Project # 200007900
Assess Resident Fish Stocks of the Owyhee and Bruneau Basins, DVIR
Response to ISRP Preliminary Review FY-03 Proposals (ISRP 2002-02)

The response should describe a scientifically sound plan.
A scientifically sound plan was developed for the work initiated in 2001. The plan was developed in coordination with the contracting laboratory (Hagerman Lab) and with the input from the scientist completing the genetic laboratory work.
The plan includes measures for sampling at sites both above and below possible fish barriers, collection of habitat data, biological data (water quality DO, Ph, temp), as well as collection of fin clips, preservation and transport to lab. There are also provision for data exchange and the protocols for completing this exchange with Stream Net.
Actual protocols attached.

Project performance to date appears to be minimal.
This project was broke up into two phases in order to assure BPA that the sampling protocols were fully developed and consistent with other ongoing work in the area. Phase one of the project began with a contract from BPA on March 23rd of 2001 and was continued until July 1st, 2001. The initial sampling did not begin until approximately mid-July 2001. The water levels were very low at this time with some water temperatures exceeding 26 degrees C, and many of the intermittent streams being dry. Many of the intermittent streams have been observed to contain possible redband/rainbow trout populations, as observed in previous sampling efforts by DVIR biologists. The remaining sites will be completed during the spring of 2002. The final sites to be completed include 6 streams that were not completed in the fall 2001 due to low water levels and elevated water temperatures on the DVIR. These waters will be completed in early spring 2002 and sent to Hagerman laboratory for analysis. Upon completion of these results further sampling locations will be developed and mapped. Habitat conditions on streams completed to date have been entered in Tribal database for possible submission to STREAM NET. The last of the streams to be sampled in 2002 will also have habitat evaluations completed. This data will also be entered in Tribal database and sent to STREAM NET for inclusion in database. Final results of habitat evaluation and genetic information from the laboratory will be included in Annual Report to BPA and uploaded to BPA web-server. Also, to date we have sent 8 monthly reports to BPA. The actual time the project has been on the ground is only 8 months.

Justify the level of effort for the last two years and indicate how the current proposal and budget will remedy the situation.

This project did not receive initial funding until March 2001. The project has been ongoing for less than 1 year. The work included development of standardized sampling protocols that are consistent with work being completed by Idaho Department of Fish and Game and the Idaho BLM in proximity to the Reservation. The sampling protocols developed are similar to those developed by these two agencies and was reviewed by these agencies prior to the beginning of Phase II (sampling) of our contract. To date sampling has been complete on 5 streams with habitat data entered in Tribal database for submittal to STREAMNET. The current budget is set for one years work for a biologist and two technicians/aides. The time line is very similar to the work that is currently being completed.

Explain the relationship to similar ongoing projects which overlap the DVIR.
There are no other genetic assessments of native resident fish currently ongoing which overlap the DVIR. Project 1999800200 is scheduled to complete work in the Bruneau and Owyhee subbasins but they are not planning on entering NV or getting close to the DVIR (personal communication Kevin Meyer IDFG 3/12/02).
Similar project that overlap the DVIR are project 1997011 (Habitat Enhancement and Protection- DVIR), which involves collecting water quality and habitat information on the Owyhee and Bruneau River tributaries. The only other work of this type that is occurring in this general area is by the Idaho BLM. That work is taking place in Shoofly and Little Jacks Creek, which is approximately 50 miles downstream of the DVIR. There are no other resident fish assessment projects ongoing that overlap the DVIR.

Project should be coordinated with Project 199800200 "Snake River Native Salmonid Assessment" to ensure that the same random site selection procedures are used, as far as possible, and the same data collection procedures are used.
This project has been in coordination with project 199800200 as well as proposed project 33001. The data collection procedures are very similar to what IDFG is proposing. The protocols developed between the Tribes and BPA were in coordination with IDFG, ODFW, and USFWS in order to ensure accuracy and repeatability throughout the Snake River Basin. IDFG sampled 40-50 sites on the Lower Owyhee River in 1999 and is planning on moving into the Bruneau River subbasin in 2003-2004 (personal communication Kevin Meyer IDFG 3/12/02). Also, project 3301 is planning on sampling in the Bruneau and upper Snake River tributaries. Protocols utilized in project 33001 are very similar to protocols developed for this project (personal communication Matt Campbell 12/01) as the project lead for 33001 helped developed our protocols when working for Hagerman Laboratory.

The response needs to identify specific sample areas, data collection methods, sampling frequency and intensity. A map of sampling sites and schedule for accomplishing work should be given.

A map showing proposed sites, methods, and sampling frequency and intensity for FY-03 are attached.

The schedule for completing this work will be expedited in order to ensure that Project 32001 has sufficient data to proceed with the best available scientific knowledge.

We are proposing to begin this project as early as possible (October 2002) in order to have sufficient data to make recommendations for project 32001.

Experimental Design

Genetic investigation of East Fork Owyhee River and Bruneau River drainage redband trout populations will be done using non-lethally collected fin tissue samples. Study objectives will be addressed using Restriction Fragment Length Polymorphisms (RFLP) analysis of one nDNA intron gene, RFLP analysis of two mtDNA gene regions, and a screen of six microsatellite loci. Shoshone-Paiute Tribal biologists will collect all necessary samples during the 2002/03 field season. Samples will be stored in lysis buffer or ethanol, and shipped to the laboratory for analysis.

Sample Collection

Non-lethal fin clips will be collected from 10 populations within the East Fork Owyhee River and Bruneau River Drainage. Fin clips will be stored at room temperature in lysis buffer (EDTA 0.5M, 2M Tris, 5M NaCl, SDS 20%, dH_2O) or 95% ethanol prior to genetic analysis.

DNA Extraction-

Mitochondrial DNA and nuclear DNA will be extracted from non-lethally collected fin-clip samples using methods described by Paragamian et al. (1999), adapted from protocols by Sambrook et al. (1989) and Dowling et al. (1990).

CRA Amplification and restriction digestion of Nuclear DNA gene region-

DNA isolated from each sample will be amplified using the Polymerase Chain Reaction (PCR) with primers specific for the Protoncogene 53 (p53) nDNA gene region. Variation in allele frequency at the p53 locus has been observed in rainbow/redband trout populations from the Upper Columbia River drainage and rainbow trout from the Hayspur Hatchery (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, stained with ethidium bromide, and fluoresced under UV-light to visualize alleles.

PCR AAmplification and Restriction Enzyme digestion of mtDNA gene region-

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*). Eight of these restriction enzymes have been used in combination with the ND2 region in previous studies examining introgressive hybridization within redband trout populations (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 visualized as band patterns (fragments) when stained with ethidium bromide and fluoresced under UV-light. The fragment size in base pairs was estimated by comparison to a size standard, a pUC-19 marker (Bio-Synthesis).

Microsatellite Amplification and allele scoring-

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 collected samples. Expected number of alleles observed, allele size range (bp), and PCR conditions are as follows:

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

Assessments of genetic subpopulation structure will be made using the statistical genetic computer programs GENEPOP version 1.2 (Raymond and Rousset 1995). 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 (version 3.5c; Felsenstein 1993) will be used to calculate Nei's (1978) standard distance (D) and Cavalli-Sforza and Edwards' (1967) chord distance (CSE) for all populations. The neighbor-joining algorithm in the NEIGHBOR program (in PHYLIP) will be used to generate dendograms from these distance matrices. Maximum likelihood distance matrixes and dendograms will be calculated using the program CONTML (PHYLIP). Robustness of tree topologies will be assessed using the programs SEQBOOT and CONSENSE (PHYLIP). Dendrogram diagrams will be created in the programs DRAWTREE and DRAWGRAM (PHYLIP).

Above information is part of ongoing work being implemented in project 2000-079 with the Shoshone-Paiute Tribes and Hagerman Genetics Laboratory.

Project Objectives:
Objective 1
Sign and manage a subcontract for genetic analysis with the Laboratory.

Tasks

Task 1-a
Submit a signed copy of the professional services agreement (subcontract) to BPA

Task 1-b
Manage subcontract and coordinate with Lab.

Deliverable:
(1) Report from the genetics laboratory in hard copy and electronic form containing … (list specifics as contained in subcontract)
(2) Database spreadsheet in MS-Excel on a floppy, Zip disk or CD-R.

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Objective 2
Determine type and location of resident fish in the Owyhee/Bruneau Subbasin portion of the DVIR

Task 2-a
Collect fish samples in 12 streams/year. See map

Crew will collect tissue samples from trout collected in streams identified by biologist. Samples will be collected above and below blockages in streams (i.e.: water falls, log jams, etc). Trout collected from approximately 10 streams and two sites per stream (approx. 30-60 samples per site)

Task 2-b
Collect habitat information (see Appendix)

During collection take photos and conduct baseline habitat information on areas of collection. Fill out EPA habitat characterization forms/or appropriate habitat forms (PFC) one for each stream.
Task 2-c
Submit samples to genetics laboratory as per subcontract for appropriate electrophoretic and/or DNA analysis

Deliverable:
Task 1
30-60 samples per stream for 12 streams.
Task 2

Habitat forms for each stream or sample location for use in analysis and annual report.
Task 3
Samples delivered to genetics lab as per subcontract

Objective 3
Manage data from sample collection, lab analysis, results analysis and organize for reporting.

Tasks

Task 3-a
Enter data from field notes, GPS, laboratory (if lab data needs to be manipulated), EPA-Habitat evaluation forms, if needed, into spreadsheets. Use separate spreadsheet for sampling locations, Barrier locations and Barrier codes, as discussed with StreamNet.

Task 3-b
Format or adapt data spreadsheets from 8-1 and organize for use in analysis or reporting and submittal to BPA, StreamNet or STORET, as appropriate. Include relevant data tables, forms and field notes for use as appendices in the Annual report.

Deliverables:

Task 3-a
GPS coordinates for sampling locations and barriers. Spreadsheets or tables if appropriate for other field or analytical information.
Task 3-b
Adapted spreadsheet data if needed for analysis. Tables and forms for use in the Annual report.

Data sheets are attached. Field data books will also be utilized to record other data or information that may be pertinent once out in the field. Information will then be transferred to database for inclusion in StreamNet or other data management system.

The following was adopted after contact with StreamNet, EPA, and Idaho Fish and Game. These formats are compatible with what is occurring in the Owyhee and Bruneau Subbasin.

Data Exchange Formats (DEF)

1. Data collected from sampling will be entered into Tribal database in MS Excel, with non-confidential information being entered into STORET /StreamNet (coordination with B. Butterfield, IDFG StreamNet)
2. Data to be entered will included at a minimum: MS Excel to be able to coordinate with Tribes GIS system

-Water quality data (
Temp (degrees Celsius)
O2 (parts per million)
Conductivity (microseimens)
-Habitat data (riparian type, pool, run, riffle, woody debris/jams, width/depth of stream channel, substrate composition, overhead cover, bank stability)
-stream order
-elevation
All habitat information will be collected in meters or metric equivalent.

3. Salmonid densities, population size-structure, and number of species sampled will be included in the database
4. Genetic composition of salmonids (once received from lab) entered in database
5. Latitude and longitude coordinates and elevations entered into database
6. Electro-fishing effort will be entered into database

All data collected (biological and habitat) will be sent to StreamNet in either MSAccess or in Excel (pers. Comm. Mike Banach). The information will either be sent in spreadsheet form or will be extracted from Annual report.

Objective 4
Evaluate and analyze data results from genetics laboratory to determine levels of introgression with hatchery salmonids.

Data collected from this study will assure the Tribes ability to determine where there are "pure" redband trout populations present. The amount of genetic differentiation will be estimated using Nei's (1978) measure of standard genetic distance. Genetic distance will be calculated with the BIOSYS-1 program (Swofford and Sclander 1981). Differences among populations will be examined using GENEPOP (Raymond and Rousset 1981).

This work will be completed using the laboratory contracted.

Assessment of Stock Status
Genetic relationships of Redband trout and bull trout will be assessed for the Duck Valley Indian Reservation through 1) Compilation of baseline/historical electrophoretic/intron analysis data where available, 2) Implementation of baseline genetic inventory using electrophoretic techniques and intron analysis, 3) Compilation and analysis of life history and genetic data.

Tasks

Task 4-a
Document results of genetic data in final report and relate it to other aspects of the study.

Task 4-b

Relate current resident fish composition to abundance and population estimates conducted in previous years and with other information available on species.

Task 4-c

Use data to update subbasin summaries and to develop Subbasin plans for the DVIR and Owyhee Basin.

Deliverable:

Task 4-a & 4-b

Analysis of habitat characteristic data, fish species distribution, richness, density, relative abundance, and genetic composition.
Task 4-c
Recommendations for native species restoration in the Owyhee/Bruneau Subbasin.

Objective 5

Complete annual and project reports required by BPA with possible submission to peer review journal for publication.

Use same approach as in work statements from other SPT projects. Task for annual report. Task for Data submission, property inventory, lessons learned, etc. can be combined with annual submitting Final quarterly addressing Bippy (or contract) requirements.

Tasks

5-a

Submit 6 - month report to BPA with information on status of collections and laboratory information to date on genetic samples.

5-b

Submit Annual (project technical) report to BPA. Hard copy of this report will be sent to COTR. Annual report will contain whatever is required in the contract and the Bonneville Purchasing Instructions such as methodology, lessons learned, etc. The property report for this project will be part of the Annual report, the final quarterly report or a separate submittal due with the Annual.

Upon completion of project a final report will be submitted to BPA. The information gathered from the project will be utilized for future projects and planning on the reservation.

5-c

Submit project database spreadsheets to BPA for eventual upload to BPA server, StreamNet or EPA-STORET. Databases can be on floppy, Zip disk or CD-R or e-mailed

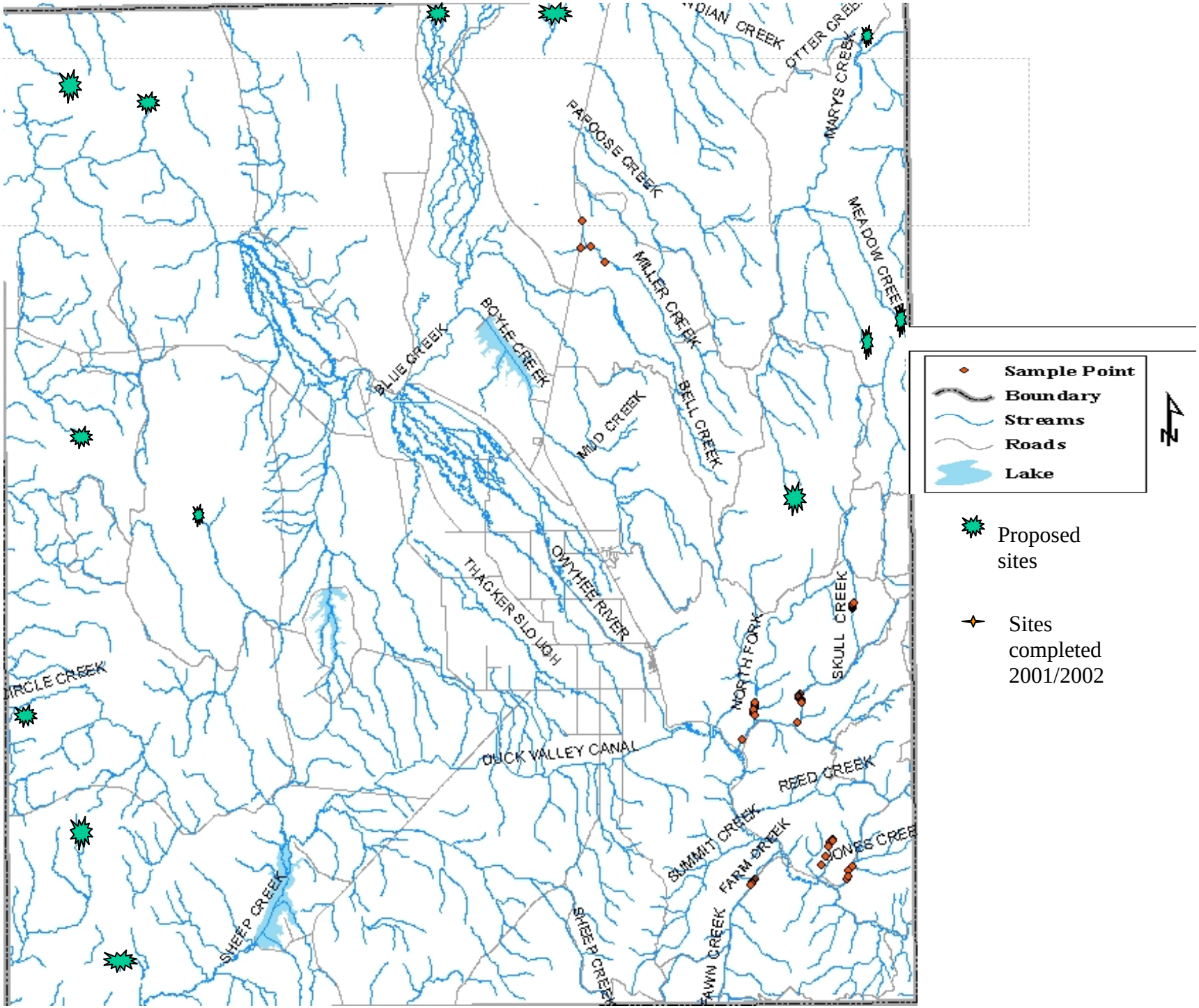
to BPA COTR. Data to reside on StreamNet can be uploaded or sent directly to StreamNet.

Schedule:

Task 5-a	180 days after Phase 2 start date. Or specify dates if project start date is fixed.
Task 5-b	60 days of contract end date (specify date if established by this work statement) or other date approved by BPA COTR.
Task 5-c	Databases due to BPA within 60 days after contract end.

Schedule of work to be accomplished
Tasks are same as tasks listed above in Methods and Objectives Section
Starting October 2002

Date		*Oct*	*Nov*	*Dec*	*Jan*	*Feb*	*March*	*April*	*May*	*June*	*July*	*Aug*	*Sept*
Objective	Task												
1													
	a		X										
	b		X	X	X	X	X	X	X	X	X	X	X
2													
	a							X	X	X			
	b							X	X	X			
	c								X	X			
3													
	a								X	X	X	X	X
	b								X	X	X	X	X
4													
	a								X	X	X	X	X
	b								X	X	X	X	X
	c								X	X	X	X	X
5													
	a	X	X	X	X	X	X	X	X	X	X	X	X
	b												X
	c											X	X


OTTER CREEK
MARYS CREEK
PAPOOSE CREEK
MEADOW CREEK
MILLER CREEK
BOYLE CREEK
BLUE CREEK
BELL CREEK
MUD CREEK
THACKER SLOUGH
OWYHEE RIVER
CIRCLE CREEK
NORTH FORK
SKULL CREEK
DUCK VALLEY CANAL
REED CREEK
SUMMIT CREEK
FARM CREEK
SHEEP CREEK
Sample Point
Boundary
Streams
Roads
Lake
N
Proposed sites
Sites completed 2001/2002