
PART I - ADMINISTRATIVE

Section 1. General administrative information

Title of project

Analyzing Genetic And Behavioral Changes During Salmonid Domestication

BPA project number: 20045
Contract renewal date (mm/yyyy): Multiple actions?

Business name of agency, institution or organization requesting funding
Washington State University

Business acronym (if appropriate) WSU

Proposal contact person or principal investigator:

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NPPC Program Measure Number(s) which this project addresses
7.1.F.2, 7.2.A.1, 7.4.D.1

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

Other planning document references

Technical Recommendations 4 and 5 from Executive Summary, "Spirit of the Salmon"

Short description

Analyze genetic changes occurring during domestication in chinook salmon and steelhead trout by studying selection on mapped DNA markers under wild and hatchery conditions and analyze behavioral and physiological changes using standardized tests.

Target species

Chinook salmon (*Oncorhynchus tshawytscha*), Steelhead trout (*Oncorhynchus mykiss*)

Section 2. Sorting and evaluation

Subbasin

Systemwide

Evaluation Process Sort

CBFWA caucus	Special evaluation process	ISRP project type
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Mark one or more caucus	If your project fits either of these processes, mark one or both	Mark one or more categories
<input checked="" type="checkbox"/> Anadromous fish <input type="checkbox"/> Resident fish <input type="checkbox"/> Wildlife	<input checked="" type="checkbox"/> Multi-year (milestone-based evaluation) <input type="checkbox"/> Watershed project evaluation	<input type="checkbox"/> Watershed councils/model watersheds <input type="checkbox"/> Information dissemination <input type="checkbox"/> Operation & maintenance <input type="checkbox"/> New construction <input checked="" type="checkbox"/> Research & monitoring <input type="checkbox"/> Implementation & management <input type="checkbox"/> 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
	Intracytoplasmic Sperm Injection: Genetic Retrieval from Sperm	also part of UI/ WSU Fish Reproduction program grant
	Endocrine Control of Ovarian Development in Salmonids	also part of UI/ WSU Fish Reproduction program grant
	Induction of Precocious Sexual Maturity and Enhanced Egg Production in Fish	also part of UI/ WSU Fish Reproduction program grant
	Motility and Fertility of Salmonid Gametes	also part of UI/ WSU Fish Reproduction program grant
	Viral Vaccines and Effects on Reproductive Status	also part of UI/ WSU Fish Reproduction program grant

Section 4. Objectives, tasks and schedules

Past accomplishments

Year	Accomplishment	Met biological objectives?
	New project	

Objectives and tasks (FY 2000)

Obj 1,2,3	Objective	Task a,b,c	Task
1	Develop a genetic map of microsatellite markers for chinook salmon.	a	Test primers from other salmonid species for amplification in chinook salmon
		b	Prepare mapping cross for chinook salmon
		c	Identify markers that are polymorphic in

			mapping cross
		d	Map markers to develop chinook salmon map
2	Conduct tests for three behavior patterns in strains of chinook salmon and steelhead trout with varying histories of domestication which have been raised in a common environment from the egg stage.	a	Test for differences in use of water column
		b	Test for differences in startle response
		c	Test for differences in aggressive interactions
3	Investigate physiological responses to acute and sudden environmental stressors in the chinook salmon and steelhead trout with varying histories of domestication.	a	Test for differences in cortisol levels related to startle response
		b	Test for differences in cortisol levels related to handling and restraint
		c	Test for variation in cortisol level in eggs

Objective schedules and costs

Obj #	Start date mm/yyyy	End date mm/yyyy	Measureable biological objective(s)	Milestone	FY2000 Cost %
1	10/1999	09/2002	Develop chinook salmon genetic map	200 marker map	44.8
2	10/1999	09/2004	Identify behavior differences related to domestication	Behavioral assay for domestication	28.7
3	10/1999	09/2004	Identify stress response differences related to domestication	Cortisol assay for domestication	26.5
4	10/2000	09/2004	Correlation of DNA markers with survival in hatchery and wild environments	Possible major gene effects identified related to domestication	0
5	10/2000	09/2004	Measure levels of selection in hatchery and wild environments	Information on selection levels in hatchery & wild environments	0
				Total	100

Schedule constraints

Completion date

09/2004

Section 5. Budget

FY99 project budget (BPA obligated): \$ 0

FY2000 budget by line item

Item	Note	% of total	FY2000
Personnel	0.5 month salary (summer) each for Thorgaard, Schwabl and Verrell (co-PIs), two postdoctoral fellows (12 months each), one semester support for graduate research assistant	33.8	70,924
Fringe benefits	Benefits for the above six employees	9.9	20,874
Supplies, materials, non-expendable property	Molecular biology supplies for genetic mapping studies (\$15,000), Materials for behavior testing and fish rearing (\$3,000), Supplies for stress hormone testing (\$6,000)	11.4	24,000
Operations & maintenance			-
Capital acquisitions or improvements (e.g. land, buildings, major equip.)	PCR machine for genetic mapping studies (\$4,000), Video equipment for behavior studies (\$10,000)	6.7	14,000
NEPA costs			-
Construction-related support			-
PIT tags	# of tags:		-
Travel	Travel to collect gametes and attending a scientific meeting	0.5	1,000
Indirect costs	45% of direct costs excluding equipment and student tuition	28.6	59,922
Subcontractor			-
Other	Costs of aquaculture core lab (\$15,000) and administrative core for Fish Reproduction Program (\$4,000)	9.1	19,000
TOTAL BPA FY2000 BUDGET REQUEST			209,720

Cost sharing

Organization	Item or service provided	% total project cost (incl. BPA)	Amount (\$)
Total project cost (including BPA portion)			

Outyear costs

	FY2001	FY02	FY03	FY04
Total budget	203,549	211,741	220,120	229,120

Section 6. References

Watershed?	Reference

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PART II - NARRATIVE

Section 7. Abstract

A serious concern in regional salmon and steelhead management programs is that hatchery rearing may select genetically for fish with reduced viability in the wild. It is important to understand the types of genetic and behavioral changes that may be occurring during the domestication process. We propose to investigate domestication-related genetic and behavioral changes using controlled crosses of strains of chinook salmon and steelhead trout with varying degrees of hatchery ancestry. A genetic map of microsatellite markers will be developed for chinook salmon and this map, together with an already-developed map for steelhead, will be used in crosses that test for DNA markers associated with the ability of chinook salmon and steelhead trout to survive in hatchery or wild environments. We will also measure the level of selection occurring in populations of chinook salmon and steelhead trout in hatchery and wild environments by measuring the levels of fluctuating asymmetry of the same populations reared in these environments. Behavior patterns and physiological responses to acute and sudden environmental stressors of wild and hatchery strains of both species which have been raised in a common environment from the egg stage will be characterized. On completion of this study, we will have initiated the characterization of the types of genetic changes associated with domestication in chinook salmon and steelhead trout, and will have identified standard behavioral and physiological tests that can be used to monitor levels of domestication in these species.

Section 8. Project description

a. Technical and/or scientific background

Domestication in salmonids and its possible impact on survival in nature

It is currently estimated that at least 300 salmonid strains are at risk of extinction in the Pacific Northwest (Allendorf et al. 1997). Factors responsible include overexploitation, introduction of exotic taxa, pollution, degradation of breeding habitats and especially the disruption of migratory routes, primarily by the effects of dams on downstream migration of smolts. Consequences of extinction include erosion of a resource of considerable economic value, cultural disturbance (especially for Native Americans) and loss of biodiversity. Disruption at the level of the ecosystem also is likely, given the role of many salmonids as keystone species in food webs (e.g., Willson & Halapka 1995).

Much effort and many dollars are being spent to conserve dwindling stocks of salmonids in our region. Rearing of young fish in hatcheries is an important component of this effort; the goals have been to mitigate the effects of habitat changes and to augment natural populations by reintroduction or translocation. Recently, captive breeding programs which involve propagation in hatcheries through entire life cycles have also been initiated for some endangered populations. However, rearing fish in hatcheries may carry certain costs (Conway 1980; Foose 1986; Lyles & May 1987; Kohane & Parsons 1989). Environmental conditions in hatcheries differ greatly from those in the wild, and fish may undergo a process of genetic domestication in response to selection in captivity during early life (for general discussions of behavioral domestication, see Hafez 1962; Boice 1973; Clutton-Brock 1992). In a typical hatchery, fish are reared at high densities with large quantities of food and few, if any, predators. Such conditions likely will, over successive generations, select for genotypes that differ from those of young fish produced by wild parents. Artificially-selected genotypes may actually hinder, not help, in attempts to augment natural populations. If domestication results in competitively superior fish, then released fish might 'swamp' their natural counterparts demographically and genetically. If domesticated fish are competitively inferior after release (the most likely outcome), then they may fail to thrive and survive. And, if such domesticated fish carry alleles that decrease fitness in the wild and if they breed with wild fish, then hybrid/backcross individuals may suffer decreased survivorship (a phenomenon akin to outbreeding depression: Templeton et al. 1986). There is indeed some evidence that hatchery strains of anadromous salmonids may not survive as well in nature as do wild strains (Reisenbichler and McIntyre, 1977; Leider et al., 1990). We believe that it is crucial to seek to understand the process of genetic domestication from genetic, behavioral and physiological standpoints. We seek support to genetically characterize differences between hatchery and wild genotypes of chinook salmon and steelhead trout, and to develop tests to measure aspects of organismal performance that likely are changed by domestication.

Genetic mapping and analysis of the genetic control of complex traits

Recent advances in molecular genetic technology have made it possible to detect high levels of genetic variation in animal populations (Avisé, 1994). The availability of such DNA markers has made it possible to make detailed comparisons among salmonid populations (Ferguson et al., 1995). These methods have also enabled researchers to produce detailed genetic maps for numerous species of plants and animals. Such maps allow valuable comparisons of the arrangements of genes on chromosomes to be made

among species (Lyons et al., 1997). However, their greatest benefit may lie in the ability to follow the inheritance of the markers in controlled genetic crosses to better understand the nature of the genes controlling complex traits. A large number of genetically-mapped markers can be simultaneously tested for association with a trait of interest that is segregating in a test population. This approach of quantitative trait locus (QTL) analysis has made it possible to begin to characterize how many genes are responsible for differences between strains of plants and animals, and where those genes are located on the chromosomes (Lander and Botstein, 1989; Lynch and Walsh, 1998; Paterson, 1998).

QTL studies are in their infancy for salmonids (e.g., Jackson et al., 1998). Genetic maps are beginning to be developed. The Thorgaard laboratory at Washington State University has this year published the most detailed genetic map to date for a salmonid, the rainbow trout (Young et al., 1998). The intent is to characterize the genetic basis of traits of evolutionary and aquacultural importance in rainbow and steelhead trout using this map as a foundation.

We propose to similarly develop a genetic map for chinook salmon and to use QTL analysis to seek to identify major genes associated with the domestication process in chinook salmon and steelhead trout. The chinook salmon genetic map could also serve as a foundation for genetic analysis of other complex traits in that species.

Fluctuating asymmetry and selection in hatchery and wild environments

Fluctuating asymmetry refers to random differences in meristic counts observed between measures on the left and right side of an animal. This measure has been shown to increase when animals are inbred (Leary et al., 1985). In general, the level of fluctuating asymmetry is correlated with heterozygosity as detected by protein electrophoresis (Leary et al., 1983).

Fluctuating asymmetry can be used to document the extent of selection occurring on fishes in hatchery and wild environments (Moran et al., 1997). A recent study in Spain on Atlantic salmon (*Salmo salar*) demonstrated that, in a group of fish divided into hatchery and stream environments, the surviving hatchery-reared fish showed much higher levels of asymmetry. This result implies that greater selection was occurring in the wild than in the hatchery. We propose during the later years of this project to conduct a similar study for chinook salmon and steelhead while at the same time testing for DNA markers correlated with viability in hatchery and wild environments.

Behavioral changes associated with domestication in salmonids

Salmonid hatcheries likely select genetically for young fish that are bold and hard to frighten relative to their wild counterparts. In reviewing the literature on this topic, we deliberately excluded studies in which hatchery-raised fish were derived from completely wild parents (e.g., work on *Salmo salar* by Fenderson and Carpenter 1971). In such instances, differences between hatchery- and wild-raised fish are most likely due to experiential factors rather than domestication (Kleiman 1989). We also excluded studies that did not clearly state the geographic origin(s) of the fish involved (e.g., work on *Oncorhynchus clarki* by Mesa 1991). We repeat Moyle's (1969) plea for inclusion of full documentation of strain histories in published reports.

The resulting six studies of four taxa involved hatchery fish with different histories of genetic domestication, varying from as few as four generations to as long as

90 years! Despite differences in testing protocols, at least four of these studies attempted to 'match' hatchery and wild fish by location of origin, and all raised the two types of fish under common conditions (to control for confounding experiential effects). The major results obtained in these studies are summarized in Table 1. Despite considerable methodological heterogeneity among these studies, their results provide support for our prediction that domestication increases the boldness of young fish. There is also evidence for increased aggressiveness in hatchery fish, although this effect may be at least partially size-dependent. We believe that the results presented in Table 1 justify continued research on the behavioral effects of domestication in salmonid fishes.

TABLE 1. Results of six studies of four salmonid species in which behavioral performances of the progeny of hatchery and wild parents were compared.

Salvelinus fontinalis (brook trout)

Hatchery fish were less likely to use cover and more likely to orient to the surface (Vincent 1960).

Hatchery fish were more likely to move through the whole water column (rather than remain near the bottom), and were more aggressive (Moyle 1969).

Salmo trutta (brown trout)

Hatchery fish were less likely to be alarmed by a trout predator (Johnsson et al. 1996)

Oncorhynchus kisutch (coho salmon)

Hatchery fish were more aggressive (Swain and Riddell 1990).

Oncorhynchus mykiss (steelhead trout)

Domesticated fish* were less likely to be alarmed by a trout predator (Johnsson and Abrahams 1991).

Hatchery fish were more aggressive if given a modest size advantage over wild fish (Berejikian et al. 1996).

* Domesticated fish were hybrids between steelhead and domesticated rainbow trout.

After reviewing the work of other 'behavioral conservationists' we believe that the time is ripe for the development of tests of domestication's effects on organismal performance in young salmonids that meet the following criteria:

- 1) They must be straightforward operationally, requiring minimal specialist training of personnel.
- 2) They must be standardized, repeatable and applicable across taxa.
- 3) They must assay performance traits of clear significance with respect to fitness (e.g., Arnold, 1988).

Our goal is to work collaboratively to develop tests that will produce data to both guide and inform managers in making decisions about the likely impacts of releasing

domesticated fish. We identify three aspects of organismal performance whose assay will facilitate our goal:

- 1) Use of the water column, where tendency to orient toward the surface indicates 'boldness' (perhaps beneficial in terms of food intake, but detrimental in terms of predation risk).
- 2) Behavioral and endocrine 'startle responses' to an environmental stimulus that is stressful (perhaps influencing predation risk and/or energy balance).
- 3) Intensity of aggressive interactions (which may result in injury, and may influence predation risk and/or energy balance).

Our null hypotheses are that the progeny of hatchery-raised and wild parents will not differ in boldness, startle response or aggressive behavior. After *in vitro* fertilization, young fish will be reared under 'common garden' conditions before testing to truly isolate genetic domestication from, say, experiential influences on performance (see McLean 1997). Successful rejection of some, any or all of these null hypotheses should, we suggest, alert managers to possible undesirable consequences of releasing domesticated fishes and could indicate that objective tests of domestication levels might be applied to salmonid populations.

Physiological changes associated with domestication of salmonids

The brain-pituitary-adrenal axis of tetrapods (homologous to the hypothalamic-pituitary-interrenal axis in fishes) is an important determinant of performance in response to environmental challenges. Cortisol, the major teleost corticosteroid released during stress, modifies metabolic and behavioral processes (Schreck, 1993; Sumpter, 1997). We propose that domestication might have influenced this axis, resulting in behavioral and metabolic differences. Modified stress responses might affect the behavior of the adult fish as well as the development of fry, because it has been shown that maternal steroids are transferred into fish eggs (Schreck et al., 1991) and that maternal cortisol is transferred into eggs, where it influences larval development (McCormick 1998).

b. Rationale and significance to Regional Programs

Hatchery propagation of salmon and steelhead is one of the central approaches being used in the efforts to supplement and restore populations in the Columbia River system. The nature of genetic and behavioral changes that take place in hatcheries and under captive breeding protocols appears to be one of the central controversies in fish management in the region. Some believe that the changes are minimal and unlikely to have significant impacts if hatchery fish interbreed with wild fish. Others believe that the changes are substantial and that interbreeding could be highly detrimental to wild fishes. We are seeking to address the problem in a systematic, experimental fashion using approaches of genetic, behavioral and physiological analysis which have not been exploited to date.

c. Relationships to other projects

This project could have significant interactions with other BPA projects. For example, Dr. Jeffrey Hard of the National Marine Fisheries Service is investigating genetic effects of captive broodstock rearing, and our studies on genetic and performance changes occurring during the domestication process would interface

with his project. Similarly, Dr. Reg Reisenbichler of the U.S.. Geological Survey is investigating supplementation with field studies (BPA Project 9005200), and some of the work we propose would interface with his project. Interactions with other projects in the UI/ WSU Fish Reproduction program would also be significant.

d. Project history (for ongoing projects)

New project

e. Proposal objectives

(1) **Develop a genetic map of microsatellite markers for chinook salmon.** This genetic map will be used in objective (4) in the assessment of genetic changes associated with domestication in this species. Numerous microsatellite markers have been developed for salmonids and we will seek to map at least 200 of these markers in a cross that should be highly polymorphic for such markers.

(2) **Conduct tests for three behavior patterns in strains of chinook salmon and steelhead trout with varying histories of domestication which have been raised in a common environment from the egg stage.** Comparisons would be made regarding use of the water column, startle response and aggressive interactions for the various strains. The hypothesis is that fish with hatchery ancestries will be more bold than fish with wild ancestries for all the tests.

(3) **Investigate physiological responses to acute and sudden environmental stressors in the chinook salmon and steelhead trout with varying histories of domestication.** The hypothesis is that fish with wild ancestries will show a greater physiological response to environmental stressors than will fish with hatchery ancestries. We propose to measure cortisol levels, as an endpoint of hypothalamic-pituitary-interrenal activation, in wild and hatchery chinook salmon and steelhead trout in response to acute and sudden environmental stressors. We also propose to measure levels of cortisol in the eggs of females reared in a common environment. That will allow us to assess whether domestication resulted in a modification of the brain-pituitary-adrenal axis which might influence adult behavior and metabolism. Finally, since cortisol is deposited in eggs we propose to measure levels of this corticosteroid in freshly laid, undeveloped eggs of wild and domesticated fish. This will allow us to assess whether domestication may result in changes of phenotype caused by maternal hormone exposure.

(4) **Test for DNA markers associated with the ability of chinook salmon and steelhead trout to survive in hatchery or wild environments.** We will first produce progeny of steelhead trout and chinook salmon that are the offspring of wild X hatchery parents backcrossed to hatchery parents. These backcross progeny will be raised in two environments: a typical hatchery environment, and a contained wild environment. After mortality has occurred in both environments, we will monitor DNA marker segregation in the surviving progeny. The hypothesis is that markers in particular chromosome regions may be associated with ability to survive in the hatchery or wild environments. This portion of the project relies on prior development of the chinook salmon genetic map, and would be initiated in FY 2001.

(5) Measure the level of selection occurring for chinook salmon and steelhead trout in hatchery and wild environments using fluctuating asymmetry measurements.

We will test the levels of fluctuating asymmetry in the backcross progeny raised in the two environments. The hypothesis is that fluctuating asymmetry may be lower in the wild environment than in the hatchery environment due to more intense selection in the former. We will test the hypothesis by statistically comparing the levels for the same groups of fish raised in the two environments. This portion of the project would also be initiated in FY 2001.

f. Methods

We propose a collaborative, interdisciplinary effort in our project to understand fundamental processes involved in domestication in salmonid fishes. Gary Thorgaard is a geneticist who has considerable expertise with salmonid fishes. He has recently become involved with the genetic mapping of salmonids and with efforts to elucidate the genetic control of complex traits (Young et al., 1998; Robison et al., in press). Paul Verrell is an animal behaviorist who has conducted numerous manipulative and quantitative studies of behavior in lower vertebrates since 1979. Hubert Schwabl is a vertebrate endocrinologist interested in environmental effects on physiological processes. Ted Bjornn of the University of Idaho has indicated an interest in participating in outyear objectives (4 and 5) that involve field studies. He is a salmonid ecologist with extensive experience on studies of fish in both natural and artificial environments. We believe that we can make progress through cooperation that none of us could achieve independently.

Our goal is to compare genetic, behavioral and physiological measures of performance between juvenile spring chinook salmon and steelhead trout with varying histories of hatchery rearing. Four strains will be studied for each species; one pair of wild and hatchery fish of coastal ancestry and one pair of inland ancestry. At this time, although we are open to discussing these issues with experienced managers, we believe that the following pairs of strains may be the best choices for study: (1) coastal spring chinook: domesticated: White River, wild: Skagit River, (2) inland spring chinook: domesticated: Rapid River, wild: Imnaha River, (3) coastal steelhead: domesticated: Chambers Creek, wild steelhead: Snow Creek, (4) inland steelhead: domesticated: Dworshak hatchery, wild steelhead: Selway or Lochsa. We have a history of contact with a number of the relevant agencies and management personnel already, but will need to make specific, detailed arrangements if project funding is confirmed. These strains are selected to maximize the chances that we will detect differences attributable to domestication. Spring chinook are chosen because of concerns for their conservation and the longer time that they may spend in hatcheries compared to fall chinook.

Artificial fertilizations will be initiated in the falls of both 1999 and 2000 for chinook and the springs of both 2000 and 2001 for steelhead. Eggs will be obtained from at least three females per hatchery strain. Sperm will be collected from both hatchery and wild fish and will be stored using standard protocols until used in fertilizations. The experimental comparisons will be between pure hatchery fish (domesticated X domesticated) and hybrids with wild fish (domesticated X wild). In this manner, no wild females will be required for the experiments, greatly facilitating collection and permit approval. This will also ensure that genetic and not maternal effects will be responsible

for any differences detected, and that fish of the same developmental stages will be compared.

Juveniles of the eight groups (coastal hatchery, coastal hybrid, inland hatchery, inland hybrid for chinook; coastal hatchery, coastal hybrid, inland hatchery, inland hybrid for steelhead) will be reared under identical conditions ("common gardens") to ensure that any differences detected are genetic in origin. We acknowledge that detailed studies of individual families are of potential interest, but we will focus on interstrain differences in the present work. Year 1 of our study will be devoted to perfecting and validating our experimental protocols and assays. In subsequent years we will be positioned to compare all eight groups of fishes, and collect definitive data of high quality. If the assays prove to be highly repeatable and successful early in the project, we envision applying them to develop a domestication inventory of basin stocks in the later years of the project.

Specific experimental approaches will be described for each of the experimental objectives.

(1) Develop a genetic map of microsatellite markers for chinook salmon.

The chinook salmon genetic map will be developed using methods that we have already used successfully for rainbow trout (Young et al., 1998). We will obtain sperm in the fall of 1999 from two male chinook salmon, one from a coastal population and one from an inland population, both known to have high levels of protein heterozygosity. These fish will be used to produce androgenetic diploid progeny as we have done for rainbow trout. The progeny should be available for mapping beginning in February 2000. We would seek to produce at least 100 progeny from each of the two males for the mapping study; this should be a sufficient number since an excellent map was produced using 67 progeny in our rainbow trout study. Such homozygous fish greatly facilitate the interpretation of the inheritance of DNA markers. The use of outbred males to produce such homozygous progeny is analogous to mapping efforts by Allendorf with pink salmon (Allendorf and Spruell, personal communication) and Kocher with tilapia (Kocher et al., 1998). We will use the Mapmaker computer program to construct the map, as we have already done for rainbow trout (Young et al., 1998). We hope to map 200 microsatellite markers and anticipate that this will take three years of effort, with completion of this objective expected in September 2002. A similar number of microsatellite markers are now being mapped in rainbow trout at the University of Guelph (R. Danzmann, personal communication) and we would plan to use the same microsatellite primers being used in that study.

(2) Conduct tests for three behavior patterns in strains of chinook salmon and steelhead trout with varying histories of domestication which have been raised in a common environment from the egg stage

Fish will be reared under standard conditions long in use in Thorgaard's laboratory. Utmost care will be taken to ensure that conditions of rearing are identical between strains and between species. We believe it important to conduct all trials in an experiment on fish of comparable post-emergence age and body size. If necessary, we will achieve such matching by manipulation of developmental rate through temperature, as done by Berejikian et al. (1996). Individual fish will be used only once (i.e., in a single trial). This will avoid confounding effects that may result from repeated testing, and also preserve statistical independence in our data sets.

Behavioral observations will be made in glass aquaria, the walls of which will be marked with horizontal lines at equidistant intervals. Thus the position of a fish in the water column will be observable when viewed laterally. Behavioral trials will be observed directly (from behind a screen) and also videotaped for later analysis. Unless dictated by the experiment, fish will be placed singly into aquaria in the absence of food.

Experiment a. Ho: domestication has no effect on use of the water column

Each fish will be tested alone, with each trial lasting for a total duration of 60 min under constant illumination. Two treatments will be included in this experiment: (1) No food provided throughout the trial, and (2) Food provided 30 min after introduction of the fish, remotely sprinkled onto the water surface and left there for the remaining 30 min. Experiments would be conducted with at least 15 fish per strain, or a minimum total of 120 individuals.

For all fish, the amount of time spent at each level in the water column will be recorded continuously. In addition, the number of discrete feeding trips to the surface will be counted for fish in the second treatment. Comparisons of position in the water column during the first 30 min of the observation period will be made between strains within species, using the nonparametric Mann-Whitney test. This will be one-tailed because we predict that domesticated fish will orient more to the surface and less to the bottom than wild fish. A similar analysis will be conducted for the second 30 min period, comparing strains within species for the use of space with food provided. We again predict greater surface orientation in domesticated fish. Finally, we will compare between strains the use of space for individual fish before and after provision of food, using the Wilcoxon test. This will be one-tailed for we predict less of a change in the behavior of domesticated versus wild fish once food is made available.

Experiment b. Ho: domestication has no effect on the behavioral 'startle response'

For each strain, an individual fish will be tested singly in one of two treatments: (1) No change in illumination 20 min after introduction into the aquarium, and (2) A sudden and brief change in illumination at the 20 min mark. Experiments would be conducted with at least 15 fish per strain, or a minimum total of 120 individuals.

For each fish, use of the water column will be compared in the 10 min period preceding and 10 min period following the 20 min mark. Comparisons between groups of fishes within strains (startled or not) will be made using the Mann-Whitney test (one-tailed). Comparisons of activity scores for individual fish before and after startling will be made with the Wilcoxon test (one-tailed). We predict that domesticated fish will show no or a reduced behavioral response to a sudden environmental change (no change in use of the water column or a faster return to control/prechange levels).

Experiment c. Ho: domestication has no effect on aggressive interactions between pairs of fish

In this experiment, two fish matched for age and body size will be placed together in an aquarium. Because all trials will be videotaped, the individual activities of each fish will be tracked by following its position on a video monitor. Experiments would be conducted with at least 15 fish per strain, or a minimum total of 120 individuals.

Three sets of trials will be conducted per species: (1) Reciprocal homotypic encounters: domesticated X domesticated and domesticated X wild, and (2) Heterotypic encounters: domesticated X hybrid.

Within a trial, fish will be introduced simultaneously and the identity of each indicated for the video camera. We will record continuously for a period of 30 min all events occurring in the aquarium (position of each fish in the water column and the aggressor and aggressee in any physical interactions). Comparisons between sets of trials will be made using the Mann-Whitney test (one-tailed). We predict that domesticated fish will show higher rates of aggression than the hybrid wild fish (comparing the two types of reciprocal homotypic encounters). We also predict that, in heterotypic encounters, domesticated fish will deliver the highest and receive the lowest frequencies of aggressive nips.

Overall, we expect that, relative to the progeny with hybrid ancestry, domesticated fish will: (1) Use more levels of the water column, and show less orientation to the bottom and more to the surface, (2) Show no or a reduced behavioral startle response to a sudden environmental stress (no change in use of the water column or a faster return to control levels), and (3) Be more aggressive in both homotypic and heterotypic encounters with other young fish.

(3) Investigate physiological responses to acute and sudden environmental stressors in the chinook salmon and steelhead trout with varying histories of domestication.

Ho: domestication has no effect on the physiological 'stress response'

a) Using the same general protocol as in Experiment b for Objective 2 above, fish from each strain will be tested individually in one of two treatments: (1) No change in illumination 20 min after introduction into the aquarium (control), or (2) A sudden and brief change in illumination at the 20 min mark (experimental). Experiments would be conducted with at least 10 fish per strain per time point.

In the control group (no change at 20 min), fish will be rapidly sacrificed and bled into heparinized tubes at 19 min, 21 min and 40 min post-introduction. In the experimental group (with change at 20 min), fish will be sacrificed and bled at 21 min, 30 min and 40 min. Standard radioimmunoassay procedures will be used to determine plasma concentrations of cortisol in all fish, and so reveal the magnitude of cortisol response (levels typically become elevated within a few minutes in vertebrates) and the time course of recovery to control levels. We will reject our null hypothesis if comparisons of cortisol concentrations (using Mann-Whitney tests) yield the following results:

- 1) Control at 19 min = control at 21 min = control at 40 min.
- 2) Control at 19 min < experimental at 21 min.
- 3) Control at 21 min < experimental at 21 min.
- 4) Experimental at 21 > experimental at 30 > experimental at 40 min (to chart the time course of recovery).

We expect that, relative to the progeny of wild parents, those of domesticated fish will show no or a reduced endocrine startle response to a sudden environmental stress (no or a reduced magnitude of cortisol increase, and/or a faster return to control levels).

b) Cortisol response of fish to handling and restraint, a potent activator of the hypothalamic-pituitary-interrenal axis.

H0: There is no difference in basal and stress-induced cortisol levels between domesticated and wild fish.

We will capture and confine fish in a net and sacrifice and blood sample them 0 (capture; basal levels), 5, 10, 20, 40, and 60 min after capture and confinement (each: n=10). We will measure cortisol in the plasma using standard RIA (radioimmunoassay) (Pankhurst and Carragher 1992). This will allow us to determine the time course of the activation of the brain-pituitary-interrenal axis in response to a potent stressor in both domesticated and hybrid chinook salmon and steelhead trout. We expect that domesticated fish will show a blunted cortisol stress response as compared to the hybrid conspecifics.

c) Cortisol content of freshly spawned eggs

H0: There is no difference in the cortisol contents of eggs from domesticated and wild fish.

We will determine the cortisol concentrations in newly spawned eggs by standard radioimmunoassay modified after McCormick (1998). Briefly, a known number (500-650, actual number will depend on cortisol levels) of eggs of 10 females of each strain will be homogenized in distilled water, vortexed and centrifuged. The supernatant will then be extracted with ethylacetate and cortisol measured in duplicates after samples are redissolved in phosphate buffer. Extraction efficiency will be determined for each batch of eggs by adding 2000cpm of cortisol prior to homogenization. We expect that cortisol levels will be lower in the eggs of domesticated than wild fish because of a blunted brain-pituitary-interrenal function in response to environmental stressors (see above). This will require obtaining a limited number of eggs from wild chinook salmon; these fish may be from non-endangered populations such as the Hanford Reach fall chinook.

(4) Test for DNA markers associated with the ability of chinook salmon and steelhead trout to survive in hatchery or wild environments.

The test for DNA marker association, to be initiated in FY 2001, will be modeled on approaches that we are already using to investigate the genetic control of development rate in rainbow trout (Robison et al., in press). These involve backcrossing a strain that is segregating (heterozygous) for a trait of interest to a uniform strain. In this case, we would cross hatchery X wild hybrid males (the same types of fish used under objectives 2 and 3) back to hatchery females for each of the four combinations (inland and coastal spring chinook, inland and coastal steelhead). The fish would be planted out into two environments: a standard hatchery environment (possibly at the University of Idaho's Hagerman research facility), and a confined wild environment. We are aware that studies are already proceeding in confined wild environments in our region (e.g., BPA project 9005200 by Reisenbichler; T. Bjornn, personal communication) and if funding is available, we will negotiate in an effort to conduct studies in cooperation and complementation with the current studies.

The experimental design will be as follows: hybrids between the hatchery and wild parents for each of the four strain pairs (inland and coastal chinook, inland and coastal steelhead) produced during FY 2000 would be reared at WSU and induced to mature at one year with hormone treatments (pituitary extract injections, 1 mg/kg body weight twice weekly). The fish would be backcrossed to the appropriate hatchery fish during FY 2001 and reared under common environmental conditions. During their first year when conditions were favorable in the confined wild environment, each group would be divided and a portion transferred to the hatchery or confined wild environment. After one year in the environments, the fish would be sampled and DNA markers tested

in groups from both environments. We expect that some markers may be associated with survival advantages in each environment and these could mark regions associated with domestication-related genes. These analyses would be conducted using the Mapmaker QTL computer program. Since the experiments would be replicated with three crosses per strain pair, two strain pairs per species, and two species, they have a reasonable chance of identifying fundamental processes associated with domestication in salmonids. We estimate that a minimum of 100 fish per cross would be needed in order for the marker association studies to be successful.

These studies would not be initiated until FY 2001, so if the first project year of funding is provided we would immediately initiate contacts to arrange to obtain the appropriate gametes and obtain approvals for transfers and field studies.

(5) Measure the level of selection occurring for chinook salmon and steelhead trout in hatchery and wild environments using fluctuating asymmetry measurements.

Levels of fluctuating asymmetry will be measured on the backcross progeny being tested for DNA marker association under objective (4). The same crosses and fish described above would be used. Forty fish from each cross would be removed at the time the crosses are split for transfer to the hatchery and wild environments, and the following bilateral meristic traits would be measured on each sacrificed individual: pectoral fin rays, pelvic fin rays, gillrakers on upper first arch, gillrakers on lower first arch. The same measures would be taken on fish after one year in the hatchery or wild environments. The mean number of asymmetric characters would be then be calculated for all individuals and compared using the Wilcoxon two-sample test. The prediction is that the number of asymmetric traits may be higher in fish from the hatchery environment than in fish from the wild environment, as has been observed for Atlantic salmon (Moran et al., 1997). This would provide a measure of the level of genetic selection that occurs on fish in the two environments. From the distribution of numbers of asymmetric characters among the early sample (before splitting) and the hatchery and wild groups it might be possible to estimate the selection differential in the hatchery and wild environments.

g. Facilities and equipment

Our laboratories are generally very well equipped for this project. Thorgaard's lab has an excellent assortment of molecular biology equipment, including PCR machines, centrifuges, water baths, and agarose gel electrophoresis units. We also have access to an ABI 377 automated DNA sequencer which would be used for the microsatellite mapping studies in chinook salmon. We are requesting one additional PCR machine as equipment to insure that overcrowding at that key experimental step does not delay progress of the project. Thorgaard's lab also has adequate fish rearing space for the juvenile rearing experiments proposed in this project. Schwabl's laboratory is fully equipped for hormone assays and no additional equipment is requested. Verrell's lab has the necessary expertise for the behavior studies; funds to purchase video equipment to be dedicated for use on this project are requested.

h. Budget

Funds are requested for one-half month summer salary for the three co-principal investigators (Thorgaard, Schwabl, Verrell). Each would also provide 5% effort during the 9-month academic year to the project at no cost to BPA. Each would assist in project planning and report preparation, focusing on their respective areas of expertise (genetics, physiology, behavior). Funds are requested for two postdoctoral fellows; one

would work full time in Thorgaard's lab on the genetic mapping project (objective 1) and the other would work between the Schwabl and Verrell labs on the behavior and physiology objectives (2 and 3). Funds for one semester of support for a graduate student working on the behavioral and physiological objectives of the project, Megan Hines, are also requested. Funds for travel to collect gametes and to attend a scientific meeting are also requested. Equipment requested during year one includes a PCR machine (for Thorgaard lab, objective 1) and video equipment (for Verrell lab, objective 2). Supply funding is requested for molecular biology costs associated with genetic mapping in chinook salmon (\$15,000), costs of physiological tests including cortisol assays (\$6,000), and cost of modification of fish rearing facilities for behavior tests and of videotape and computer supplies connected with the behavior tests (\$3,000). Costs for the aquaculture core facility (\$15,000; for rearing of fish, including personnel costs) and the administrative core facility (\$4,000) are included, as well indirect costs at the negotiated indirect costs rate for Washington State University.

Section 9. Key personnel

Name: Gary H. Thorgaard

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Born: December 19, 1950 in Portland, Oregon

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Citizenship: USA

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Degrees:

1973 B.S. Zoology, Oregon State University, Corvallis, Oregon

1977 Ph.D. in Genetics, University of Washington, Seattle, Washington

Current employment:

1991-present Professor and Chair, Department of Zoology, Washington State University. A member of the WSU faculty since 1979.

Previous employment:

1978-79. Postdoctoral research geneticist, University of California, Davis, California. Research on chromosomal variation in rainbow trout populations.

Current responsibilities:

Teaching: General Genetics, Fish Genetics, Fish Biology, Conservation Genetics

Research: Genetics of salmon and trout

Administration: As department chair, I administer a department with 20 faculty, 25 graduate students and 125 undergraduates. This position ends in August 1999.

Expertise:

My expertise is in the genetics of trout and salmon. Particular areas of expertise include the study of chromosomal variation, the utilization of DNA marker methods for differentiating populations, and the development of genetic maps.

Five Publications Relevant to the Proposed Project:

1. Thorgaard, G.H, 1992. Application of genetic technologies to rainbow trout. Aquaculture 100: 85-97.
2. Cloud, J.G. and G.H. Thorgaard, eds., 1993. Genetic Conservation of Salmonid Fishes. NATO ASI Series A: Life Sciences Vol. 248. Plenum Press, New York, 314 pp.
3. Palti, Y., J.E. Parsons and G.H. Thorgaard, 1997. Assessment of genetic variability among strains of rainbow and cutthroat trout using multilocus DNA fingerprints. Aquaculture 149: 47-56.
4. Cummings, S.A., E.L. Brannon, K.J. Adams and G.H. Thorgaard, 1997. Genetic analyses to establish captive breeding priorities for endangered Snake River sockeye salmon. Conservation Biology 11: 662-669.
5. Young, W.P., P.A. Wheeler, V.H. Coryell, P. Keim and G. H. Thorgaard, 1998. A detailed linkage map of rainbow trout produced using doubled haploids. Genetics 148: 839-850.

Name: Hubertus Georg Schwabl

Department of Zoology, Washington State University, Pullman WA 99164-4236
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Born: March 17, 1951 in Bad Reichenhall, Germany

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Citizenship: German, US permanent resident

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Degrees:

1979 Diplom in Biology, Ludwig-Maximilian-University, Munich, Germany.

1981 Ph.D. in Zoology, Ludwig-Maximilian-University, Munich, Germany.

1989 Habilitation in Zoology, Ludwig-Maximilian-University, Munich, Germany.

Current employment:

1996- Associate Professor, Department of Zoology, Washington State University.

Research: Environmental Physiology and Development.

Previous employment:

1984-89. Fellow of the Deutsche Forschungsgemeinschaft and the Max-Planck-Society at the Max-Planck-Institute for Behavioral Physiology, Seewiesen, Germany. Research into the mechanisms of seasonal and circadian physiological and behavioral rhythms, and the environmental and hormonal control of behavior.

1990-96. Assistant Professor at The Rockefeller University Field Research Center for Ecology and Ethology. Research in environmental endocrinology: Hormonal and behavioral responses to environmental changes; role of hormones in development and brain function.

Current responsibilities:

Teaching: Human physiology, mammalian physiology.

Research: Environmental physiology, endocrinology.

Expertise:

My expertise is in the area of vertebrate endocrinology, neuroendocrinology, and environmental biology. We use radioimmunoassays to measure steroid and peptide hormones, and molecular techniques of neuroanatomy (in situ hybridization, immunocytochemistry).

Five Publications Relevant to the Proposed Project:

1. Schwabl, H., F. Bairlein and E. Gwinner. 1991. Basal and stress-induced corticosterone levels of garden warblers, *Sylvia borin*, during migration. *J. Comp. Physiol. B.* 161: 576-5801.
2. Gwinner E., M. Zeman, I. Schwabl-Benzinger, S. Jenni, L. Jenni and H. Schwabl. 1992. Corticosterone levels of passerine birds during migratory flight. *Naturwissenschaften* 79: 276-278.
3. Schwabl, H. 1993. Yolk is a source of maternal testosterone for developing birds. *Proc. Natl. Acad. Sci. USA* 90: 11439-11441.
4. Schwabl, H. 1995. Individual variation of the acute adrenocortical response to stress in the white-throated sparrow. *Zoology*, 99:113-120.
5. Schwabl, H., D. Mock, and J. Gieg. 1997. A hormonal mechanism of parental favouritism. *Nature* 386: 231.

Name: Paul A. Verrell

Department of Zoology, Washington State University, Pullman WA 99164-4236

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Born: May 20, 1958 in London, England

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Citizenship: British, US permanent resident

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Degrees:

B.Sc. (Honors) in Zoology, University of London (England) 1979

Ph.D. in Animal Behavior, The Open University (England), 1982

Current Employment:

1992-present, Assistant Professor, Washington State University, Pullman, WA 99164

Previous employment:

Research Associate (Assistant Professor), University of Chicago, 1989-1992

Current responsibilities:

My responsibilities consist of teaching, research and service. I teach a core of three upper-division courses, supplemented with occasional undergraduate and graduate seminars. My lab's research focuses on the function and evolution of animal behavior.

Expertise:

I have been conducting both observational and manipulative studies of animal behavior since 1979. Most of these have involved aquatic lower vertebrates as subjects. In addition, most have been highly quantitative, involving statistical analyses of data.

Five Publications Relevant to the Proposed Project:

1. Arnold, S. J., Verrell, P. A. & Tilley, S. G. 1996. The evolution of sexual isolation: a model and a test case. *Evolution* 50, 1024-1033.
2. Verrell, P. A. 1998. Geographic variation in sexual behavior: sex signals and speciation. Pp. 262-286 in Foster, S. A. & Endler, J. A. (Eds), *Geographic Variation in Behavior: Perspectives on Evolutionary Mechanisms*. Oxford University Press, New York.
3. Verrell, P. & Krenz, J. 1998. Competition for mates in the mole salamander, *Ambystoma talpoideum*: tactics that maximize male mating success. *Behaviour* 135, 121-138.
4. Verrell, P. & Pelton, J. 1996. The sexual strategy of the central long-toed salamander, *Ambystoma macrodactylum columbianum*, in southeastern Washington. *J. Zool.* 240, 37-50.
5. Vinnedge, B. & Verrell, P. 1998. Variance in male mating success and female choice for persuasive courtship displays. *Anim. Behav.* 56, 443-448.

Section 10. Information/technology transfer

Results will be published in suitable journals, including journals widely read by fisheries researchers (e.g., Canadian Journal of Fisheries and Aquatic Sciences, Transactions of the American Fisheries Society) and presented at scientific meetings, including those well-attended by fisheries professionals from the region (e.g., chapter or division meetings of the American Fisheries Society).

Congratulations!