis and implementation.

METHODS

Eagle Fish Genetics Laboratory

Lab Design and Renovation

The renovation of the Visitor Center (Figures 1, 2 and 3) located on IDFG's Eagle Fish Hatchery grounds began in early November 2001. Renovation was extensive and included the removal of five viewing tanks and the cement wall that served to support and encase the tanks (Figure 4). Since the concrete wall separating the two sides of the building also served as the main support for the ceiling, construction of a 30' x 2' wooden support beam (Figure 5) was necessary. Additional renovations included:

1. The addition of three new windows and the lowering of four existing windows (Figure 6)
2. Transformation of the two outside entrance restrooms into two laboratory rooms: a Polymerase Chain Reaction (PCR) clean room and an electrophoresis gel room (Figure 7). The construction of a PCR clean room was included to prevent environmental contamination (DNA or Dnases) of consumable products used for PCR. The construction of a separate electrophoresis gel room was included to isolate ethidium bromide, a mutagen and carcinogen used to stain DNA.
3. Installation of three sinks and an overhaul of existing plumbing.
4. Installation of a water softener and deionizer system for production of lab quality water.
5. Installation of phone and Internet cable lines.
6. Installation of 110v and 220v outlets.

7. Installation of new doors at the west, east, and south entrances (double doors).
8. Installation of a stainless steel center island workbench.
9. Installation of extensive cabinetry for lab bench and storage space.
10. Construction of office for genetics lab manager (Figure 8).



Figure 1. Old Visitor Center, Eagle, Idaho (front room, facing west).



Figure 4. Cement wall that housed viewing tanks.



Figure 5. Wooden support beam.



Figure 6. Window lowering.



Figure 7. Southeast wall prior to cement cutting to access outside entrance restrooms (on right).



Figure 8. Office construction.

IDFG staff performed most of the actual physical renovation of the facility. This included wall removal, PCR clean room and gel room construction, window lowering, installation of new windows, beam construction and installation, framing, glass-board installation, cabinet installation, plumbing renovation, and painting. Electrical work was completed by an IDFG certified electrician.

Lab Equipment Research and Purchasing

Equipment necessary to make the lab functional was researched and acquired or purchased by IDFG Eagle Genetic Laboratory and IDFG Fish Health Laboratory staff. Technical advice regarding equipment options and capabilities were provided by Brent Boyle (Fisher Scientific, Research Division Sales Representative), Joyce Faler (Senior Scientific Aide, University of Idaho, Moscow, Idaho), and Dr. Matt Powell (Geneticist, University of Idaho, Hagerman, Idaho).

Microsoft® Access Genetic Database

The genetic database was developed in the software program Microsoft® Access 2000, following procedures recommended by the Executrain Microsoft® Access 2000 Manual (ExecuTrain Corporation 1999). Genetic database development was initiated with the construction of an outline describing all of the sample information to filter, query, analyze, and display in the database (Table 1).

Table 1. Database Outline.

| Access Outline | | |
|---|---|--|
| Tables: | Sample Location | Genetics Lab (who is running sample) |
| 1. Sample Location (State, Region, County, Drainage Basin, Stream/River, Stretch, River-mile), must be GIS and ArcView® compatible. | <i>Agency</i> | 1. Idaho Department of Fish and Game, Eagle, ID |
| 2. Agency | <i>Species</i> | 2. University of Idaho, Moscow, ID |
| 3. Species | <i>Contact (Biologist/Manager)</i> | 3. University of Idaho, Hagerman, ID |
| 4. Contact (Biologist/Manager) | Contact (Genetics Lab) | 4. University of Montana, Missoula, MT |
| 5. Contact (Genetics Lab) | 1. Bruce Baker/Washington Department of Fish and Wildlife | 5. Washington Department of Fish and Wildlife, Olympia, WA |
| 6. Project Title | 2. Matthew Campbell/Idaho Department of Fish and Game | 6. Alaska Department of Fish and Game, Anchorage, AK |
| 7. Project Question | 3. Joyce Faler/University of Idaho | 7. Other |
| 8. Status | 4. Robb Leary/University of Montana | |
| | 5. Jennifer Nielsen/Alaska Department of Fish and Game | |
| | 6. Matt Powell/University of Idaho | |
| • Progress Reports | | Tissue Type |
| • Final Reports | | 1. Fin clip |
| 9. Sample Storage Location | | 2. Muscle tissue |
| 10. Genetics Lab | | 3. Blood |
| 11. Genetic Technique | Project Question | 4. Sperm |
| 12. Raw data table | 1. Hybridization/Introgression | 5. Heart |
| • A table for each genetic technique used (mtDNA RFLPs, nDNA/Intron RFLPs, microsatellites) | 2. Genetic Diversity/Differentiation | |
| Tissue Type | | 6. Eye |
| 13. Sample Storage Media | 3. MU/ESU identification/designation | 7. Liver |
| 14. Budget (budget number) | 4. Hatchery Broodstock Selection | 8. Kidney |
| | 5. Phylogeography | |
| | 6. Pathogen detection and identification | 9. Other |
| | 7. Sex identification | |
| Info to be included in Master Table: | | Sample Storage Media |
| 1. Sample Location (State, Region, County, Drainage Basin, Stream/River, Stretch, River-mile). | Status | 1. Lysis Buffer |
| 2. Agency | 1. Have not received samples | 2. Ethanol |
| 3. Species | 2. Have received samples | 3. Frozen |
| 4. Contact (Biologist/Manager) | | 4. Dried |
| 5. Contact (Genetics Lab) | 3. DNA extracted | 5. Other |
| 6. Project Title | 4. Samples completed | |
| 7. Project Question | 5. Preparing final report | Genetic Technique |
| 8. Status | 6. Final report completed | 1. mtDNA RFLP |
| 9. Sample number/label | Sample Storage Location | 2. nDNA Intron RFLP |
| 10. Date Collected | 1. Eagle Fish Hatchery, Eagle, ID | 3. Microsatellites |
| 11. Sample Storage Location | 2. University of Idaho, Moscow, ID | 4. Allozyme electrophoresis |
| 12. Genetics Lab | 3. University of Idaho, Hagerman, ID | 5. RAPDs |
| 13. Genetic Technique | 4. IDFG stations/sites | 6. Quantitative PCR |
| 14. Tissue Type | | 7. Other |
| 15. Sample Storage Media | | |
| 16. Budget (budget number) | | |

The database should include passwords on raw data and reports so that only the project manager/PI could review info. Parts of this database should be very restrictive with regards to who has access to raw data, etc. Generally, raw data is reviewed by the lab manager and P.I. and is not released to biologists, managers, etc. (because it is raw data that has not been verified).

Primary requirements:

1. Ability to inventory and organize all genetic samples collected within the state.
2. Query compatibility with current IDFG Access databases.
3. Compatible with GIS and ArcView® mapping software programs.

Yellowstone Cutthroat Trout and Rainbow Trout Genetics Proposal

After discussions with IDFG management and research staff, it was determined that detailed genetic information on Yellowstone cutthroat and redband trout populations throughout the Middle and Upper Snake River basins was needed to guide IDFG management plans and decisions. A project proposal to develop genetic information was developed to complement the demographic and ecological information collected under IDFG's current Snake River Native Salmonid Assessment project and provide managers the genetic information needed for effective conservation and restoration efforts. Project proposal goals and objectives were agreed upon by IDFG research staff (Matthew Campbell, Steve Yundt, and Paul Kline) and Dr. Matt Powell (Geneticist, University of Idaho, Hagerman, Idaho). Genetic methods were reviewed and agreed upon by Matthew Campbell and Matt Powell.

Genetics Work Plan (Current and Proposed Fishery Genetic Projects)

The development of a genetics work plan of current and proposed fishery genetic projects for the State of Idaho was initiated through a request for information regarding current projects and proposals. The request for information was e-mailed to 49 project managers/leaders in Idaho and asked the following questions:

1. What projects are you currently working on in your region that involves collecting tissue samples for genetic analysis?
2. What projects/samples have funding?
3. What agencies/individuals are you cooperating with on sample collection/genetic projects?
4. What questions are you trying to address with genetic information?
5. What tissue samples have you or your cooperators collected?
6. Where are the tissues currently stored?
7. How are the tissues stored (in alcohol, in lysis buffer, frozen)?
8. What genetics lab(s) have you worked with in the past?
9. Which lab(s) are you currently working with?
10. Who is the genetic lab contact (name and phone number) on these projects?
11. Are there genetic reports that have been completed on your projects that I could read?
12. What projects do you see as high priority?
13. Do you need sample collection tubes for the upcoming field season?

Manager/project leaders were asked to respond to questions through e-mail, phone, or directly in person and were contacted by phone or directly in person for clarification and further information.

Individual projects were described in 16 sections as follows: Region, Manager/Project Leader, Project Title, Population/Species, Questions/Objectives, Genetic Analyses, Sample Locations/Populations and Samples to be Analyzed, Cooperating Agencies, Genetic Lab Performing Work, Sample Storage Location, Time Frame, Proposed Cost, Funding Source, Relationship to Other Projects, Current Status of Project, and Comments.

Suggestions for genetic analyses and methods for genetic analyses came from current literature reviews (including Campbell et al. 2002; Baker et al. 2002; Shaklee and Young 2002) as well as discussions with Dr. Matt Powell (Geneticist, University of Idaho, Hagerman, Idaho), and Dr. James Shaklee (Geneticist, Washington Department of Fish and Game, Olympia, Washington). Project costs were estimated using a cost worksheet developed at the Eagle Genetics Laboratory based on current prices of consumable supplies and technician hourly wages (Appendix A).

RESULTS

Eagle Fish Genetics Laboratory

The IDFG Eagle Fish Genetics Laboratory was completed in May 2002. The 700 square foot facility has separate rooms for clean PCR master-mix preparation (Figure 9) and for Gel Electrophoresis (Figure 10). The clean PCR room contains its own refrigerator and -20°C freezer, pipettes, and storage cabinets. The Gel Electrophoresis room has a sink with distilled and deionized water, pipettes, and chemical hood. The main lab (Figures 10, 11, and 12) houses a PCR U-V hood for final PCR DNA amplification set-up, two built-in sinks with D.I. water, two storage refrigerators, a -20°C freezer for chemical storage, a -80°C freezer for long-term sample storage, a computer for data analysis, and bench space for up to four technicians. The lab is outfitted with all of the equipment necessary for mitochondrial DNA Restriction Fragment Length Polymorphism (RFLP) analysis, nuclear intron DNA RFLP analysis, microsatellite amplification and purification, and dye-terminator sequencing amplification and purification. This includes two 96 well MJR PTC-100 PCR machines, two horizontal agarose gel systems, and two vertical acrylamide gel systems (a full equipment inventory is listed in Appendix B). Products from microsatellite and sequencing reactions will still have to be run out on the University of Idaho's ABI 3100 sequencer in Hagerman, Idaho.

The IDFG Eagle Fish Genetics Laboratory is currently capable of addressing questions pertaining to genetic purity, diversity, and structure of native fish populations and can provide PCR-based technology for confirmation testing of samples for Whirling Disease and Bacterial Kidney Disease.



Figure 9. PCR clean room.



Figure 10. Electrophoresis gel room



Figure 11. Main lab including stainless steel workbench (looking east).



Figure 12. Main lab (looking northwest).



Figure 13. Main lab (looking east).

Microsoft® Access Genetic Database

The construction of the Microsoft® Access Genetic Database was finished in the spring of 2002. The database allows for the inventorying of genetic samples collected by projects throughout the State. It also allows for the recording, organizing, and retrieval of genetic results that the Eagle Fish Genetics Lab will generate in the future, as well as the ability to query results from multiple projects or multiple analyses. Perhaps most importantly, the database is designed to share information with other Microsoft® Access databases currently being used by IDFG and be compatible with GIS and ArcView® mapping software programs. An example of the main genetic table in which sample information is entered is shown below (Figure 14). Over 125 sample locations and nearly 5000 samples from six separate projects have been entered into the Microsoft® Access Genetic Database as of June 30, 2002.

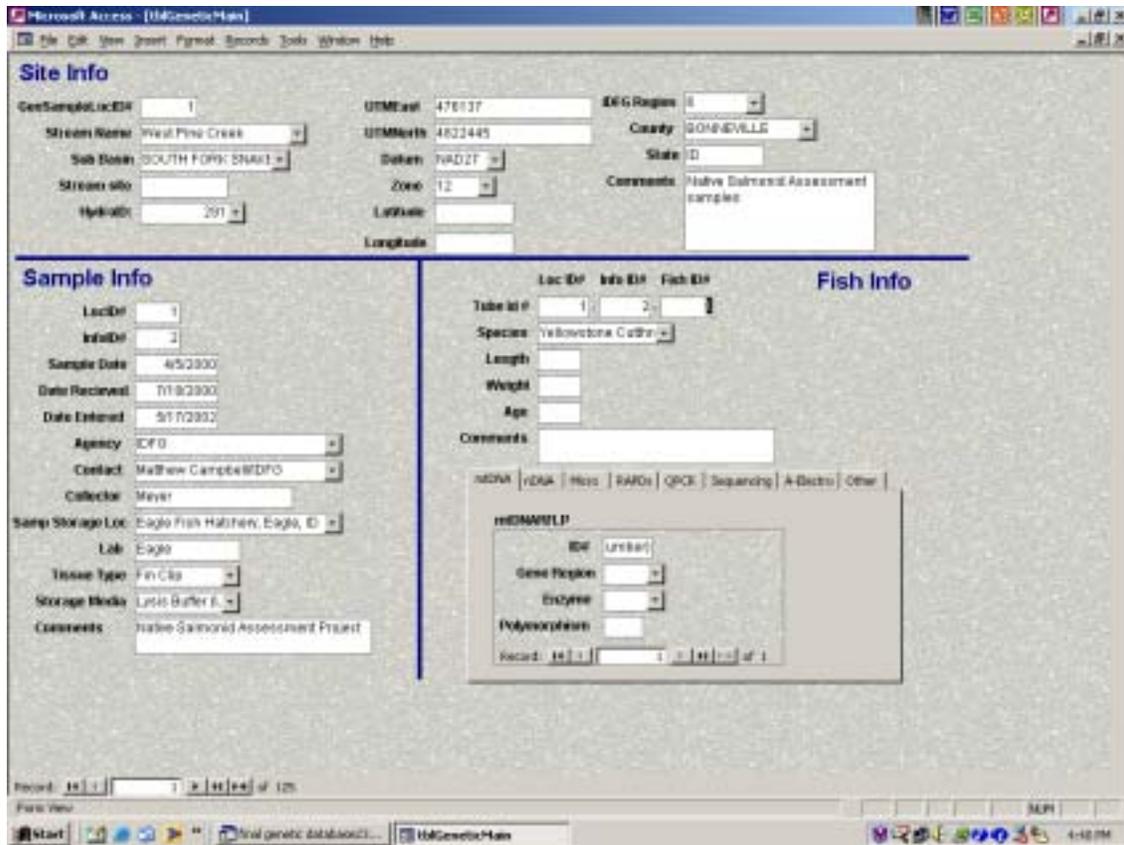


Figure 14. Main genetic table in Microsoft® Access.

Yellowstone Cutthroat Trout and Rainbow Trout Genetics Proposal

A proposal focused on the assessment of genetic population structure and risk of introgression and hybridization to Yellowstone cutthroat trout and redband trout in the Middle and Upper Snake River basins was completed in mid-December 2001 (Appendix C).

Genetics Work Plan (Current and Proposed Fishery Genetic Projects)

As of June 30, 2002, 20 projects from 18 different project managers/leaders have been identified and outlined in the Genetics Work Plan (Draft, Appendix D). Proposed projects include investigations on Westslope cutthroat *Oncorhynchus clarki lewisi*, Yellowstone cutthroat *Oncorhynchus clarki bouvieri*, rainbow trout/steelhead *Oncorhynchus mykiss*, bull trout *Salvelinus confluentus*, Chinook salmon *Oncorhynchus tshawytscha*, sockeye salmon *Oncorhynchus nerka*, lamprey *Lampetra tridentata* and shorthead sculpin *Cottus confusus*. Current proposed projects range in time frame from two weeks to three years with a total estimated cost of over 1.5 million dollars.

ACKNOWLEDGEMENTS

First and foremost, a tremendous thanks must be extended towards Doug Marsters and Larry Gardner for their expertise and care in the construction of the genetics lab. Many thanks also to Chris Cegelski, Carla Hogge, Keith Johnson, Sharon Landon, Brian Malaise, and Roberta Scott for their help with lab design, equipment investigations, and purchasing. The entire Eagle Fish Hatchery staff should be recognized for their help with lab construction. Thanks to Jim Davis for his useful suggestions in the design of the Microsoft® Access database and to Tony Lamansky for construction of the database. Matt Powell's assistance in the development of the proposal is greatly appreciated, as well as Steve Yundt's direction on work priorities and project development. Finally, a big thank you to Paul Kline for his guidance during the past year and his help on every project described in this report.

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APPENDICES

Appendix A. Equipment Inventory List

| Item | Company | Price |
|-------------------------------------|--------------------|--------------|
| 2.5 Cubic foot isotemp incubator | Fisher Scientific | \$1,122.01 |
| 5.0 Cubic foot isotemp incubator | Fisher Scientific | \$1396.15 |
| -80°C ultra-low freezer | NuAire, Inc. | \$4,752.00 |
| Acrylamide gel casting units | Bio-Rad | \$355.00 |
| Top loading balance (0-600g) | Denver Instruments | \$388.93 |
| Office chairs (4) | Staples | \$519.96 |
| Chest freezer | RC Wiley | \$159.99 |
| Corning Hotplate-Stirrer | Fisher Scientific | \$243.60 |
| Dell computer w/flat-screen monitor | Dell | \$1,805.00 |
| Electrophoresis gel casting system | Owl Scientific | \$900.00 |
| -20°C Freezer | Danby | \$144.99 |
| 24 place Z180M Microcentrifuge | Hermle | \$1495.00 |
| Minicentrifuge-PCR strip-tubes (2) | Fisher Scientific | \$425.00 |
| Microwave | RC Wiley | \$250.00 |
| 8 multi-channel pipette | Eppendorf | \$483.75 |
| Acumet pH Meter | Fisher Scientific | \$477.27 |
| Paramount HEPA-Filtered hood | Labconco | \$7,200.00 |
| Stand for HEPA-filtered hood | Labconco | \$1,500.00 |
| GE Refrigerator | RC Wiley | \$449.97 |
| Refrigerator | RC Wiley | \$639.99 |
| Set of single-channel pipettes | Eppendorf | \$819.00 |
| PTC-100 Thermocycler (2) | MJ Research, Inc. | \$9180.50 |
| Microcentrifuge tube shaker | Labquake | \$280.65 |
| UV dead-air box for PCR set-up | Airclean Systems | \$1,495.00 |
| UV 120V lamp | Fisher Scientific | \$324.90 |
| Vacuum pressure station | Barnant Company | \$150.38 |

Appendix B. Estimated Cost Analysis

| | Cost of Reagents for 100 (\$)* | With Time (\$) ** |
|---|--------------------------------|-------------------|
| Ethanol tubes | \$13.30 | \$17.30 |
| Lysis buffer tubes | \$13.30 | \$40.00 |
| Chloroform: Salt extraction | \$45.00 | \$180.00 |
| Test Gel (1% agarose) | \$0.38 | \$7.70 |
| Test Gel (3% agarose) | \$1.00 | \$8.50 |
| Acrylamide gel | \$2.75 | \$15.00 |
| PCR reaction (1 gene region) | \$22.00 | \$40.00 |
| Sequencing (1 gene region) | \$600.00 | \$3000.00 |
| Restriction enzyme digest (1 gene region) | \$21.50 | \$35.00 |
| Restriction Enzyme Digests (6 loci: 5 nuclear + 1 mtDNA) Includes PCRs, gels, and set-up time | \$1700.00 | \$1940.00 |
| Fragment Analysis (Visual identification of bands) Database Management | | |
| Microsatellite Analysis (6 loci) Includes PCRs, gels, and set-up time Includes PCRs, gels, and set-up time Fragment Analysis (GeneScan, Genotyper®) Database Management | \$850.00 | \$1500.00 |

* Estimated using current tube and chemical distributor prices June 30, 2002

**Estimated using hourly wage of \$15.00

Appendix C. Proposal: “Assessment of genetic population structure and risk of introgression and hybridization to native trout in the Middle and Upper Snake River basins”

a. Abstract

This project seeks to detect and quantify levels of introgression from hatchery-produced *O. mykiss* within native Yellowstone cutthroat trout populations and native redband trout populations. This project will also assess genetic diversity and genetic population structure within Yellowstone cutthroat and redband trout throughout the Middle and Upper Snake River basins. This project will provide the genetic information fisheries managers require to assess risk and to protect and restore these two ecologically and economically important native species. Specifically, this genetic information will assist in prioritization of populations for conservation and management purposes, as well as identifying suitable populations for translocations, reintroductions, and all currently proposed or ongoing broodstock development programs.

b. Background

Management and conservation of Yellowstone cutthroat trout *Oncorhynchus clarki bouvieri* populations and native, redband trout *Oncorhynchus mykiss gairdneri* populations have become high priorities for many state and federal agencies due to dramatic population declines throughout their historic native ranges (Williams et al. 1996; Thurow et al. 1988; Behnke 1992; May 1996). Both species have been petitioned for listing as threatened under the Endangered Species Act (ESA) (Biodiversity Legal Foundation et al. 1994; 1998), and both species are recognized as a “species of special concern” or a “sensitive species” by the U.S. Fish and Wildlife Service, the U.S. Forest Service, the Bureau of Land Management, the American Fisheries Society, and all states throughout their historic range (Thurow et al. 1988; IDFG 2000; Clancy 1988; Wyoming Game and Fish Department 2000; Gresswell 1995).

Population declines of both species are due to a variety of complex, contributing factors, including habitat degradation, overfishing, and the extensive stocking of nonnative, hatchery-produced, rainbow trout *Oncorhynchus mykiss*, which have hybridized with or replaced native Yellowstone cutthroat trout and native, redband trout populations throughout their historic native range (Varley and Gresswell 1988; Behnke 1992). Management response to these declines has likewise been complex and has involved numerous private, state, and federal agencies working together for the purpose of outlining recovery and conservation strategies. The results of these collaborations have been the production of detailed management planning documents that have stressed as goals and objectives the importance of:

- Identifying and conserving remaining pure, native, trout populations and the genetic diversity present within them, and
- Increasing the number of trout populations within their native range through habitat improvements, translocations, and hatchery supplementation.

However, current management plans are severely hampered in reaching these objectives by the lack of required genetic information needed for effective conservation and restoration efforts. This project serves to provide detailed genetic information with regards to levels of hybridization and introgression, genetic diversity, and genetic population structure of Yellowstone cutthroat and redband trout populations throughout the Middle and Upper Snake River basins. The information gained from this project should help managers directly in:

- Assessing current and future genetic risks,
- Assessing the predictive power of Idaho Department of Fish and Game's (IDFG) Historical Stocking Database in quantifying hybridization and introgression levels,
- Preserving existing genetic variability,
- Delineating and prioritizing populations for conservation and management purposes,
- Estimating effective population size,
- Understanding genetic population structure,
- Identifying suitable populations for translocations and reintroductions,
- Identifying suitable populations for broodstock development, and
- Addressing genetic concerns in future ESA petitions.

In this study, three different but complementary genetic techniques will be used to address the proposal objectives. The first phase of this project will involve a genetic screen for rainbow trout introgression using mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) markers within designated sample locations throughout the range of Yellowstone and redband trout in the Middle and Upper Snake River basins. This screen will allow an assessment of the predictive power of the Idaho Historical Stocking Database in quantifying hybridization and introgression rates (and, therefore, a good predictor of risks to cutthroat trout populations from historical rainbow trout stocking), and will allow identification of nonintrogressed populations for further genetic analysis. The second phase of this project will involve a more detailed and comprehensive genetic study using an array of microsatellite loci to determine genetic diversity and genetic population structure of Yellowstone cutthroat trout and redband trout populations in the Middle and Upper Snake River basins.

c. Rationale and Significance to Regional Programs

The rationale behind this project is to provide critically needed genetic information to aid state and federal agencies in the protection, restoration, and prioritization of native resident trout populations in the Upper and Middle Snake River basins. The genetic information obtained from this project will directly assist managers in meeting the goals and objectives for resident fish outlined in the 2000 Fish and Wildlife Program (NPPC 2000) that state:

“Restore native resident fish species (subspecies, stocks, and populations) to near historic abundance throughout their historic ranges where original habitat conditions exist and where habitats can be feasibly restored.”

And: “Complete assessments of resident fish losses throughout the basin resulting from the hydrosystem, expressed in terms of the various critical population characteristics of key resident fish species.”

This project also addresses goals and objectives directly outlined for resident fish in the 1994 Fish and Wildlife Program (FWP), Section 10 (NPPC 1994) or goals and objectives that the Council “believes should be applied to resident fish” (Section 7.1). The 1994 FWP states that a:

“Thorough and comprehensive approach to conserving genetic diversity is needed for native species” (Section 10.2B)

And requests a recommendation for the: “Approach to identify provisional genetic conservation units for production and harvest, and rules for taking action with regard to those conservation units” (Section 7.1B.1).

Numerous additional state and regional conservation and management summaries have identified the need for genetic information with regards to hybridization and introgression, genetic diversity, and genetic population structure of native resident trout populations. The most notable examples of these requests for genetic information are outlined below:

1. Middle and Upper Snake River Basin Summaries 2001 (NPPC 2001).

The Statements of Fish and Wildlife Needs in the Subbasin summaries for the Middle and Upper Snake Basins clearly identify the need for the genetic work outlined in this proposal:

“Use genetic markers to detect and quantify levels of hatchery produced *O. mykiss* introgression within native Yellowstone cutthroat trout populations and to delineate genetic population structure of Yellowstone cutthroat trout throughout their historic range. This fundamental genetic information with regards to introgressive hybridization and genetic population structure is needed to identify remaining pure populations, preserve existing genetic variability, and identify population segments for the development of management plans and the designation of conservation units/management units.”

“Compare rates of hybridization and introgression between hatchery-produced *O. mykiss* and native populations of Yellowstone cutthroat, redband trout, and westslope cutthroat trout. A greater understanding of the phenomenon of hybridization and introgression observed within *Oncorhynchus* populations throughout the Middle and Upper Snake River basins should allow a better assessment of the impacts of past hatchery-produced *O. mykiss* introductions and allow a better evaluation of the possible future genetic risks native *Oncorhynchus* populations face with regards to hybridization and introgression.”

“Develop genetic-DNA markers for redband trout so that the degree of introgression with introduced rainbow trout can be quantified and the degree of variability between and among populations of redband trout can be determined.”

2. Memorandum of agreement for conservation and management of Yellowstone cutthroat trout among Montana, Idaho, Wyoming, Nevada, Utah, U.S. Forest Service, Yellowstone National Park, Grand Teton National Park (MOA 2000).

This memorandum of agreement between the above resource agencies explicitly states as its goals and objectives that the agencies:

“Ensure the persistence of the Yellowstone cutthroat subspecies within its historic range. Manage YCT to preserve genetic integrity and provide adequate numbers and populations to provide for protection and maintenance of intrinsic and recreational values associated with the fish.”

“Identify genetic purity of existing populations. Prioritize populations based on genetic purity, population size, unique characteristics, and management goals. Secure and if necessary enhance all known and suspected genetically pure YCT populations, and high priority introgressed populations.”

“Increase the number of stream populations by restoring YCT within their native range.”

3. “Cutthroat Trout Management: A Position Paper: Genetic Considerations Associated with Cutthroat Trout Management. Publication Number 00-26” (UDWR 2000).

This position paper developed by the U.S. Fish and Wildlife Service, U.S. Forest Service, Colorado Division of Wildlife, Idaho Department of Fish and Game, Montana Fish, Wildlife and Parks, Nevada Division of Wildlife, New Mexico Game and Fish, Utah Division of Wildlife Resources, and Wyoming Game and Fish Department explicitly states as its goals and objectives that:

“The primary management goal for conservation populations is to preserve and conserve unique genetic, ecological, and behavioral characteristics of the subspecies that exist on a population by population basis.”

“The primary management goal for core conservation populations is to facilitate long-term persistence of each subspecies in a genetically pure condition.”

“Core conservation populations will serve as the primary source for gametes for introductions and reintroductions through transplants and brood stock development.”

“Identification of core populations will require complete genetic analysis to validate purity.”

4. Yellowstone cutthroat trout *Oncorhynchus clarki bouvieri* Status Review, USDA Forest Service (May 1996).

In this status review the author clearly outlines specific needs for Yellowstone cutthroat management including:

“Yellowstone cutthroat populations need to be screened for genetic purity. This is especially true for populations in Idaho and Wyoming where only limited testing has occurred to date.”

“Information on genetic status will provide a clearer understanding of the need for protection.”

“Consideration should be focused on genetic restoration of hybridized populations through repeated introductions of genetically pure individuals. Population specific genetic information will be needed to evaluate the applicability of this option.”

d. Relationships to other projects

This proposed project is a logical extension to the Snake River Native Salmonid Assessment Project (199800200) being conducted by IDFG. The primary goal of the Snake River Native Salmonid Assessment Project is the protection and restoration of populations of native salmonids in the Middle and Upper Snake River basins to self-sustaining, harvestable levels. The project has focused on measuring the abundance and status of native salmonid populations in the Snake River and describing and measuring the habitat characteristics in which these populations are found. The overall plan is to use this population and habitat information to identify life history and habitat needs, causes for population declines, and opportunities for restoration. While this project has collected fin tissue samples for genetic analysis to identify pure and introgressed populations, a genetic component to this project has been limited, both in scope and coverage. We believe that a comprehensive, genetically-based approach for native salmonid conservation and management is needed to complement the demographic and ecological approaches outlined in the current BPA-funded project 199800200. Not only will this new project actually perform the needed genetic analyses to detect and quantify levels of rainbow trout introgression, it will also provide the fundamental genetic information on population genetic variability and structure that will allow managers to meet goals of long-term persistence of Yellowstone cutthroat and redband trout populations in the Middle and Upper Snake River basins.

The Shoshone-Bannock/Shoshone Paiute Joint Culture Facility Project (199500600) is also a logical collaborator with this proposed project. Project 199500600 includes as study objectives:

1. To provide baseline information on genetic variation within and among populations of redband trout in the Duck Valley Indian Reservation, and
2. To assess the extent of hatchery introduced rainbow trout introgression within these populations.

Importantly, the genetic work for project 199500600 is being performed by University of Idaho (Dr. Madison Powell) and is using the exact same nuclear and mitochondrial DNA markers to investigate rainbow trout introgression and the exact same microsatellite markers to investigate genetic population structure as this proposed project. Managers of project 199500600 have agreed to share genetic information gained from their project with this project to allow a complete and comprehensive analysis of genetic population structure of redband trout populations throughout the Middle and Upper Snake River basins.

e. Proposal Objectives, Tasks And Methods

This project will be carried out in two separate but complementary phases. The first phase involves genetic screening for rainbow trout introgression within designated locations throughout the range of Yellowstone cutthroat and redband trout in the Middle and Upper Snake River basins. Part of this phase will involve additional genetic analysis of samples screened in a preliminary study initiated by the IDFG and the University of Idaho. However, genetic analysis of samples from locations that have not previously been examined will also be required to raise the level of statistical significance for hypothesis testing. The overall goals of this phase of the project will be to assess the predictive power of stocking records in quantifying hybridization and introgression and to identify nonintrogressed populations for further genetic analyses of population structure and divergence.

Genetic work proposed for the first phase of this study that focuses on the detection and quantification of introgressive hybridization from introduced hatchery rainbow trout will involve restriction fragment length polymorphism (RFLP) analysis of both mtDNA and nDNA gene regions. This work requires no special interpretation for interspecific hybrids since the nDNA and mtDNA markers used are fixed between Yellowstone cutthroat and rainbow trout (Campbell et al. In Press).

However, identification of intraspecific hybridization and introgression between native redband trout and introduced rainbow trout of hatchery origin is problematic. Analytical results are complicated, since currently there are no known protein, mtDNA, or nDNA markers that exhibit fixed differences between native and hatchery produced *O. mykiss* populations. Nevertheless, the utility of mtDNA RFLP analysis in assessing intraspecific *O. mykiss* hybridization has been previously demonstrated. Williams and Jaworski (1995) and Williams et al. (1996) examined mtDNA diversity in native trout populations from the Kootenai River in northern Idaho and from several native and nonnative trout populations in southern Idaho. They concluded that introgressed or admixed populations often exhibit higher mtDNA diversity than non-admixed populations and frequently exhibit multiple, dominant mtDNA haplotypes. Additionally, their findings show mtDNA haplotype divergence was higher within introgressed populations (0.9%-1.5% sequence divergence) than within nonintrogressed populations (less than 0.5% sequence divergence).

We will employ both nDNA (one intron gene region) and mtDNA analysis (two gene regions, 10 restriction enzymes each) to assess hybridization between hatchery rainbow trout and native redband trout and to identify nonintrogressed redband trout populations for further genetic study.

The overall utility of microsatellites for intraspecific hybridization investigations remains largely unknown. As such, we also plan to screen microsatellite results obtained from the second phase of this project to identify any possible fixed allelic differences between redband trout populations and reference hatchery rainbow trout populations.

The second phase of this project involves a detailed and comprehensive genetic study using microsatellite loci to determine genetic diversity and genetic population structure of Yellowstone cutthroat trout and redband trout populations in the Middle and Upper Snake River basins. Genetic information obtained from the first phase of this project will be used to identify sample locations for further population structure analysis. The salient point of phase one is to identify sampling locations that are free of rainbow trout introgression, since introgressed populations will confound genetic variability and population structure estimates. Detailed descriptions of each of the two phases of this project are described below.

Phase One:

Objective 1. Assess the predictive power of Idaho's Historical Stocking Database in quantifying hybridization and introgression levels, and identify and prioritize nonintrogressed populations for additional genetic work.

Yellowstone cutthroat trout samples

As part of the Snake River Native Salmonid Assessment project number 199800200, and in cooperation with the U.S. Forest Service, the U.S. Fish and Wildlife Service, and the Bureau of Land Management, IDFG has collected over 6000 non-lethally collected fin tissue samples of Yellowstone cutthroat trout from over 200 sampling locations throughout their entire native range in the Upper Snake River Basin (Table 1; Figure 1). The IDFG has also secured over 200 samples of Yellowstone cutthroat from Yellowstone Lake, Wyoming and over 100 samples of Yellowstone cutthroat trout (fine-spotted form) from the Jackson National Fish Hatchery for use as comparison and reference populations. The task will be the identification of a subset of these 6000 samples to examine for introgressive hybridization and population genetic structure work. In 2000, IDFG and the University of Idaho began a screen for rainbow trout introgression within Yellowstone cutthroat samples from 40 locations that had been sampled up to that point. This work was initiated to meet the 90-day review of the 1998 petition to list Yellowstone cutthroat as threatened under the ESA. An assessment of rainbow trout introgression within these sample locations was done using one mtDNA RFLP marker and two nDNA intron RFLP markers diagnostic between cutthroat trout and rainbow trout. Preliminary results identified rainbow trout mtDNA and/or nDNA in 17 of these populations (Table 2). We propose that work on these samples be finished by increasing the sample size to 60 at each sample location, which will insure 95% confidence of detecting rainbow trout mtDNA haplotypes present within the population at a frequency of 5% or greater. We also suggest that three additional nDNA markers that have previously been shown to have fixed allele differences between rainbow and cutthroat trout be run on these same samples (Campbell et al. In Press). This additional work will provide increased power in detecting introgression and should help with our ability to determine the type and extent of introgression within these populations. Finally, we suggest that four additional sample locations/populations from each of the ten major drainages sampled be selected for a screen of rainbow trout introgression and a test of the historical stocking database (4 locations X 10 major drainages X 60 samples = 2400 samples). Of these four sample locations within each major drainage, two will be selected from streams that have never received rainbow trout stocking according to the historical stocking database. The remaining two additional sample locations will be selected from streams that have had substantial rainbow trout stocking according to the

database. Not only will these additional samples allow an *a priori* selection of populations to test the stocking database, it will also provide a more complete, comprehensive geographic range from which to select nonintrogressed populations for study of population genetic structure. This sampling scheme will allow for a category 2-type analysis of 2x2 contingency table (test of homogeneity) and is a favorable method of hypothesis testing for independence (Zar 1999).

Table 1. Major drainages sampled for Yellowstone cutthroat and specific sampling locations.

| | |
|--|--|
| <p>1. Henry's Lake Duck Creek Howard Creek Targhee Creek Tygee Creek</p> <p>2. Teton River Canyon Creek Canyon Creek Canyon Creek (lower) Canyon Creek (middle) Canyon Creek (upper) Fish Creek (lower) Fish Creek (upper) Game Creek (lower) Garner Creek (lower) Garner Creek (middle) Horseshoe Creek Little Pine Creek Mahogany Creek Mike Harris Creek (lower) Mike Harris Creek (middle) Moose Creek North Fork Horseshoe Creek (lower) North Fork Horseshoe Creek (upper) North Fork Mahogany Creek North Fork Packsaddle Creek (lower) North Fork Packsaddle Creek (upper) North Leigh Creek North Leigh Creek (Wyoming) North Moody Creek (lower) North Moody Creek (middle) Sob Canyon (lower) Sob Canyon (upper) South Fork Badger Creek (middle) South Fork Badger Creek (upper) South Fork Canyon Creek (lower) South Fork Canyon Creek (Wyoming) South Fork Horseshoe Creek (upper) South Fork Mahogany Creek (lower) South Fork Mahogany Creek (middle) South Moody Creek State Creek</p> | <p>3. South Fork Snake River Bear Creek Big Elk Creek Black Canyon Burns Canyon (lower) Burns Canyon (upper) Burns Creek Canyon Creek Fall Creek Fall Creek (lower) Fall Creek (upper) North Fork Pine Creek (confluence) North Fork Pine Creek (upper) Palisades Creek Palisades Creek Pine Creek Pine Creek (lower) Pine Creek (upper) Pritchard Creek Rainey Creek Rainey Creek (lower) Rainey Creek (middle) Rainey Creek (upper) Rapid Creek West Pine Creek West Pine Creek</p> <p>4. Willow Creek Alley Lyons Creek (middle) Alley Lyons Creek (upper) Brockman Creek Gray's Lake Outlet Homer Creek Lava Creek Mill Creek (upper) Mill Creek (lower) North Fork Lava Creek (middle) North Fork Lava Creek (upper) Sellars Creek Sellars Creek (lower) Sellars Creek (middle) Sellars Creek (upper) South Fork Sellars Creek (middle)</p> |
|--|--|

Appendix C. Table 1. (Continued.)

| | |
|---|---|
| <p>Teton River, mainstem Trail Creek Warm Creek (lower)</p> <p>5. Salt River (upper South Fork Snake) Barnes Creek Clear Creek Crow Creek Fish Creek Horse Creek Jensen Creek McCoy Creek Squaw Creek Tin Cup Creek Tin Cup Creek, South Fork</p> <p>6. Blackfoot River Blackfoot River, upper mainstem Angus Creek Bacon Creek (upper) Bacon Creek (middle) Blackfoot River (main) Browns Canyon (upper) Browns Canyon (middle) Brush Creek (lower) Brush Creek (middle) Horse Creek Miner Creek (lower) Miner Creek (middle) Rawlins Creek (upper) Rawlins Creek (lower) Sheep Creek (lower) Sheep Creek (middle) Timber Creek Timothy Creek Inman Creek (upper) Mink Creek Mink Creek (WF-lower) Mink Creek (WF-middle) Pebble Creek, North Fork Rapid Creek Robber Roost Creek (lower) Robber Roost Creek (middle) Robber Roost Creek (upper) Toponce (MF-lower) Toponce (MF-middle) Toponce (MF-upper) Toponce (SF-lower) Toponce (SF-middle) South Fork Sellers Creek (upper) South Fork Sellers Creek (lower) Tex Creek Walker Creek (lower) Walker Creek (Middle) Webb Creek (lower) Webb Creek (middle) Fall Creek</p> | <p>Webb Creek (upper)</p> <p>7. Portneuf River Bell Marsh Creek (lower) Bell Marsh Creek (middle) Bell Marsh Creek (upper) Big Springs Creek Dempsey Creek East Bob Smith Creek (middle) East Bob Smith Creek (upper) Gibson Jack Creek (lower) Gibson Jack Creek (middle) Gibson Jack Creek (upper) Goodenough Creek (upper) Goodenough Creek (lower) Harkness Creek Inman Creek (upper) Inman Creek (middle) Inman Creek (lower) Middle Fork Toponce Creek Middle Fork Toponce Creek (upper) Middle Fork Toponce Creek (lower) Mink Creek Pebble Creek, North Fork Portneuf (main) Rapid Creek Right Hand Fork Marsh Creek Robber Roost Creek (upper) Robbers Roost Creek (middle) Robbers Roost Creek (lower) South Fork Toponce Creek (upper) South Fork Toponce Creek (middle) South Fork Toponce Creek (lower) Toponce Creek Walker Creek (upper) Walker Creek (lower) Webb Creek (upper) Webb Creek (middle) Webb Creek (lower) West Fork Mink Creek (upper) West Fork Mink Creek (lower)</p> <p>8. Bannock Creek Crystal Creek Midnight Creek (lower) Midnight Creek (upper)</p> <p>9. Raft River Almo Creek Cassia Creek (lower) Clyde Creek Cold Creek Cottonwood Creek Dry Creek Edwards Creek Eight Mile Creek (upper) Eight Mile Creek (lower) Cassia Creek (upper)</p> |
|---|---|

Appendix C. Table 1. (Continued.)

Flat Canyon Creek
Grape Creek
Green Creek
Gross Creek
Lake Fork (upper)
Lake Fork (lower)
New Canyon Creek
Six Mile Creek (upper)
Six Mile Creek (lower)

10. Goose Creek

Big Cottonwood Creek (upper)
Big Cottonwood Creek (lower)
Birch Creek
Dry Creek
Ecklund Creek (upper)
Ecklund Creek (lower)
Goose Creek (lower)
Goose Creek (upper-Nevada)
Sawmill Creek (middle)
Sawmill Creek (lower)
Trout Creek

11. Yellowstone Lake, Wyoming

Arnica Creek outlet
Breeze Point
Grant Village Marina
Outlet Trail Creek
Pearle Island South Arm

12. Jackson National Fish Hatchery

1998
1999

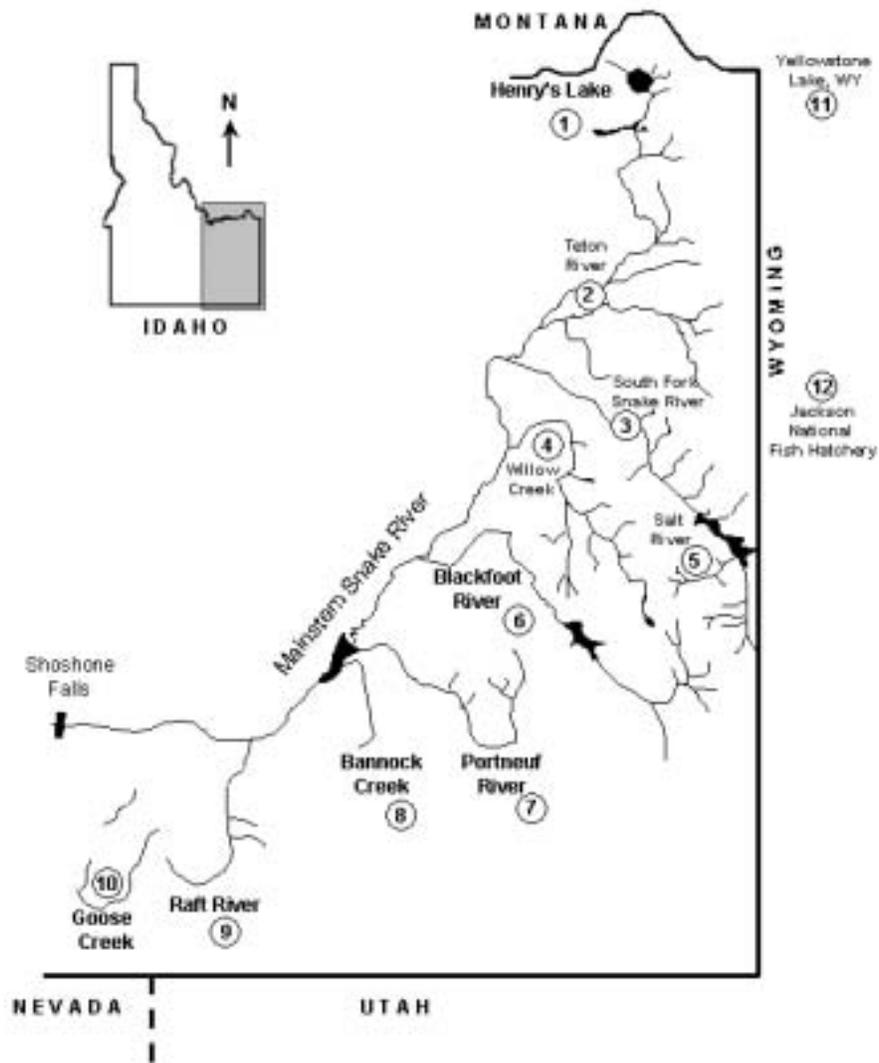


Figure 1. Map of major drainages sampled for Yellowstone cutthroat trout.

Appendix C. (Continued.)

Table 2. Nuclear DNA and mitochondrial DNA rainbow trout introgression observed in 40 populations/sample locations of Yellowstone cutthroat trout.

| Yellowstone Cutthroat Population | Year | Nuclear DNA Individuals with RBT alleles/n | Mitochondrial DNA Individuals with RBT mtDNA/n |
|---|-------------|---|---|
| Almo Creek | 1999 | 0/23 = 0.0% | 0/23 = 0.0% |
| Big Cottonwood Creek | 1999 | 1/9 = 11.1% | 0/9 = 0.0% |
| Blackfoot River | 1997 | 0/42 = 0.0% | 0/48 = 0.0% |
| Blackfoot River | 2000 | 6/24 = 25.0% | 5/24 = 20.8% |
| Clear Creek | 1999 | 0/48 = 0.0% | 0/48 = 0.0% |
| Crow Creek | 1999 | 0/44 = 0.0% | 0/44 = 0.0% |
| Goose Creek | 1999 | 0/37=0.0% | 0/37 = 0.0% |
| Harkness Creek | 1999 | 0/48 = 0.0% | 0/48 = 0.0% |
| High Bridge | 1999 | 0/46 = 0.0% | 0/46 = 0.0% |
| Horse Creek | 1999 | 0/48 = 0.0% | 0/48 = 0.0% |
| Lava Creek | 1999 | 0/22 = 0.0% | 0/59 = 0.0% |
| McCoy Creek | 1999 | 0/38 = 0.0% | 0/40 = 0.0% |
| Palisades | 1999 | 0/43 = 0.0% | 1/43 = 2.3% |
| Pebble Creek (N. F.) | 1999 | 1/47 = 2.1% | 0/47 = 0.0% |
| Rainey Creek (N. F.) | 1999 | 0/48 = 0.0% | 0/48 = 0.0% |
| Robbers Roost | 1999 | 0/40 = 0.0% | 0/40 = 0.0% |
| Sellers Creek | 1999 | 0/48 = 0.0% | 0/48 = 0.0% |
| Teton River (inclusive) | 1999 | 16/64 = 25.0% | 11/62 = 17.7% |
| Teton River (-RBT) | 1999 | 9/57 = 15.8% | 4/62 = 6.5% |
| Tin Cup Creek (S. F.) | 1999 | 0/48 = 0.0% | 0/48 = 0.0% |
| Tin Cup Creek (upper) | 1999 | 0/48 = 0.0% | 0/48 = 0.0% |
| Pine Creek | 1999 | 11/48=22.9% | 6/46=13.0% |
| Burns Creek | 1999 | 3/48=6.25% | 1/47=2.1% |
| Tyghee Creek | 1999 | 0/60=0.0% | 0/60=0.0% |
| Howard Creek | 1998 | 7/60=11.7% | 0/60=0.0% |
| Duck Creek | 1998 | 1/60=1.7% | 0/60=0.0% |
| Targhee Creek | 1998 | 13/60=21.7% | 0/60=0.0% |
| 6-Mile Creek | 1999 | 20/20=100.0% | 12/20=60.0% |
| 8-Mile Creek | 1999 | 0/20=0.0% | 0/20=0.0% |
| Barnes Creek | 1999 | 0/45=0.0% | 0/48=0.0% |
| Big Elk Creek | 1999 | 0/30=0.0% | 0/30=0.0% |
| Big Springs Creek | 1999 | 2/48=4.3% | 0/48=0.0% |
| Fish Creek | 1999 | 0/48=0.0% | 0/48=0.0% |
| Grays Lake Outlet | 1999 | 0/6=0.0% | 0/6=0.0% |
| Homer Creek | 1999 | 0/4=0/0% | 0/4=0.0% |
| Jenkins Creek | 1999 | 0/30=0.0% | 0/30=0.0% |
| Mill Creek | 1999 | 0/14=0.0% | 0/14=0.0% |
| Pritchard Creek | 1999 | 1/48=2.1% | 0/48=0.0% |
| Squaw Creek | 1999 | 1/27=3.7% | 0/40=0.0% |
| Tex Creek | 1999 | 0/10=0.0% | 0/10=0.0% |

Redband trout samples

As part of the Snake River Native Salmonid Assessment project 199800200, and in cooperation with the U.S. Forest Service, the U.S. Fish and Wildlife Service, and the Bureau of Land Management, the IDFG has collected or obtained over 1200 non-lethally collected fin tissue samples of redband trout from over 35 sampling locations throughout their entire native range in the Snake River basin between Hells Canyon Dam, Idaho and Shoshone Falls, Idaho (Table 3; Figure 2). The IDFG also has also secured over 200 samples of hatchery rainbow trout (consisting of several strains) from the Hayspur and Mackay hatcheries and over 500 redband trout samples from the Salmon River and Little Salmon River drainages for use as comparison and reference populations.

In 1999, the University of Idaho began an investigation of intraspecific hybridization and introgression between native redband trout and introduced hatchery-produced rainbow trout within five streams (Castle Creek, Big Jacks Creek, Little Jacks Creek, Shoefly Creek, and Sinker Creek, 10 sample locations, upper and lower reaches X 50 samples = 500 samples) in the Bruneau River drainage. Rainbow trout introgression within these sample locations was investigated using a mtDNA gene region (ND2) combined with a RFLP screen of eight separate restriction enzymes and a nDNA intron RFLP marker (p53) that has previously yielded allele frequency variation between redband trout populations and hatchery rainbow trout populations (University of Idaho, unpublished data). We propose that work on the ten sample locations listed above is continued by expanding the screen of restriction enzymes from eight to ten and by adding an additional mitochondrial region (Cyt B) from which to screen with restriction enzymes. Furthermore, we suggest that four additional sample locations/populations from each of the five major drainages sampled thus far be examined with the same mtDNA RFLP screen and nDNA RFLP marker (4 locations X 5 major drainages X 60 samples = 1200 samples). Of these four sample locations within each major drainage, two will be selected from streams that have never received rainbow trout stocking according to the historical stocking database. The remaining two additional sample locations will be selected from streams that have had substantial rainbow trout stocking according to database. These additional sample locations will allow a better test of the historical stocking database capabilities of predicting rainbow trout hybridization rates and will also allow a more complete, comprehensive geographic range from which to select nonintrogressed populations for study of population genetic structure.

Table 3. Major drainages sampled thus far for redband trout and specific sampling locations.

| | |
|---|---|
| 1. Little Wood River Grays Creek Little Wood-Main (lower) Little Wood-Main (middle) Little Wood-Main (upper) Slide Canyon Creek | 6. NF Boise River Pike's Fork Creek Upper NFBR? |
| 2. Big Wood River Red Warrior Creek Adams Gulch Castle Creek East Fork Baker Creek Greenhorn Creek Hyndman Creek (North Fork) Hyndman Creek (upper) Trail Creek (lower) Trail Creek (upper) Upper Deer Creek | 7. SF Boise River SFBR above Anderson Res. SFBR below Anderson Res. (canyon) Willow Creek (below) Smith Creek (below) Rattlesnake Creek (below) NF Lime Creek (above) Whiskey Jack Creek (above) Big Smokey Creek (above) Little Smokey Creek (above) Ross Creek (above) |
| 3. Malad River Malad River (upper) Malad River (middle) Malad River (lower) | 8. Bruneau Castle Creek (upper) Castle Creek (lower) Big Jacks Creek (upper) Big Jacks Creek (lower) Little Jacks Creek (upper) Little Jacks Creek (lower) Shoefly Creek (upper) Shoefly Creek (lower) Sinker Creek (upper) Sinker Creek (lower) |
| 4. Jarbidge Jarbidge (East Fork) Jarbidge (Main) | |
| 5. MF Boise River Roaring River Upper MFBR | |

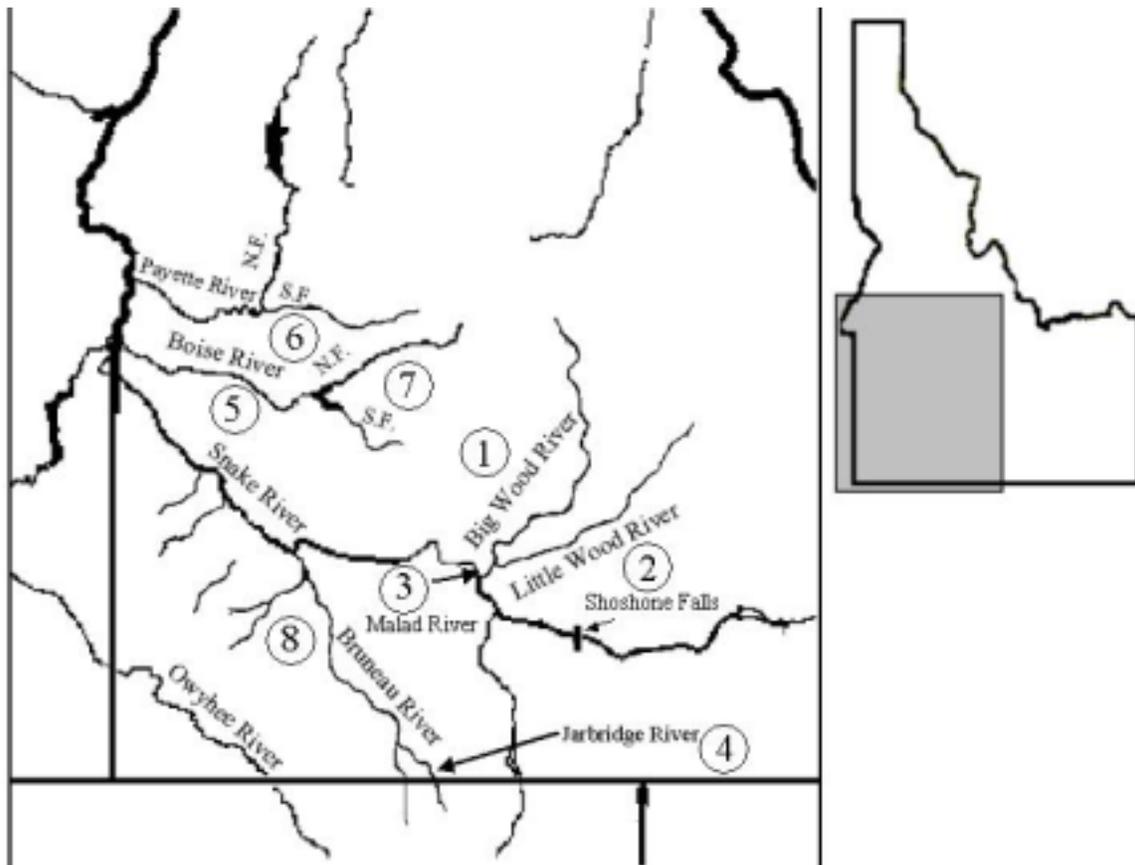


Figure 2. Map of major drainages sampled for redband trout.

Task 1. Conduct mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) intron Restriction Fragment Length Polymorphism (RFLP) analysis to quantify levels of rainbow trout hybridization and introgression.

DNA Extraction—Mitochondrial DNA and nuclear DNA will be extracted from nonlethally collected fin-clip samples using methods described by Paragamian et al. (1999), adapted from protocols by Sambrook et al. (1989) and Dowling et al. (1996).

PCR Amplification and restriction digestion of Nuclear DNA gene regions—DNA isolated from each sample will be amplified using the Polymerase Chain Reaction (PCR) with primers specific for five nuclear intron gene regions: Recombination activation gene (RAG 3'), Ikaros gene (IK), Protocogene 53 (p53), Insulin Growth Factor 2 gene (IGF-2), and Heat-shock cognate gene 71 (HSC 71). All five loci are diagnostic between rainbow trout and cutthroat trout when digested with a particular restriction enzyme (Campbell et al. In Press). Variation in allele frequency at the p53 locus has been observed between redband trout populations and hatchery-produced rainbow trout populations and has been used previously to examine intra-specific hybridization projects (University of Idaho, unpublished data). Digests will be electrophoresed on 3% agarose gels with tris-acetate-EDTA buffer or 6% acrylamide gels with tris-borate-EDTA and stained with Ethidium bromide and fluoresced under UV-light to visualize alleles.

PCR Amplification and restriction digestion of Nuclear DNA gene regions—DNA isolated from each sample will be amplified with primers specific for two gene regions of the mitochondrial genome (ND2 and Cyt B) and digested with 10 restriction enzymes (Ava-I, Dde-I, Dpn-II, Hae-III, Hha-I, Hinf-I, Mse-I, Msp-I, Rsa-I, and Taq-I). Previous studies have demonstrated that the digestion of Cyt B with restriction enzymes (Hae-III, Hinf-I, and Rsa-I) yields diagnostic polymorphisms between cutthroat trout and rainbow trout (Mays 2001). Eight of the ten restriction enzymes listed above have been used in combination with the ND2 gene region in previous studies to examine introgressive hybridization within redband trout populations (Silver Creek, University of Idaho 2000). Digests will be electrophoresed on 3% agarose gels with tris-acetate-EDTA buffer or 6% acrylamide gels with tris-borate-EDTA and visualized as band patterns (fragments) when stained with Ethidium bromide and fluoresced under UV-light.

Task 2. Compare stocking variables with observed rates of introgression.

A pilot test of independence of stocking (stocked, not stocked) versus introgression (introgressed, not introgressed) of the 40 Yellowstone cutthroat trout sample locations preliminarily investigated, indicates that stocking and introgression are not independent (Chi Square, corrected for continuity, $\chi^2 = 5.6884$; $0.01 < P < 0.025$), (University of Idaho, IDFG, unpublished data). This suggests that Idaho's stocking database may be useful in predicting hybridization and introgression levels and therefore a good predictor of genetic risks to resident trout populations from historical rainbow trout stocking.

The following stocking variables available from Idaho's historical stocking database (used in combination or alone) will be tested against observed rates of rainbow trout introgression for their predictive ability in assessing hybridization and introgression rates:

- Whether any rainbow trout have been stocked or not (yes or no),
- Number of total fish stocked,
- Size at stocking,
- Age at stocking,
- Month stocked,
- Strain of stocked fish,
- Number of pounds stocked,
- Number of years stocked and,
- Number of years since last stocking.

We plan to statistically analyze these stocking variables against observed levels of rainbow trout introgression through three statistical methods:

1. Tests for independence of stocking vs. introgression (hypothesis testing of independence using χ^2 corrected for continuity [Zar 1996]).
2. Tests for correlations between stocking criteria and introgression (using two-tailed χ^2 and r_n analyses [Zar 1996; Motulsky 1995] to test for significant, positive and negative correlations).
3. Tests to assign predictiveness to stocking criteria (regression analysis, parametric and non-parametric tests and Bayesian prediction [Motulsky 1995]).

Phase Two:

Objective 2. Determine genetic population structure of native Yellowstone cutthroat trout populations and redband trout populations within the Middle and Upper Snake River basins.

Genetic information on rainbow trout introgression obtained from the first part of this project will be used to identify nonintrogressed Yellowstone cutthroat and redband trout sample locations to screen with microsatellite markers to investigate genetic population structure. The overall goal will be to examine populations throughout their geographic range within the Middle and Upper Snake River basins.

Task 1. Conduct microsatellite DNA analysis of Yellowstone cutthroat and redband trout samples collected from populations within the Middle and Upper Snake River basins.

Allele frequency variation at six microsatellite loci (*Ocl 1*, *Ocl 2*, *Ocl 3*, *Ocl 4*, *Ocl 8*, and *Ocl 9*) will be examined on all Yellowstone cutthroat trout samples. These highly polymorphic microsatellite loci have been used previously to successfully describe genetic population structure within coastal cutthroat trout, and have been amplified successfully within Yellowstone cutthroat trout (Wenburg 1998). Expected number of alleles observed, allele size range (bp), and PCR conditions are described by Wenburg (1998).

Appendix C. (Continued.)

Allele frequency variation at six microsatellite loci (*Ots-3*, *Omy-77*, *Ots-103*, *Ots-100*, *Ots-1*, and *Ots-108a*) will be examined on all redband trout samples. These same microsatellite loci are currently being used to examine introgressive hybridization and genetic population structure in redband trout populations on the Duck Valley reservation as part of the Shoshone-Bannock Paiute Joint Culture Facility Project (199500600). Expected number of alleles observed, allele size range (bp), and PCR conditions are shown in table 3 below:

Table 3. Note all PCRs have an initial 5-cycle 1°C/cycle touchdown, followed by 38 cycles at specified annealing temp, and ended with a 30 min final extension at 72°C.

| Locus | Primer repeat size | # alleles conc. | allele label | PCR observed | size range (bp) | annealing temp. |
|-----------------|---------------------------|------------------------|---------------------|---------------------|------------------------|------------------------|
| PCR-1 | | | | | | |
| <i>Ots-3</i> | dinucleotide | 0.3 µM | ned | 6 | 79-89 | 50°C |
| <i>Omy-77</i> | dinucleotide | 0.3 µM | hex | 20 | 99-149 | 50°C |
| PCR-2 | | | | | | |
| <i>Ots-103</i> | tetranucleotide | 0.2 µM | hex | 9 | 59-93 | 55°C |
| <i>Ots-100</i> | tetranucleotide | 0.35 µM | ned | 12 | 165-213 | 55°C |
| PCR-3 | | | | | | |
| <i>Ots-1</i> | dinucleotide | 0.2 µM | 6-fam | 15 | 163-246 | 50°C |
| PCR-4 | | | | | | |
| <i>Ots-108a</i> | tetranucleotide | 0.3 µM | 6-fam | 25 | 97-269 | 46°C |

Levels of genetic variation in each sample location/population will be estimated three ways:

1. The average number of alleles per locus (total number of alleles detected at the six loci analyzed in each sample divided by six);
2. Observed and expected heterozygosities at each locus (expected heterozygosity at a locus in a sample is one minus the sum of the squared allele frequencies. These values summed over all loci and divided by six yields average expected heterozygosity) and;
3. The proportion of polymorphic loci in a sample (the number of loci at which evidence of genetic variation is detected divided by six).

Assessments of genetic subpopulation structure will be made using the statistical genetic computer programs Arlequin version 2.0 (Excoffier 2000), Genepop version 1.2 (Raymond and Rousset 1995) and GDA (Lewis and Zaykin 1999). Heterozygosity components (H_i , H_s , H_r and H_T) calculated from generated allelic frequency data will be used to partition gene diversity within populations (F_{IS}), between populations within regions ($F_{SR(T)}$), among regions (F_{RT}), and overall among populations (F_{ST}). The program GENDIST in the statistical software package PHYLIP will be used to generate dendograms from these distance matrices. Maximum likelihood distance matrixes and dendograms will be calculated using the program CONTL (PHYLIP). Robustness of tree topologies will be assessed using the program CONSENSE in PHYLIP. Dendogram diagrams will be created in the programs DRAWTREE and DRAWGRAM (PHYLIP).

Appendix C. (Continued.)

Previous studies using allozymes have suggested that Yellowstone cutthroat trout exhibit relatively low levels of genetic diversity in comparison to other subspecies and to rainbow trout (Allendorf and Leary 1988). However, research has shown that “Yellowstone cutthroat trout display adaptations to different environments and biotic communities that have resulted in ecotypes displaying characters as variable as those commonly found between subspecies or even between species of trout” (Varley and Gresswell 1988). These previous scientific findings pose interesting questions:

- Does microsatellite analysis yield congruent results regarding genetic variability?
- How much genetic diversity is present within the species that has not been accounted for?

The genetic information gained from this project (described in detail above) will be used to address these two questions as well as the following pertinent issues regarding both Yellowstone cutthroat and redband trout populations:

- How is genetic diversity partitioned throughout these populations?
- What are appropriate conservation/management units for these species within the Middle and Upper Snake River Basins?
- What is the genetic effective population size of these populations?
- What populations are at immediate genetic risk?
- What populations are most appropriate for use in translocations, re-introductions, or broodstock development purposes?
- What management strategies would pose genetic risks to these populations?
- Can population abundance estimates be used to predict population genetic variability?

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Appendix C. (Continued.)

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Appendix D. Genetics Work Plan. Idaho Department of Fish and Game, Current and Proposed Genetic Projects.



Eagle Fish Genetics Laboratory, June 2002

Region:

Nampa/Eagle Research

Manager/Project Leader:

Matt Campbell (939-6713)

Project Title:

Assessment of genetic population structure and risk of introgression and hybridization to native trout in the Middle and Upper Snake River basins (genetic work for Kevin Meyer's Native Trout Assessment Project).

Populations/species:

Oncorhynchus mykiss and *Oncorhynchus clarki*

Questions/Objectives:

- Assess current and future genetic risks,
- Assess the predictive power of Idaho Department of Fish and Game's (IDFG) Historical Stocking Database in quantifying hybridization and introgression levels,
- Preserve existing genetic variability,
- Delineate and prioritize populations for conservation and management purposes,
- Estimate effective population size,
- Understand genetic population structure,
- Identify suitable populations for translocations and reintroductions,
- Identify suitable populations for broodstock development, and
- Address genetic concerns in future ESA petitions.

Genetic Analyses:

- Yellowstone cutthroat: Six microsatellite loci (*Ocl 1*, *Ocl 2*, *Ocl 3*, *Ocl 4*, *Ocl 8*, and *Ocl 9*).
- Redband trout: Six microsatellite loci (*Ots-3*, *Omy-77*, *Ots-103*, *Ots-100*, *Ots-1*, and *Ots-108a*).
- Yellowstone cutthroat and Redband trout: Mitochondrial analyses-ND2 and CytB (12 restriction enzymes each).

Sample Locations/Populations and Samples to be Analyzed:

See proposal.

Cooperating Agencies:

University of Idaho

Genetic Lab Performing Work:

IDFG Eagle Genetics Lab

Sample Storage Location:

IDFG Eagle Genetics Lab

Time Frame:

3 years

Proposed Cost:

Approximately \$250,000/year for 3 years

Funding Source:

Relationship To Other Projects:

Native Resident Trout Assessment

Current Status of Project:

IDFG began genetic investigation of 40 Yellowstone cutthroat populations in 2000. Results are found in proposal.

Comments:

Waiting on funding status.

Region:

Nampa Research

Manager/Project Leader:

Alan Byrne (465-8404)

Project Title:

Steelhead Supplementation Studies in Idaho Rivers

Populations/species:

Oncorhynchus mykiss

Questions/Objectives:

Determine the evolutionary significance and genetic population structure of steelhead in the Snake, Salmon and Clearwater drainages. The hypotheses to be tested under this study are:

HO1—Unique evolutionary and biogeographic structure occurs in natural populations of steelhead in Idaho. Steelhead tissues collected from Idaho for this study contain distinct genetic allelic structure when compared to other coastal and interior steelhead populations. Tests of this hypothesis could be used to look at genetic substructure within and between river basins in comparison with sample collections from other parts of the distribution of *O. mykiss* throughout their range.

HO2—Introgression by straying hatchery-produced steelhead has had no major effect on the natural genetic diversity found in Idaho steelhead. Hatchery fish used for supplementation in the same geographic area may carry diminished genetic diversity due to bottleneck effects induced through common husbandry practices.

Genetic Analyses:

Microsatellite Analyses (15 loci),

| Locus | Locus |
|--------------|--------------|
| Ogo1a | Ogo3 |
| Ogo4 | Ots1 |
| Ots3 | Ots4 |
| Ots100 | Oneu10 |
| Omy27 | Omy77 |
| Omy207 | Omy325 |
| Oneu8 | Oneu11 |
| Oneu14 | |

mtDNA RFLPs (2 regions)

ND5/6 (cut with *Dde*-I, *Hinf*-I, *Hae*-III)

Cyt B (cut with *Dde*-I, *Hinf*-I, *Hae*-III, *Hinf*-I)

Appendix D. (Continued.)

Sample locations/populations and samples to be analyzed. Juvenile steelhead fin samples collected by IDFG for DNA analysis during summer 2000. All samples were collected fly-fishing except where noted during July & August 2000.

| Stream | Drainage | Code | Number of Samples | Notes |
|---------------------------------|-----------------|-------------|--------------------------|--|
| Big Smoky Creek | Boise | BOIR | 60 | |
| Big Canyon Creek | Clearwater | BCAN | 52 | Collected electrofishing in March 2001 |
| EF Potlatch River | Clearwater | EPOT | 52 | |
| Jacks Creek | Clearwater | JACK | 41 | Collected electrofishing |
| Little Bear Creek | Clearwater | LBRC | 58 | |
| Mission Creek | Clearwater | MISS | 52 | Collected electrofishing |
| Dworshak | Hatchery | DWOR | 102 | Collected in September 2000 |
| EF Salmon "B-run" | Hatchery | EFRB | 103 | Collected in September 2000 |
| Oxbow | Hatchery | OXBW | 101 | Collected in September 2000 |
| Pahsimeroi | Hatchery | SIMH | 102 | Collected in September 2000 |
| Sawtooth | Hatchery | SAWT | 93 | Collected in September 2000 |
| Boulder Creek | Little Salmon | BOUL | 63 | |
| Hazard Creek | Little Salmon | HAZC | 61 | |
| Little Salmon upstream of falls | Little Salmon | LSR1 | 68 | |
| Little Salmon, Pinehurst area | Little Salmon | LSR2 | 68 | |
| Rapid River | Little Salmon | RAPR | 61 | Collected upstream of hatchery |
| Boulder Creek | Lochsa | BLDK | 61 | |
| Brushy Fork Creek | Lochsa | BRUS | 60 | |
| Canyon Creek | Lochsa | CANY | 59 | |
| Colt Creek | Lochsa | COLT | 69 | |
| Crooked Fork Creek | Lochsa | CFCK | 57 | |
| Deadman Creek | Lochsa | DEAD | 59 | |
| Fish Creek (summer collection) | Lochsa | FISH | 62 | Collected fly-fishing in July |
| Fish Creek (fall migrants) | Lochsa | FSCT | 70 | Collected at screw trap in the fall |
| Hungry Creek | Lochsa | HUNC | 66 | |
| Lake Creek | Lochsa | LAKE | 59 | |
| Papoose Creek | Lochsa | PAPO | 55 | |
| Storm Creek | Lochsa | STRM | 70 | |
| Warm Springs Creek | Lochsa | WARM | 60 | Downstream of falls |
| Weir Creek | Lochsa | WEIR | 67 | |
| Clear Creek | MF Clearwater | CLRC | 60 | |
| Bear Valley Creek | MF Salmon | BVAC | 62 | |
| Big Creek (lower) | MF Salmon | BIG1 | 78 | |
| Big Creek (upper) | MF Salmon | BIG2 | 49 | |
| Camas Creek | MF Salmon | CAM1 | 69 | |
| Loon Creek | MF Salmon | LON1 | 67 | |
| Marsh Creek | MF Salmon | MARC | 62 | |
| Pistol Creek | MF Salmon | PIST | 30 | |
| Rapid River | MF Salmon | RRDR | 62 | |
| Sulphur Creek | MF Salmon | SULP | 67 | |
| Collins Creek | NF Clearwater | CNLC | 59 | |
| MF Payette River | Payette | PAYR | 56 | |

Appendix D. (Continued.)

| | | | | |
|----------------------------------|---------------|------|--------------|---|
| Bargamin Creek | Salmon | BAR1 | 61 | |
| Basin Creek | Salmon | BASC | 61 | |
| Chamberlain Creek | Salmon | HAM1 | 69 | |
| Horse Creek | Salmon | HRSC | 62 | |
| Lemhi River | Salmon | LEMR | 53 | Collected at screw trap in the fall |
| Morgan Creek | Salmon | MORG | 62 | |
| Owl Creek | Salmon | OWLC | 61 | |
| Sheep Creek | Salmon | EEPC | 22 | |
| Slate Creek (near Whitebird) | Salmon | SLAT | 58 | |
| Valley Creek | Salmon | VALC | 52 | Collected electrofishing |
| Warm Springs Creek | Salmon | WSCK | 55 | |
| Whitebird Creek | Salmon | WHBC | 61 | |
| Pahsimeroi River | Salmon | PAHR | 70 | Collected at screw trap in the fall |
| WF Yankee Fork | Salmon | WIFY | 58 | |
| | | | | |
| Bear Creek | Selway | BEAR | 59 | |
| EF Moose Creek | Selway | EMOS | 66 | |
| Gedney Creek | Selway | GEDC | 71 | |
| Meadow Creek | Selway | MEDC | 54 | |
| Mink Creek | Selway | MINK | 59 | |
| NF Moose Creek | Selway | NFMO | 67 | |
| O'Hara Creek | Selway | OHAR | 60 | |
| Pettibone Creek | Selway | PETB | 58 | |
| Three Links Creek | Selway | 3LNK | 61 | |
| | | | | |
| Johns Creek | SF Clearwater | JOHN | 50 | |
| Ten Mile Creek | SF Clearwater | MILE | 50 | |
| Red River | SF Clearwater | REDR | 50 | Collected at screw trap in the fall & spring 2001 |
| | | | | |
| EF SF Salmon River | SF Salmon | EFSF | 67 | |
| Johnson Creek | SF Salmon | JSON | 67 | |
| Lick Creek (lower) | SF Salmon | LIK1 | 69 | |
| Lick Creek (upstream of barrier) | SF Salmon | LIK2 | 70 | |
| Poverty Flat area | SF Salmon | POVF | 60 | |
| Secesh River | SF Salmon | SECR | 70 | |
| Stolle Meadow | SF Salmon | STOL | 52 | Collected at Knox bridge screw trap |
| | | | | |
| Captain John Creek | Snake | CAPJ | 57 | |
| Granite Creek | Snake | GRAN | 50 | |
| Sheep Creek | Snake | SHPC | 50 | |
| | | | | |
| Little Weiser River | Weiser | WEIS | 59 | |
| Total number collected: | | | 4,913 | |

Cooperating Agencies:

Dr. Jennifer L. Nielsen, Fisheries Supervisory Team Leader, USGS Alaska Biological Science Center

Genetic Lab Performing Work:

USGS Alaska Biological Science Center

Sample Storage Location:

USGS Alaska Biological Science Center

Time Frame:

Progress report completed March 22, 2002. Final report due December 2002.

Proposed Cost:

Approx. \$300,000

Funding Source:

BPA

Relationship To Other Projects:

This project is tied closely to BPA project #198909600: Monitor and evaluate genetic characteristics of supplemented salmon and steelhead (NMFS).

Current Status of Project:

Started. Should be completed by the end of 2002.

Comments:

Samples from Middle Fork Salmon River may assist in hybridization questions (between *O. clarki* and *O. mykiss*). However, sampling as part of this project excluded fish that were not phenotypically identified as *O. mykiss*.

Microsatellite markers from this study should be considered for use in other studies involving *O. mykiss* populations in Idaho.

Region:

Nampa Research

Manager/Project Leader:

Paul Kline

Project Title:

Identification of steelhead runs in Squaw Creek (upper Salmon River)

Populations/species:

Oncorhynchus mykiss

Background:

B-run steelhead—there were no B-run steelhead in the upper Salmon River historically. A B-run steelhead program was initiated on the upper Salmon River in the mid-1980's to provide larger fish for anglers. This involved moving Dworshak B-run steelhead eggs to the Pahsimeroi Hatchery where they were raised until smolts, and then releasing them into the East Fork Salmon River. Eventually, the Magic Valley Hatchery took over rearing duties from the Pahsimeroi Hatchery. Currently, Dworshak traps B-run adults, spawns fish, and sends eggs to the Clearwater Hatchery for Clearwater River drainage programs and to the Magic Valley Hatchery for upper Salmon River programs.

In 2002, the following Dworshak B-run smolts (raised at the Magic Valley Hatchery) were released in the Upper Salmon River Drainage:

100,000 to Squaw Creek Pond

200,000 to Squaw Creek

225,000 to the lower end of the East Fork Salmon River.

Besides releasing Dworshak B-run smolts (raised at the Magic Valley Hatchery), any B-run adults that are trapped at EFSR trap, Slate Creek Pond, and Squaw Creek Pond are taken to the EFSR trap and spawned. Eyed eggs are taken to the Magic Valley Hatchery for rearing and then returned to the upper Salmon River as smolts. In 2002, 80,000 smolts were released in Squaw Creek.

A-run steelhead—Pahsimeroi and Sawtooth hatcheries have A-run programs. Adults trapped at Pahsimeroi are spawned and resulting eggs are sent to the following hatcheries:

Magic Valley

Niagra Springs

Hagerman National Fish Hatchery

Adults trapped at the Sawtooth Hatchery are spawned and resulting eggs are sent to the following hatcheries:

Magic Valley

Hagerman National Fish Hatchery

Pahsimeroi and Sawtooth smolt distribution back to the upper Salmon River:

120,000 to Salmon River between North Fork Salmon River and Lemhi (from PAH eggs)

140,000 to Lemhi River (from PAH eggs)

220,000 to Salmon River between Lemhi River and Pahsimeroi River (from PAH eggs)

Appendix D. (Continued.)

800,000 to Salmon River between North Fork Salmon River and Lemhi (from PAH eggs)
230,000 to Pahsimeroi River (from PAH eggs)
100,000 to Yankee Fork Salmon River (from SFH eggs)
630,000 to Salmon River at Sawtooth Weir (from SFH eggs)

There is a small run of unmarked A-run steelhead (all based on length) that returns to the East Fork Salmon River trap. NMFS is requiring IDFG to manage this group of fish separately. Unmarked A-run adults collected at the EFSR weir are spawned within group. Eggs go to Magic Valley hatchery and are released as smolts at the EFSR weir. In 2002, 4000 smolts were released.

Problems:

A-run and B-run fish are considered 1-ocean and 2-ocean, respectively. However, there are 1-ocean B-run adults and 2-ocean A-run adults. Currently A's and B's are distinguished by length.

In 2002, 165 steelhead were trapped in Squaw Creek. Forty-four were B-run size fish (only B-run smolts are released into Squaw Creek). Six returning adults had PIT tags, three were big (met B-run size requirement), and three were small (met A-run size requirements). All six had been released as B-run smolts. All six of these fish were included in the spawning design, but the remaining 118 fish were excluded from spawning based on small size. How many excluded fish were actually B-run fish?

Questions/Objectives:

What are the origins of adults that return to Squaw Creek? Do they all represent fish that were originally released as B-run smolts? Do they represent an admixture of returning B-run adults and straying A-run adults?

What are the consequences of excluding B-run fish as broodstock based on length?

Is the unmarked A-run steelhead that return to the East Fork Salmon River trap genetically distinct from adults that return to the Pahsimeroi and Sawtooth hatcheries?

Genetic Analyses:

To make genetic comparisons between the adult populations that return to Squaw Creek, Dworshak, and the East Fork Salmon River, microsatellite analysis (~15 loci) will have to be performed.

| Locus | Locus |
|--------------|--------------|
| Ogo1a | Ogo3 |
| Ogo4 | Ots1 |
| Ots3 | Ots4 |
| Ots100 | Oneu10 |
| Omy27 | Omy77 |
| Omy207 | Omy325 |
| Oneu8 | Oneu11 |
| Oneu14 | |

mtDNA RFLPs (2 regions)
ND5/6 (cut with *Dde*-I, *Hinf*-I, *Hae*-III)
Cyt B (cut with *Dde*-I, *Hinf*-I, *Hae*-III, *Hinf*-I)

Sample Locations/Populations and Samples to be analyzed:

Source population samples for A and B Steelhead runs have already been obtained in Alan Byrne's study. This includes 102 samples from Dworshak Hatchery, 103 from the East Fork Salmon River, and 102 from the Pahsimeroi River Hatchery. Approximately 165 adults returned to Squaw Creek in 2002. If genetic samples were collected on these adults then all should be analyzed with the same genetic markers used on Alan Byrne's study. Genetic samples should be collected from all adults returning to Squaw Creek during the next several years.

Juvenile steelhead fin samples collected by IDFG for DNA analysis during summer 2000. All samples were collected fly-fishing except where noted during July and August 2000.

| Stream | Drainage | Code | Number of Samples | Notes |
|-----------------|-----------------|-------------|--------------------------|-----------------------------|
| Dworshak | Hatchery | DWOR | 102 | collected in September 2000 |
| EF Salmon B-run | Hatchery | EFRB | 103 | collected in September 2000 |
| Oxbow | Hatchery | OXBW | 101 | collected in September 2000 |
| Pahsimeroi | Hatchery | SIMH | 102 | collected in September 2000 |
| Sawtooth | Hatchery | SAWT | 93 | collected in September 2000 |

Cooperating Agencies:

Possibly Dr. Jennifer L. Nielsen, Fisheries Supervisory Team Leader, USGS Alaska Biological Science Center

Genetic Lab Performing Work:

Not determined

Sample Storage Location:

USGS Alaska Biological Science Center, Nampa Research

Time Frame: Not determined.

Proposed Cost:

Microsatellite analysis of 165 samples would cost about \$5,000.00.

Funding Source:

Not determined.

Relationship To Other Projects:

Samples from Alan Byrne's project should be used as reference populations in this project.

Current Status of Project:

Proposal idea.

Comments: None

Region:

Nampa Research

Manager/Project Leader:

Dan Schill (465-8404)

Project Title:

Bruneau River Redband trout (Dan Schill's Ph.D. project)

Populations/species:

Oncorhynchus mykiss

Questions/Objectives:

How much hybridization and introgression has occurred between native redband trout and introduced hatchery rainbow trout (what populations are pure)?

How is genetic diversity partitioned throughout these populations and how much gene flow occurs between "populations"?

What is the genetic effective population size (N_e) of these populations?

Genetic Analyses:

A mtDNA RFLP screen on the ND2 and CytB gene regions (12 restriction enzymes each) should be used to detect intraspecific hybridization and introgression within these populations. The assessment of genetic population structure will involve using 10-15 hyper-variable microsatellite loci (including *Ots-3*, *Omy-77*, *Ots-103*, *Ots-100*, *Ots-1*, and *Ots-108a*), possibly also incorporating microsatellite markers currently employed on Alan Byrne's project.

Sample Locations/Populations and Samples to be analyzed:

15 sample locations within the Bruneau River Drainage (approx. 60 samples at each location, ~N=900 samples).

Cooperating Agencies:

None

Genetic Lab Performing Work:

IDFG Eagle Genetics Lab

Sample Storage Location:

Currently at Nampa Research, but samples will be sent to Eagle.

Time Frame (Lab Work):

3 months of **full-time** (40 hours/week) lab work.

Proposed Cost:

900 samples X 12 microsatellite markers = approx. \$30,000

Funding Source:

Some DJ. Mostly from Kevin Meyer's Native Resident Trout Assessment Project.

Relationship To Other Projects:

Ties closely with Kevin Meyer's Native Resident Trout Assessment and the new BPA proposal "Assessment of genetic population structure and risk of introgression and hybridization to native trout in the Middle and Upper Snake River Basins."

Current Status of Project:

Most of the samples have been collected.

Comments:

DNA extraction, microsatellite amplification would be carried out at the Eagle Genetics Lab. Microsatellites would have to be run out on the ABI 3100 sequencer in Hagerman.

Region:

Nampa Research

Manager/Project Leader:

Paul Kline / Catherine Willard / Matt Powell (Major Prof.)

Project Title:

Reproductive success of captive-reared Redfish Lake Sockeye Salmon (Catherine Willard's M.S. Project).

Populations/species:

Oncorhynchus nerka

Project Background:

During the fall of 2000, 118 anadromous hatchery adults and 46 captive-reared adults (fish that spent their entire lifecycle in the hatchery) were released into Redfish Lake for wild spawning. In the spring of 2002, juvenile out-migrants produced from wild-spawning adults (non-ad clipped) and juvenile out-migrants resulting from hatchery production released into the lake (ad-clipped) were randomly sampled as they out-migrated from Redfish Lake (~N=300).

Questions/Objectives:

Determine the relative contributions of anadromous hatchery adults and captive-reared adults to 2002 juvenile out-migrant production.

Determine the genetic effective population size (N_e) of the Redfish lake sockeye population.

Genetic Analyses:

10 hypervariable microsatellite loci (including One-1, 2, 8, 11, 14) and a mtDNA screen of four regions: ND2 (cut with Apa-I, Hae-III, Kpn-I, Stu-I), Cyt B (cut with Apa-I, Hae-III, Kpn-I, Stu-I), ND 5/6 (cut with Apa-I, Kpn-I, Stu-I, Taq-I), ND1 (cut with Apa-I, Dpn-II, Hae-III, Kpn-I, Rsa-I, Stu-I) to obtain unique genotypes on all prospective parents ($n = 164$).

Sample Locations/Populations and Samples to be analyzed:

118 anadromous hatchery adults
46 captive-reared adults
~300 out-migrants
Total = ~450 samples

Cooperating Agencies:

None

Genetic Lab Performing Work:

IDFG Eagle Genetics Lab.

Sample Storage Location:

Currently samples are stored at three locations: Aquaculture Research Institute, Hagerman Fish Culture Experiment Station, and IDFG Eagle Genetics Lab.

Time Frame (Lab Work):

2 year Graduate Project.

Proposed Cost:

~450 samples = approx. \$18,000.00.

Funding Source:

Redfish Lake Sockeye BPA project.

Relationship To Other Projects:

Genetic questions and analyses very similar to the project: "Reproductive success of supplementation Chinook Salmon in the Pahsimeroi River and Salmon River @ Sawtooth weir upstream (Brian Leth's M.S. Project)."

Current Status of Project:

All samples have been collected.

Comments:

DNA extraction, microsatellite amplification would be carried out at the Eagle Genetics Lab. Microsatellites would have to be run out on the ABI 3100 sequencer in Hagerman.

Region:

Nampa Research

Manager/Project Leader:

Jeff Lutch / Brian Leth

Project Title:

Reproductive success of “supplementation” Chinook salmon in the Pahsimeroi River and Salmon River @ Sawtooth weir upstream (Brian Leth’s M.S. Project)

Populations/species:

Oncorhynchus tshawytscha

Questions/Objectives:

Evaluate reproductive success of “supplementation” Chinook salmon in the Pahsimeroi River and Salmon River @ Sawtooth weir upstream.

Background:

The Idaho Supplementation Studies (ISS) was developed in 1991 to evaluate the effects of supplementation with hatchery-reared pre-smolts and smolts (products of hatchery X wild crosses) on naturally produced pre-smolt and smolt numbers and resulting spawning escapement of naturally-producing Chinook salmon. This project plans to evaluate the relative contributions of “supplementation” adults and wild adults on the production of out-migrants from the Pahsimeroi and Salmon Rivers. “Supplementation” adults are returning adults that are products of wild X hatchery crosses raised until smolts at the hatchery. Wild adults are returning adults that are products of wild X wild matings, wild X “supplementation” matings, or “supplementation” X “supplementation” matings.

Genetic Analyses:

To obtain unique genotypes on all prospective parents (Pahsimeroi, ~n = 160, Sawtooth, ~n = 140) both microsatellite analysis and mtDNA RFLP analysis should be performed. The microsatellite analysis should probably screen between 10-15 hypervariable microsatellite loci (loci used on the University of Idaho/CRITFC Methow River Chinook project include: Ots1, Ots2, Ots3, Ots9, Ots10, OMM1020, and Omy77). The mtDNA RFLP screen should probably follow previous work at the University of Idaho examining mtDNA diversity within Chinook salmon populations. Currently, the Aquaculture Research Institute screens two mtDNA gene regions: ND1 (cut with Ase-I, Dde-I, Hae-III, and Rsa-I) and ND 5/6 (cut with Bstu-I and Dpn-II). Out-migrating smolts will be randomly sampled (n = 300, from both populations) to estimate the relative contributions and success of wild and supplementation adults.

Sample Locations/Populations and Samples to be analyzed:

Pahsimeroi (approximately 160 wild and supplementation adults combined*).

Sawtooth (approximately 140 wild and supplementation adults combined*).

Approximately 300 out-migrants from each population.

*These are current predictions of adult returns. Returns may be higher, which would probably make current objectives infeasible due to time and budget constraints. Samples should be collected regardless of adult return numbers. However, objectives and study design would have to change if adult returns are high in number.

Cooperating Agencies:

Nez Perce, Shoshone-Bannock Tribes, USFWS

Genetic Lab Performing Work:

University of Idaho's Hagerman Fish Culture Experiment Station and IDFG Eagle Genetics Lab.

Sample Storage Location:

Eagle Genetics Lab, Nampa Research

Time Frame:

2 years.

Proposed Cost:

900 samples, Approximately \$32,500.00.

Funding Source:

BPA

Relationship To Other Projects:

Genetic questions and analyses very similar to the project: "Reproductive success of captive-reared West Fork Yankee Fork Chinook Salmon" and "Reproductive success of captive-reared Redfish Lake Sockeye Salmon (Catherine Willard's M.S. Project)."

Current Status of Project:

Samples will be collected during this spawning season for analysis this fall/winter.

Comments:

None

Region:

Nampa Research

Manager/Project Leader:

Dave Venditti / Paul Kline

Project Title:

Reproductive success of captive-reared West Fork Yankee Fork Chinook Salmon

Populations/species:

Oncorhynchus tshawytscha

Questions/Objectives:

Evaluate reproductive success of out-planted (captive-reared) adults.

Genetic Analyses:

Considerations: In order to evaluate the reproductive success of captive-reared adults out-planted into the West Fork Yankee Fork, genetic samples would have to be obtained from three different groups: 1) all prospective captive-reared parents, 2) prospective wild parents, and 3) out-migrants from the West Fork Yankee Fork. If all prospective parents are sampled, theoretically, multi-locus genotyping of parents and offspring could be used to determine relative contributions of wild and captive-reared parents. The difficulty in this project is the inability to account for all prospective wild parents. Since captive-reared adults and wild adults come from the same spawning population, it would be impossible to rule out unsampled wild adults as parents. However, a large microsatellite screen should still be able to exclude captive-reared adults as parents.

Sample Locations/Populations and Samples to be analyzed:

Approximately 90 adult captive broodstock (brood years 1997, 1998, 1999).

Approximately 12 wild carcasses.

Approximately 200 parr.

Cooperating Agencies:

Genetic Lab Performing Work:

Not determined.

Sample Storage Location:

University of Idaho (Aquaculture Research Institute), Nampa Research

Time Frame:

Not determined

Proposed Cost:

Not determined

Funding Source:

BPA

Relationship To Other Projects:

Genetic questions and analyses very similar to the projects: "Evaluate reproductive success of "supplementation" Chinook salmon in the Pahsimeroi River and Salmon River @ Sawtooth weir upstream" and "Reproductive success of captive-reared Redfish Lake Sockeye Salmon (Catherine Willard's M.S. Project)."

Current Status of Project:

Proposal idea

Comments:

None

Region:

Eagle

Manager/Project Leader:

Keith Johnson / Carla Hogge

Project Title:

Development of an RFLP marker to distinguish *M. Cerebralis* from an unknown Myxosporean.

Populations/species:

Myxobolus cerebralis, Unknown Myxosporean

Background:

DNA-based tests to detect the presence of *Myxobolus cerebralis* (the causative agent of whirling disease) have been pursued for two reasons. First, it allows for the detection of the parasite in all stages of its lifecycle, an attribute not available in ELISA and analogous antibody-based recognition methods. Second, it should allow for the differentiation of many different *Myxobolus* species that have very similar spore shape and size.

In 1992 researchers developed a DNA-based test for the detection of *M. cerebralis* using a nested-PCR amplification of the 18s rRNA gene. The Eagle Fish Health lab currently includes this test in their disease investigations. During the last two years, the IDFG Eagle Fish Health Lab has isolated spores of similar shape and size to *M. cerebralis* from the nervous tissue of *Oncorhynchus mykiss* and *Oncorhynchus clarki* samples. While it was originally speculated that this unknown neurotropic spore was *M. neurobius*, sequencing of a smaller region (296 base pairs) of the 18s rRNA gene from new primers developed at the Eagle lab, indicated high sequence divergence between the unknown neurotropic spore and samples of both *M. cerebralis* and *M. neurobius*.

Questions/Objectives:

The purpose of this research project is to:

Develop an RFLP marker to differentiate *M. cerebralis* from the currently unknown neurotropic form.

Continue to investigate and describe the currently unknown neurotropic form.

Genetic Analyses:

Using the new primers developed this fall at the Eagle Fish Health Lab, amplify 296 base pairs of the 18s rRNA gene and digest with two restriction enzymes, Dde-I and Mse-I, which based on sequence comparisons should yield diagnostic banding (fragments) patterns.

Sample Locations/Populations and Samples to be analyzed:

10 known *M. cerebralis* controls.

10 putative “neurotropic form” Myxosporean samples.

Cooperating Agencies:

Possibly Dr. Karl Andree (University of California, Davis, California)

Genetic Lab Performing Work:

IDFG Eagle Fish Health Lab/IDFG Eagle Genetics Lab

Sample Storage Location:

IDFG Eagle Fish Health Lab/IDFG Eagle Genetics Lab

Time Frame:

Approx. 2 weeks

Proposed Cost:

\$1,000.00

Funding Source:

Keith Johnson (internal)

Relationship To Other Projects:

None

Current Status of Project:

Work in progress

Comments:

None

Region:

Southwest Region

Manager/Project Leader:

Dale Allen (208) 634-8137

Project Title:

Fishery Restoration of Gold Fork River, Idaho (Genetic characteristics of bull trout populations in the Payette River Basin)

Populations/species:

Salvelinus confluentus

Questions/Objectives:

To determine whether the remaining group of bull trout in the North Fork Gold Fork River (NFGFR) actually represents a single, randomly mating population.

To identify the extent of hybridization between bull trout and brook trout within the NFGFR bull trout population,

To determine the genetic variability and the genetic effective population size of this remaining population (in order to determine whether the population is at immediate genetic risk), and,

To determine whether this population would be appropriate for use in reintroductions into areas of suitable habitat within the Gold Fork River watershed that have been eradicated of brook trout.

Comprehensively identify the genetic characteristics of sampled populations of bull trout within the Payette River Basin; including identifying possible hybridization with brook trout and identifying genetic variation both within and between populations.

Genetic Analyses:

The Washington Department of Fish and Wildlife's genetics laboratory (Directed by Dr. James Shaklee) has agreed to do the genetic analyses of bull trout samples collected in this study (if this project receives BPA funding). Importantly, the DNA analyses that will be run on these samples are the same DNA analyses proposed for the Genetic Inventory of Bull Trout and Westslope Cutthroat Trout in the Pend Oreille Subbasin project (24008) that will be completed by the WDFW lab. Specifically, microsatellite DNA analysis will be conducted for approximately 6-12 loci (*Oki-10*; *Omy-77*; *One-7*; *Ocl-1*; *Ocl-2*; *Ots-101*; *Ots-107*; *Ogo-2*; *Ogo-3*; *Ogo-4*; *Sco-1*; *Sco-2*; *Sco-19*; *Sfo-8*; *Sfo-18*; *Str-60*; *Str-73*; *Ssa-197*; *FGT-3*). Samples will also be screened for diagnostic RFLPs at two mtDNA regions.

Sample Locations/Populations and Samples to be analyzed:

NF Gold River (N = 60), 10 additional populations of bull trout in the Payette River Basin (N = 60 for each).

Cooperating Agencies:

WDFW genetics laboratory in Olympia, Washington. Kalispell Tribe (Natural Resource Department).

Genetic Lab Performing Work:

WDFW genetics laboratory in Olympia, Washington

Sample Storage Location:

WDFW genetics laboratory in Olympia, Washington when collected.

Time Frame:

2 years. Samples will be run concurrently with samples from the Kalispell tribe's Pend Oreille Subbasin project.

Proposed Cost:

\$33,000 asked for in BPA proposal budget.

Funding Source:

BPA funding.

Relationship To Other Projects:

Similar questions to the project: "Hybridization and introgression and genetic population structure of Bull trout and Westslope cutthroat trout in the Priest River Drainage, Idaho" and the Kalispell tribe's Pend Oreille bull trout project. The same genetic analyses will be run on all projects (WDFW genetics laboratory).

Current Status of Project:

Waiting funding status. Samples to be collected this field season.

Comments:

None

Region:

Clearwater Region

Manager/Project Leader:

Christopher Claire / Tim Cochnauer (799-5010)

Project Title:

Red River Lamprey Genetic Study

Populations/species:

Lampetra tridentata

Questions/Objectives:

Determine whether lamprey from the Red River (a tributary to the SF Clearwater) are genetically distinct from other lamprey populations in the Columbia basin.

Investigate whether lamprey exhibit homing fidelity.

The hypotheses to be tested under this study are:

HO1—There is no genetic differentiation between Pacific Lamprey from the Red River and other Pacific Lamprey populations in the Columbia River Basin.

HO2—Populations of Pacific Lamprey throughout the Columbia River Basin represent one spawning aggregate.

Genetic Analyses:

Background—Matt Powell (University of Idaho) performed a limited mtDNA RFLP screen on populations of Pacific Lamprey from the Red River, Rogue, Deschutes, Willamette, and Walla Walla. Results indicated significant haplotype frequency differences between samples from the Red River and the other sample locations (Matt Powell, Personal Communication 6/12/02). No written report was submitted to IDFG on genetic work. Christopher Claire said that Matt Powell told him that genetic work was inconclusive (6/12/02). Unclear of whether any protein work was completed on samples from the Red River.

Possible future work—Sequencing an mtDNA region (Cyt B) may provide the resolution needed to address the above questions. IDFG would have to cooperate with the University of Idaho and perhaps the Umatilla tribe and USGS to obtain lamprey samples from other locations in the Columbia Basin for comparison purposes. Matt Powell seemed interested in collaborating, although he does not currently have funding secured (6/12/02).

USGS is currently having the University of Wisconsin develop microsatellite loci to investigate Pacific Lamprey. When these are developed and tested, they may provide a powerful means of addressing questions of population genetic structure and homing fidelity.

Sample Locations/Populations and Samples to be analyzed:

Red River, Idaho (n = 20). Possibly Rogue, Deschutes, Willamette, and Walla Walla (n = 20 for each).

Cooperating Agencies:

Possible: Dave Close (Confederated Tribes of the Umatilla Indian Reservation), Dr. Jim Sealy and Jen Bayer (USGS), Matt Powell (University of Idaho).

Genetic Lab Performing Work:

Not determined.

Sample Storage Location:

University of Idaho (Hagerman Fish Culture Experiment Station)

Time Frame:

Sequencing of one mtDNA region on 20 samples from the Red River, Rogue, Deschutes, Willamette, and Walla Walla (n = 100 total) would require three weeks of full-time (40 hour week) work.

Proposed Cost:

Sequencing of one mtDNA region on Red River samples (n = 20) would cost about \$600.00. Total cost of all samples would be approximately \$3,000.00.

Funding Source:

Internal, possibly shared with University of Idaho.

Relationship To Other Projects:

None.

Current Status of Project:

Limited genetic work done by U of I. Still in proposal phase.

Comments:

None

Region:

Clearwater Region

Manager/Project Leader:

Jody Brostrom / Tim Cochnauer (208) 799-5010, Katherine Thompson (208) 983-1950

Project Title:

Characterization of Intraspecific Genotypic Variation At The Subbasin Scale: A Preliminary Proposal for Westslope Cutthroat and Steelhead/Redband Trout

Populations/species:

Oncorhynchus mykiss and *Oncorhynchus clarki*

Questions/Objectives:

Has hybridization of Westslope cutthroat trout occurred from past stocking of Yellowstone and hatchery rainbow trout in high mountain lakes? Have different stocking regimes resulted in different patterns of hybridization? Is outbreeding continuing where stocking has ceased?

How do the genetic characteristics of Westslope cutthroat trout in the Selway basin vary spatially among populations? What proportion of that variation is contained within and among populations?

Do the genetic characteristics of the juvenile steelhead and redband trout phenotypes differ significantly or suggest a measure of divergence?

Genetic Analyses:

A Master Challenge Cost Share Agreement was made between the University of Montana (UMT), the Idaho Department of Fish and Game (IDFG), and the Forest Service to work on this collaborative genetic project on Westslope cutthroat trout in the Selway Drainage. The University of Montana has agreed to do all genetic analysis on this project (Microsatellite loci and P.I.N.E.S.). As of May 20, 2002, the University of Montana had not started any work on this project and had not received any money from the Forest Service to do the work (Paul Sprewell, Personal Communication, May 20, 2002). IDFG has ½ a fin clip from eight of the sample locations listed below [Selway River Main (n = 50), Stripe Cr. (n = 7), Surprise Cr. (n = 17), Swet Cr. (n = 22), Thirteen Cr. (n = 20), Three Lakes Cr. (n = 13), White Cap Cr. (n = 30), and Wilkerson Cr. (n = 30)].

Cooperating Agencies:

University of Montana (UMT) and the U.S. Forest Service.

Genetic Lab Performing Work:

University of Montana, perhaps IDFG Eagle Genetics Lab.

Sample Storage Location:

University of Montana, ½ a fin clip from eight of these locations (Selway River Main (n = 50), Stripe Cr. (n = 7), Surprise Cr. (n = 17), Swet Cr. (n = 22), Thirteen Cr. (n = 20), Three Lakes Cr. (n = 13), White Cap Cr. (n = 30), and Wilkerson Cr. (n = 30) are stored at IDFG Eagle Genetics Lab.

Appendix D. (Continued.)

Sample Locations/Populations and Samples to be Analyzed:

| Watershed | Sample Location | Year | N | Comments |
|-------------------|----------------------|------------|-----|---|
| Selway | Burnt Knob | 2000-2001? | 2 | 1/2 clip fin clip to UofM 1/2 clip fin clip to UofI |
| Selway | Canyon Creek | 2000-2001? | 30 | 1/2 clip fin clip to UofM 1/2 clip fin clip to UofI |
| Selway | Deep Creek | 2000-2001? | 30 | 1/2 clip fin clip to UofM 1/2 clip fin clip to UofI |
| Selway | Eagle | 2000-2001? | 19 | 1/2 clip fin clip to UofM 1/2 clip fin clip to UofI |
| Selway | Salamander | 2000-2001? | 27 | 1/2 clip fin clip to UofM 1/2 clip fin clip to UofI |
| Selway | Schofield | 2000-2001? | 29 | 1/2 clip fin clip to UofM 1/2 clip fin clip to UofI |
| Selway | Selway River | 2000-2001? | 50 | 1/2 clip fin clip to UofM 1/2 clip fin clip to UofI |
| Selway | Stripe | 2000-2001? | 7 | 1/2 clip fin clip to UofM 1/2 clip fin clip to UofI |
| Selway | Surprise | 2000-2001? | 17 | 1/2 clip fin clip to UofM 1/2 clip fin clip to UofI |
| Selway | Swet | 2000-2001? | 22 | 1/2 clip fin clip to UofM 1/2 clip fin clip to UofI |
| Selway | Thirteen | 2000-2001? | 20 | 1/2 clip fin clip to UofM 1/2 clip fin clip to UofI |
| Selway | Three Lakes | 2000-2001? | 13 | 1/2 clip fin clip to UofM 1/2 clip fin clip to UofI |
| Selway | White Cap Cr. | 2000-2001? | 30 | 1/2 clip fin clip to UofM 1/2 clip fin clip to UofI |
| Selway | Wilkerson | 2000-2001? | 30 | 1/2 clip fin clip to UofM 1/2 clip fin clip to UofI |
| | | | 326 | |
| Bear Creek Drain. | Wahoo Cr. | 2000-2001? | ? | All samples to UofM |
| East Moose Drain. | Pettibone Cr. | 2000-2001? | ? | All samples to UofM |
| East Moose Drain. | Cedar Cr. | 2000-2001? | ? | All samples to UofM |
| N.F. Moose Drain. | N. Moose Cr. | 2000-2001? | ? | All samples to UofM |
| N.F. Moose Drain. | W. Moose Cr. | 2000-2001? | ? | All samples to UofM |
| N.F. Moose Drain. | Wounded Doe Cr. | 2000-2001? | ? | All samples to UofM |
| Mainstem Bear Cr. | Three Links Cr. | 2000-2001? | ? | All samples to UofM |
| Mainstem Bear Cr. | Marten Cr. | 2000-2001? | ? | All samples to UofM |
| Mainstem Bear Cr. | Mink Cr. | 2000-2001? | ? | All samples to UofM |
| Mainstem Bear Cr. | Spruce Cr. | 2000-2001? | ? | All samples to UofM |
| Mainstem Bear Cr. | Flat Cr. | 2000-2001? | ? | All samples to UofM |
| Mainstem Bear Cr. | Little Clearwater R. | 2000-2001? | ? | All samples to UofM |

Time Frame:

3 weeks of **full-time** (40 hours/week) lab work for mtDNA and nDNA RFLP analysis.

Proposed Cost:

\$3,000.00 for 189 samples with mtDNA and nDNA RFLP screen.

Funding Source:

U.S. Forest Service, Internal

Relationship To Other Projects:

Questions and objectives are the same as those proposed by IDFG on the Middle Fork of the Salmon River (Mike Petersen's Graduate Project and "effects of stocking high mountain lakes" project in the MFSR).

Current Status of Project:

A Master Challenge Cost Share Agreement has been made between the University of Montana (UMT), the Idaho Department of Fish and Game (IDFG), and the Forest Service to work on this collaborative genetic project on Westslope cutthroat trout in the Selway Drainage. All samples have been collected. No samples have been genetically analyzed.

Comments:

A genetic screen for rainbow trout introgression using mtDNA and nDNA RFLP markers on all of the samples IDFG has tissue for (n = 189) may provide information needed to respond to Judge Sullivan's decision regarding possible listing of Westslope cutthroat under the ESA.

Region:

Salmon Region

Manager/Project Leader:

Steve Yundt

Project Title:

Determining natural levels of hybridization between *O. mykiss* and *O. clarki* in the Middle Fork Salmon River (Mike Peterson's M.S. project)

Populations/species:

Oncorhynchus mykiss and *Oncorhynchus clarki lewisi*

Questions/Objectives:

Identify a "baseline" level of natural hybridization and introgression between *O. mykiss* and *O. clarki* in areas that have not been stocked with hatchery rainbow trout that can be compared to areas that have been stocked.

Sampling location requirements:

From areas in which the two species exist sympatrically.

From areas that have had no history of stocking rainbow trout (either directly in the stream/tributary or in headwater lake).

Genetic Analyses:

A diagnostic mtDNA RFLP marker screen will be performed first to determine the degree of hybridization and introgression. This will include the use of five diagnostic intron RFLP markers that exhibit fixed diagnostic differences between *O. mykiss* and *O. clarki lewisi*. If hybridization is found, a microsatellite screen will be performed. Five highly polymorphic microsatellite loci (Ots-101, Ots-107, Oki-10, Ogo-3, and FGT-3-Shaklee et al. 2000) will be used to determine if we are sampling more than one population.

Sample Locations/Populations and Samples to be analyzed:

Big Creek Drainage*

Big Creek Mainstem (Upper n = 60, Middle n = 60, Lower n = 60).

Cabin Creek (n = 60) (We sampled 52 *Oncorhynchus sp.* in 2000. It would be nice to increase this to at least 60).

Rush Creek (n = 60) (We sampled 35 *Oncorhynchus sp.* in 2000. It would be nice to increase this to at least 60).

Marble Creek Drainage

Marble Creek Mainstem (Upper n = 60, Middle n = 60, Lower n = 60).

Trail Creek (n = 60)

Big Cottonwood Creek (n = 60)

Indian Creek Drainage

Indian Creek Mainstem (Upper n = 60, Middle n = 60, Lower n = 60).

Little Indian Creek (n = 60)

Cooperating Agencies:

None

Genetic Lab Performing Work:

IDFG Eagle Genetics Lab

Sample Storage Location:

IDFG Eagle Genetics Lab

Time Frame:

This is a 2-year masters project; however, all of the lab work and analysis may be performed within one year of time.

Proposed Cost:

\$27,000 including mtDNA/Intron RFLPs and microsatellite analysis.

Funding Source:

Internal. Graduate student work.

Relationship To Other Projects:

Genetic questions and analyses are very similar to a number of IDFG projects examining hybridization and introgression between cutthroat and rainbow trout. It will be important to run the same suite of mtDNA and nDNA RFLP markers and the same microsatellite loci on all projects.

Current Status of Project:

Samples to be collected July 18 – July 26.

Comments:

None

Region:

Westslope Hybridization Work in the Middle Fork Salmon River

Manager/Project Leader:

Steve Yundt

Project Title:

Effects of high mountain lake of high mountain lake stocking of rainbow trout on Westslope cutthroat populations.

Populations/species:

Oncorhynchus mykiss and *Oncorhynchus clarki lewisi*

Questions/Objectives:

Has past stocking of Yellowstone cutthroat and hatchery rainbow trout in high mountain lakes led to hybridization and introgression of Westslope cutthroat trout populations below these lakes?

Genetic Analyses:

The genetic analyses are similar to the previous study. An mtDNA diagnostic RFLP marker screen will be performed first to determine the degree of hybridization and introgression. This will include the use of five diagnostic intron RFLP markers that exhibit fixed diagnostic differences between *O. mykiss* and *O. clarki lewisi*. If hybridization is found, a microsatellite screen will be performed. Five highly polymorphic microsatellite loci (Ots-101, Ots-107, Oki-10, Ogo-3, and FGT-3-Shaklee et al. 2000) will be used to determine if we are sampling more than one population.

Sample Locations/Populations and Samples to be analyzed:

Both streams in which no stocking has occurred and streams in which the headwater lake has been stocked will be included in the sampling design.

Streams that have had no lake stocking in headwaters (and have had no other direct stocking of rainbows):

Elkhorn Creek (Previously sampled 20, would like to increase to N = 60)

Garden Creek (Previously sampled 20, would like to increase to N = 60)

Soldier Creek (Previously sampled 10, would like to increase to N = 60)

Roaring Creek (Previously sampled 10, would like to increase to N = 60)

Streams that have had lake stocking in their headwaters:

Little Pistol Creek (N = 60)

Cache Creek (Previously sampled 31 in 1996-1997, would like to increase to N = 60)

Wilson Creek (Previously sampled 35 in 2001, would like to increase to N = 60)

Float Creek *Sampling done. 20 samples in 1999, 40 samples in 2001.

Cooperating Agencies:

None

Genetic Lab Performing Work:

IDFG Eagle Genetics Lab

Sample Storage Location:

IDFG Eagle Genetics Lab

Time Frame:

This is a 2-year masters project; however, all of the lab-work and analysis may be performed within one year of time.

Proposed Cost:

\$16,000 including mtDNA/Intron RFLPs and microsatellite analysis.

Funding Source:

Internal. Graduate student work.

Relationship To Other Projects:

Determining natural levels of hybridization between *O. mykiss* and *O. clarki* in the Middle Fork Salmon River (Mike Peterson's masters project)

Current Status of Project:

Comments:

Samples to be collected July 18 – July 26. Genetic questions and analyses are very similar to a number of IDFG projects examining hybridization and introgression between cutthroat and rainbow trout. It will be important to run the same suite of mtDNA and nDNA RFLP markers and the same microsatellite loci on all projects.

Region:

Salmon Region

Manager/Project Leader:

Tom Curet / Greg Schoby (208) 756-2271

Project Title:

Seasonal Movement, Habitat Use Patterns, and Baseline Genetic Profiles of Fluvial Trout Populations in the Upper Salmon River Basin, Idaho (Greg Schoby's M.S. Project).

Populations/species:

Salvelinus confluentus, *Oncorhynchus mykiss*, and *Oncorhynchus clarki lewisi*

Questions/Objectives:

To identify the migration patterns of fluvial trout in the upper Salmon River basin.

To identify habitat variables associated with the migration patterns of fluvial trout in the upper Salmon River basin.

To identify distinct fluvial trout populations in the upper Salmon River basin through genetic analysis.

Methods:

50 rainbow trout, 50 Westslope cutthroat trout, and 50 bull trout will be radio tagged during the spring and summer of 2002 and 2003 on the mainstem Salmon River. Once fish are tracked to staging or spawning areas, subsequent genetic samples will be taken from 50 additional adults within the "designation" stream. Samples within and between "designation" streams will be analyzed for hybridization and introgression, genetic variability, and genetic population structure.

Genetic Analyses:

Diagnostic mtDNA and nDNA RFLP markers should be screened first to determine the degree of hybridization and introgression between rainbow trout and westslope cutthroat trout and between bull trout and brook trout. Microsatellite analysis will have to be used to address questions concerning genetic population structure, genetic variability, and gene flow between populations.

Suggested loci (Westslope cutthroat): Ots-101, Ots-107, Oki-10, Ogo-3, and FGT-3-Shaklee et al. 2000

Suggested loci (Bull trout): Oki-10; Omy-77; One-7; Ocl-1; Ocl-2; Ots-101; Ots-107; Ogo-2; Ogo-3; Ogo-4; Sco-1; Sco-2; Sco-19; Sfo-8; Sfo-18; Str-60; Str-73; Ssa-197; FGT-3

Sample Locations/Populations and Samples to be analyzed:

Not determined. Possibly 3-4 Westslope cutthroat populations, 3-4 rainbow trout populations, and 3-4 bull trout populations (all in the Salmon River Basin). Depends on where tagged fish return to spawn; population number may be higher or lower.

Appendix D. (Continued.)

If four populations are sampled for each species ($4 \times 3 = 12$), and 60 samples per populations, then sample size may be $60 \times 12 = 720$.

Cooperating Agencies:

None

Genetic Lab Performing Work:

IDFG Eagle Genetics Lab

Sample Storage Location:

IDFG Eagle Genetics Lab

Time Frame:

2 years

Proposed Cost:

For 720 samples, \$25,000 including mtDNA/Intron RFLPs and microsatellite analysis.

Funding Source:

Internal. Graduate student work.

Relationship To Other Projects:

Determining natural levels of hybridization between *O. mykiss* and *O. clarki* in the Middle Fork Salmon River (Mike Peterson's masters project)

Current Status of Project:

Bull trout will be tagged and sampled for genetic analyses during this field season.

Comments:

Region:

Southeast Region

Manager/Project Leader:

Dave Teuscher (208-232-4703)

Project Title:

Hybridization and introgression between Yellowstone cutthroat and rainbow trout in the Blackfoot River, Idaho.

Populations/species:

Oncorhynchus clarki bouvieri

Questions/Objectives:

What is the level of hybridization and introgression between Yellowstone cutthroat and introduced hatchery rainbow trout in the upper Blackfoot River?

How is rainbow trout introgression partitioned throughout the Blackfoot River?

Do multiple "populations" of Yellowstone cutthroat trout exist in the Blackfoot River and are some more susceptible to hybridization and introgression?

For example: There may be reproductive isolation between adfluvial cutthroat in tributaries that flow into the upper Blackfoot and rainbow trout, as well as hybrids that exist in the middle and lower Blackfoot River.

Previous Work:

In 2000, an mtDNA RFLP marker and two nDNA RFLP markers was used to investigate hybridization and introgression in the Blackfoot River. Analysis of 24 samples indicated that six of the 24 fish examined had rainbow trout mtDNA and/or rainbow trout nDNA (<http://www.cbwfw.org/files/province/uprsnake/projects/33001.htm>). Managers were concerned whether these samples could have come from multiple populations, making definitive conclusions about the purity of Yellowstone cutthroat from the Blackfoot River difficult.

Current Proposed Work:

Randomly sample 150 spawning adults (no phenotypic selection against hybrids, rainbow trout) at the Blackfoot weir. Determine levels of rainbow trout introgression. Determine if samples come from multiple populations.

Genetic Analyses:

Genetic suggestions:

(First)

mtDNA diagnostic RFLP marker

Five diagnostic intron RFLP markers (These exhibit fixed diagnostic differences between *O. mykiss* and *O. clarki clarki*.)

We are looking for genotypes indicative of hybrids. If we fail to find any hybrids, then we do not need to proceed with the microsatellite screen.

(Second, if genotypes indicative of hybrids are found)
Six highly polymorphic microsatellite loci (*Ocl 1*, *Ocl 2*, *Ocl 3*, *Ocl 4*, *Ocl 8*, and *Ocl 9*) to determine if we are sampling more than one population.

Sample Locations/Populations and Samples to be analyzed:

150 samples collected at the Upper Blackfoot Weir.

Cooperating Agencies:

None

Genetic Lab Performing Work:

IDFG Eagle Genetics Lab.

Sample Storage Location:

IDFG Eagle Genetics Lab.

Time Frame (Lab Work):

6 weeks of **full-time** (40 hours/week) lab work (if both RFLP and microsatellite work were to be done). 3 weeks of **full-time** (40 hours/week) lab work (if only mtDNA and nDNA RFLP work were to be done).

Proposed Cost:

\$4,500.00 for 150 samples for mtDNA, nDNA RFLP screen, and 6 microsatellites.

\$2,500.00 for 150 samples for only mtDNA and nDNA RFLP screen.

Funding Source:

Internal

Relationship To Other Projects:

Ties closely with Kevin Meyer's Native Resident Trout Assessment and the new BPA proposal "Assessment of genetic population structure and risk of introgression and hybridization to native trout in the Middle and Upper Snake River Basins."

Current Status of Project:

Samples have been collected and sent to IDFG Eagle Genetics Lab.

Comments:

DNA extraction, microsatellite amplification would be carried out at the Eagle Genetics Lab. Microsatellites would have to be run out on the ABI 3100 sequencer in Hagerman.

Region:

Upper Snake Region

Manager/Project Leader:

Jim Fredericks / Scott Host (208-525-7290)

Project Title:

Hybridization and introgression between Yellowstone cutthroat and rainbow trout in the South Fork Snake River, Idaho.

Populations/species:

Oncorhynchus clarki bouvieri

Questions/Objectives:

Determine if weirs and phenotypic selection can be used as an effective method to reduce hybridization between native YCT and nonnative RBT and their hybrids in the South Fork Snake River, Idaho.

Determine the extent of temporal isolation between rainbow trout, Yellowstone cutthroat trout, and their hybrids in the South Fork Snake River, Idaho.

Project Design:

Set up weirs on four tributaries to the South Fork Snake River. Allow only adults above weir that phenotypically are identified as "Yellowstone cutthroat." At each tributary, randomly sample adults over the entire migration period that are allowed to pass over weir (60 per week) and adults that are phenotypically identified as "hybrid" and not allowed to pass over weir (60 per week). Randomly sample YOY out-migrating from each tributary in the summer/fall (60 per week). Genetically test (for rainbow trout introgression) in all three sample groups (adults allowed to pass weir, adults not allowed to pass weir, and out-migrants).

Genetic Analyses:

Genetic suggestions:

mtDNA diagnostic RFLP marker

Five diagnostic intron RFLP markers (These exhibit fixed diagnostic differences between *O. mykiss* and *O. clarki lewisi*.)

Sample Locations/Populations and Samples to be analyzed:

4 tributaries * X adults per week * 6 weeks X 2 sample groups (adults allowed above weir, adults not allowed above weir) = 1000 (Scott said that he collected approximately 1000 adults.

4 tributaries * 60 fry per week * X weeks = ? Total = approx. 2000.

Cooperating Agencies:

None

Genetic Lab Performing Work:

IDFG Eagle Genetics Lab.

Sample Storage Location:

Idaho Falls Office. Will be sent to IDFG Eagle Genetics Lab.

Time Frame (Lab Work):

6 months of **full-time** (40 hours/week) work.

Proposed Cost:

\$32,000.00 for approx. 2000 samples for mtDNA and nDNA RFLP screen

Funding Source:

Internal (Graduate Student Fund?)

Relationship To Other Projects:

Ties closely with Kevin Meyer's Native Resident Trout Assessment and the new BPA proposal "Assessment of genetic population structure and risk of introgression and hybridization to native trout in the Middle and Upper Snake River Basins."

Current Status of Project:

Adult samples have been collected. Scott will start juvenile collections in a few weeks.

Comments:

DNA extraction, microsatellite amplification would be carried out at the Eagle Genetics Lab.

Region:

Panhandle Region

Manager/Project Leader:

Ned Horner / Joe Dupont (208-769-1414), Joe Maroney (Kalispell Tribe (509-445-1147)).

Project Title:

Hybridization and introgression and genetic population structure of bull trout and Westslope cutthroat trout in the Priest River Drainage, Idaho.

Populations/species:

Salvelinus confluentus and *Oncorhynchus clarki lewisi*

Questions/Objectives:

Determine levels of hybridization and introgression between bull trout and brook trout and between Westslope cutthroat trout and rainbow trout in the Priest River Drainage, Idaho.

Determine genetic population structure of bull trout and Westslope cutthroat trout in the Priest River drainage, Idaho.

Background:

Some limited genetic analyses were run on Westslope cutthroat trout (n = 36) and bull trout (n = 136) from Priest River Drainage in 2001 (Hagerman Fish Culture Experiment Station Report-Matt Powell to Jim Fredericks-June 12, 2002). Diagnostic mtDNA and nDNA (1) RFLP markers failed to detect rainbow trout introgression in any of the Westslope cutthroat samples. The genetic analyses performed were incapable of examining bull X brook trout hybridization and were too limited to adequately describe genetic population structure for either Westslope cutthroat trout or bull trout.

In 2001 the Kalispell tribe (Joe Maroney-project leader), were approved for BPA funding of project #24008 "Genetic Inventory of Bull Trout and Westslope Cutthroat Trout in the Pend Oreille Subbasin." As part of this project, the Kalispell tribe has agreed to include the above samples (from IDFG) in its larger genetics review. They have also agreed to examine additional bull trout and Westslope cutthroat trout samples collected by IDFG from the Upper Priest River Drainage (including the Middle Fork of the East River-a tributary to the Priest River) during this field season (summer 2002). All of the genetic analyses for this project will be performed at the WDFW genetics laboratory in Olympia, Washington (Dr. Jim Shaklee geneticist), and have been agreed upon by IDFG biologists.

Genetic Analyses:

Conduct microsatellite analysis for approximately 6-12 loci (*Oki-10*; *Omy-77*; *One-7*; *Ocl-1*; *Ocl-2*; *Ots-101*; *Ots-107*; *Ogo-2*; *Ogo-3*; *Ogo-4*; *Sco-1*; *Sco-2*; *Sco-19*; *Sfo-8*; *Sfo-18*; *Str-60*; *Str-73*; *Ssa-197*; *FGT-3*) to determine genetic relationships between populations and estimate genetic variation within and among those populations.

Conduct microsatellite (same loci listed above) and/or PINES analysis to estimate the extent of hybridization between bull trout and brook trout and between westslope cutthroat and rainbow trout.

Sample Locations/Populations and Samples to be analyzed:

30 Bull trout populations, 55 Westslope cutthroat trout populations from 60 sampling locations in the Pend Oreille basin in Washington, Idaho, and Canada (From Kalispell tribe proposal). This will include IDFG Upper Priest River samples.

Cooperating Agencies:

Kalispell Tribe, Washington Department of Fish and Wildlife

Genetic Lab Performing Work:

WDFW genetics laboratory in Olympia, Washington.

Sample Storage Location:

All samples, when collected, will be stored at the WDFW genetics laboratory in Olympia, Washington.

Time Frame (Lab Work):

2 years.

Proposed Cost:

Approximately \$400,000

Funding Source:

BPA funding

Relationship To Other Projects:

Ties closely with other Westslope cutthroat work proposed in the Middle Fork Salmon River and Lochsa River drainages.

Current Status of Project:

Funding has been secured. Samples are currently being collected.

Comments:

We should strive to use the same suite of microsatellite loci in all Westslope cutthroat investigations if possible.

Region:

Panhandle Region

Manager/Project Leader:

Jody Walters (208-769-1414)

Project Title:

Hybridization and introgression and genetic population structure of bull trout and Redband trout in the Kootenai River, Idaho.

Populations/species:

Salvelinus confluentus and *Oncorhynchus mykiss*

Questions/Objectives (From Jody)

Are the fluvial fish (which appear to spawn mainly in tributaries upstream of Bonners Ferry) "pure" redband (inland) rainbow trout? The University of Idaho (Matt Powell) may have 60 samples collected from tributaries upstream of Bonners Ferry.

Does the fluvial population differ genetically from the adfluvial population (from Kootenay Lake B.C.), which spawns in Deep Cr? Genetic analysis of samples from Deep Cr indicated an admixture of both coastal and inland forms, although IDFG never received a final report from the University of Idaho.

Does the fluvial population in Idaho differ genetically from the fluvial population upstream of Kootenai Falls, MT? How much recruitment to Idaho originates in Montana?

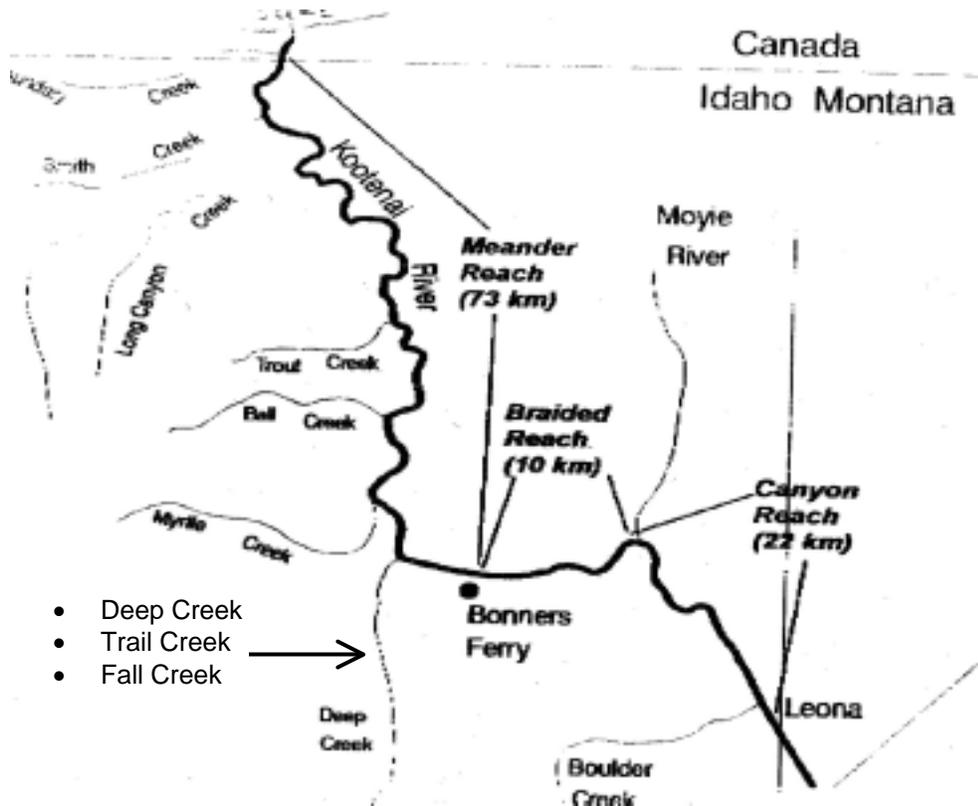
Does the bull trout population in Idaho differ genetically from bull trout in Kootenay Lake (where fishing is allowed) and from bull trout upstream of Kootenai Falls, MT?

What is the extent of hybridization and introgression between Westslope cutthroat and *O. mykiss* in the Kootenay River Drainage?

What is the genetic population structure of Westslope cutthroat in the Kootenay River Drainage?

Background:

In 1998, the University of Idaho examined 56 rainbow/redband trout *Oncorhynchus mykiss* from the Idaho reach of the Kootenai River using mtDNA RFLP analysis (ND2 gene region cut with Dde-I, Dpn-II, Hae-III, Hha-I, Hinf-I, Mse-I, Msp-I and Rsa-I). Results suggested that the population was probably an admixture of both coastal hatchery rainbow trout and redband trout (University of Idaho, unpublished data, Personal Communication, Matt Powell).



Genetic Analyses:

To begin with, the Kalispell tribe (Joe Maroney-project leader) should be contacted regarding whether they would be interested in obtaining bull trout and Westslope cutthroat trout genetic samples from the Kootenai/Kootenay River Drainage (call and e-mail placed to Joe M. on June 25, 2002).

To address the first question concerning the genetic purity of fluvial *O. mykiss* populations above Bonners Ferry, the same ND2 eight enzyme screen should be performed on the ~60 samples collected in (1998?) that was performed on samples from Deep Creek. (These samples can also be compared to the Deep Creek samples and to samples from the fluvial population upstream of Kootenai Falls, MT, to address questions 2 and 3.)

To address question 4, samples of bull trout from Kootenay Lake (n = 60) (where fishing is allowed) and samples of trout upstream of Kootenai Falls, MT (n = 60) would have to be collected and analyzed with the same microsatellite loci being used by the Washington Department of Fish and Wildlife Genetics Lab on the Kalispell tribe's bull trout project (*Oki-10*; *Omy-77*; *One-7*; *Ocl-1*; *Ocl-2*; *Ots-101*; *Ots-107*; *Ogo-2*; *Ogo-3*; *Ogo-4*; *Sco-1*; *Sco-2*; *Sco-19*; *Sfo-8*; *Sfo-18*; *Str-60*; *Str-73*; *Ssa-197*; *FGT-3*). This would allow the assessment of genetic relationships between populations and estimate genetic variation within and among those populations.

Appendix D. (Continued.)

To answer question 5, regarding the extent of hybridization and introgression between Westslope cutthroat and *O. mykiss* in the Kootenay River Drainage, 60 samples per population, would have to be collected and analyzed with a mtDNA RFLP marker and five diagnostic intron RFLP markers that exhibit fixed diagnostic differences between *O. mykiss* and *O. clarki lewisi*.

To answer question 6, regarding genetic population structure of Westslope cutthroat in the Kootenay River Drainage, Westslope cutthroat samples would have to be analyzed with five microsatellite loci Ots-101, Ots-107, Oki-10, Ogo-3, and FGT-3-Shaklee et al. 2000.

Sample Locations/Populations and Samples to be analyzed:

~60 samples of *O. mykiss* collected above Bonners Ferry (1998 samples stored at U of I). Most of the sample locations/populations to sample have not been determined.

Cooperating Agencies:

Possibly the Kalispell Tribe and the Washington Department of Fish and Wildlife. Possibly Dr. Eric Taylor from UBC.

Genetic Lab Performing Work:

Not determined

Sample Storage Location:

IDFG Eagle Genetics Lab, University of Idaho Aquaculture Research Institute.

Time Frame (Lab Work):

Not determined

Proposed Cost:

Not determined

Relationship To Other Projects:

Ties closely to the work that the Kalispell tribe is doing in the Pend Oreille and Priest River drainages.

Current Status of Project:

Comments:

Out of Department Projects:

Region:

Forest Service (Lost River Ranger District)

Manager/Project Leader:

Bart Gammet

Project Title:

The colonization of shorthead sculpin *Cottus confusus* in the Lost River Drainages of Southeastern Idaho

Populations/species:

Cottus confusus

Questions/Objectives:

From what drainage (i.e. Salmon, Snake, Missouri) did the Lost River shorthead sculpin populations originate?

What is the amount of genetic drift or genetic change that has occurred since the isolation of this population from its source population?

How long has this population been isolated from its source population?

Genetic Analyses:

Sequencing an mtDNA region (Cyt B) may provide the resolution needed to address the above questions. Samples will be extracted in the Eagle Genetics Lab and sequenced on the ABI 310 at Hagerman.

Sample Locations/Populations and Samples to be analyzed:

Twenty samples will be obtained from each of the three possible source drainages (Missouri, Snake, and Salmon drainage; $n = 60$). Twenty samples will also be obtained from each of the four sink drainages of interest ($n = 80$). Outgroups will also be used. Samples will be obtained from Northern Idaho ($n = 20$), Western Washington ($n = 20$), and Southwest Idaho ($n = 20$). A total of 200 samples will be analyzed for this study.

Cooperating Agencies:

University of Idaho

Genetic Lab Performing Work:

IDFG Eagle Genetics Lab

Sample Storage Location:

IDFG Eagle Genetics Lab

Time Frame:

3 weeks of full-time (40 hour week) work.

Proposed Cost:

Approximately \$3,600 for all samples.

Funding Source:

U.S. Forest Service. The University of Idaho and Idaho Fish and Game will also provide some cost sharing.

Relationship To Other Projects:

None

Current Status of Project:

Samples are currently being collected for the study.

Comments:

Sequencing would have to be run out on the ABI 310 sequencer in Hagerman.

The IDFG Eagle Fish Genetics Lab has already developed a mtDNA RFLP screen to distinguish three species of sculpin for the U.S. Forest Service (confuses, beldingi, and bairdi).

Prepared by:

Matthew Campbell
Fisheries Research Biologist

Approved by:

IDAHO DEPARTMENT OF FISH AND GAME

Virgil K. Moore, Chief
Bureau of Fisheries

Steve Yundt
Fisheries Research Manager

Non-native species homogenise ecosystems and, in some instances, cause populations of native species to plummet to the point of local extinction. The true impact of non-native species on ecosystems may take time, even decades, to become obvious, by which point it is often too late to reverse negative impacts from these intruders. This presents a problem for the entire ecosystem, including the humans who rely on ecosystem services for their well-being.