DEPARTMENT OF THE INTERIOR
U.S. FISH AND WILDLIFE SERVICE
REGION 1

ENVIRONMENTAL CONTAMINANTS PROGRAM

OFF-REFUGE INVESTIGATIONS SUB-ACTIVITY

Interim Report- FY 2004

NV - Lower Truckee River Contaminant Assessment

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by

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II. INTRODUCTION

II.A. Background and Justification

In 1990, Congress enacted the Truckee-Carson-Pyramid Lake Water Rights Settlement Act (Title II of Public Law [P.L.] 101-618) to resolve conflicts associated with increasing water demands in the Truckee and Carson River basins in western Nevada. P.L. 101-618 recognized that recovery of the federally endangered cui-ui (*Chasmistes cujus*) and federally threatened Lahontan cutthroat trout (LCT; *Oncorhynchus clarki henshawi*) in the Truckee River basin was imperative to the success of the settlement agreement required under P.L. 101-618 and directed the Fish and Wildlife Service (Service) to "expeditiously revise, update, and implement plans for the conservation and recovery of cui-ui and Lahontan cutthroat trout." P.L. 101-618 further mandated substantial effort in the rehabilitation of aquatic and riparian habitats in the lower Truckee River. Since the enactment of P.L. 101-618, the Service and its partners have made significant progress in the rehabilitation of the lower Truckee River. Aggressive riparian habitat improvement efforts have promoted the geomorphic stabilization of the lower river and substantial improvement of instream and riparian habitat conditions. Washoe County and the cities of Reno and Sparks have also agreed to acquire water to augment lower river flows for the benefit of water and habitat quality and the listed fishes. Significant progress has also been made toward the recovery of listed species. These efforts have included considerable modification of Marble Bluff Dam to allow the more efficient passage of the listed fishes from Pyramid Lake to the lower Truckee River. In 1998 and 1999, record numbers of cui-ui were passed from Pyramid Lake to spawning areas in the lower river via Marble Bluff Dam. The Service, Pyramid Lake Paiute Tribe, and other cooperators have also initiated efforts to reestablish a self-sustaining population of LCT in the Truckee River basin. These efforts have included the annual stocking of 50,000 LCT in the lower Truckee River. However, widespread sediment contamination has recently been identified in the lower Truckee River. The Service and other cooperators are concerned that this contamination has the potential to compromise lower river rehabilitation and listed species recovery efforts.

In 1998, the U.S. Geological Survey (USGS) National Water Quality Assessment Program (NAWQA) reported elevated concentrations of a variety of metals and polycyclic aromatic hydrocarbons (PAH) in Truckee River sediment collected in and downstream of the Reno-Sparks metropolitan area (Bevans et al. 1998). Contamination extended from Reno to at least Tracy (Figure 1). Metal and PAH concentrations were greater than the national 75th percentile reported by NAWQA and, in several instances, exceeded published biological effects criteria. NAWQA scientists also documented elevated contaminant concentrations in fish and aquatic invertebrates and noted a higher incidence of lesions, hemorrhagic septicemia, and external parasites in fish collected in this reach. In 1999, Service biologists monitoring LCT survival and stocking success in the lower Truckee River also reported a high incidence of external anomalies in fish from the reach of river downstream of Reno (William Cowan, Fishery Biologist, USFWS, Reno, NV, pers. comm. 2000). External anomalies again included external lesions, hemorrhagic septicemia, and

external parasites.

Several potential sources of contaminants in lower Truckee River sediments have been identified. Elevated contaminant concentrations have been documented in permitted discharges to the river, including treated municipal waste water, commercial sump pumping discharges, and dewatering operations (Nevada Division of Environmental Protection file information, 2000). Under low flow conditions, these permitted discharges (particularly treated municipal waste water) account for the majority of flow in the lower Truckee River. Several non-point source discharges (stormwater runoff, urban runoff, agricultural return flows, and groundwater inflow) have also been identified as potential contaminant sources in the lower Truckee River. However, the relative contribution of contaminants in point and non-point source discharges is uncertain. Additional research is needed to characterize the extent and severity of sediment contamination, the contribution of potential contaminant sources, and the occurrence and nature of effects to aquatic species.

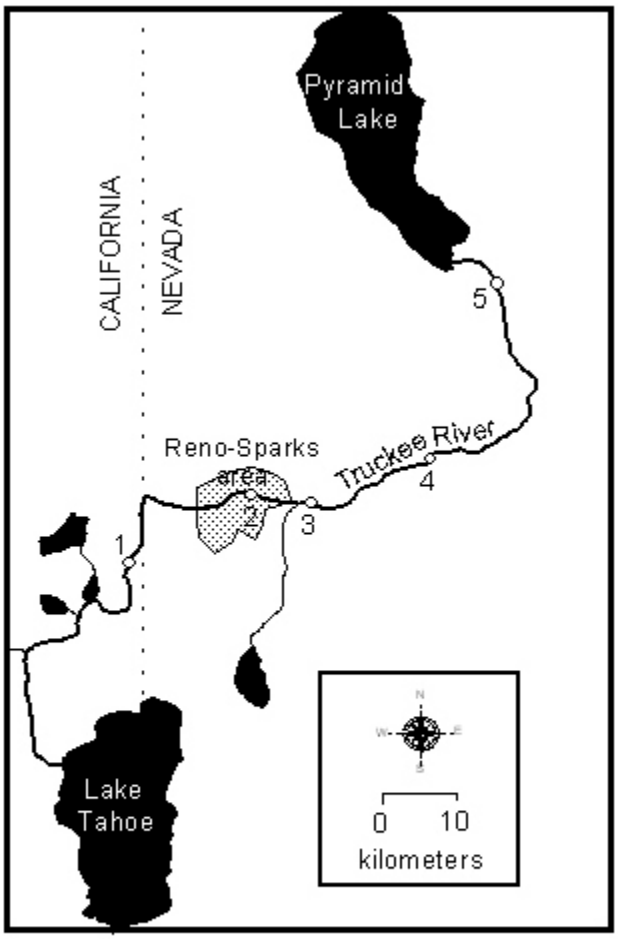


Figure 1. Map of the Truckee River drainage, Nevada and California, showing proposed sampling sites including: 1) Upstream of Reno, 2) Reno, 3) Lockwood, 4) Tracy, and 5) Nixon.

In fiscal year 2002, a number of activities will be initiated or continued in the lower Truckee River which offer the Service the opportunity to partner with other agencies to obtain the necessary information on contamination in lower Truckee River. The Pyramid Lake Paiute Tribe, in conjunction with the Nevada Division of Wildlife and the Service will continue the evaluation of the success of LCT reintroduction efforts. These efforts will include the monitoring of LCT and other fish populations in the lower river. The Pyramid Lake Paiute Tribe is in the process of developing a water quality program. Key components of the program will include the monitoring of water quality and biological characteristics (e.g., invertebrate and riparian community composition and structure). The Pyramid Lake Paiute Tribe, EPA, Nevada Division of Environmental Protection, and the University of Nevada, Reno, have also initiate efforts to evaluate and develop bioassessment protocols and biocriteria in the Truckee River basin. The USGS will reinitiate contaminant assessment activities under the NAWQA Program.

The goal of NAWQA efforts is to assess the status and trends of water quality in the region and to document the effects of human actions and natural factors on water quality conditions. It is anticipated that NAWQA activities will again include the collection of water, sediment, and

biological samples for analyses of organic and inorganic contaminants. Finally, water quality monitoring by the Nevada Division of Environmental Protection and the USGS and required monitoring of point source discharges will provide additional water quality information for the lower Truckee River. However, none of the planned efforts will assess the condition of fish or fisheries.

This proposal requests funding to enable the Nevada Fish and Wildlife Office to partner with other agencies in the assessment of contaminant concerns in the Truckee River. The primary contributions of the Service would be to evaluate the occurrence and severity of environmental contaminant effects to listed fishes and the potential for these contaminants to compromise survival and reproduction in the lower Truckee River. This information will enable the Service to more effectively meet responsibilities pursuant to the Endangered Species Act and P.L. 101-618.

II.B. Scientific Objective(s)

The primary goal of the Service's component of this cooperative effort would be to determine if contaminants are affecting or have the potential to affect fish health, survival, or reproductive potential in the lower Truckee River. Specific Service objectives would include: 1) evaluation of fish diversity, abundance, and community structure; 2) assessment of the external condition of fish; 3) detailed evaluation of salmonid health (i.e., internal/external condition, histology, cytology, disease, and parasites); 4) characterization of fish contaminant exposure and accumulation; and 5) screening for indicators of endocrine disruption. Data generated cooperatively by the Service and investigation cooperators would also be used to: 1) characterize the severity and extent of contamination in the lower Truckee River; 2) evaluate chemical concentrations in water, sediment, and biological tissues; 3) identify potential contaminant sources and source areas; 4) evaluate relations between environmental contaminants and invertebrate community structure; 5) develop bioassessment protocols and biocriteria in the Truckee River.

II.C. Management Action(s)

Information generated through this cooperative effort would provide several benefits to the management of the lower Truckee River and recovery of listed species. The investigation would characterize contaminant effects to aquatic organisms and ecosystems and identify potential impediments to successful LCT reintroduction and cui-ui recovery. This information would be provided to the Truckee River Recovery Implementation Team for use in recovery planning and implementation. Local- and landscape-scale factors affecting water and habitat quality would also be characterized and contaminant sources and source areas would be identified. This information would be provided for use in recovery and land use planning efforts. Information generated through this effort would be used to evaluate and, if necessary modify point source and urban runoff quality

requirements and monitoring. The information would also be used during the triennial review process (as required under the Clean Water Act) to evaluate and, if necessary modify water quality standards (via Section 7 consultation) to ensure adequate protection of listed species. Study findings will also be applied during Section 7 consultations for projects that have the potential to affect the quality or quantity of water in the Truckee River basin. Finally, information generated through this cooperative investigation would be used in the development of biological criteria and assessment protocols for the Truckee River (as required under the Clean Water Act). Information generated by Service's components of the investigation would ensure that fish health, aquatic community structure, and requirements of listed species are fully considered during criteria development. The Truckee River is the first water body in Nevada for which biological criteria will be developed. Therefore, criteria developed for this system will set a precedent for all other water bodies in the State.

III. METHODS

III.A. Data Collection and Analysis

A team consisting of scientists from the USGS NAWQA, EPA REMAP, Nevada Fisheries Resource Office, and Nevada Fish and Wildlife Office would evaluate five sites (Figure 1), including 1) upstream of Reno (background), 2) Reno (known contaminated reach), 3) Lockwood (known contaminated reach), 4) Tracy (known contaminated reach), and 5) Nixon (cui-ui spawning area). The proposed investigation would include five tasks conducted by the Service. Methods used for each task are discussed below.

1) Fish Community Structure

Index of Biological Integrity (IBI) methodologies provide a reproducible method to evaluate and rank relative condition of stream fish communities on a geographic scale and to assess changes over time (Miller et al. 1988; Plafkin et al. 1989). Study teams would use IBI methodologies to assess stream fish community condition at each study site. It is anticipated IBI methodologies previously developed for the Sacramento/San Joaquin drainage would be applied in the proposed study. Representative reaches of stream at each study site would be systematically electroshocked to collect fish. Methods presented in Kolz et al. (1995) would be followed during electrofishing. During collection, captured fish would be placed temporarily in a live well or 5-gallon bucket then transferred to a live car in the stream. All fish would be identified to species level and counted. To assess population size distribution, up to 50 fish of each species would be weighed and measured. Otoliths would be taken from sacrificed fish to assess age distribution and age-size relationships. Appropriate permits would be obtained prior to collection of fish.

2) Fish Condition Assessment

Environmental stress can affect growth rate and general condition of fish. Condition factors, such as Fulton's condition factor, provide a relative measure of nutritional state or "well being" of individual fish and populations (Anderson and Gutreuter 1983). Such factors may also be used to compare relative condition of populations and to monitor environmental change over time (Ney 1993). To assess the fish health and general condition, up to 50 fish of each species from each site would be measured, weighed, and assessed for indicators of disease, parasites, and external anomalies. Fish to be assessed would be selected at random. Length and weight data would be used to calculate Fulton's condition factor for each fish and the species for each site. Methods described in Anderson and Gutreuter (1983) would be used. Examination of external condition of fish would be adapted from procedures provided in Meyer and Barclay (1990) and methods of external fish condition assessment provided in Foott (1990) and Goede and Barton (1987). Fish species, length, weight, and any abnormalities would be recorded on a separate form for each site. All fish, with the exception of trout collected for health assessment or chemical analyses, would be released back to the site from which they were collected.

3) Assessment of Salmonid Health

Up to 10 trout from each site would be sacrificed for organosomatic assays, blood chemistry, microbiological assessment, and histological evaluation. Organosomatic assay methodologies developed by Goede and Barton (1987) as modified by Foott (1990) will be used. An organosomatic assay is a method for the ordered observation of gross morphological features of general appearance, morphology of selected organs, and size criteria and presents a general indication of organism health. For each observational endpoint, a ranking of the severity of impact is established and assigned a numerical value. Each fish is evaluated in terms of the predetermined ranking criteria and the scores are recorded. Trout would be collected during electrofishing described above. The initial 10 fish of appropriate size (200 - 300 mm) collected from each site would be assessed. If possible, Lahontan cutthroat trout would be collected for health assessments and other assessments described below.

Blood samples would be collected at ten sites with suspected environmental contamination and placed into lithium-heparinized vacutainers and microhematocrit sample tubes. The blood samples would be centrifuged at 10,000 RPM for 10 minutes for measurement of hematocrit, leucocrit, and collection of blood plasma. Differential leukocyte counts would be performed on "diff-quick" stained cytospin preparations (Stoskopf 1993 as cited in Rice and Schlenk 1995). An aliquot of blood plasma would be collected, frozen on dry ice and stored at -80ºC. If health concerns are detected, a variety of clinical tests may be performed on plasma.

A microbiological assay media (TSA) would be inoculated with a swab from either the kidney or spleen and incubated at room temperature for 3 days. Isolated colonies would be identified by standard biochemical methods. If the specimens are salmonids, a 0.5 -1.0 gram sample of kidney would be collected for *R. salmoninarum* antigen enzyme-link immunosorbent assay and the head frozen for whirling disease tests (Pepsin-Trypsin Digest for *M. cerebralis* spores, PCR confirmation). Two to five inch sections of the lower intestine would be placed into 70 % alcohol for later examination for helminths. Liver, testes, spleen, gill, and kidney samples would be collected within 3 minutes after death, fixed in Davidson's fixative (Humason 1979),

transferred to 70% ethanol, processed for 5 μm paraffin sections and stained with hematoxylin and eosin. Special stains may also be used to aid in rating the composition and severity of any endogenous pigments such hemosiderin, ceriod, and lipofuscin, which are often linked to environmental stressors. Tissue abnormalities and parasite infections would be analyzed by light microscopy for evaluation of histological effects which may be associated with exposure to environmental contaminants. Whole livers would be weighed for calculation of hepatosomatic index values. Histological, bacteriological, virological, and parasitological assessments would be performed at the California-Nevada Fish Health Center in Anderson, California under the direction of Dr. Scott Foott. Samples of liver and muscle tissue will be retained and analyzed for metals and trace elements.

4) Fish Contaminant Exposure and Accumulation

To assess contaminant exposure and accumulation, five fish from each site would be randomly selected from the fish sacrificed for assessment of salmonid health (above). Upon opening of the each fish, bile would be extracted from the gallbladder with a sterile syringe and placed in a pre-cleaned 10 ml vial with a teflon-lined closure. Bile samples would be stored on ice in the field and refrigerated upon return to the laboratory. Following organosomatic assays, the remaining carcass of each of the five fish will be placed in a plastic bag, stored on ice in the field, and frozen upon return to the laboratory. A total of 25 bile samples and 25 whole fish samples would be submitted to a laboratory specified by the Patuxent Analytical Control Facility for determination of concentrations of aromatic metabolites (in bile) and metals (whole fish).

Instruments and working surfaces used for dissections would be cleaned with a brush and mild detergent, rinsed with a dilute nitric acid solution, and triple rinsed with deionized water prior to use on each fish.

Samples of water, sediment, and biological tissues at each site will be collected by REMAP and NAWQA scientists using appropriate agency protocols.

5) Screening of Endocrine Disruption

Several studies have associated municipal waste water discharges with endocrine system effects in fish (Bevans et al. 1996, Flomar et al.1996, Harries et al. 1997). Because treated municipal waste water represents a significant component of flows in the lower Truckee River, we propose to screen indicators of endocrine disruption in fish from the lower Truckee River. To evaluate endocrine system effects, up to 5 cubic centimeters (cc) of whole blood will be collected from the caudal peduncle of each of the 10 fish collected for assessment of salmonid health (above) and placed in lithium-heparinized vacutainer sample tubes. The blood samples will be centrifuged at 10,000 RPM for 10 minutes. Plasma will then be decanted and placed in a 2 ml cryotubes. Blood plasma samples will be frozen on dry ice and submitted to the Florida Carribean Science Center for analysis of sex steroid (17B-estradiol and 11-ketotestosterone) concentrations and ratios, concentrations of the synthetic hormone ethinyl estradiol, and vitellogenin concentrations. Hormone concentrations would be determined by radioimmunoassay procedures. Vitellogenin concentrations would be determined by enzyme-linked immunosorbent assay. Detailed procedures for these assays may be found in Goodbred et. al. (1997).

Data Analysis

Geographic conditions and trends within the study area would be evaluated using Geographic Information System methodologies. **Relations among study sites, physical and chemical variables, aquatic community condition ranking scores, and measures of fish health would be evaluated by FWS scientists using two-way analysis of variance and Tukey's multiple comparison tests. Regression analysis would be used to examine relations between water quality variables and trace element concentrations in water, sediment, and biological samples.** Additionally, water quality variables and concentrations of contaminants in water, sediment, and biological tissues would be compared to aquatic life effect concentrations from published literature.

The Pyramid Lake Paiute Tribe will provide information on physical, chemical, and biological conditions in the river and riparian areas and will provide information on trends in water quality, aquatic invertebrate community structure, and instream and riparian condition on tribal lands.

The NAWQA scientists will contribute information on the status and trends of water quality, including the documentation of chemical concentrations in sediment and biological samples, and document the effects of human actions and natural factors on water quality conditions. These data will again be used to assess sources and source areas of contaminants that have the potential to adversely affect fish and fisheries.

Information gained through the cooperative efforts of the study partners will be used to 1) identify local- and landscape-scale factors that affect water and instream and riparian habitat quality; 2) develop cost effective environmental and ecological monitoring tools; and 3) develop of management tools to improve water and habitat quality within affected watersheds and stream reaches. The Service will work with cooperators to examine the relations between local- and landscape- scale factors and the condition of fish and fisheries, identify sources and source areas of contaminants that adversely affect fish and fisheries, and evaluate measures of fish health and community structure that may be used for biological monitoring and the establishment of biological criteria.

III.B. Proposed Schedule of Milestones

Existing information on water and sediment quality and contaminant concentrations would be compiled in a single comprehensive database from October to December 2002. Field data collection would be conducted from July to September 2002 (depending on runoff conditions). A draft report would be submitted in March 2003. The final report would be completed within 90 days of receipt of reviewers comments.

IV. INTERIM REPORT

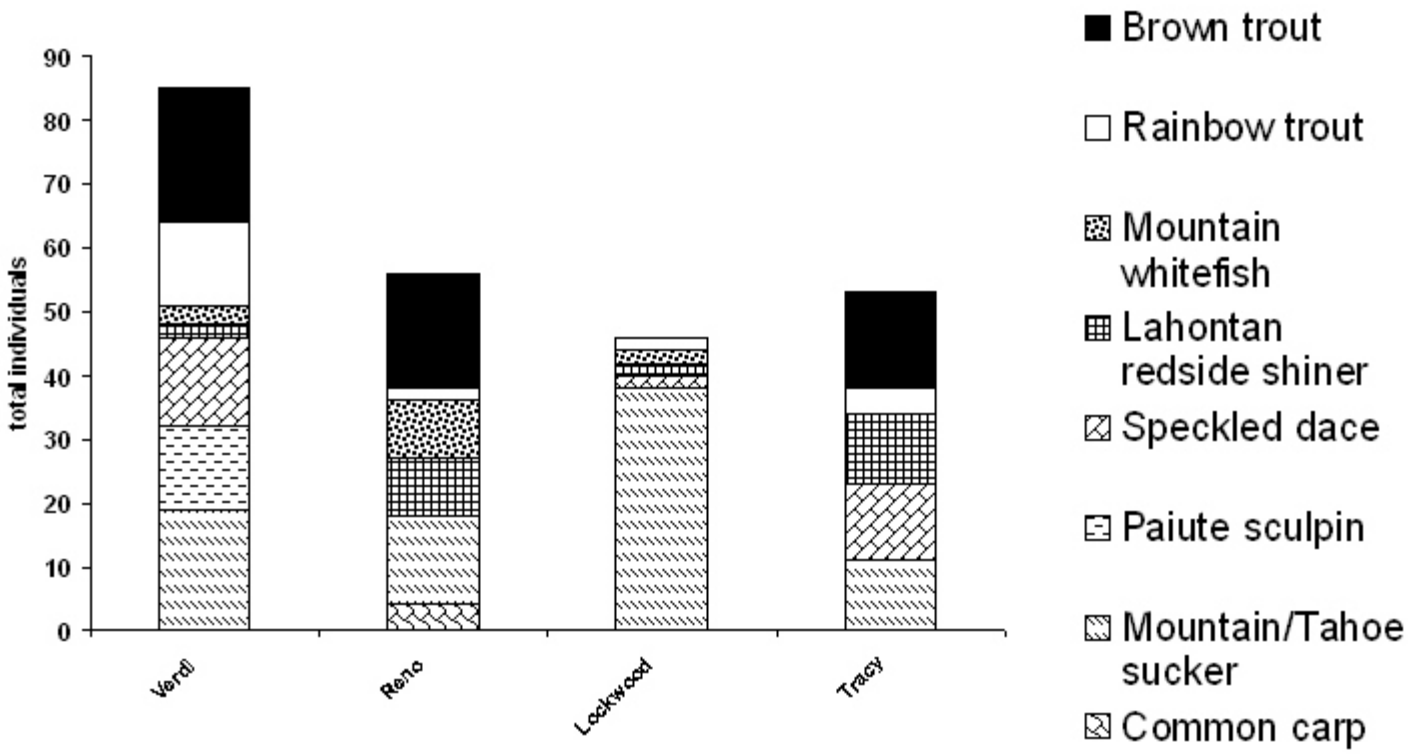
IV.A. Results to Date

All fieldwork activities for this investigation were conducted in August 2002. Personnel from USGS NAWQA program assisted the NFWO in collecting fish at all five sites identified in this investigation. USGS also collected their own fish samples for mercury analysis and conducted habitat surveys using established NAWQA protocols. The Nevada Division of Wildlife and Pyramid Lake Paiute Tribe assisted the NFWO in electroshocking and collecting fish at the four sites in Nevada for tissue collection as well as histological and organosomatic assays.

1) Fish Community Structure

Total fish abundance decreased at sites within and below the Reno-Sparks urban area compared to the upstream site at Verdi (Figure 1). Community composition of fish was noticeably different at the Lockwood site immediately below the Reno-Sparks urban area demonstrating a shift toward more tolerant fish species that are herbivorous and a dramatic reduction in salmonid species (Figure 1). These results were presented to the Truckee River Bioassessment Workgroup in their 2004 annual meeting.

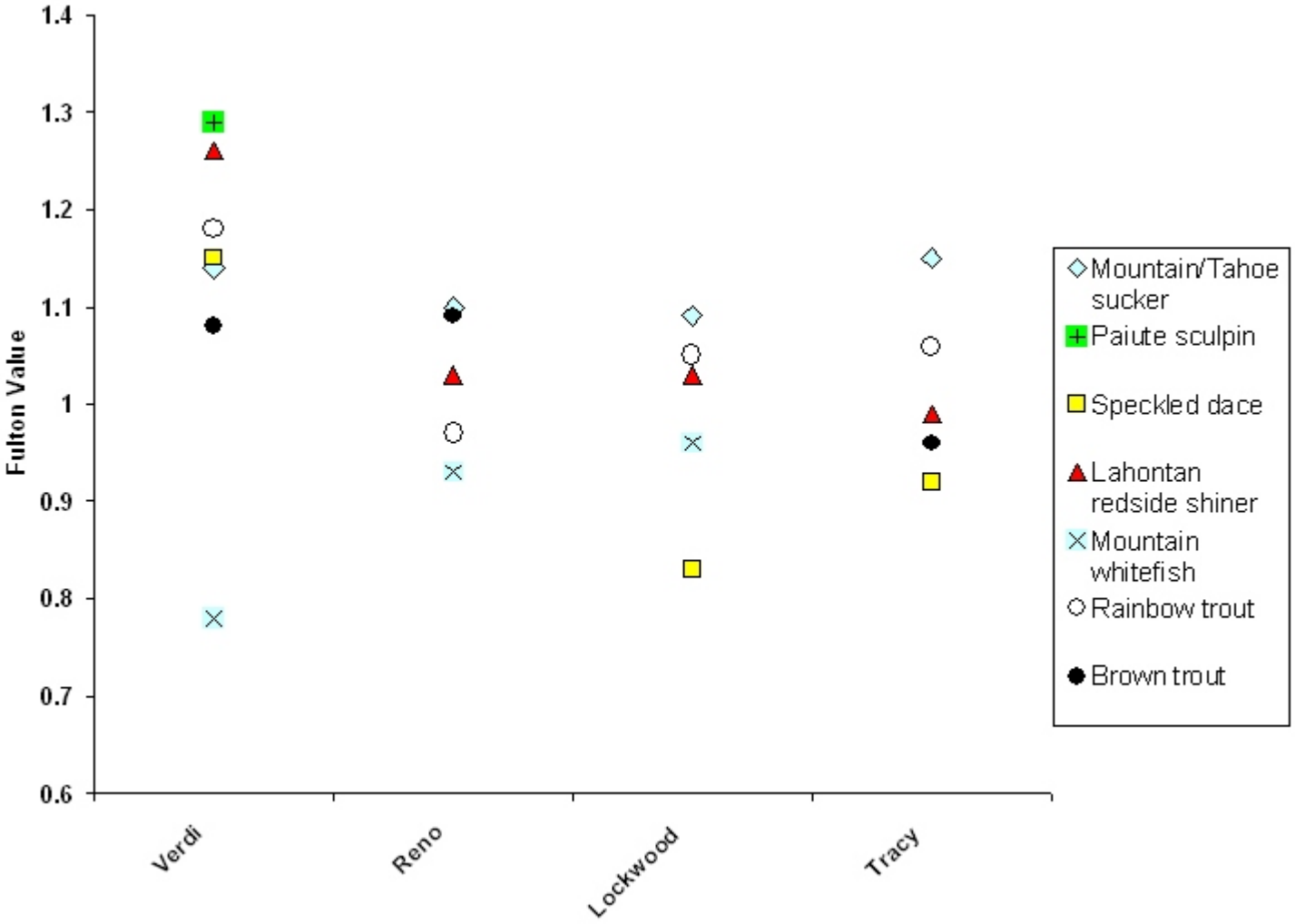
Figure 1. Total number of fish captured and composition of species in the Truckee River, Nevada, August 12-15, 2002.



2) Fish Condition Assessment

Fish condition data utilizing Fulton's factor illustrated a general decreasing trend in all species, with the exception of mountain whitefish, within and downstream of the Reno-Sparks urban area (Figure 2).

Figure 2. Average Fulton fish condition factors for fish species collected from the Truckee River, Nevada, August 12-15, 2002.



3) Assessment of Salmonid Health

a. Organosomatic Assays

As a result of mixed species, sexes, maturity levels and number of fish collected per site; statistical analysis was limited. The range of lengths and weights are listed in Table 1. Condition factor (Weight {g} / fork length {mm}^{3} x 100000) ranged from 0.936 and 1.839 (Table 2). No obvious low condition fish or site trend in condition factor detected in the sampled groups. The variation in female condition factor was influenced by their ovarian mass. Two brown trout (BRN) females at the Reno site had high gonadosomatic indices (8 and 11 %) indicative of preparation for spawning in late fall or winter. All males examined had immature testes as indicated by gonadosomatic indices $\leq$ 1 (Table 2). When species are combined, no statistically significant differences in KFL or GSI was detected between similar sex fish of the 3 sites (ANOVA, $P<0.05$). The Hepatosomatic index of the 4 Tracy male trout were significantly higher than the 7 males at Verdi (ANOVA, $P=0.01$).

Table 1. Total length and weight of fish used in the organosomatic assays (RBT= rainbow trout; BRN= brown trout)

		Verdi	Reno	Tracy
Total RBT		8	1	0
Female	(no.)	3	1	0
	Length Range (mm)	265-298	360 (fork length)	NA
	Weight Range (g)	178-392	829	NA
Male	(no.)	5	0	0
	Length Range (mm)	210-315	NA	NA
	Weight Range (g)	100-402	NA	NA
Total BRN		2	9	7
Female	(no.)	0	7	3
	Length Range (mm)	NA	228-583	250-282
	Weight Range (g)	NA	115-1874	161-234
Male	(no.)	2	2	4
	Length Range (mm)	295-325	230-260	246-262
	Weight Range (g)	372-393	129-301	139-185

Table 2. Mean (Std. Dev.) data for condition factor (KFL), hepatosomatic index (HIS), and gonadosomatic index (GSI) by sex at each of the 3 sample sites. Species data is combined.

	Verdi	Reno	Tracy
Female (no.)	3	8	3
KFL	1.469 (0.272)	1.295 (0.313)	1.057 (0.017)
HIS	0.661 (0.088)	0.950 (0.410)	1.348 (0.143)
GSI	0.617 (0.272)	4.27 (4.82)	2.029 (0.467)
Male (no.)	7	2	4
KFL	1.357 (0.274)	1.443 (0.439)	1.008 (0.046)
HIS	0.688 (0.256) b	0.725 (0.180) ab	1.519 (0.519) a
GSI	0.063 (0.201)	0.033	0.237 (0.313)

* Letters indicate statistic significant differences

Visceral Fat was rated on each sampled fish on a scale of 0 – 3, 0 = no visible visceral fat, 1 = some visible visceral fat (<50%), 2 = visceral fat (>50%) around pyloric caeca, and 3 = visceral fat (>90%) throughout the peritoneal cavity (Table 3).

Table 3. Visceral fat scores of salmonid fish collected for organosomatic assays in the Truckee River, Nevada, August 12-15, 2002.

	Verdi					Reno					Tracy				
Score	No. Fish	0	1	2	3	No. Fish	0	1	2	3	No. Fish	0	1	2	3
Total RBT	8					1					0				
Female	3		1	1	1	1			1		0				
Male	5		4	1		0					0				
Total BRN	2					9					7				
Female	0					7		1	5	1	3			3	
Male	2	1		1		2			2		4			3	1

b. Hematology data

Hematology data collected on all fish used in organosomatic assays revealed normal blood values. Hematocrit (% packed erythrocyte vol.), Leukocrit (% packed white blood cell vol.), and plasma protein (g/dL) data is given in ranges (Table 4). Blood data is separated by the three (3) sites, species, and further by sex. Accuracy of the plasma protein data is questionable, as samples were inadvertently held at room temperature overnight. The white blood cell profiles were normal for salmonids (Table 5). There is some difficulty in distinguishing between activated thrombocytes (round form) and small lymphocytes. The low number of granulocytes (neutrophils) observed correlated with the low prevalence of microbial infection in all sampled groups.

Table 4. Blood Data.

	Verdi	**Reno**	**Tracy**
Total RBT	8	1	0
Female	3	1	0
Hematocrit Range (%)	30.77 – 53.97	52.38	NA
Leukocrit Range (%)	1.29 – 1.38	1.29	NA
Plasma Protein (g/dL)	4.4 – 4.6	5.2	NA
Male	5	0	0
Hematocrit Range (%)	18.33 – 47.54	NA	NA
Leukocrit Range (%)	0.93 – 1.52	NA	NA
Plasma Protein (g/dL)	3.9 – 4.8	NA	NA
Total BRN	2	9	7
Female	0	7	3
Hematocrit Range (%)	NA	32.08 – 40.00	41.94 – 57.14
Leukocrit Range (%)	NA	0.62 – 1.80	1.94 – 3.00
Plasma Protein (g/dL)	NA	3.8 – 6.1	4.0 – 4.4
Male	2	2	4
Hematocrit Range (%)	39.68 – 43.94	32.00 – 44.62	34.38 – 65.00
Leukocrit Range (%)	1.05 – 1.24	0.65 – 1.32	1.36 – 3.75
Plasma Protein (g/dL)	4.1 – 5.8	3.1 – 4.7	3.3 – 4.6

NA not applicable

Table 5. Differential Leukocyte Count.

	Lymphocyte (%)	Thrombocyte (%)	PMN (%)
Verdi Site			
0	62.70 ± 24.34	39.20 ± 24.72	0.10 ± 0.32
Range	21 - 92	7 - 79	0 - 1
Reno Site			
0	55.40 ± 22.87	45.40 ± 24.20	1.33 ± 3.04
Range	28 - 95	5 - 81	0 – 9
Tracy Site			
0	73.43 ± 26.38	23.57 ± 28.08	0.14 ± 0.38
Range	26 - 100	0 - 73	0 - 1

c. Microbiological assays

Microbiological assay data revealed no bacteria were isolated from fish at the Verdi site. Motile gram-negative rod bacteria, belonging to the motile aeromonad group (ie *Aeromonas hydrophila, Pseudomonas sp.*), were isolated from 2 of 10 trout from the Reno site and 1 of 7 trout at the Tracy site. It is not uncommon to isolate these aquatic bacteria from apparently healthy fish as they are residents in their gastrointestinal tracts. No virus was detected from any of the sampled fish. Of the 27 fish tested for *R. salmoninarum* antigen by ELISA, only one kidney sample from a Reno site trout showed high antigen concentration indicating an active infection. No clinical signs of Bacterial Kidney Disease were seen in this fish. Six kidney samples had low *R. salmoninarum* antigen values and were considered suspect (1 = Verdi, 4 = Reno, and 1 = Tracy). Confirmatory PCR tests are pending. The FHC will assay the heads for *Myxobolus cerebralis* spores when otolith aging work is completed by the Reno FWO.

d. Histology

In terms of histology, no significant abnormalities or parasite infection was observed in sections of liver, kidney, spleen, testes or gill from any of the sampled trout. The kidney sections had varying degrees of melanomacrophage aggregates in the interstitum. These aggregates also contained small quantities of hemosiderin (iron from recycled erythrocytes) and lipofuscin ("age") pigments. Moderate quantities of these endogenous pigments are normal in adult fish. No trend by sample location was obvious in pigment quantity or type. All 9 males collected in the study had immature testes. Only one fish (#10 Verdi) had primary spermatocytes within its testes with most specimens showing only spermatogonia.

4) Contaminant Exposure and Accumulation

Data from tissue samples collected for analyses of metal concentrations were submitted in FY2003 and received in February 2004. The raw data report from PACF for inorganic constituents is summarized in Attachment 1. A summary of data from the organic analyses of fish bile samples is presented in Table 6. Full statistical analysis and interpretations of inorganic and organic concentrations in fish tissues will be completed and summarized in the final report to be completed later this fiscal year.

Table 6. Summary of results from organic analyses (polycyclic aromatic hydrocarbons) of salmonid bile collected from the Truckee River, Nevada, August 12-15, 2002.

Sample ID	Sample site	benzo(a)pyrene (ppm)	naphthalene (ppm)	phenanthrene (ppm)	fish weight (g)
001FB001	Verdi	0.1	39	9.4	392
001FB002	Verdi	0.3	49	15	393
001FB003	Verdi	0.2	51	18	368
001FB004	Verdi	0.2	26	11	402
001FB005	Verdi	0.4	100	32	305
002FB01	Reno	0.1	140	49	129
002FB02	Reno	N/A	N/A	N/A	N/A
002FB03	Reno	0.2	73	27	132
002FB04	Reno	0.6	190	74	1115
002FB05	Reno	0.4	120	43	378
004FB01	Tracy	<.100	3.1	1.5	142
004FB02	Tracy	0.3	95	37	223
004FB03	Tracy	0.6	110	42	234
004FB04	Tracy	0.4	76	30	173
004FB05	Tracy	0.4	91	39	139

5) Screening of Endocrine Disruption

Fish bile samples collected in this investigation for the screening of endocrine disruptors were collected and shipped to the USGS Florida Carribean Science Center in September 2002. Results from the fish bile samples were received in January 2004 and the results are provided in Table 7. Interpetation and statisitical analyses of these results will be presented in the final report to be completed later this fiscal year.

Table 7. Raw data of hormone analysis in fish bile collected from salmonids in the Truckee River, Nevada, August 12-15, 2002.

Sample ID	Site	Species	Sex	Vitellogenin (ug/ml)	Estradiol (pg/ml)	11-Keto-testosterone (pg/ml)	ethnyl/11-Keto-testosterone (pg/ml)
LTR001FPH01	Verdi	Rainbow Trout	F	484.4	536	199	2.69
LTR001FPH02	Verdi	Brown Trout	M	ND	246	927	0.27
LTR001FPH03	Verdi	Rainbow Trout	F	218	627	315	1.99
LTR001FPH04	Verdi	Rainbow Trout	F	22.2	901	349	2.58
LTR001FPH05	Verdi	Rainbow Trout	M	ND	237	410	0.58
LTR001FPH06	Verdi	Brown Trout	F	529.2	535	463	1.16
LTR001FPH07	Verdi	Rainbow Trout	M	9.8	NS	NS	NS
LTR001FPH08	Verdi	Rainbow Trout	M	ND	134	237	0.57
LTR001FPH09	Verdi	Rainbow Trout	F	53509	1035	52	19.90
LTR001FPH010	Verdi	Rainbow Trout	M	ND	153	564	0.27
LTR002FPH01	Reno	Brown Trout	M	ND	101	340	0.30
LTR002FPH02	Reno	Brown Trout	F	22	652	341	1.91
LTR002FPH03	Reno	Brown Trout	F	54	853	318	2.68
LTR002FPH04	Reno	Brown Trout	F	759.2	1561	395	3.95
LTR002FPH05	Reno	Brown Trout	F	718.8	1461	446	3.28
LTR002FPH06	Reno	Brown Trout	F	442.3	1615	645	2.50
LTR002FPH07	Reno	Brown Trout	F	738.8	1456	386	3.77
LTR002FPH08	Reno	Brown Trout	F	638.3	1582	384	4.12
LTR002FPH09	Reno	Rainbow Trout	F	215.8	1459	327	4.46
LTR002FPH10	Reno	Brown Trout	M	ND	472	505	0.93
LTR004FPH01	Tracy	Brown Trout	M	6.4	143	420	0.34
LTR004FPH02	Tracy	Brown Trout	F	503.3	1418	621	2.28
LTR004FPH03	Tracy	Brown Trout	F	178.3	1007	394	2.56
LTR004FPH04	Tracy	Brown Trout	M	12.5	441	806	0.55
LTR004FPH05	Tracy	Brown Trout	M	ND	120	401	0.30
LTR004FPH06	Tracy	Brown Trout	F	8.5	385	65	5.92
LTR004FPH07	Tracy	Brown Trout	M	ND	200	660	0.30

IV.B. Significant Changes to Previous Proposal

Due to drastically reduced flows and elevated water temperatures in the Truckee River during the 2002 sampling season, no salmonids were captured at the Nixon sampling site. Obtaining samples from the Nixon site at a different time period would be inappropriate due to different hydrologic conditions and would cause improper statistical comparisons among sites. Therefore the Nixon site was removed from this investigation.

Budgetary constraints and technical issues with Patuxent Analytical Control Facility in FY2003 caused delays in shipping tissue samples and receiving results from those analyses. Additionally, hormone results from bile samples submitted to the USGS Florida Carribean Science Center were more than one year overdue which contributed to the delay in finalizing the report as scheduled.

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VI. ROLES, RESPONSIBILITIES, AND PARTNERSHIPS

VI.A. Roles and Responsibilities

The Nevada Fish and Wildlife Office would be the primary Service contact and would be responsible for ensuring completion of Service tasks. Dr. Scott Foott (Director, CA-NV Fish Health Center, Andersen, CA) will be responsible for completion of salmonid health assessment. Dan Mosley of the Pyramid Lake Paiute Tribe will be responsible for monitoring and development of standards and biocriteria on tribal lands. Mike Lico (USGS, Nevada Basin and Range Study Leader, NAWQA, Carson City, NV) will be the primary USGS contact and will be responsible for ensuring completion of all NAWQA tasks.

VI.B. Partnerships

The proposed investigation will include the Pyramid Lake Paiute Tribe is expected to be an active partner in this investigation. The budget for the development of the tribal water quality program in 2002 is $167,000. The USGS NAWQA Program is also expected to take an active role in the investigation. The NAWQA budget is currently under development with a final budget estimate expected in June 2001. Contributions in the form of water quality and invertebrate community assessment data outside of tribal lands collected by the Nevada Division of Environmental Protection and the Reno-Sparks Water Reclamation Facility will also be utilized in this investigation.

VII. BUDGET					
EXPENDITURES	**Year 1 FY 2002**	**Year 2 FY 2003**	**Year 3 FY 2004**	**Year 4 FY 2005**	**All Years**
Field Operations					
Personnel - Field[1]	12,000.00				12,000.00
Personnel - Data Analysis	5,000.00				5,000.00
Personnel - Report Writing		10,000.00			10,000.00
Travel					
Supplies	1,000.00				1,000.00
Equipment					
Non-PACF Analytical[2]	3,000.00				7,000.00
Other (specify)[3]	5,000.00				
Operational Subtotal	$26,000.00	$10,000.00			$36,000.00
PACF Funding					
Analytical Subtotal	13,250.00				13,250.00
TOTAL FUNDING	$39,250.00	10,000.00			$49,250.00

[1] Estimated personnel costs include preparation time (3 days), field sample and data collection (2 biologist X 12 days), and sample processing and management (6 days).

[2] Non-PACF analytical costs include analyses of hormone and vitellogenin concentrations by the Florida Carribean Science Center.

[3] Other costs include costs of the California Nevada Fish Health Center for the assessment of salmonid health.

VIII. REVIEW AND APPROVAL

Submitted by: _____ Date: _____
Contaminant Specialist, Field Office

Reviewed by: **N/A** Date: _____
Refuge Manager, (required for On-Refuge Investigations)

Reviewed by: _____ Date: _____
Assistant Field Supervisor- Ecological Services

Reviewed by: _____ Date: _____
Environmental Contaminants Coordinator

Approved by: _____ Date: _____
Regional Director

Attachment 1.

Patuxent Analytical Contol Facility's
Inorganic Results Report from Catalog #1070067