PART I - ADMINISTRATIVE

Section 1. General administrative information

Title of project

Document Native Trout Populations

BPA project number: 9802600
Contract renewal date (mm/yyyy): 5/1999 ☐ **Multiple actions?**

Business name of agency, institution or organization requesting funding
Washington Trout

Business acronym (if appropriate) WT

Proposal contact person or principal investigator:

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NPPC Program Measure Number(s) which this project addresses
10.0, 10.2A1, 10.2B1-5, 10.2C, 10.5A, 10.8, 10.8A, 10.8B.19.

FWS/NMFS Biological Opinion Number(s) which this project addresses

Other planning document references

Short description
Photo-Document native trout populations in Columbia Basin in WA state and collect tissue samples for DNA analysis.

Target species
westslope cutthroat (O.clarki lewisi), redband rainbow (O.mykiss gairdneri), and bull trout (Salvelinus confluentis).

Section 2. Sorting and evaluation

Subbasin
Little White Salmon, Big White Salmon, Klickitat, Wenatchee, Entiat, Chelan, Okanogan, Spokane, Crab, Tucannon, Asotin, and Grande Ronde.

Evaluation Process Sort

CBFWA caucus	Special evaluation process	ISRP project type
Mark one or more caucus	If your project fits either of these processes, mark one or both	Mark one or more categories
☐ Anadromous fish ☒ Resident fish ☐ Wildlife	☒ Multi-year (milestone-based evaluation) ☐ Watershed project evaluation	☐ Watershed councils/model watersheds ☐ Information dissemination ☐ Operation & maintenance ☐ New construction ☒ Research & monitoring ☐ Implementation & management ☐ Wildlife habitat acquisitions

Section 3. Relationships to other Bonneville projects

Umbrella / sub-proposal relationships. List umbrella project first.

Project #	Project title/description

Other dependent or critically-related projects

Project #	Project title/description	Nature of relationship

Section 4. Objectives, tasks and schedules

Past accomplishments

Year	Accomplishment	Met biological objectives?
1998	Sampled 12 tributaries in Yakima/Naches subbasin; photo-documented and collected tissue samples for DNA analysis from 11 of these (the 12th had been taken over by brook trout (S.	Yes. Results from DNA analysis not available at time of writing this proposal.

	fontinalis).	

Objectives and tasks

Obj 1,2,3	Objective	Task a,b,c	Task
1	Catalogue and photo-document native trout	a	Collect specimens by non-lethal means: angling, electroshocking, snorkeling.
2	Collect tissue samples for later DNA analysis.	b	Measure speciment length to nearest mm. Weigh to nearest gm.
		c	Take taxonomic-quality photographs of collected live specimens on-site.
		d	Take adipose or caudal fin tissue sample for later DNA analysis
		d	Take adipose or caudal fin tissue sample for later DNA analysis
		e	Release live specimens to capture site.
3	Photograph stream and adjacent landscape habitats at sampling sites.	f	Photograph stream and associated landscape at collection site.
		g	Record, label, take field notes, record latitude/longitude with GPS, identify location on maps.
4	Submit collected and preserved tissue samples to laboratory for microsatellite DNA analysis.	h	Submit samples to laboratory. Laboratory peroform DNA analyses and report results.
5	Publicize results.	i	Analyze and publish results in journals, reports, Web sites. Produce slide shows. Provide data to regional databases.

Objective schedules and costs

Obj #	Start date mm/yyyy	End date mm/yyyy	Measureable biological objective(s)	Milestone	FY2000 Cost %
1	6/2000	10/2002	Photo record of resident morphs,phonotypes.	Reports 04/2001 and 04/2003.	30.00%
2	6/2000	10/2002	DNA analysis; correlation of photoed phenotypes with pure	Reports 04/2001 and 04/2003.	30.00%

			and hybrid individuals/populations.		
3	6/2000	10/2002	Photo record of variety of habitat conditions where native trout are still present.	Reports 04/2001 and 04/2003.	10.00%
4	6/2000	10/2002	Microsatellite analysis will determine which populations are pure and which are hybridized.	Reports 04/2001 and 04/2003.	14.00%
5	10/2000	04/2003	Communication of results to the community of scientists, stakeholders, and managers.	Reports 04/2001 and 04/2003.	16.00%
				Total	100.00%

Schedule constraints
Weather may alter start and/or finish date of field work each year. this contingency is factored into the timeline: A field season of ~90 days is given a 150 day window.

Completion date
Field work: 10/2002; final DNA results and Report, 04/2003.

Section 5. Budget

FY99 project budget (BPA obligated): $52,300

FY2000 budget by line item

Item	Note	% of total	FY2000
Personnel	Field, analysis, communication and report writing.	%40	24,390
Fringe benefits	25%	%10	6,097
Supplies, materials, non-expendable property	Film&processing, cryovials, office, postage/copying/communications	%5	2,800
Operations & maintenance	Vehicle mainenance	%2	1,000
Capital acquisitions or improvements (e.g. land, buildings, major equip.)	Temp. loggers	%2	1,200
NEPA costs		%0	
Construction-related support		%0	
PIT tags	# of tags:	%0	

Travel	Mileage, food, lodging, pack trips	%10	6,225
Indirect costs	20% of non-subcontract total	%14	8,489
Subcontractor	U of Montana; DNA analysis	%16	10,000
Other	Software upgrades	%1	500
TOTAL BPA FY2000 BUDGET REQUEST			$60,701

Cost sharing

Organization	Item or service provided	% total project cost (incl. BPA)	Amount ($)
	Discount Dr. Trotter's consulting rate of $60/hr.	%20	14,850
		%0	
		%0	
		%0	
Total project cost (including BPA portion)			$75,551

Outyear costs

	FY2001	FY02	FY03	FY04
Total budget	$62,000	$65,000		

Section 6. References

Watershed?	Reference
☐	Anderson, W.Gary, R.Scott McKinley, and Maria Colavecchia. 1997. The Use of Clove Oil as an Anesthetic for Rainbow Trout and Its Effect on Swimming Performance. North American Journal of fisheries management 17: 301-307.
☐	Behnke, Robert J. 1992. Native Trout of Western North America. American Fisheries Society Monograph 6.
☐	Gresswell, Robert E. (editor). 1988. Status and Management of Interior Stocks of Cutthroat Trout. American Fisheries society Symposium 4.
☐	Leary, Robb F. 1997. Hybridization Between Introduced and Native Trout in Waters of the Colville National Forest. Wild Trout and Salmon Genetics Laboratory Report 97/3. Division of Biological Sciences, University of Montana, Missoula, Montana.
☐	Mullan, James W., Kenneth Williams, Granville Rhodus, Tracy W. Hillman, and John D. McIntyre. 1992. Production and Habitat of Salmonids in Mid-Columbia River Tributary Streams. U.S. Fish and Wildlife Service, Monograph I.
☐	Proebstel, Don S. and Sandra M. Noble. 1994. Are "Pure Native Trout in the Mid-Columbia River Basin?" In Wild Trout V.

☐	Trotter, Patrick C. 1987. Cutthroat: Native Trout of the West. Colorado Associated University Press.
☐	Trotter, Patrick C. and Peter A. Bisson. 1988. History of the Discovery of the Cutthroat Trout. In Gresswell 1988.
☐	Trotter, Patrick C. 1989. Coastal cutthroat trout: A life history compendium. Transactions of the American Fisheries Society 118: 463-473.
☐	Trotter, Patrick C., P.A. Bisson, and B. Fransen. 1993. Status and Plight of the Searun Cutthroat Trout. In Cloud, Joseph G., and Gary H. Thorgaard (Eds.) Genetic Conservation of Salmonid Fishes. Plenum Press 1993.
☐	Trotter, P.C. 1997. Sea-Run cutthroat Trout: Life History Profile. Pages 7-16 in J.D. Hall, P.A. Bisson, and R.E. Gresswell, editors. Sea-Run Cutthroat Trout: Biology, Management, and Future Conservation. Oregon Chapter. American Fisheries Society, Corval
☐	G. Watson and T.W. Hillman, 1997. Factors Affecting the Distribution and Abundance of Bull Trout: An Investigation at Hierarchical Scales. North American Journal of Fisheries Management v.17, #2 May 1997, pp.237-253.
☐	

PART II - NARRATIVE

Section 7. Abstract

The 1994 Fish and Wildlife Program is to recover and preserve the health of native resident fish. Among the native resident species of concern noted in the Resident Fish section are bull trout, redband trout, and westslope cutthroat trout. All are or are likely candidates for actions under the ESA.

Before at-risk populations can be protected, their presence and status must be documented. Where introgression from introduced species is a concern, as in the case of both westslope cutthroat and redband rainbow, genetic issues must be addressed as well. Most of the remaining pure populations of these species are in relatively remote, headwater drainages which present surmountable, but not slight, logistical obstacles to properly documenting/assessing such populations.

Washington Trout proposes to conduct field visits, to remote habitats throughout the Washington State sub-basins of the Columbia still occupied by these native trout populations, for the purpose of cataloguing and photo-documenting the remaining native trout ecotypes. Taxonomy-quality photographs of living specimens will be made with the aid of a portable field aquarium. In addition, non-lethal methods will be used to take and preserve adipose or caudal fin tissue samples in a manner suitable for subsequent genetic analysis using DNA microsatellites.

Dr. Pat Trotter will lead this fieldwork. He has extensive experience in, and credentials for, doing the work proposed. Fieldwork was begun in 1998 and proposed to extend over the course of 5 field seasons. Results will be written up by Dr. Trotter for peer reviewed publication.

Section 8. Project description

a. Technical and/or scientific background

The present state of knowledge regarding both the original post-glacial distribution and the present distribution/status of native populations of westslope cutthroat trout (*Onchorynchus clarki lewisi)*, redband rainbow trout (*Onchorynchus mykis gairdneri*), and bull trout (*Salvelinus confluentis)* is disturbingly limited, if slowly growing. Both the original and current distributions of westslope cutthroat in the Columbia Basin between the John Day River and the Okanogan River appear surprisingly patchy. Behnke (1992) is only sure of apparent pure populations in the upper Methow Basin and in the North Fork of the John Day River, either presently or originally. This condition stems as much, if not more, from lack of evidence and lack of looking as from loss of an original more widespread number of populations.

Behnke (1992) further notes, however, extensive reduction in the known original range of westslopes in Montana and Idaho due to introgression from introduced non-native rainbow and Yellowstone cutthroat trout (*Onchorynchus clarki bouvieri)* and to displacement from these introductions and from introductions of non-native brook trout (*Salvelinus fontinalis)*. Combined with habitat loss, alteration, and destruction, pure strains of westslope are almost exclusively, if not now entirely, remnant populations confined to cold, low-order headwater streams. Such populations have retreated to their last fortress where they are especially vulnerable to genetic, demographic, and environmental stochasticities, including human-caused landscape impacts.

Habitat alteration and hybridization with non-native strains of rainbow trout, and with introduced populations of Yellowstone Cutthroat trout, have similarly resulted in the significant loss of pure redband populations, including the mid- and upper-Columbia Basin in Washington State (See Behnke 1992, Chapter 10; and Leary 1997 and 1998).

Bull trout have suffered extensively from the same impacts. Fluvial and adfluvial populations have particularly labored under the impacts of loss of connectivity in their migratory pathways due to river regulation and the disruption of large river and lacustrine food chains by introduction of non-native char. Headwater resident populations constitute a major remaining outpost for the species throughout much of its range, including the Columbia Basin in Washington State. Many of these headwater populations are threatened by hybridization with introduced non-native brook trout. (See, for example, Mullan et al, 1992, Chapter 5, p. 115 and Appendix K)

If remaining populations of these three species are to be preserved and managed consistently with the concerns and provisions of Sections 7 and 10 of the 1994 Fish and Wildlife Program, their current distribution must be established. We must know where they are, if we are to properly manage them. Since the highest probability of finding existing pure populations of these species, and of documenting remaining among-population genetic and phenotypic differences, lies in relatively remote, difficult to access catchments of low-order streams, we believe that it is imperative to undertake field research into such catchments for the purpose of documenting these likely remaining pure populations.

The principal field research will involve photo-documenting representative specimens of individual populations of the species in situ, and taking non-lethal fin tissue samples from up to 20 individuals from each population for subsequent microsatellite DNA analysis of proteins. Taxonomy-quality photographs of individual fish will be taken using a portable field aquarium designed by the Principal Investigator for just this purpose.

A concomitant of such research will be the documentation of introgressed, hybridized populations. Among other motivations, such knowledge will be extremely valuable, and likely indispensable, to managers who are and will be confronted with ESA petitions and/or listings concerning these species in the Columbia Basin in the immediate future.

Field work under this Project was conducted in the Yakima and Naches sub-basins in 1998. 12 tributaries were suveyed and photos and tissue samples of putative native resident trout were collected from 11 (10 westslope and one redband). Results from the DNA analysis of these populations are not available at the time of writing. (See also Subsection (d) below.

The Principal Investigator for the Proposed Project, Dr. Patrick C. Trotter, has considerable experience and expertise in both the biology/life history of the three species of focus, and in field work of exactly the kind proposed, including the taking and proper preservation of tissue samples for later DNA analysis. Dr. Trotter's monograph, Cutthroat: Native Trout of the West , was the result in large part of this kind of field research. Other relevant publications of Dr. Trotter's are listed in subsection g below. Mr. Bill McMillan will continue to assist Dr. Trotter in the field with occasional additional assistance from the Project Director. FY2000 will initiate the third consecutive year of the Project and the field research team will have considerable experience working together on the Project.

b. Rationale and significance to Regional Programs

The need to document the present distribution of remaining pure indigenous populations of westslope cutthroat, redband trout, and bull trout has been discussed above, as has the related need to document the extent and degree of hybridization within these species and the extent and degree of displacement by non-indigenous salmonids. All are pre-requisites to assessing present and potential future threats to the continued existence of these native resident salmonids in the Columbia Basin. Assessment of such threats, based in part upon the information expected to result from the Proposed Project, is essential to the choice, design, and implementation of protection and recovery strategies which may, and very likely will, be necessary in order to implement and maintain consistency with the sections and sub-sections of the 1994 Fish and Wildlife Program noted and/or commented upon below.

The introduction to Section 10 (Resident Fish) of the Program (p.10-1) explicitly notes the three trout species which are the focus of the Proposed Project as "resident fish species of special interest…". Sub-section 10.1A. calls for "the identification of resident fish mitigation objectives and, to the extent appropriate, associated rebuilding schedules, survival targets and performance standards." 10.2A requires relevant parties to "accord highest priority to weak, but recoverable, native populations injured by the hydropower

system…" and to "accord high priority to areas of the basin where anadromous fish are not present."

With regard to these provisions, we note several relevant considerations. First, we must locate all remaining native resident populations before we can protect and/or rebuild them in a systematic manner that is both biologically and financially efficient. (See also, Section 8 below.)

Second, impacts from the hydropower system upon these populations, and others which have previously been lost due to the development of the hydropower system encompass more than simply the loss of fluvial habitats due to flooding caused by reservoir construction on the Columbia and Snake mainstems. They include flooding and habitat discontinuity impacts from construction of irrigation diversions on innumerable tributaries which development of the hydropower system on the mainstem significantly helped to make possible. And they include impacts on resident salmonid populations that are the direct and indirect result of declines and losses of anadromous salmonid populations, some of which we are only recently becoming aware of and understanding. Nutrient loading from salmon carcasses is perhaps the most obvious and important example.

Third, mitigation can and must include special efforts to protect headwater populations of native resident salmonids whose remaining small populations represent a vastly shrunken vestige of a much more widespread series of resident meta-populations. These remaining populations retain the genetic legacy of the wider metapopulation, which was cut down due in large measure to the development of the hydropower system. As noted above, these populations are particularly subject to genetic and demographic stochasticities. Mitigating for the existence of these stochasticities, which result from the reduction and loss of meta-population structure, are genuine mitigation responsibilities under Section 10. Mitigation in these cases require preservation of the remaining population, even though particular headwater populations may have suffered no loss directly attributable to reservoir construction or irrigation development. This is consistent with the introduction to sub-section 10.8 and with 10.8A.

Numerous other sub-sections of sections 7 and 10 support the Proposed Project.

Section 7.7, which calls for cooperative and comprehensive watershed management, stresses the need to "enhance and expedite implementation of actions by clearly identifying gaps in programs and knowledge." The Proposed Project is conceived to address significant gaps in relevant knowledge.

Sub-section 10.2B addresses natural and artificial propagation and the preservation of genetic diversity of native resident salmonids. It calls explicitly for "a thorough and comprehensive approach to conserving genetic diversity…for native species." Sub-section 10.2B.1, 10.2B.2, and 10.2B.5 explicitly extend genetic policy measures for anadromous salmonids called stated in sub-sections 7.1, 7.2, and 7.4 to resident salmonids.

Sub-section 7.1C notes that obtaining "base-line information that will improve management and conservation of wild and naturally spawning populations is needed." The Proposed Project is conceived to achieve precisely this for resident populations. Sub-section 7.1C.2 explicitly calls upon Bonneville to fund a study of a sort to which the proposed Project is directly relevant in the case of resident fish.

Sub-section 7.1D states that "explicit priority" must be given to "conserve, manage, and rebuild the basin's remaining wild and naturally spawning populations…" The Proposed Project is also germane to the implementation of the development of a Pacific Northwest Biodiversity Institute called for in sub-section 7.11.
Finally, we point out that one of the unique features of the Proposed Project is obtaining taxonomy-quality photographs of live specimens of the three species of concern, in situ. Significant in its own right for detailing unique morphology and coloration of individual populations of the Basin's remaining native resident trout species, this provides a unique complement to the genetic material which will also be obtained for each population.

c. Relationships to other projects

A similar project to collect tissue samples for DNA analysis of westslope cutthroat in the Clearwater Basin in Idaho has recently been initiated by the Nex Perce Nation. Dr. Paul Spruell of the University of Montana's Wild Trout and Salmon Genetics Laboratory (WTSGL) (see "d" and "f' below) is conducting the DNA analysis for this project. He is also conducting the analyses for the present project. Dr. Robb Leary, also of the WTSGL, has recently analyzed samples of rainbow from the Colville National Forest (CNF) using protein electrophoresis. Our work in the Colville National Forest scheduled for the 1999 field season will contribute to the research which the CNF has initiated with Dr. Leary. These projects are synergistic with one another.

d. Project history (for ongoing projects)

This Proposal is for the continuation of Project #98-026, field work for which was begun in June, 1998. Project #98-026 was origianlly proposed as a five-year field data collection project. The current proposal, accordingly, is for the remaining three (3) years of the original project.
During the recently-completed 1998 field season, a total of twelve (12) tributary (second to fourth-order) streams in the Yakima and Naches subbasins were sampled. Representative specimens were photographed and tissue samples collected from populations from eleven (11) of these. Ten (10) of these contained putative westslope cutthroat populations and one (1) (Cooke Creek) contained putative redband rainbow. Bull trout were encountered and observed in one (1) of the streams containing cutthroat.The tissue samples from all 11 populations are currently in the possession of Dr. Paul Spruell of the Wild Salmon and Trout Genetics Laboratory at the University of Montana. Analysis of DNA microsatellite sequences from these samples was begun in late November 1998 and results will be available by March 1999.
The 12th tributary, Coleman Creek, which joins Cooke Creek at the northern edge of the floor of the Kittitas Valley and eventually enters the Yakima River as part of the Wilson Creek system at the entrance to the Yakima Canyon, was overrun by brook trout. Cursory evidence from WDFW stocking records and from personal communication with Geoff McMichaels of the Yakima Species Interaction Studies Team indicate that this constituted a recent and rapid displacement of a previously existent population of westslope cutthroat. No brook trout were (legally) stocked since the early 1980s and

westslope were observed to be common, in the reaches where only brook trout now are found, as recently as 1991.

The ten (10) putative westslope populations surveyed inhabit a range of riparian/channel habitat conditions from pristine montane to regrowing, open-canopy reaches recovering from recent timber harvest on private and federal forest lands, at elevations ranging from 1000 to 3700 feet. Very qualitatively, all populations surveyed prior to baseflow conditions in mid-September were abundant enough for two angler/researchers using dry flies to catch the desired sample size of 20 in no more than 90 minutes. In addition, within the limits of the size of the samples, all samples fit a normal distribution based on length.

Three distinct spotting patterns were observed among the cutthroat, one of which is nearly identical to the stereotypical pattern of westslope found, for example, in the upper Flathead Basin. The other two appear to be variants of the "classic" westslope pattern, one of which is quite striking in being devoid of any spotting forward of the posterior margin of the dorsal fin.

Pending the results of the DNA analysis, this phenotypic diversity would appear to be an important subject for additional research. We conjecture that the isolation of these headwater populations, and the absence of significant fishing or other predatory pressures, may permit sexual selection to play a larger role in the expression of color and spotting pattern than is normally the case. Other hypotheses are surely possible.

In 1999, the Project will survey streams in the Colleville National Forest, including tributaries of the Kettle and Pend O'reille subbasins, in response to requests from the Kalispell Tribe (Jason Scott) and Colleville National Forest (Tom Schuda). A $5000 cost-share from the Colleville National Forest has already been secured to assist with this work. The cost-share is a mix of field assistance, lodging for the Washington Trout team, and up to $2000 for the analysis of additional tissue samples which the cost-share will enable us to collect. We expect to be able to sample 15 to 20 populations of westslope and redband rainbow.

The final budget expenditure for FY1998 is estimated to be $40,000 to $42,000, which is approximately $10,000 under the budget projection. This was due primarily to having to undertake only one (1) horsepack trip, and a significant reduction in the anticipated food and lodging budget. This latter reduction was due both to favorable weather which occasioned little delay/disruption in scheduled surveying, efficient survey scheduling, and to the fact that most of the surveying in the Yakima subbasin could be accomplished with day trips from Washington Trout headquarters in Duvall, Washington. The mileage budget, however, was accordingly 36% above budget ($1689 vs. $1240). Personnel costs for field work, including benefits, was also below budget ($11725 vs. $20,000).

The budget for FY1999 is anticipated to be as proposed ($52,300), not including the $5000 match from Colleville national Forest, and will include a modest increase in salary (hourly rate) for the field crew.

The Project Report for FY1998 will be completed in the spring of 1999 after the results of the DNA analysis are received and analyzed. This Report is proposed to serve as a Milestone for the five-year project, with a second Milestone report in Spring of 2001, and a final Report in spring of 2003.

Pending the results of the DNA analysis, we are reasonably sure that pure westslope cutthroat populations exist the upper Yakima and Naches subbasins and that one or more

unique redband rainbow populations exist in the Wilson Creek system. Once the DNA analysis confirms the purity of specific populations of westslope, we believe that one or more comparative studies of some of these populations will suggest themselves.
Based upon the relative ease of securing the desired sample size of 20 from all populations sampled prior to baseflow conditions in mid-September and the normal length distribution of the samples, as noted above, the populations appear to have reasonable levels of abundance. In view of the noted diversity of existing habitat (in-channel and riparian) conditions in which these populations occur the following questions arise: -- what kinds of habitat conditions are necessary for the exisence of healthy populations of westslope cutthroat?

-- are some ranges or types of habitat conditions more "productive" of native resident fish populations than others?

-- do some ranges or types of habitat conditions provide for the existence of more secure populations of resident native trout than others?; if so, can this be documented/quantified?

-- brook trout were encountered in several of the streams in which (putative) westslope cutthroat appear to dominate and appear to have recently completely displaced a previous westslope population in Coleman Creek. What factors lead to the successful displacement of native residents by brook trout? Can an assessment of the risk which brook trout pose to the integrity of native resident trout be developed which would apply on a tributary or even a reach scale?

The immediate purpose of the Project is to identify the existence (the occurrence) of remaining pure native resident trout and to produce a photo-documentary record of them. Beyond this, however, the purpose is to identify priorities for preservation, including identifying opportunities for additional research the results of which will further our ability to protect native resident trout populations in the Columbia Basin.
Both of these objectives have been furthered by the results of the FY1998 field season and will be further clarified when the results of the DNA analyses are received. Research projects of the kind suggested have been identified and will be proposed for funding in related proposals for FY2000 and FY2001.

e. Proposal objectives

1. To systematically document the presence (i.e., the occurrence) of indigenous populations of bull trout (*S. confluentis*), redband trout (*O. mykis gairdneri*), and westslope cutthroat trout (*O. clarki lewisi*) in subbasins of the Columbia Basin in Washington State. Selected subbasins include: Wind River, Little and Big White Salmon rivers, Klickitat River, Walla Walla river, Yakima River, Wenatchee River, Entiat river, Methow river, Okanogan River, Kettle River, Spokane River,Tucannon river, and Grande Ronde River.

2. To document and assess the extent of hybridization between indigenous redband rainbow and introduced, non-indigenous salmonids, particularly Yellowstone cutthroat trout (*O. clarki bouvieri*) and coastal rainbow trout (*O. mykis irideus*).

3. To document and assess the extent of hybridization between indigenous westslope cutthroat and introduced, non-indigenous salmonids, particularly Yellowstone cutthroat, redband rainbow, and coastal rainbow.

4. To document and assess the extent of hybridization between indigenous bull trout and introduced, non-indigenous brook trout.

These objectives will be achieved by photographing individual specimens belonging to pure and/or hybridized populations of the three primary species on-site in their native habitats, taking length and weight measurements of each fish, and taking adipose or caudal fin tissue samples for subsequent micro-satellite DNA analysis.

Results will be written up in several formats. Some of the results and photographs will be used by the Principal Researcher, Dr. Pat Trotter, to revise and update his 1987 monograph Cutthroat: Native Trout of the West. Some results pertaining to each of the principal species will be written up by Dr. Trotter, in some cases in collaboration with Dr. Paul Spruell of the Wild Trout and Salmon Genetics Laboratory which has been contracted to do the DNA analysis (see subsection "f" below), in fisheries journals such as the Transactions of the American Fisheries Society. Semi-Annual "Milestone" Progress Reports will also be written and submitted to CBFWA and BPA. The precise location of each fish photographed and each fish from which a tissue sample was taken will be located on USGS 7.5-minute maps with the use of compass and GPS for later incorporation intoWashington Trout's GIS system and CBFWA databases.

Pending the results of DNA analyses, several in-season reports/presentations, including slide-shows can be made available to fisheries managers of sub-basin tribes, WDFW, and USF&WS, and to various other interested groups, organizations, and agencies within the sub-basins and across the state. Copies of photos and slides documenting the presence of the species of concern and their habitats, together with field notes and map locations, including GPS logged data can be made available to managers within days of their recording. Updates of the Project will be regularly published in Washington Trout's regular newsletter The Washington Trout Report and regularly updated on WT's web site together with slides of specimens and sites. Copies of the Report will be freely distributed to interested parties in the relevant subbasins.

f. Methods

1. Redband, westslope cutthroat, and bull trout specimens will be collected non-lethally, principally by fly angling with single barbless hooks. This method is particularly well suited to the lower order streams in which the bulk of the collecting is to be done and to the generally warm temperatures between mid-June and October during which the filed work will be conducted.

Additionally, in the few envisioned cases (e.g., bull trout >12 inches fork length or excessive turbidity) in which angling does not result in the minimum desired number

of fish per population for tissue sample collection of 10, backpack electroshockers will be used.

After each fish is caught, it is placed in a 10-gallon bucket, which has been lined with a large heavy gauge dark plastic garbage and filled to within 3 inches of the lip with fresh stream water. The garbage sack provides shade, darkness, and some texture all of which helps to settle the fish by providing a sense of cover. When the fish being collected are under 10 inches (254 mm) in fork length, as is usually the case with redband, westslope, and bull trout in the lower order streams to be sampled (see, e.g., Mullan et al. 1992, p.114 and Appendix K, p.409), up to 8 individuals can be safely detained for up to 30 minutes in such a bucket.

A portable streamside aquarium measuring approximately 22x16x4 inches is set up on a portable table at streamside where the sampling is to take place. The aquarium has an inlet port attached to the lower portion of one of the narrow sides to which a tube connected to a battery-driven pump is attached. This allows for fresh stream water to be continuously circulated through the aquarium, providing both well-oxygenated cool water and a mild current into which the fish faces thereby facilitating a realistic and high taxonomic quality photograph of the fish to be taken. An outlet port on the upper portion of the opposite narrow side to which a drain tube is attached allows for a constant level just short of overflowing to be maintained as water is continuously pumped from the stream and circulated through the aquarium.

Collection is undertaken from approximately 50 yards upstream and downstream of the location of the aquarium. Depending upon fish density, this normally allows for up to 8 individuals per sampler to be collected and transported to the position of the aquarium within 30 minutes of the first sample being placed into a bucket. When necessary fresh stream water is easily replaced in the bucket if collection or photographing will exceed the 30-minute period.

Each fish is removed, one at a time, from the bucket using a large soft-meshed aquarium dip-net and placed into the aquarium. A series of taxonomy-quality photographs of the fish holding its station while facing the flow are taken. The fish is then removed from the aquarium with the dip-net and placed into a 5-gallon bucket containing fresh stream water and Clove Oil to anesthetize it. (The use of Clove Oil as an anesthetic as reported by Anderson, McKinley, and Colavecchia 1997, was extensively evaluated against MS-222 during the 1998 field season and found to be as effective.)

After the fish has been mildly anesthetized it is removed from the bucket and placed onto a V-shaped rule and its fork length measured and recorded to the nearest millimeter. It is then placed into an appropriately-sized pre-weighed “Zip-Loc” bag and weighed with a spring scale to the nearest gram and its weight recorded. A section of its adipose or caudal fin approximately ¼ inch square is then removed with a pair of small surgical scissors. The tissue sample is placed into a small plastic screw-capped cryo-vial containing 95% denatured ethyl alcohol, the vial capped, and the fish released into another bucket filled with fresh stream water and allowed to recover before being released into the capture reach.

The vial containing the fresh tissue sample is labeled externally with a pre-assigned alphanumeric code, which is unique to that fish. In addition, the code is written on a waterproof label which is then placed inside the vial along with the tissue sample. The code is recorded in a waterproof field logbook together with the fish’s length, film

role number and exposure numbers, date and stream location information. The vial is placed in a lidded rack specifically designed to contain the vials, the lid placed on the rack thus firmly securing the individual vials inside the rack, and the rack placed in an iced cooler.

2-4. The vials containing the labeled tissue samples will be delivered to Dr. Paul Spruell of the University of Montana's Wild Trout and Salmon Genetics Laboratory for microsatellite DNA analysis.All samples will be analyzed blind. Samples will be segregated and vials grouped as "same species collected from one stream or stream reach". This will be the only information given to the analyst in addition to the unique alphanumeric identification written indelibly on each vial and on the label placed inside the vial at the time of collection.

Based upon evidence collected by the Principal Investigator, Dr. Pat Trotter, in 1992 in a subbasin of the Yakima River and results reported in 1994 for subbasins of the Methow River (Proebstel and Noble, 1994), and sampling in Montana and Idaho over the past decade (reported, e.g., in Behnke 1992, in Gresswell 1988 and by Watson and Hillman, 1997), we expect to document both small, pure populations of westslope cutthroat, redband rainbow, and bull trout principally in small headwater streams and upstream of natural barriers. We also expect to find evidence of hybridization of the types noted in subsection "b" above in all three species. We further expect to document evidence of disruption and displacement of some populations of each of these three species by non-indigenous species, particularly brook trout.

Based upon preliminary fieldwork in 1997 in the Yakima Basin, we expect to document the presence of several small headwater refuge populations of pure westslope cutthroat trout. In conjunction with results from other fieldwork to be conducted by the proposed project, we expect that this will contribute to a significant expansion and refinement to our knowledge of the original extent of post-glacial colonization of the mid-Columbia Basin by westslope cutthroat.

In combination, all of the above will significantly increase our understanding of the impacts of euroamerican colonization, including the development of the hydropower system, on the native resident trout species of the Columbia Basin in Washington State. Such an understanding is fundamental to assessing the current risks facing the remaining pure native populations of these species and to undertaking appropriate measures to insure their continued existence and recovery.

(Replace this text with your response in paragraph form)

g. Facilities and equipment

All Photographic equipment, collection equipment and vehicles for field use, camping equipment, office space and equipment, including computers are already in-hand by Washington Trout and/or the Field Investigators.

h. Budget

The budget includes a modest but much-needed increase in hourly rate for the Principal Investigator, Dr. Trotter, and the primary field assistant Bill McMillan, and provision for

time invested in the direction of the Project and assistance in some of the field work by Project director, Nick Gayeski. Dr. Trotter will be paid at the rate of $25/hr. for all field work (estimated at 390 hours) and $30/hr. for report writing (estimated at 40 hours). Dr. Trotter's normal consulting fee for the type of work which he is performing under the Project is $60/hr., which is the basis for calculating his match to the project of $14850.
During FY1998, Mr. McMillan was involved in sorting slides taken in the field and in summarizing his field notes to facilitate their entry into the project database. The budget includes 20 hours of Mr. McMillan's time for assistance with sorting slides and development of informational slide shows, in addition to 390 field hours.
The 390 hours are a downward revision from the FY 1998 and FY 1999 proposals of 500 hours, based upon the experience from the 1998 field season. Distance travelled in 1999 and 2000 will be greater than in 1998, so travel time will take a greater portion of total time than in 1998. 390 hours are estimated as follows: 15 field surveys at 10 hours each, plus travel to and from the site or region of the surveys. Travel from Duvall, Washington to regions beyond the Yakima Basin essentially involve one days time each way. 30 travel days of 8 hours each are estimated for the 15 days field time. It can be expected that the 15 days field time estimate is too optomistic, and that 2 days travel per day in the field is too generous. The two balance and provide a reasonable upper bound on field crew hours.
The Project Director will assist with approximately 7 of the 15 surveys, both to increase the efficiency of the project and to directly observe the field work to maintain its efficiency and assist Dr. Trotter and Mr. McMillan in suggesting ways to improve the details of the project. This part of the budget is calculated at 182 hours ($7/15^{th}$ of 390) at $20/hr. Travel time for the Project Director will not be budgeted.
In addition to assistance with field work, the Project Director's time is budgeted for project planning and procurement, development of slide shows and related communication tasks, and for assistance with Report writing tasks, including data entry and statistical analysis. A total of 80 hours are calculated for these tasks at $20/hr.
The travel budget is reduced somewhat over that contained in proposals for FY1998 and FY 1999. Based on experience from 1998 and the longer distances which will be involved during FY2000 in comparison to FY1998 mileage is increased to 7500 miles from 4000 in FY1998. Food and lodging costs, however, are reduced from the FY 1998 budget based on experience from FY1998, and fewer horse pack trips are planned (3 down from 8 in the FY1998 proposal).
$10,000 is budgeted for DNA analyses. The 1998 rate was $30/sample, with a sampling goal of 20 per population. The $10,000 covers expected increases in this fee and would cover the analysis of 20 samples from 15 populations, which is an ambitious target.
The budget also includes 6 Optic Stowaway 32k temperature loggers, to facilitate the project's ability to collect temperature data at survey sites during and after sampling. This enables the project to gather additional significant data on the conditions of the aquatic habitats surveyed, making efficient use of the field time and geographic extent of the project.

Section 9. Key personnel

Dr. Pat Trotter is self-employed as a fisheries science consultant. He has a Ph.D. in Chemistry from Lawrence University in Appleton, Wisconsin. Five especially relevant publications are listed in Section 6. Dr. Trotter has a decade and a half experience with the kind of photo-documentation involved in this project and in the collection of fish tissue samples.

Dr. Trotter will be assisted in the field work principally by Mr. Bill McMillan. Mr. McMillan is a gifted naturalist and fisheries field worker, and an experienced and outstanding nature photographer. Among his relevant experience is the initiation of systematic and still ongoing wild summer steelhead snorkel surveys on the Wind and Washougal rivers in the late 1970s. Recently, in 1995 and 1996 he was a leader of a remote camp on the Kamchatka peninsula of Russia for the Wild Salmon Center's Kamchatka Steelhead Project, a scientific joint venture between the Wild Salmon Center and Moscow State University (Russia) which also involves Dr. Robert Behnke of Colorado State University.

In 1996/97 Mr. McMillan led a two-person field crew in a systematic survey of tributaries of the Tolt and Snoqualamie Rivers in order to assess fish presence and stream type classification, and culvert problems. This work involved a minimum of four 10-hour days per week from late October through June. This work requires excellent woodsmanship and map-reading skills. It also involves accurately transcribing field data onto Washington State Forest Practice Base Maps which must be submitted to the Department of Natural Resources to document change of stream type.

Both Dr. Trotter and Mr. McMillan are skilled in the use of backpack electroshockers. Employees of Washington Trout have been certified by Washington Department of Fish and Wildlife to conduct training courses in the use of backpack electroshockers. Mr. McMillan has been one of the instructors for this course.

These two individuals worked together extensively under this Project during 1998 and will do so again in 1999.

Nick Gayeski, the Project Director has a Masters Degree In Philosophy from the University of Washington where his primary philosophic interest was in the philosophy of science. He has had a longstanding avocational interest in aquatic biology, including macroinvertebrate ecology, salmonid life history and population biology. He has extensive experience in the collection of freshwater macroinvertebrates and has taught numerous field courses in macroinvertebrate identification for fly anglers for professional flyfishing retail stores.

For the past three years he has been employed fulltime by Washington Trout, where his primary duties have been grant writing and public fisheries and landscape mangement policy evaluation. He has been the Director of the current project since its inception and wrote the original grant proposal. Recent additional experience/training includes organizing and hosting a day-long public/scientific forum on "Addressing Risk and Uncertainty in Salmonid Harvest Management" held in Olympia, Washington in January of 1998, including contacting professional fisheries biologists from throughout the northwest and east coast and arranging for their participation. In September of 1998, he attended a training course on "Ground Water-Surface Water Interaction" conducted by Dr. Jack Stanford at the University of Montana's Flathead Lake Biological Station.

Section 10. Information/technology transfer

In addition to peer reviewed scientific publications and annual/semi-annual progress reports or "milestone" reports to CBFWA, results from the project will be provided to managers and other interested parties in the Basin through on-line database transfers. Results, details, and slides/photographs of native resident trout from the subbasins will be made available on Washington Trout's web site, in Washington Trout's regular newsletter, the Washington Trout Report, and in special publications. Slide shows will be developed and presented to a variety of audiences throughout the state, including flyfishing clubs, environmental groups, Fish and Wildlife Commissions, and subbasin watershed councils.

Congratulations!