

99F-102

4.5 PSP Cover Sheet (Attach to the front of each proposal)

Proposal Title: Health monitoring of hatchery and natural fall-run chinook in SJ River
 Applicant Name: J. SCOTT GOOT
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Amount of funding requested: \$ 37,850 for 1 years

Indicate the Topic for which you are applying (check only one box).

- Fish Passage/Fish Screens
- Habitat Restoration
- Local Watershed Stewardship
- Water Quality
- Introduced Species
- Fish Management/Hatchery
- Environmental Education

Does the proposal address a specified Focused Action? yes no

What county or counties is the project located in? MEACRO, San Joaquin

Indicate the geographic area of your proposal (check only one box):

- Sacramento River Mainstem
- Sacramento Trib: _____
- San Joaquin River Mainstem
- San Joaquin Trib: _____
- Delta: _____
- East Side Trib: _____
- Suisun Marsh and Bay
- North Bay/South Bay: _____
- Landscape (entire Bay-Delta watershed)
- Other: _____

Indicate the primary species which the proposal addresses (check all that apply):

- San Joaquin and East-side Delta tributaries fall-run chinook salmon
- Winter-run chinook salmon
- Late-fall run chinook salmon
- Delta smelt
- Splittail
- Green sturgeon
- Migratory birds
- Other: _____
- Spring-run chinook salmon.
- Fall-run chinook salmon
- Longfin smelt
- Steelhead trout
- Striped bass
- All chinook species
- All anadromous salmonids

Specify the ERP strategic objective and target (s) that the project addresses. Include page numbers from January 1999 version of ERP Volume I and II:

Artificial fish propagation p 921
Water quality (Temperature & Contaminants) p 54 & 921

Indicate the type of applicant (check only one box):

- | | |
|--|--|
| <input type="checkbox"/> State agency | <input checked="" type="checkbox"/> Federal agency |
| <input type="checkbox"/> Public/Non-profit joint venture | <input type="checkbox"/> Non-profit |
| <input type="checkbox"/> Local government/district | <input type="checkbox"/> Private party |
| <input type="checkbox"/> University | <input type="checkbox"/> Other: _____ |

Indicate the type of project (check only one box):

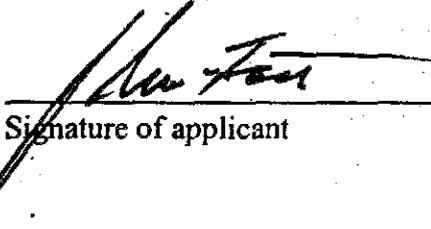
- | | |
|--|---|
| <input type="checkbox"/> Planning | <input type="checkbox"/> Implementation |
| <input checked="" type="checkbox"/> Monitoring | <input type="checkbox"/> Education |
| <input type="checkbox"/> Research | |

By signing below, the applicant declares the following:

- 1.) The truthfulness of all representations in their proposal;
- 2.) The individual signing the form is entitled to submit the application on behalf of the applicant (if the applicant is an entity or organization); and
- 3.) The person submitting the application has read and understood the conflict of interest and confidentiality discussion in the PSP (Section 2.4) and waives any and all rights to privacy and confidentiality of the proposal on behalf of the applicant, to the extent as provided in the Section.

J. Scott Foot

Printed name of applicant



Signature of applicant

99F-102

Health monitoring of Hatchery and Natural Fall-run Chinook Juveniles in the San Joaquin River and Delta, April - June 2000.

Primary Contact: J. Scott Foott, PhD
U.S. Fish & Wildlife Service
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Type of Organization / Tax status:
Federal Government / Tax exempt

Executive Summary

This project would characterize the health and physiological condition of both natural and hatchery juvenile chinook (*Oncorhynchus tshawytscha*) in the San Joaquin River and Delta. Sampling would occur in April - July 1, 2000 and be in conjunction with the Interagency Ecological Program (IEP) bio-sampling program. The requested funds total \$37,860. The California-Nevada Fish Health Center has conducted similar health and physiological monitoring of chinook smolts since 1991 in both the Klamath and Sacramento River basin (attached report summaries).

Project Description

Weekly samples would be collected from a variety of San Joaquin River sites and the Delta (Chipp's Island trawl) in conjunction with the IEP bio-sampling effort. Laboratory tests for selected salmonid pathogens and physiological indicators of energy reserves, immunodefences, and smolt development would be conducted by the California-Nevada Fish Health Center. An individual fish database would be utilized in the study in order to track disease and physiological disfunction to specific stocks, water temperatures of a given location, and sample date. Quarterly and a final report would be produced by the principal investigator as well as oral presentation(s) of the study results.

Location

Fish collections will occur at the California Department of Fish & Game Merced River

Fish Facility (MRFF), at various Interagency Ecological Program (IEP) beach seining sites on the San Joaquin River between the confluence of the Stanislaus River and Dois Reis Park (ca. river mile 50 - 74), and from Chipp's Island trawls conducted by IEP biologists.

MRFF	Snelling, Merced Co.	One pre-release sampling
SJ River	RM 50 - 74, San Joaquin Co.	Bi-weekly, April - June30
Chipp's	RM 18, Solano/Contra Costra Co.	Bi-weekly, May - June30

Ecological / Biological Objectives

Declining chinook populations in the Central Valley has prompted an intense restoration effort of this valuable resource and a key element of the State's aquatic biodiversity. Health and fitness of juvenile salmon out-migrants ("smolts") are major determinates of their performance and survival. Infectious disease can influence survival due to both direct mortality and reduced physical performance (predator avoidance, saltwater adaptation, etc.). No comprehensive fish pathogen survey has been conducted on San Joaquin River chinook to ascertain the effect of fish disease on this population. Contaminants and elevated water temperature have been identified in the CALFED process as stressors for salmonids in the San Joaquin River and Delta. Both of these stressors would have the potential for immunosuppressive effects. Hatchery - wild fish interaction is a controversial topic in natural resource management. The criteria used to define a quality hatchery fish is being reviewed and debated among hatchery and fish biologists. It will be important to profile the physiological condition of the natural population in order to compare with hatchery fish. Similarly, a comparison of the pathogens present in both populations is needed to either support or refute the charge that hatchery fish spread disease to natural populations.

Linkage

No comprehensive fish pathogen survey or physiological evaluation of juvenile Fall-run chinook in the San Joaquin River and Delta have been reported to date. This project is primarily directed at the topic of fish management / hatchery operations of a CALFED priority species (pg 421, Artificial Fish Propagation), however, it also addresses the following ERP topics (Feb. 1999 revised ERP Vol.1 and 2):

- a) **Water quality** - evaluation of the biological processes governed by stream temperature. (pg64). *Specifically, any correspondence of disease incidence or impaired physiological performance to elevated water temperatures.*
- b) **Water quality** - evaluation of the effects of contaminants on Fall-run chinook juveniles in San Joaquin River and Delta (pg 421). *Specifically, the occurrence of histological lesions in liver and kidney that are reported biomarkers of contaminant exposure.*

Systemwide ecosystem benefits

Data from this project will complement the quantitative efforts of the IEP bio-sampling program by providing qualitative in-sight into the health and condition of the juvenile chinook population. Data can be used by the CDFG in development of optimal hatchery operations for the basin.

Technical feasibility and Timing

Lethal sampling of up to 600 juvenile chinook (90 natural and 510 marked hatchery fish) will be required for this project as only internal organ samples can be used for pathogen assays. At the time of writing, the only listed chinook stock in California which would require a NMFS take permit (section 10) are Winter-run chinook. We do not expect to encounter this stock in the San Joaquin River. Marked fish, **already lethally sampled by IEP biologists for tag recovery**, will be used for this project. We anticipate that our project will be added to the IEP collection permits. Upon acceptance of this proposal, both CDFG inland fisheries branch and the National Marine Fisheries Service will be contacted to communicate the intent of the project and coordinate any amendment to the Stockton Office IEP permits. Extreme weather conditions or high river flow situation may pose a constraints for any fish collection activity. The ability to collect adipose fin clipped (cwt) chinook will vary considerable with sample period and site.

Methodology

Proposed sites and schedule of sampling: The study will begin in mid-April and end in early July. This time frame was chosen to target fish > 70 mm in fork length, so as to accommodate the various assays and encompass the main smolt migration period. Beach seining, random net "grabs" from hatchery rearing units, and trawl methods will be used for fish collection. The sample goal for each general site (river or trawl) per week will be 30 fall chinook juveniles (as identified by collecting biologists through size range criteria). The *maximum* sample size are outlined below:

San Joaquin R. <u>naturals</u> (April - time of hatchery release)	~ 90
San Joaquin R. marked hatchery	~ 200
Chipp's trawl marked hatchery	~ 240

It is important to note that the number of marked fish collected per week may vary considerably. Total sample numbers and methods were primarily selected to provide statistically valid pathogen prevalence data. The sample size necessary ***to collect*** one affect fish, in a population at least 100,000 in size with a presumed prevalence of infection $\geq 2\%$ and with a confidence level of $p=0.95$, is 147 (Ossiander & Wedemeyer, 1973). The number of samples to actually ***detect*** the agent is related to the sensitivity of the test and tend to increase the collection numbers. Physiological measurement sub-samples will be from 6 - 12 fish per general site on a bi-weekly

basis. A 30 - 60 fish sample will be collected from the Merced R. Fish Facility prior to the May release.

Field - Captured chinook will be quickly killed in an overdose of benzocaine. After a brief external examine for organosomatic characteristics, the caudal peduncle will be cut and blood collected in a heparinized microhematocrit tube. The blood sample will either be centrifuged (10,000 RPM, 10 min.) for measurement of hematocrit, leukocrit, and collection of plasma. Plasma samples will be held on ice and later transferred to -80° C. Kidney tissue will be inoculated onto BHI agar (bacterial assay). Portions of the kidney and liver will be collected for 2-fish pooled viral assays. The posterior kidney will be removed for *R. salmoninarum* ELISA (fish > 100 mm FL) or Fluorescent antibody test on imprints (< 100 mm FL). Gill, intestine, pyloric caecae, kidney, and liver from a subset of the collection will be preserved in Davidson's fixative for histological processing. Carcasses (Head removed for CWT reading) from a subset of the collection group will be frozen for later lipid analysis. Each fish will be identified with a unique number for tracking of lab samples and CWT identity (IEP activity). Heads from the natural chinook will be tested for *Myxobolus cerebralis* spores. Heads from marked chinook will be given to IEP biologist for stock identification. Refrigerated samples will be expressed shipped to CA-NV Fish Health Center twice per week basis. Target sample numbers per assay are presented in Table 1.

Laboratory - Manual chemistry tests will be used to test specific plasma samples for total protein (5 μ l plasma in microplate assay, Pierce BCA Protein Kit 23725) and electrophoretic profile (Ciba- Corning agarose gel kit - 7 fractions & Albumin: Globulin ratio). Plasma protein electrophoresis will be performed with a 7 μ L sample run on a CIBA agarose gel (1M barbital buffer, 90V for 45 min.). The stained gels will be scanned , the percent area of each fraction determined with Seprascan™ software, and Analysis of Variance performed on percent area values for each fraction (or combined fractions).

Histological samples will be processed for 5 μ m Hematoxylin and Eosin slides. Evaluations will key on any liver lesions (Fatty changes of hepatocytes, Degenerative nuclei, Hepatocyte hypertrophy, Hepatocytomegaly, Basophilic regeneration zones, abnormal tissue pigments, Neoplastic changes). Kidney tissue will also be closely examined for necrotic changes to the nephron and interstitium. Gill Chloride cells will be enumerated and their size recorded. The anterior 1/3 of the fish minus the head will be processed for virological assays and *R. salmoninarum* ELISA or DFAT tests will be performed on posterior kidney. One method for comparing the groups could involve determining the lowest quartile of each measurement type and the number of fish in each sample which fall below it (e.g. if 0.500 is determined to be the lowest quartile for all leukocrit values, how many fish in a given sample fall below this value are judged abnormal).

Table 1. Assays

Pathogens:

R. salmoninarum
cultured bacteria
virus
parasites

General method

ELISA or Fluorescent antibody test
BHI agar, biochemical identifications
cell culture, dot blot identifications
histology, pepsin-trypsin digest (head)

Physiological Measurements:

Energy reserves

% lipid, condition factor, hepatosomatic index,
visceral fat scores

Immune defense

leukocrit, plasma protein (total & specific fraction)

Smolt development

Gill ATPase assay, Gill Chloride cell indices

Contaminant

Liver and kidney histological examination

Table 2 Target sample sizes for each assay per collection site / week.

Test	sample No. / collection	Total sample No. for study
Virus - pooled kidney sample / EPC - PEG pretreatment Bacteria - kidney / BHIA <i>R. salmoninarum</i> ELISA or DFAT kidney sample	2 fish pool x 10 = 20	20 collections x20 = 400
Histology - lesions / parasites in gill, intestine / pyloric cecae, kidney, liver	10 fish	200 fish
Organosomatic Analysis: Hot, Lot, externals, Wt/Lng, HSI	20 fish	20 collections x20 = 400
Energy Measurements: % lipid, water, Condition factors, visceral fat score	5 fish	100 fish
Immune defenses: leukocrit, plasma protein concentration - albumin/ globulin or Electrophoretic profile	5 plasma tests	100 fish
Smolt Development: gill cl. cells, ATPase	8- 10 fish	160 - 200 fish

Local involvement

Collections will be in conjunction with IEP monitoring activities and with the permission of the CDFG (Merced R. Fish Facility). No local entity involvement or impacts are foreseen.

Cost

Total request for the 5 month (field, lab, data/ reporting) project is \$37,860. Categorical breakdown is as follows:

Personnel:

(GS9 biologist, GS5 technician) 560 hrs + 20hrs O/T estimate	\$20,000
Supplies / sample shipments:	\$15,000
Travel:	\$ 1,750
3% FWS overhead charge	\$ 1,110

Table 3. Budget

task	Labor hrs	salary /bene.	service contracts	materials	misc.	O/H 3%	total
1.	160	\$ 2600	\$0	\$15000	\$ 500	\$545	\$18645
2	480	\$11100	\$0	\$0	\$1000	\$365	\$12465
3/4	480	\$ 5800	\$0	\$0	\$0	\$175	\$5975
5	40	\$ 500	\$0	\$0	\$0	\$15	\$515
6	**	\$ 0	\$0	\$0	\$ 250	\$10	\$260
totals	1160hr	\$20,000	\$0	\$15,000	\$1750	\$1110	\$37860

Table 4. Quarterly Budget

task	oct-dec99	jan-mar00	apr-jun00	jul-sep00	oct-dec00	totals
1	\$0	\$0	\$16800	\$0	\$0	\$0
2	\$0	\$0	\$11100	\$0	\$0	\$0
3	\$0	\$0	\$8090	\$0	\$0	\$0
4	\$0	\$0	\$1110	\$0	\$0	\$0
5	\$0	\$0	\$500	\$0	\$0	\$0

306-308.

Foott, JS and RP Hedrick. 1990. Blood parameters and immune status of rainbow trout with proliferative kidney disease. *J Aquatic Animal Health*, 2: 141-148.

Appendix 1

FY96 Investigational Report :

Health and Physiology Monitoring of Coleman NFH Fall-run Chinook Smolts (FCS-BCW-95-COL) Component of 1996 Marked Out-migrant Study.

J.S. Foott and J.W. Williamson

Summary: During March - May 1996, the California - Nevada Fish Health Center (FHC) sampled juvenile Fall-run chinook salmon (FCS) at Coleman National Fish Hatchery (CNFH) prior to their release and at 3 sites downriver. A limited number of natural chinook juveniles were also sampled from the Upper Sacramento River. Fish were examined for disease, energy reserves, smolt development, plasma proteins, and an organosomatic analysis. There were 3 FCS release groups (14MAR, 29MAR, 23APR) of approximately 4 million each, of which 300,000 were marked with coded wire tags. The most significant disease detected was Infectious Hematopoietic Necrosis Virus (IHNV), which caused high mortalities in some FCS raceways and advanced the release time of the 1st two groups. The two early release groups were smaller (mean 66.5 and 70.5 mm FL) than the normal "smolt" release size. In spite of severe IHNV infection, marked fish of the 3rd release group had the highest Delta recovery. This data could indicate that large size influences recovery (migration survival) more than group health status. Infected fish, swimming with healthy cohorts, were recovered 183 km from CNFH. There were no marked changes in physiological measurements during the short time span between CNFH release and re-capture at Glen-Clousa Irrigation pumps (2-4 days) or Knight's Landing (6-7 days). Energy measures declined slightly in fish captured at Knight's Landing and Chipp's Island (Delta), however, they appear sufficient for the migration. Two weeks post-release may be the point where the smolt's energy balance, while still substantial, is starting to decline rapidly. Smolt development appeared to be more advanced in the 3rd group and improved in lower river captures. The natural chinook had lower condition factors (leaner) than 2 of the release groups yet had plasma triglyceride levels comparable to the fed hatchery fish. While no significant infectious diseases were detected in these fish, degenerative changes were observed in the livers of parr caught near Redding. Monitoring of the health and physiology of the marked groups will allow for better analysis of tag recovery data.