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SANTA BARBARA • SANTA CRUZ

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JUL 22 1997

CALFED Bay-Delta Program Office
1416 Ninth Street, Suite 1155
Sacramento CA 95814

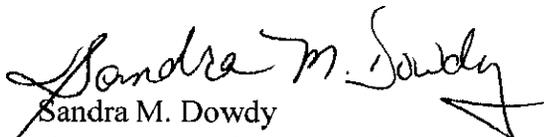
Research Proposal Entitled
**"Analysis of Tag Performance: Physiological and Biochemical Effects
of Marking Systems on Juvenile Chinook Salmon"**
RFP: 1997 Category III Ecosystem Restoration Projects and Programs
Principal Investigator - Ernest S. Chang

Dear Colleague:

It is our pleasure to present for your consideration the referenced proposal in response to **the CALFED Bay-Delta Program RFP.**

Please call on the principal investigator for scientific information. Administrative questions may be directed to me or my assistant, René Domino, at the above address and phone number. We request that correspondence pertaining to this proposal and a subsequent award be sent to the Office of Research and to the principal investigator.

Sincerely,


Sandra M. Dowdy
Contracts and Grants Analyst

Enclosure

cc: E. S. Chang

DWR WAREHOUSE

97 JUL 24 PM 2:21

Proposal to: CALFED Bay Delta Program Office
1416 Ninth Street, Suite 1155
Sacramento, California 95814

Title of Project: Analysis of Tag Performance: Physiological and Biochemical Effects of Marking Systems on Juvenile Chinook Salmon

Total Amount Requested: 383,431

Requested Start Date: 01/01/98

Proposed Duration of Project: 3 Years

Applicant Information:

The Regents of the University of California
Office of the Vice Chancellor for Research
410 Mrak Hall
University of California
Davis, California 95616.8671

Principal Investigator Information:

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Kristen D. Arkush	kdarkush@ucdavis.edu	707-875-2062
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PO Box 247, Bodega Bay, CA 94923

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Type of Organization and Status: Non-profit Public Institution of Higher Education

Tax Identification Number: 94.6036494.W

Technical Contact:

Ernest S. Chang	eschang@ucdavis.edu	707-875-2061
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Administrative/Contractual/Financial Contact:

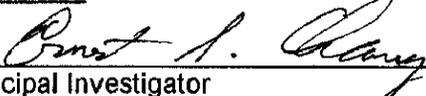
Shelley Macdonald	skmacdonald@udavis.edu	707-875-2007
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Participants/Collaborators in Implementation:

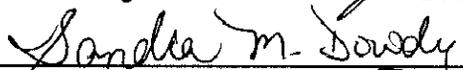
Lee Weber, University of Nevada, Reno
H. Lee Blankenship, Washington Department of Fisheries

RFP Project Group: (3) Other Services

Approvals


Principal Investigator Date 7/21/97


Department Chair Date 21 July 97


Official Signing for Organization Date JUL 22 1997
Sandra M. Dowdy
Contracts and Grants Analyst

P1 007

I. Executive Summary

Analysis of Tag Performance: Physiological and Biochemical Effects of Marking Systems on Juvenile Chinook Salmon

Ernest S. Chang, Kristen D. Arkush, James S. Clegg and Paul A. Siri

Supplementation of wild salmonid stocks through the mitigation efforts of hatcheries has led to increasing concern over the relative risks and benefits of introducing fish into a sensitive ecosystem like the Sacramento River drainage, which provides habitat to an endangered race and a USFWS species of concern, the winter-run and spring-run chinook salmon, respectively. The ability to discriminate hatchery-propagated from wild fish in the fishery is critical to the success of wild stocks. Mass marking hatchery fish is the first step towards a selective fishery. Efficient mass marking also increases the ability to monitor salmon migration through the Delta and other parts of the estuary. A variety of new tags are in the R&D stage but have not been adequately tested to determine their longevity or the benefits/risks and economics for their use in mass marking. Fishery management requires mass marking tags that are economical, data rich (i.e., specific to source and family) and easy to access and read. This proposal seeks funding to examine the sub-lethal consequences of both conventional tagging techniques and the most promising new generation of visible implants as a means to determine the most efficient, cost-effective and safe method for stock discrimination. Measures of stress will include stress hormone (cortisol) levels, metabolite concentrations, and alteration of stress protein (SP) expression.

This project has several goals. The primary task is to determine if routinely used tags such as coded wire (CWT) and passive integrated transponder (PIT) tags actually reduce the competency of salmon during the intense physiological challenge of smoltification as compared to select, alternative tagging methods (e.g., visible implant tags). In year 1, we will develop protocols for sampling (e.g., standardize blood collection methods, determine appropriate tissues to sample for SP analysis). Next, we will conduct pilot studies implementing the tagging methods and the selected protocols. In year 2, we will begin replicate studies (tagged vs. untagged) using hatchery-produced fall-run chinook salmon reared through smoltification. All experiments will be conducted at the Bodega Marine Laboratory. In year 3, we propose to repeat the replicate studies as in year 2, in an effort to reduce potential experimental variation due to differences among year classes. If SP induction is evident in tagged fish, experiments in year 3 will include evaluation of SP levels/isoforms following elevated temperature challenges. Additionally, other potentially lethal stressors (e.g., disease challenge) may be coupled with tagging to determine if tagging effects are sub-lethal yet synergistic (combination of the two stress events is lethal).

The research proposed here supports a potential restoration action and an improved population management action (CALFED 1997 RFP Category III, Attachment C, items 7 and 8). The work also helps to evaluate current hatchery marking practices and assess new hatchery needs for marking (CALFED 1997 RFP Category III, Attachment C, item 10). Additionally, the tasks listed here are consistent with the August 1996 Pacific Fisheries Management Council Research and Data Needs for mass marking assessment and selective fishery development - both

listed as high priorities. A significant goal of this project is to describe the biological consequences and ecosystem impacts of mass marking using salmon from the Sacramento River as a model.

<i>Proposed Budget:</i>	<u>Jan-Dec 1998</u>	<u>Jan-Dec 1999</u>	<u>Jan-Dec 2000</u>
Salaries & Benefits	27,442	28,258	29,100
Supplies and Expense:	42,722	31,722	31,722
Total Subcontracts:	26,016	26,637	27,289
Total Equipment:	16,500	0	0
Travel:	2,800	2,800	2,800
Fee Remission:	<u>8,982</u>	<u>8,982</u>	<u>8,982</u>
Total Direct Costs:	124,462	98,399	99,893
Indirect at 25.5% MTDC:	<u>25,240</u>	<u>18,059</u>	<u>17,378</u>
Total Budget:	149,702	116,458	117,271

There are no anticipated negative third party impacts. The proposed work may have positive implications for hatchery practices if it identifies a tagging method that is superior (e.g., has no deleterious effect on the fish, contains more information) to current marking systems.

Kristen D. Arkush: Over 5 years of experience in working with fish diseases and rearing. Is currently the co-principal investigator on two projects dealing with salmonid aquaculture and conservation at BML.

H. Lee Blankenship: Has worked for over 24 years on salmonid biology and management. Has recently participated in several studies on the physiological effects of hatchery tagging.

Ernest S. Chang: Has conducted research on endocrinology for over 19 years. Has developed radioimmunoassays and enzyme-linked immunoassays for various steroid and peptide hormones. Has measured glucose and other metabolites in several species of aquatic animals.

James S. Clegg: Has worked for over 36 years in cellular biology. Has worked on stress proteins in aquatic animals during the past 7 years and has become one of the primary researchers in biochemical adaptation.

Paul A. Siri: Has extensive background in managing and operating aquatic systems, particularly with fish. Consultant to a number of educational, state, and federal agencies. Currently project leader for the Winter-Run Chinook Captive Broodstock Project at Bodega Marine Lab.

Lee A. Weber: Has worked on cellular biochemistry for over 22 years and is one of the primary researchers on the expression of stress proteins in fish.

Full technical and financial reports will be provided at the end of each year funded. All scientific publications will be included in those documents.

Protecting wild salmon stocks in the Sacramento River system (winter-run, spring-run, and late-fall run chinook salmon are identified as priority species) directly supports the CALFED mission by providing a tool by which to restore ecological health.

III. Project Description

Project Description and Approach

In recent years the evidence that hatchery fish pose a competitive threat to wild stocks has been increasing. Whether this problem is linked to reduction of the effective population size or increase in the frequency of disease due to the large number of cultured fish entering the system, the ability to identify hatchery fish versus wild fish remains of paramount importance if wild stocks are to persist. Mass marking hatchery fish is the first step towards a selective fishery. Efficient mass marking also increases the ability to monitor salmon migration through the Delta and other parts of the estuary. A variety of new tags are in the R&D stage but have not been adequately tested to determine their longevity or the benefits/risks and economics for their use in mass marking.

Fishery management requires mass marking tags that are economical, data rich (i.e. specific to source and family) and easy to access and read. The new generation of tags, generically described as visible implant tags, would allow the marking of all hatchery fish in ways more beneficial to current management needs than coded wire tags (CWT) alone or in combination with adipose fin clips. One type of marking system, the visible implant fluorescent elastomer (VIE) method, involves implanting the elastomer material beneath transparent or translucent tissue (e.g., periocular tissue). The material is injected as a liquid that quickly cures into a pliable, bio-compatible solid. The small volume of material required and its high retention rate combine to make the VIE superior to other commercially available marking systems such as the visible implant alphanumeric tags, which may be shed more frequently in fish smaller than 150 mm forklength. The VIE tag has been used to mark reef fishes as small as 8 mm standard length (Frederick, 1997) and is available in four colors, allowing for up to 420 unique codes (two marks per specimen using six body locations). Such codes could be used to identify run, year class, and hatchery of origin. These tags are visually identified, eliminating the need for specific equipment to interrogate hidden tags (e.g., passive integrated transponder, or PIT, tags) or time and effort in tag recovery (e.g., CWT). Although promising as a potential alternative, the photonic tag (latex microbead containing fluorescent dye) is still under development. One of us (Arkush) has demonstrated inferior tag retention rates in this application 4 months post-tagging, owing to the variation in bead size and ability of the host's macrophages to engulf and relocate the particles.

This proposal seeks funding to examine the sub-lethal consequences of both conventional tagging techniques and the most promising new generation of visible implants. This project has several goals. The primary task is to determine if routinely used tags such as CWT and PIT tags actually reduce the competency of salmon during the intense physiological challenge of smoltification. There is ample evidence in the literature that demonstrates how tagging and handling increase cortisol levels. The process of smoltification requires a precise sequence consisting of the onset of cortisol production that is mirrored by the rise and fall of gill ATPase activity and thyroxine. An interruption or perturbation of the smolting endocrine cascade by other stressors, such as the artificial combination of physiological responses to tagging stress, can create a situation whereby a salmon's ability to move through the estuary is seriously compromised.

The initial tests will examine the performance of tagged juvenile presmolts and untagged

controls. Performance will be measured by conventional corticosteroid and glucose assays and by the evaluation of levels and induction of specific proteins immediately following tagging and at defined intervals post-tagging through the process of smoltification.

All vertebrate animals respond to a variety different stresses by secreting corticosteroid hormones from their adrenal glands. The stresses can be environmental (temperature, osmotic), physical (handling, injury), or psychological (visual or auditory disturbances). The corticosteroids are a group of steroid hormones that mediate a wide range of physiological effects. These effects include alterations in cellular metabolism, developmental morphology, and mineral balance. The most important corticosteroid related to stress in fish is cortisol (see Iwata, 1995, for review).

Other biochemical measures of stress in fish are the blood levels of glucose and lactate. Elevation of blood glucose is a response to elevated cortisol, which itself is elevated upon exposure to the various stresses described above. Cortisol promotes the breakdown of proteins and fats, which are then converted into carbohydrates and eventually to glucose (gluconeogenesis). Lactate is the usual end product of anaerobic metabolism and can accumulate under stressful conditions. This accumulation is due to the inability of the organism to cope with an increased metabolic load via aerobic metabolism. We propose to measure blood levels of cortisol, glucose, and lactate as sublethal indicators of chronic stress. These measures will likely be valuable indicators of subtle, long-term stressors, such as various tagging methods.

It is well known that a suite of specific proteins, called "stress proteins" (SP) here, is synthesized by virtually all cells in response to a wide variety of sub-lethal stresses, including temperature fluctuations, osmotic changes and exposure to a wide variety of chemicals. The literature in this area is massive, but good coverage can be found in books edited by Morimoto et al. (1994) and Feige et al. (1996). These proteins have been used as indicators of stress prior to visually-obvious damage, in a wide variety of aquatic species (Sanders 1993). We propose to examine the effects of several tagging procedures on the levels of selected stress proteins. Our search of the literature indicates that nothing has been published on any SP in fish that have been tagged by any procedure. Therefore, the proposed research will provide completely new information that could be useful in other types of studies on salmon, such as evaluating the presence of stressful conditions in the field. We will study two families of stress proteins, SP-30 and SP-70, that have been identified as the most likely to be involved in the stress response of chinook salmon. Changes in the levels of these proteins, and the appearance or disappearance of specific "isoforms" (different molecular forms within a SP family) will serve as sensitive indicators of stress induced by tagging, as well as indicate the ability of tagged fish to mount an effective stress response.

A second stage of the trials will include an evaluation of performance through the period of smoltification, using plasma sodium levels as a measure of saltwater competency. By comparing CWT versus new tag technologies we can determine if conventional stock management tools are contributing to salmon losses, especially when fish are responding to multiple endogenous and environmental stressors.

We propose to conduct these experiments over a three year period. In year 1, we will develop protocols for sampling (e.g., standardize blood collection methods, determine appropriate tissues to sample for SP analysis). Next, we will conduct pilot studies implementing the tagging

methods and the selected protocols. In year 2, we will begin replicate studies (tagged vs. untagged) using hatchery-produced fall-run chinook salmon reared through smoltification. All experiments will be conducted at the Bodega Marine Laboratory (BML). In year 3, we propose to repeat the replicate studies as in year 2, in an effort to reduce potential experimental variation due to differences among year classes. If SP induction is evident in tagged fish, experiments in year 3 will include evaluation of SP levels/isoforms following elevated temperature challenges. Additionally, other potentially lethal stressors (e.g., disease challenge) may be coupled with tagging to determine if tagging effects are sub-lethal yet synergistic (combination of the two stress events is lethal).

Location and Geographic Boundaries of Project

All of the hatchery-reared fish will be held at BML. Experimental work will be carried out at BML and the University of Nevada-Reno. Although focusing on the Sacramento River fall-run chinook salmon, the project has general application in the management of all 4 Sacramento River runs. In its design, the proposed work encompasses the freshwater, estuarine, and early seawater phases of the fall-run chinook salmon, and thus simulates conditions in the mainstem Sacramento River, the Delta, and the San Francisco Bay.

Expected Benefits

The expected benefits of mass marking all hatchery fish are enormous. A selective fishery will be the most effective means to manage wild salmon stocks. This study will provide the framework for informed selection of the most effective tag that is safe, compatible and informative. Recovery of fish that are externally marked with such tags, either through Delta sampling programs or in the terminal fishery will enable resource agencies to precisely monitor fish movement, translating into more effective management of water resources. Protecting wild salmon stocks in the Sacramento River system (winter-run, spring-run, and late-fall run chinook salmon are identified as priority species) directly supports the CALFED mission by providing a tool by which to restore ecological health. In the short-term, this work will provide basic biological information on the effects of tagging, comparing both old and new technologies (primary benefit). The longer term (secondary) benefits include movement towards rational decision-making in the development of a selective fishery that ultimately benefits all involved parties (fishermen, resource managers, water contractors, and environmentalists) in this often contentious environment.

Background and Biological/Technical Justification

The research proposed here supports a potential restoration action and an improved population management action (CALFED 1997 RFP Category III, Attachment C, items 7 and 8). The work also helps to evaluate current hatchery marking practices and assess new hatchery needs for marking (CALFED 1997 RFP Category III, Attachment C, item 10). Additionally, the tasks listed here are consistent with the August 1996 Pacific Fisheries Management Council Research and Data Needs for mass marking assessment and selective fishery development - both listed as high priorities. A significant goal of this project is to describe the biological consequences and ecosystem impacts of mass marking using salmon from the Sacramento River as a model. An important benefit of the proposal is that it will incorporate a novel yet universal cellular response, the production of stress proteins, in response to sub-lethal stress. This

physiological marker may prove to be an excellent indicator of stress, in advance of lethal or reduced fitness (reduced fecundity) consequences already known for races of Sacramento River chinook salmon (e.g., effects of high water temperatures on egg viability for the endangered winter-run chinook salmon).

Proposed Scope of Work

Fish Tagging: Hatchery-produced fall-run chinook salmon will be obtained in early February as fingerlings for this study. Once acclimated to the life support systems at BML, the fish will be tagged by one or a combination of the following tag types: CWT, adipose fin clip, PIT, VIE. All of the tagging procedures will be coordinated by Lee Blankenship, who has extensive experience testing a variety of marking systems for the Washington Department of Fisheries (see Blankenship and Tipping, 1993). Beginning in March, the fish will be allowed to undergo smoltification through acclimation to increasing salinities (up to full strength seawater). BML has life support systems designed to monitor and control salinities through sensors linked to a programmable logic computer (PLC) with data logging capabilities. The system can be controlled and monitored remotely via an Internet connection. Sampling for hormones, metabolites, and stress proteins (see below for detail) will occur prior to tagging, immediately after tagging, and at defined intervals post-tagging. Three types of controls will be used: fish that have been tagged but not smolted, fish that have not been tagged but are smolted, and fish that have not been tagged or smolted. In addition to providing a basis for interpreting the results on the effects of tagging, these controls will evaluate the effects of smoltification, per se, on hormone, metabolite, and stress protein profiles.

Efforts in year 1 will focus on developing protocols for sampling prior to, and immediately following, tagging. We will evaluate stress hormones and metabolite production post-tagging, and determine appropriate tissues to sample for stress protein quantification. During year 1, we will conduct pilot studies implementing the tagging methods and selected protocols. In year 2, we will tag hatchery-produced fall-run chinook salmon and evaluate stress hormones, metabolites, and stress proteins post-tagging and through smoltification. In year 3, we will repeat the studies as in year 2, to minimize potential experimental variation due to differences among year classes. Additionally, the effects of elevated temperatures (simulating low flow conditions in the Sacramento River and Delta) on stress protein levels/isoforms will be evaluated. We will examine stress protein production in tagged and untagged fish exposed to mild heat stress (3-5 °C above ambient), and more extreme thermal shock (5-10 °C above ambient) to mimic normal and extreme river temperature fluctuations, respectively. Finally, we propose to incorporate disease challenge as another stressor to determine if tagging effects are sub-lethal yet synergistic. Interestingly, it has been shown that coho salmon infected with *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease (BKD), express SP-70 (Forsyth et al., 1997). If SP expression is induced following tagging or exposure to elevated water temperatures, it will be particularly important to correlate it with the imposed stressor, not BKD, which is ubiquitous in some stocks in the Sacramento River. We intend to incorporate disease detection and diagnosis, particularly for BKD (by immunofluorescence), as an important feature of this project.

Stress Hormones and Metabolites: Fish will be quickly netted with a minimum of disturbance and they will be placed into buckets containing a lethal concentration of MS-222 (200

mg/l buffered with 500 mg/l NaHCO₃). Blood will be collected from the severed caudal peduncle and placed into heparinized tubes on ice until the tubes can be centrifuged. The resulting plasma will be frozen at -80°C.

Plasma cortisol concentrations will be determined by means of either an established radioimmunoassay (RIA) or by an enzyme-linked immunoassay (ELISA) that we will develop during the first year of this project (modification of procedure by Barry et al., 1993; Chang et al., unpublished). The RIA will follow the procedure of Redding and Schreck (1983) based upon methods developed by Foster and Dunn (1974). Serum or standards are added to tubes. A standard series for cortisol is constructed. Radiolabeled cortisol is added to each tube. A commercially available antiserum is added and incubated for 2 h at 25°C. The tubes are then cooled and a hemoglobin-coated charcoal suspension is added (Dunn and Foster, 1973), which separates the bound from the unbound hormone. After centrifugation, aliquots of the supernatant are transferred to scintillation vials for analysis in a scintillation spectrometer. The percentage bound of the standards is used to construct a standard curve. From this standard curve, the amount of cortisol in the sample sera can be determined.

Glucose is measured by a previously described colorimetric assay (Chang et al., 1990) with modifications. Blood serum is mixed with ethanol. The precipitated proteins are pelleted and the supernatants (0.1 ml/well) are loaded onto 96-well microplates. The plates are read on a microplate reader at 490 nm after reaction with a mixture of glucose enzyme reagents and color reagents, and the results are compared to standards.

To measure lactate, serum is added to trichloroacetic acid. The tubes are centrifuged and the supernatant or standards are added to a lactate dehydrogenase reagent solution. These solutions are then measured in a spectrophotometer at 340 nm and the samples quantified by comparison to the standards. Aspects of the hormone and metabolite measurements will be conducted in collaboration with Dr. Carl Schreck (Oregon State University), a leading researcher in the area of stress physiology in fish.

Stress protein (SP) analysis: As mentioned, SP-30 and SP-70 will be measured in selected tissues of previously-tagged salmon. SP-30 should be a particularly useful indicator because it is only present in fish that have previously experienced stress; that is, SP-30 is "inducible" (Hargis et al., 1997). The methodology needed for stress protein analysis is well-developed and used routinely in our laboratories (Norris et al., 1997; Hargis et al., 1997; Clegg et al., 1995; Liang et al., 1997). Fortunately, antibodies against these stress proteins in chinook salmon have already been produced in Weber's laboratory and used in his and Clegg's laboratories. That is a very important and significant advantage in the case of SP-30 since production of anti-SP-30 "from scratch" would require a lot of time and effort. Unlike other SP families, "small" stress proteins like SP-30 are usually not recognized by commercially-available antibodies produced against the stress proteins of other species. Thus, the proposed research can be initiated immediately.

Previous studies have indicated that skeletal muscle, skin and ventral fin are the tissues of choice for study (Weber, unpublished results). However, those results will be examined further during the first year when we will be establishing conditions for the final experimental protocols. Although the first year will be devoted chiefly to working out these protocols, initial studies on

the effects of the various tags on SP-30 and SP-70 will also be carried out. At designated times after tagging, fish will be sacrificed, and the selected tissues quickly dissected, weighed and homogenized using a buffer developed to stabilize the organelles of animal cells (see Clegg et al., 1995). Homogenates will be prepared for analysis (see Clegg et al., 1995) and either used at once, or stored at -70 °C, before electrophoresis (SDS-PAGE) and immunoblotting (western analysis). Quantification of stress proteins, and estimation of their molecular weights (sub-units) will be achieved by scanning the films from immunoblotting into a laser-scanning densitometer.

As indicated in Section 1, research during year 2 will be designed on the outcome of the pilot study done during the first year, and year 3 will be a replicate to evaluate another year class. In addition, the effects of temperature elevation on SP profiles and levels will be examined during year 3. A great deal of research on the effects of temperature variation on salmonids has been carried out by Weber and his colleagues, and this part of the research will be guided by that knowledge and information.

Personnel at BML have over 6 years experience in rearing and maintaining the endangered winter-run chinook salmon in a captive broodstock program. Facilities staff are proficient at designing and fabricating life support systems for salmonids and integrating alarms for critical variables such as water flow and temperature. Currently, BML has a 12 tank (100 L each) system designed specifically for controlling salinity, enabling the monitored introduction of smolts to seawater conditions. The laboratory recently completed a freshwater treatment system to supply 200 gpm water from a well source. Both the freshwater and seawater systems are maintained by BML's physical plant staff on a 24 hour basis. Kristen Arkush is also collaborating with Dr. Maxwell Eldridge (NMFS) and Dr. Peter Klimley (BML) on a proposal for funding through NOAA/NMFS to evaluate the use of archival tags in monitoring adult salmon in seawater (off the California coast). Their work promises to complement our proposed project in that both provide information critical to managing a selective fishery.

Full technical and financial reports will be provided at the end of each year funded. All scientific publications will be included in those documents.

Monitoring and Data Evaluation

The presence of stress will be indicated by increases in the relative amounts of SP-70, and the appearance of SP-30 (inducible). These data, obtained by scanning densitometry, will be subjected to an analysis of variance (ANOVA) to evaluate statistical significance of results from the various control and experimental groups of fish. Similar statistical tests will be used to analyze the stress hormone and metabolite data.

Implementability

No adverse implementation issues are anticipated. BML has an Animal Care and Use Committee on site, authorized by the University of California, Davis to approve/disapprove proposals for research on vertebrate animals. We have submitted this proposal for review and have received approval. The only anticipated delay is securing fish of the right size and age depending on contract start date.

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IV. Costs and Schedule

Salaries and benefits: The Research Assistants (RAs) will be responsible for conducting most of the laboratory experiments (hormone and metabolite assays, and protein electrophoresis). They will be graduate students and the project will comprise their thesis work. They will also assist in fish rearing and tagging. The majority of the fish rearing will be conducted by the BML Animal Care Group. We are requesting 25% of the time of one person for these tasks. Note that none of the principal investigators are requesting any funds for salaries.

Equipment: The refrigerated centrifuge and multiwell plate washer will be used for processing and assaying the blood samples for hormones and metabolites. The flame photometer will be used to measure plasma sodium levels during smoltification. This equipment will be purchased during the first year.

Travel: Travel is requested for Drs. Chang and Schreck (Oregon State University, Corvallis) to make several trips to each other's laboratory for assistance with the hormone assays. Similarly, travel funds are requested for Drs. Clegg and Weber to visit each other's laboratory during the stress protein analysis.

Supplies: Routine laboratory chemicals, glassware, and disposable items are requested. In addition, funds are requested for hazardous waste and radioactive waste removal.

Subcontracts: A subcontract for Lee Blankenship (Washington Department of Fisheries, Olympia) is requested. This will reimburse him for a two week visit to BML each year to assist with the tagging. It includes salary, travel, per diem, and overhead. A subcontract is requested for Dr. Lee Weber (University of Nevada, Reno). This will reimburse him for travel to BML and for conducting aspects of the stress protein work in his laboratory. A graduate student will work in Dr. Weber's laboratory on molecular expression of stress protein genes following tagging. The subcontract includes salary, travel, per diem, laboratory supplies, and overhead.

Contingency planning: If funds become limited, the third year of work could be eliminated, and data would cover effects of tagging on a single year class.

Third Party Impacts

There are no anticipated negative third party impacts that would result from the implementation of this project. The proposed work may have positive implications for hatchery practices if it identifies a tagging method that is superior (e.g., has no deleterious effect on the fish, contains more information) to current marking systems.

Table 1: Schedule Milestones

	1998	1999	2000
TASKS	JFMAMJJASOND	JFMAMJJASOND	JFMAMJJASOND
Tag fish	xxx	xxx	xxx
Develop sampling methods for SPs and hormones	xxxxxx	xxxxxx	xxxxxx
Conduct preliminary experiments with SPs and hormones	xxxxxxxxxxxx		
Conduct full experiment with fish and different tag types		xxxxxxxxxxxx	xxxxxxxxxxxx
Conduct salinity challenges (smoltification)		xxx	xxx
Conduct disease challenges		xx	xx

Table 2a: Budget Detail - Year I

<i>A. Personnel</i>			
EMPLOYEE/ TITLE	SALARY	BENEFITS	TOTALS
Julia Wilsey - Res. Asst.	13,321	400	13,721
TBH - Res. Asst.	13,321	400	13,721
Total Salaries and Benefits			27,442
<i>B. Supplies and Expenses</i>			17,000
<i>C. Other Direct Costs</i>			
Facilities (15,000), Labor (1,500), Feed (1,000), Animal Care (8,222) and Fee Remission for 2 Research Assistants (8,982)			17,704
<i>D. Subcontracts</i>			
University of Nevada, Reno			
Research Assistant	10,646		
Supplies & Expense	3,000		
Travel	1,276		
Indirect Costs @ 44%	6,566		
Total UNR Subcontract			21,488
Washington Department of Fisheries			
Research Assistant	2,498		
Travel	1,276		
Indirect Costs @ 20%	755		
Total Washington Dept. of Fisheries Subcontract			4,529
<i>E. Travel</i>			2,800
<i>F. Equipment</i>			
Plate Washer		4,900	
Refrigerated Centrifuge		5,600	
Flame Photometer		6,000	
Total Equipment			16,500
<i>G. Total Direct Costs</i>			124,462
<i>I. Indirect Costs @ 25.5% of MTDC*</i>			25,240
<i>J. Total Costs</i>			149,702

* MTDC is total direct costs less fee remission (8,982) and less equipment (16,500), or 124,462 - 25,482 = 98,980 x .255 = 25,240

Table 2b: Budget Detail - Year II

A. Personnel

EMPLOYEE/ TITLE	SALARY	BENEFITS	TOTALS
Julia Wilsey - Res. Asst.	13,586	543	14,129
TBH - Res. Asst.	13,586	543	14,129
Total Salaries and Benefits			28,258

B. Supplies and Expenses

17,000

C. Other Direct Costs

Facilities (5,000), Labor (500), Feed (1,000), Animal Care (8,222) and Fee Remission for 2 Research Assistants (8,982)	23,704
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D. Subcontracts University of Nevada, Reno

Research Assistant	11,077	
Supplies & Expense	3,000	
Travel	1,276	
Indirect Costs @ 44%	6,755	
Total UNR Subcontract		22,108

Washington Department of Fisheries

Research Assistant	2,498	
Travel	1,276	
Indirect Costs @ 20%	755	
Total Washington Dept. of Fisheries Subcontract		4,529

E. Travel

2,800

F. Total Direct Costs

98,399

I. Indirect Costs @ 25.5% of MTDC*

18,059

J. Total Costs

116,458

* MTDC is total direct costs less fee remission (8,982) and less portion of UNR subcontract over \$25,000 total, or $\$98,399 - 27,578 = 70,821 \times .255 = 18,059$

Table 2c: Budget Detail - Year III

<i>A. Personnel</i>			
EMPLOYEE/ TITLE	SALARY	BENEFITS	TOTALS
Julia Wilsey - Res. Asst.	13,857	693	14,550
TBH - Res. Asst.	13,857	693	14,550
Total Salaries and Benefits			29,100
<i>B. Supplies and Expenses</i>			17,000
<i>C. Other Direct Costs</i>			
Facilities (5,000), Labor (500), Feed (1,000), Animal Care (8,222) and Fee Remission for 2 Research Assistants (8,982)			23,704
<i>D. Subcontracts</i>			
	University of Nevada, Reno		
Research Assistant	11,530		
Supplies & Expense	3,000		
Travel	1,276		
Indirect Costs @ 44%	6,955		
Total UNR Subcontract			22,761
	Washington Department of Fisheries		
Research Assistant	2,498		
Travel	1,276		
Indirect Costs @ 20%	755		
Total Washington Dept. of Fisheries Subcontract			4,529
<i>E. Travel</i>			2,800
<i>F. Total Direct Costs</i>			99,893
<i>I. Indirect Costs @ 25.5% of MTDC*</i>			17,378
<i>J. Total Costs</i>			117,271

* MTDC is total direct costs less fee remission (8,982) and less portion of UNR subcontract over \$25,000 total, or \$99,893-31,743 = 68,150 x .255 = 17,378

V. Applicant Qualifications:

KRISTEN D. ARKUSH

B.S., Univ. of California, Davis, 1986 (Animal Science).

Ph.D., Univ. of California, Davis, 1994 (Comparative Pathology).

POSITIONS: Research Scientist, EPCOT Center, Walt Disney World, FL, 1992-1993; Staff Research Associate (Supervisor), Bodega Marine Lab., Univ. of California, 1994-present.

QUALIFICATIONS: Over 5 years of experience in working with fish diseases and rearing. Is currently the co-principal investigator on two projects dealing with salmonid aquaculture and conservation at BML.

H. LEE BLANKENSHIP

B.S., Univ. of Washington, Seattle, 1969 (Fisheries)

POSITIONS: Fish Biologist, Washington Dept. of Fisheries, Olympia, 1973-1986; Senior Research Scientist, 1986-present.

AWARDS AND HONORS: Washington Dept. of Fisheries Director's Award of Merit and Commendation Award.

QUALIFICATIONS: Has worked for over 24 years on salmonid biology and management. Has recently participated in several studies on the physiological effects of hatchery tagging.

ERNEST S. CHANG

A.B., Univ. of California, Berkeley, 1973 (Zoology).

Ph.D., Univ. of California, Los Angeles, 1978 (Biology).

POSITIONS: Instructor, Dept. of Biology, Univ. of California, Los Angeles, 1977; Postdoctoral Fellow, Dept. of Biochemistry, Univ. of Chicago, 1978; Assistant Professor, Bodega Marine Lab., Univ. of California, 1978-1985; Associate Professor, 1985-1991; Professor, 1991-present.

AWARDS AND HONORS: American Cancer Society Postdoctoral Fellowship, 1978; National Institutes of Health Postdoctoral Fellowship, 1978; Fellow of the American Association for the Advancement of Science, 1994.

QUALIFICATIONS: Has conducted research on endocrinology for over 19 years. Has developed radioimmunoassays and enzyme-linked immunoassays for various steroid and peptide hormones. Has measured glucose and other metabolites in several species of aquatic animals.

JAMES S. CLEGG

B.S., Pennsylvania State Univ., 1958 (Zoology).

Ph.D., Johns Hopkins Univ., 1961 (Biology).

POSITIONS: Postdoctoral Fellow, Dept. of Biology, Johns Hopkins Univ., 1961-1962; Assistant Professor, Dept. of Biology, Univ. of Miami, FL, 1962-1965; Associate Professor, 1966-1969; Professor, 1970-1985; Professor and Director, Bodega Marine Lab., Univ. of California, 1986-present.

AWARDS AND HONORS: Phi Beta Kappa, 1958; Woodrow Wilson Fellow, 1958-1959; National Institutes of Health Postdoctoral Fellow (1961-1962); Fulbright Senior Research Fellow, 1977-1978; Fellow of the American Assoc. for the Advancement of Science.

QUALIFICATIONS: Has worked for over 36 years in cellular biology. Has worked on stress proteins in aquatic animals during the past 7 years and has become one of the primary researchers in biochemical adaptation.

PAUL A. SIRI

B.S., Univ. of Hawaii, 1972 (Psychology and Zoology).

POSITIONS: Research Biologist, Naval Ocean Systems Center, Kaneohe, HI, 1972-1974; Principal, Biotic Systems, Inc., HI, 1974-1976; Staff Research Associate, Univ. of California, Berkeley, 1977-1980; Management Services Officer, Bodega Marine Lab., Univ. of California, 1980-1985; Assistant Director - Administration, 1986-1995; Associate Director, 1996-present; Expert Consultant, Food and Agriculture Organization of the United Nations, 1997.

QUALIFICATIONS: Has extensive background in managing and operating aquatic systems, particularly with fish. Consultant to a number of educational, state, and federal agencies. Currently project leader for the Winter Run Chinook Captive Broodstock Project at BML.

LEE A. WEBER

B.S., Montclair State College, Montclair, NJ, 1968 (Biology).

M.S., Univ. of Connecticut, Storrs, 1970 (Genetics).

Ph.D., Univ. of Connecticut, Storrs, 1975 (Cell Biology).

POSITIONS: Postdoctoral Fellow, State Univ. of New York, Albany, 1974-1977; Assistant Professor, Dept. of Biology, Univ. of S. Florida, Tampa, 1977-1982; Associate Professor, 1982-1990; Professor and Chair, Dept. of Biology, Univ. of Nevada, Reno, 1990-present.

AWARDS AND HONORS: National Cancer Institute Postdoctoral Fellowship, 1975-1977.

QUALIFICATIONS: Has worked on cellular biochemistry for over 22 years and is one of the primary researchers on the expression of stress proteins in fish.

VI. Compliance With Standard Terms and Conditions

See attached.

NONDISCRIMINATION COMPLIANCE STATEMENT

COMPANY NAME THE REGENTS OF THE UNIVERSITY OF CALIFORNIA

The company named above (hereinafter referred to as "prospective contractor") hereby certifies, unless specifically exempted, compliance with Government Code Section 12990 (a-f) and California Code of Regulations, Title 2, Division 4, Chapter 5 in matters relating to reporting requirements and the development, implementation and maintenance of a Nondiscrimination Program. Prospective contractor agrees not to unlawfully discriminate, harass or allow harassment against any employee or applicant for employment because of sex, race, color, ancestry, religious creed, national origin, disability (including HIV and AIDS), medical condition (cancer), age, marital status, denial of family and medical care leave and denial of pregnancy disability leave.

CERTIFICATION

I, the official named below, hereby swear that I am duly authorized to legally bind the prospective contractor to the above described certification. I am fully aware that this certification, executed on the date and in the county below, is made under penalty of perjury under the laws of the State of California.

OFFICIAL'S NAME Sandra M. Dowdy
Contracts and Grants Analyst

DATE EXECUTED JUL 22 1997 EXECUTED IN THE COUNTY OF YOLO

PROSPECTIVE CONTRACTOR'S SIGNATURE Sandra M. Dowdy
PROSPECTIVE CONTRACTOR'S TITLE

PROSPECTIVE CONTRACTOR'S LEGAL BUSINESS NAME THE REGENTS OF THE UNIVERSITY OF CALIFORNIA