

Subbasin.

Short description.

This research addresses the role disease plays in the survival of wild salmon and whether hatchery fish negatively impact wild fish either directly, by transmitting pathogens and disease, or indirectly, by reducing the fitness of wild fish.

Section 2. Key words

Mark	Programmatic Categories	Mark	Activities	Mark	Project Types
X	Anadromous fish		Construction		Watershed
	Resident fish		O & M		Biodiversity/genetics
	Wildlife		Production		Population dynamics
	Oceans/estuaries	X	Research		Ecosystems
	Climate		Monitoring/eval.		Flow/survival
	Other		Resource mgmt	X	Fish disease
			Planning/admin.		Supplementation
			Enforcement		Wildlife habitat enhancement/restoration
			Acquisitions		

Other keywords.

hatchery-wild interactions, IHNV, Ceratomyxa shasta, Bacterial cold water disease, interbreeding, genetic resistance against disease

Section 3. Relationships to other Bonneville projects

Project #	Project title/description	Nature of relationship

Section 4. Objectives, tasks and schedules

Objectives and tasks

Obj 1,2,3	Objective	Task a,b,c	Task
1	Determine the prevalence of pathogens and disease in wild salmonids in the CRB	a	Complete a literature search to compile information on pathogens and diseases of fish in the CRB

		b	Survey of pathogens in salmonid smolts
2	Determine whether hatchery and wild fish show differences in disease susceptibility	a	Obtain gametes from wild and hatchery stocks from selected locations in the CRB
		b	Quantify genetic divergence between populations
		c	Compare the susceptibility of fish from each stock to a viral, parasitic and bacterial fish pathogen
		d	Determine the effect of environmental parameters on the survival of pathogen-exposed fish
3	Determine whether fish surviving disease can transmit infection and disease to naive fish	a	Determine if cohabitation of naive and infected fish results in pathogen transmission
		b	Determine if disease survivors can transmit infection to naive fish when held under stressful conditions
4	Determine if interbreeding between hatchery and wild fish causes a change in disease resistance	a	Produce F1 crossbred progeny between hatchery and wild fish.
		b	Expose F1 progeny to pathogens
		c	Characterize genetic differences in the F1 progeny

Objective schedules and costs

Objective #	Start Date mm/yyyy	End Date mm/yyyy	Cost %
1	10/1998	2/2002	20.00%
2	10/1998	12/2000	30.00%
3	5/1999	10/2001	20.00%
4	10/2000	9/2002	30.00%
			TOTAL 100.00%

Schedule constraints.

Completion date.
2002

Section 5. Budget

FY99 budget by line item

Item	Note	FY99
Personnel	Bootland \$27,756, Bartholomew \$12,336, RA @.33 FTE \$15,688, 2 RA \$52,308	\$108,088
Fringe benefits	@41% to 51%	\$49,623
Supplies, materials, non-expendable property	PCR reagents, tissue culture reagents, ELISA kits, plasticware	\$24,000
Operations & maintenance		
Capital acquisitions or improvements (e.g. land, buildings, major equip.)		
PIT tags	# of tags:	
Travel	local travel to collection sites, partial travel to one national meeting for each PI	\$3,000
Indirect costs	@42.5%	\$84,877
Subcontracts	to USFWS	\$15,000
Other		
TOTAL		\$284,588

Outyear costs

Outyear costs	FY2000	FY01	FY02	FY03
Total budget	\$294,405	\$285,820	\$299,376	
O&M as % of total				

Section 6. Abstract

6. Abstract:

The importance of maintaining genetic diversity in the salmon stocks of the Columbia River Basin (CRB) is well recognized, but little attention has been paid to the relationship between genetic diversity and disease resistance in wild salmonid populations. It has been assumed that hatchery fish cause disease in wild fish and that interbreeding of hatchery fish with wild fish may cause adverse genetic changes, including loss of disease resistance. However, there is little scientific evidence to support either of these assumptions. **The goal of the proposed research is provide data that will establish whether or not hatchery fish adversely affect the disease status of wild fish.** Objectives have been designed to determine whether 1) there are significant

differences between hatchery and wild salmonid populations in infection and disease resistance, 2) acutely infected hatchery fish can transmit infection and disease to wild salmonids, 3) hatchery fish that survive infection can transmit pathogens and disease to wild fish when held under stressful conditions, and 4) interbreeding of hatchery fish with wild salmonids adversely affects the genetically-controlled disease resistance of progeny. Answers to these questions will be obtained over four years and will be of value in making policy and management decisions related to the use of hatchery fish within the CRB. The proposed research addresses measures 7.2A.2 and 7.2D.4 in the Fish and Wildlife Program by providing scientific data necessary to increase salmon runs in a biologically sound manner.

Section 7. Project description

a. Technical and/or scientific background.

7.a. Technical and Scientific Background:

A major goal of the Northwest Power Planning Council (NPPC) fish and wildlife program is to double salmon and steelhead runs in the CRB without the loss of genetic diversity. Achievement of this goal will require multiple approaches, including hatchery propagation of native and non-native fish. Although hatchery propagation can increase the number of fish available for harvest, straying by only a small percentage of non-native adult fish can disrupt the genetic composition of nearby wild populations (Leider 1997; Quinn 1997), many of which are already depressed in number.

Maintaining genetic diversity is vital to sustaining the ability of populations to cope with a variety of factors, including disease. The return of salmon to their natal streams provides the opportunity for genetic divergence since these populations develop adaptations, including disease resistance, in response to local environmental selection (Beacham and Evelyn 1992a). Genetic variation in disease resistance among hatchery populations is well documented for bacterial, viral, fungal and parasitic infections (Antipa and Nelson 1977; Beacham and Evelyn 1992a,b; Nilsson 1992), however little is known of disease resistance variation in wild populations.

The genetic basis of disease resistance may be altered by interbreeding. Interbreeding of disease resistant and susceptible salmon strains can result in decreased ability to combat disease in the resistant strain or increased resistance in the susceptible strain. Both of these possibilities have been demonstrated using *C. shasta* in laboratory studies with hatchery strains of salmon (Hemmingsen et al. 1986; Bartholomew 1998) and in field studies with wild and non-native hatchery fish (Currans et al. 1997). The disease consequences of changes to the genetic integrity of local fish stocks due to interbreeding of hatchery fish with fish from wild or resident populations has been little studied (McVicar 1997) but deserves immediate research attention. **Studies to investigate the variability in disease resistance of wild fish stocks and the effect of interbreeding between hatchery and wild populations on disease resistance are outlined in the proposed research.**

There are a number of important viral, bacterial and parasitic pathogens of salmon enzootic in the CRB. Studies on the distribution and effects of these pathogens have examined hatchery stocks because of the ease of sampling these populations. There is little information available on the prevalence of pathogens and disease in hatchery fish after release and in wild and resident fish populations nor on the disease effects of hatchery fish interacting with naturally reproduced fish. The presence of pathogens in non-hatchery fish has begun to receive greater attention. In a 1995 survey, erythrocytic inclusion body syndrome (EIBS) virus was found in Snake River origin hatchery (88%) and wild (50%) spring/ summer chinook salmon (*Oncorhynchus tshawytscha*) smolts sampled from barges, and *Renibacterium salmoninarum*, the cause of BKD, was isolated from 60% of the hatchery fish and 92% of the wild fish (Elliott and Pascho 1996). In Oregon, a wild fish survey found infectious hematopoietic necrosis virus (IHNV), infectious pancreatic necrosis virus (IPNV), viral erythrocytic necrosis virus and cutthroat trout virus were present and that IHNV had a wide geographic distribution in wild fish (Engelking and Kaufman 1996). It is critical to note that the occurrence of pathogen is not unusual and that infection will not necessarily lead to disease. Under normal circumstances infectious agents coexist with their host without causing disease (McVicar 1997).

In hatcheries, fish are reared at high densities, a situation not normally found in nature. This and other stressful conditions such as high water temperatures can lead to disease outbreaks when a high pathogen load is present. It is possible that wild fish in the CRB have genetically adapted to the presence of enzootic pathogens and will not suffer disease upon exposure to these pathogens unless subjected to adverse environmental conditions. However, if wild fish are susceptible to disease then, as stated by McVicar (1997), the risk with respect to the interaction between farmed and wild fish can be summed up by the generality that fish sharing water are likely to share diseases. This is potentially important when diseased hatchery fish are released into the CRB. Although there is little evidence of disease transmission from infected hatchery fish to naturally reproduced fish (reviewed by Stewart and Bjornn, 1990), there has been little research in this area. Most disease-related losses in the natural environment probably go undetected. There are a few surveys examining the prevalence of pathogens in wild fish, but no data is available on the susceptibility of wild salmonids to the diseases caused by these pathogens. There is a fundamental need to define the disease state and to differentiate this from the presence of microorganisms in healthy fish. This is a critical area that will be examined in the proposed research. Data obtained could be applied in making risk management decisions regarding the movement or release of infected hatchery fish.

The proposed research will concentrate on three pathogens enzootic to the CRB: IHNV, the parasite *Ceratomyxa shasta*, and the bacterium *Flavobacterium psychrophilum* (formerly named *Cytophaga psychrophilus*). Each of these pathogens has serious consequences on fish within the CRB.

IHNV has a complex relationship with the life cycle of its salmonid host. The virus typically causes mortalities of up to 90% in fry and fingerlings of most species of hatchery-reared salmonid fish and is detectable for up to 60 days after infection (Bootland et al. 1995). The virus then seemingly disappears, only to be detected again when fish near or reach sexual maturity. Recently, it has been shown that juvenile and yearling fish

carry the virus for a long time in a state not detectable by infectious assays, but detectable by the more sensitive polymerase chain reaction (PCR) assay (Drolet et al. 1995; Chiou et al. 1995). These findings suggest that hatchery or wild carrier fish can serve as a source of infection when brought in close contact with uninfected fish. There are five types of IHNV and the three major ones isolated in the CRB have a devastating effect on salmonid populations. Type I isolates are most virulent in kokanee and sockeye salmon (*O. nerka*), Type II isolates are more virulent in rainbow and steelhead trout (*O. mykiss*) than types I or III, and Type III isolates are usually most virulent in chinook salmon (LaPatra et al. 1990a,b, 1991, 1993). The crowded conditions present during the trucking or barging of smolts and adult salmonids around dams in the CRB would provide an excellent opportunity for the transmission of IHNV. In fact, IHNV has been detected in truck water (Batts and Winton 1989). However, it is unknown whether virus is spread from infected to uninfected fish during transport.

C. shasta is a myxosporean parasite capable of causing a high mortality disease in all species of Pacific salmonids. This parasite is important in the Pacific Northwest because it not only causes losses in hatchery-reared and wild juvenile salmonids as they migrate through enzootic waters, but also contributes significantly to prespawning mortality in adult salmon (Bartholomew et al. 1989, 1992). Although the parasite is not detected outside of the Pacific Northwest, its distribution in this region has expanded. It is not known whether this increase reflects a true spread or improved detection methods. The capacity of certain salmonids to resist infection and disease caused by *C. shasta* is well documented. Observations of differential mortality among strains suggest that intra-species differences in susceptibility develop as a result of selection pressure in enzootic waters. *C. shasta* can not be directly transmitted from fish to fish. Instead, fish become infected after coming in contact with the infective actinosporean stage of the parasite after it is released from its invertebrate polychaete host (Bartholomew et al. 1997). Once it penetrates the fish, the parasite infects and multiplies in the intestine. Although fish can become infected at 4°C, disease and mortality are temperature dependent and most frequently occur when temperature exceeds 10°C (Ratliff 1983; Udey et al. 1975).

F. psychrophilum, the cause of bacterial cold water disease (BCWD), affects most species of salmonids. The disease usually appears in the early spring when water temperatures are between 4-10°C. Clinical signs of BCWD include lesions on the peduncle, anterior to the dorsal fin or near the vent (Holt 1988). Presumptive diagnosis of BCWD is typically made by isolating bacteria from lesions on cytophaga agar and observing moist yellow colonies that microscopically contain long, thin, Gram negative rods. Confirmatory diagnosis is made serologically by IFAT or agglutination tests (Amos 1985). To induce experimental infections, giving fish a subcutaneous injection is more effective than giving an intraperitoneal injection or immersing fish in the bacteria (Holt 1988). There is considerable variation in virulence between bacterial isolates and fish species, and increases in water temperature decrease the mean day to death (Holt et al. 1989).

b. Proposal objectives.

7.b. Proposal Objectives:

To achieve the project goal, the objectives and associated hypotheses are:

1. Determine the prevalence of pathogens and disease in wild salmonids in the CRB.

Hypothesis 1. There is no significant difference in the prevalence of pathogens and disease in wild and hatchery salmonids.

2. Determine whether hatchery and wild fish show differences in disease susceptibility.

Hypothesis 2. Hatchery and wild fish are equally susceptible to disease.

3. Determine whether fish surviving disease can transmit infection and disease to naive fish.

Hypothesis 3. Infected fish that survive a disease outbreak can transmit infection and disease to naive hatchery and wild fish when held under non-stressful or stressful conditions.

4. Determine if interbreeding between hatchery and wild fish causes a change in disease resistance

Hypothesis 4. Interbreeding between hatchery fish and wild fish causes a change in disease resistance

c. Rationale and significance to Regional Programs.

7.c. Rationale and Significance to Regional Programs:

The proposed research is of significance to the NPPC Fish and Wildlife Program's goal of doubling salmon and steelhead runs in the CRB without the loss of genetic diversity and to the NPPC's system-wide sub-goal of halting declines in salmon numbers and rebuilding populations to a biologically sustainable level by 2000. Fish diseases cause devastating losses in hatchery salmonids within the CRB and it is assumed that hatchery fish negatively impact wild salmonids either directly, by transmitting pathogens and disease, or indirectly, by interbreeding and reducing the fitness of the latter. However, these hypothesized effects have rarely, if ever, been documented. Nevertheless, such effects may occur with high likelihood's if genetic differences in disease resistance and susceptibility exist between hatchery and wild populations. The goal of the proposed research is to test these hypotheses by answering the following questions: 1) is there a difference in pathogens and pathogen loads between wild and hatchery populations, 2) are hatchery and wild steelhead trout and chinook salmon equally susceptible to infection by a viral, bacterial and parasitic pathogen, 3) does transmission of pathogens between populations occur when fish are crowded or exposed to high temperatures or increasing salinity and 4) what are the effects of interbreeding of hatchery and wild fish on disease resistance. Research results should confirm or refute the assumption that hatchery fish adversely affect wild populations with respect to disease resistance. Such information is critical for making informed decisions on the use of

hatchery fish in supplementation and mitigation programs. All project objectives directly relate to measure 7.2D.4, which requires the development of programs to minimize the impact of fish diseases on wild and cultured stocks. The data obtained from the proposed research could be effectively used to further the goals of the FWP if it is incorporated into management decisions regarding the use of hatchery fish.

d. Project history

7.d Project History: This is a new project.

e. Methods.

7.e. Project Methods:

Objective 1. Determine the prevalence of pathogens and disease in wild salmonids in the CRB

Task 1.A.: Complete a literature search to compile information on pathogens and diseases of fish in the CRB:

The goal of this task is to update and expand on the literature reviewed by the Technical Working Group (TWG) on Fish Disease. The data reviewed by the TWG is over ten years old and only examined diseases in hatchery fish, yet it is known that pathogens and disease also affect wild fish in the CRB. A literature search will be completed on the prevalence of infection and disease caused by pathogens of wild and hatchery fish in the CRB. The search will include articles in peer-reviewed journals, agency technical reports, non-peer reviewed publications and interviews with fish pathologists and hatchery managers. Research will be compiled in a report and provided to the National Wild Fish Health Survey being completed by the USFW Service.

Task 1.B.: Survey of pathogens in salmonid smolts:

The goal of this task is to determine and compare the prevalence of infection in outmigrating wild and hatchery stocks of salmon smolts collected from several sites within the CRB. This task will be completed over a three year period in conjunction with project #5503800, which is evaluating the stranding of juvenile fall chinook salmon on the Hanford Reach, and will be coordinated with personnel that are sampling fish in downstream collection and holding facilities at Ice Harbor and McNary dams. Other sample sites will be used if available.

Fish Sampling: A total of 2400 salmon smolts will be examined. On each of a total of 40 days, 60 dead fish from multiple sites will be randomly sampled. Fish will be visually examined for signs of disease and attempts made to identify whether fish are from hatcheries by examining for

fin clips or tags. A blood sample will be taken, if possible, from each fish and the fish will be placed on ice and transported to Oregon State University (OSU).

Detection of Pathogens: Fish will be examined for the pathogens given highest priority by the Fish Disease TWG. Diagnostic methods for each pathogen will generally be those outlined in the Fish Health Blue Book (Thoesen 1994). Methods will include the use of a commercially available (DiagXotics, Inc.) enzyme-linked immunosorbent assay (ELISA) for *R. salmoninarum*; cell culture, immunocytochemistry and PCR for IHNV (Drolet et al. 1993; Bootland et al. 1994; Chiou et al. 1995); microscopic examination of blood smears stained with pinacyanol chloride for EIBS virus; isolation on TYE and TSA plates followed by Gram stains and serological tests for bacterial pathogens such as *F. psychrophilum* and *Aeromonas salmonicida*; and PCR for *C. shasta* (Bartholomew et al. 1997) and the cause of whirling disease, *Myxobolus cerebralis* (Andree et al. 1996).

Data Analysis: The presence of each pathogen will be tabulated and presented as a prevalence of infection. Data will be examined by analysis of variance (ANOVA) and t-tests at a 5% significance level.

Expected Results: It is likely that the above pathogens will be detected. It can not be predicted whether there will be a difference in the prevalence of infection for each pathogen between hatchery and wild fish.

Objective 2. Determine whether hatchery and wild fish show differences in disease susceptibility

In 1987, eight diseases were given high priority by the Fish Disease TWG (Williams et al. 1996). Three of these pathogens, IHNV, *C. shasta* and *F. psychrophilum* were chosen for use in the proposed research because methods of exposing fish to each pathogen and monitoring for infection and disease are well characterized and have been used by the investigators. This objective will be completed over a two year period and will examine two stocks of hatchery and wild steelhead trout and two stocks of hatchery and wild spring chinook salmon. One hatchery and one wild stock per species will be examined each year.

Task 2.A.: Obtain gametes from wild and hatchery stocks from selected locations in the CRB:

Eggs and milt will be collected from steelhead trout and chinook salmon from each of the following sites, pending permission by state and federal agencies:

<u>Source</u>	<u>Species</u>
Round Butte Hatchery	wild and hatchery stocks of summer steelhead trout
Marion Forks Hatchery	hatchery stock of winter steelhead trout
Foster Dam	wild stock of winter steelhead trout
McKenzie Hatchery	hatchery and wild stocks of spring chinook salmon
Warm Springs Hatchery	hatchery and wild stocks of spring chinook salmon

The above sites were chosen after conversations with ODFW personnel, however other sites will be chosen if necessary to ensure that fish are from pure hatchery and wild stocks. Gametes obtained from 15 males and 30 females will be kept separate, placed on ice and brought to the Salmon Disease Laboratory (SDL). Matings for each stock will use a nested design, where milt from one male is used to fertilize eggs from two females. After water hardening, the egg lots will be kept separate for wild and hatchery fish and reared in a vertical-stack incubator supplied with flowing 12°C well water. Eggs will be formalin-treated to prevent fungal infections. Resulting hatchery and wild stocks of fish will be reared in separate 100 L tanks supplied with flowing 12°C well water and fed daily with Biodiet trout pellets. Prior to pathogen challenge, fry will be shown to be pathogen-free using the assays described under task 1.B.

Task 2.B: Quantify genetic divergence between populations:

Allozymes detected by standard procedures of protein electrophoresis will be used to quantify levels of genetic divergence between hatchery and wild populations (Aebersold et al. 1987; Murphy et al. 1996). Allele frequencies will be determined at approximately 50 allozyme loci for two hatchery and two wild populations of each species (chinook salmon and steelhead trout) based on sample sizes of approximately 75 fish from each population or locality. Genetic distances between populations will be calculated by the chord distance of Cavalli-Sforza and Edwards (1967) and Nei's (1978) measure of genetic distance. Extensive genetic databases of allozyme frequencies currently exist for large numbers of chinook salmon and steelhead populations in the Pacific Northwest (e.g. Utter et al. 1995; Busby et al. 1996). These databases thus provide a baseline for evaluating levels of genetic divergence between the hatchery and wild populations to be tested in the work proposed here. Levels of divergence for disease resistance will also be quantified according to the standard methods of quantitative genetics, where hatchery and wild populations will be treated as fixed main effects, and sire and dam effects will each be treated as nested, random effects within their respective main effect populations (Becker 1984; Falconer and Mackay 1996).

Task 2.C.: Compare the susceptibility of fish from each stock to a viral, parasitic and bacterial fish pathogen:

The susceptibility of each hatchery and wild salmonid stock to the three pathogens will be determined by monitoring fish mortality, determining the prevalence of infection and by measuring pathogen load.

Subtask 2.C.a. Prepare pathogen stocks:

IHNV: Steelhead trout and spring chinook salmon will be exposed to a Type II and a Type III IHNV isolate, respectively. IHNV will be added at a low multiplicity of infection to *epithelium papillosum carpio* (EPC) cells. Infected cell monolayers will be cultured at 17°C in Eagle's minimum essential media supplemented with 10% fetal bovine serum, 1000 IU/ml penicillin G, 1 mg/ml streptomycin and 2.5 µg/ml amphotericin B and buffered to pH 7.5 with 7.5% sodium bicarbonate. Virus stocks will be titrated by plaque assays on EPC cells (Burke and Mulcahy

1980) and stored at -70°C. For use in challenge experiments the virus stock will be diluted in phosphate buffered saline (PBS).

F. psychrophilum : A recent field isolate will be obtained from Dr. R. Holt, ODFW and grown for 24 h in TYE broth at 17°C (Holt et al. 1989). Cells will be harvested by centrifugation and resuspended in PBS to an optical density at 525 nm of 1.0, which is equivalent to 10⁹ cells/ml (Holt 1988).

Subtask 2.C.b. Expose fish to each pathogen:

IHNV: Fish will be exposed to IHNV using the methods of Bootland et al. (1994). Briefly, fish stocks will be divided into six 100 L tanks of 600 fish each. When fish reach a mean weight of 0.5 g, fish in three tanks will be exposed to IHNV by lowering the water in each tank to achieve a fish density of 40g/L and adding IHNV to a final concentration of 10³ PFU/ml. As a negative control, PBS will be added to the other three tanks. A water sample from three tanks will be tested by plaque assay to confirm the virus dose. Water will be aerated with airstones and the flow resumed after 5h. The virus dose was chosen to reflect an IHNV concentration that has been found in the water from a truck transporting sockeye salmon fry (Batts and Winton 1989) and is a titer typically used to monitor IHNV virulence.

F. psychrophilum: Each fish stock will be exposed to three concentrations (10⁷, 10⁶ and 10⁵ cells/0.05 ml) of bacteria to ensure that at least one dose will cause fish mortality. Fish will be divided into twelve groups of 100 fish in 25 L tanks. When fish reach a mean fish weight of 0.5 g, individual fish will be anesthetized in 200 ppm tricaine methanesulfonate (MS222), given a subcutaneous injection of 0.05 ml of the designated bacterial concentration in the region between the adipose and dorsal fins (Holt et al. 1989) and returned to the holding tank. Fish in three tanks will be injected with 0.05 ml of PBS as negative controls. The injected bacterial dose will be confirmed by counting bacterial colonies on spread plates of Cytophaga agar.

C. shasta : Fish will be exposed to the infectious stage of *C. shasta* by holding in live cages at a site in the Willamette River, near Corvallis, OR, where the parasite is enzootic. From each stock, 600 fish (approximately 10 g) will be injected with a dye for identification before combining the groups. Fish will be exposed to the parasite for a period of 3 d, then returned to the SDL to monitor for development of disease. A group of 100 rainbow trout belonging to a susceptible strain will be exposed simultaneously as a positive control.

Subtask 2.C.c. Monitor Mortality and Infection:

Fish mortality after exposure to IHNV or *F. psychrophilum* will be monitored for 35 days and fish exposed to *C. shasta* will be monitored for 60 days. Infection will be confirmed in 10% of the dead fish. The prevalence of infection and titers in surviving fish will be calculated by examining 20% of the fish. Methods of diagnosing each pathogen are outlined below.

IHNV: Dead fish will be weighed and diluted 1/5 with Hanks balanced salt solution. After homogenization in a Stomacher, samples will be diluted two-fold in an antibiotic solution to kill

bacteria, incubated overnight at 4°C and centrifuged (1000 x g, 4°C, 15 min.). A 100 µl volume of ten fold supernate dilutions will be added to preformed monolayers of EPC cells in 24 well plates. After 1 h at 17°C, and overlaying the cells with methylcellulose, the plates will be incubated at 17°C for five days. After cells are fixed in formalin and stained with crystal violet, the number of plaques will be counted and pathogen load expressed as the virus titer (PFU/g). Virus identification will use immunocytochemistry (Drolet et al. 1993).

F. psychrophilum: To detect infection, kidney and skin lesions (if present) will be streaked onto *Cytophaga* agar plates and the plates incubated at 17°C for 4 days. For quantification, kidney tissue will be weighed, diluted 1/5 in PBS and homogenized in a Stomacher. Ten-fold dilutions (100 µl) of the homogenate will be spread plated onto *Cytophaga* agar and the plates incubated as above. *F. psychrophilum* will be presumptively identified by the growth of yellow, convex colonies with thin, spreading peripheries and confirmed using a slide agglutination test with specific rabbit antiserum (Holt et al. 1989). Pathogen load will be expressed as colony forming units/g.

C. shasta: To detect infection, wet mounts made from intestinal tissues will be examined at 250X under a microscope. Presence of the parasite will be confirmed by identification of characteristic spores (Bartholomew et al. 1989). Although difficult, attempts will be made to quantitate the number of spores as a measure of pathogen load.

Data Analysis:

Data from each fish stock will be presented as the cumulative percent mortality, the mean day to death (MDD), the prevalence of infection and pathogen load and will be statistically analyzed by ANOVA and t-tests at a significance level of 5%. Comparison of trends in mortality and MDD between the different pathogens will be conducted by simple correlation analyses and estimation of heritabilities and genetic correlations as described by Beacham and Evelyn (1992a).

Task 2.D.: Determine the effect of environmental parameters on the survival of pathogen-exposed fish:

Three parameters (fish density, water temperature and salinity) will be evaluated for their effects on the survival of fish previously exposed to fish pathogens. Fish density and temperature were chosen to reflect conditions that fish may encounter during barging or while in holding facilities. Salinity was chosen as the third parameter to determine whether fish previously exposed to the three pathogens can successfully adapt to salt water and hence survive in estuarine and ocean conditions. Preliminary data on the salt water adaptability of two stocks of fall chinook salmon that had survived IHNV infection indicated that both the fish stock and the IHNV isolate used to infect fish does affect the ability of fish to adapt to salt water (appendix, Fig. 1). Preliminary data for *C. shasta* indicated that transfer of infected Alevin steelhead trout to salt water decreased the prevalence of infection and that Big Creek coho and Round Butte chinook salmon stocks were resistant to infection when held in fresh or salt water after exposure to *C. shasta* (appendix, Table 1) (Bartholomew et al. 1992).

Fish used in this task will be the surviving fish from task 2.C. If numbers are insufficient, additional fish will be exposed to a dose that insures infection but low mortality.

Fish Density: For each stock, fish surviving exposure to IHNV and control fish will each be divided into four groups of 50 fish and reared in 25 L tanks at fish densities of 35 g/L, the density typically found during barging, and 5 g/L. Identical experiments will be completed with fish surviving infection by *C. shasta* and *F. psychrophilum*.

Temperature: For each stock, fish surviving exposure to IHNV and control fish will each be divided into four groups of 50 fish in 25 L tanks. Two groups will be reared in 18°C water and the remaining two groups will act as controls and will be reared in 12°C water. Identical experiments will be completed with fish surviving *C. shasta* and *F. psychrophilum*.

Salinity: Survivors from each pathogen group will be transported to Hatfield Marine Science Center, Newport, OR as smolts, and divided into duplicate groups of 50 fish in 350 L tanks. Duplicate groups of control fish for each stock will be similarly divided. Water salinity will be gradually increased over a five day period. Duplicate tanks of 50 fish per stock that survive exposure to each pathogen will be maintained in fresh water at the SDL as a control.

Fish exposed to varying fish densities and temperatures or exposed to salt water will be monitored for mortality for 30 days. Pathogen load will be determined using methods described under subtask 2.C.c.

Expected Results of Objective 2:

It is expected that increased temperature may enhance fish survival and decrease pathogen load after exposure to any of the pathogens. However, high fish density may negatively affect fish survival and increase pathogen load. Based on the preliminary data for salt water adaptability (Fig. 1, Table 1), it is expected that there will be a difference between stocks in fish mortality and that survivors of *C. shasta* will have a lower prevalence of infection than those maintained in fresh water.

Objective 3. Determine whether fish surviving disease can transmit infection and disease to naive fish

Transmission of pathogens from infected hatchery fish to wild stocks has been suggested to occur (Stewart and Bjornn 1990), but has yet to be confirmed. Stress may aid in the transmission of infection or exacerbate disease. There are a few times during the life cycle of salmonid fish when inescapable stresses occur, such as when smolts or adults are transported by truck or barge or held in holding ponds or collection facilities.

Task 3.A. Determine if cohabitation of naive and infected fish results in pathogen transmission:

To determine if cohabitation of naive fish with actively-infected fish reared under non-stressful conditions results in infection and mortality, 20 pathogen-free (naive) fish from wild and hatchery stocks will be differentially fin-clipped for identification and added to each tank

containing fish challenged with IHNV or *F. psychrophilum* at five days post-exposure. Cohabitation studies will not be done with fish exposed to *C. shasta* since it is known that the parasite can not be directly transmitted from fish to fish. Mortality and infection will be monitored for 25 days. At 26 days, fin-clipped fish will be killed by an overdose of MS222 and examined for infection using the methods outlined in subtask 2.C.c.

Task 3.B. Determine if disease survivors can transmit infection to naive fish when held under stressful conditions:

Naive fish from wild and hatchery stocks will be differentially fin-clipped and 20 fish from each stock will be added to each tank from Task 2.D. Fish will be fed and monitored for mortality daily for 30 days. Infection and pathogen load in all fish mortalities and in 10% of the fish killed at 30 days will be determined as described in subtask 2.C.c.

Expected Results of Objective 3:

This objective should provide evidence that confirms or refutes the ability of infected hatchery fish to transmit infection and disease to wild fish and vice versa and it is expected that the three stressors will aid in infection and disease transmission.

Objective 4. Determine if interbreeding between hatchery and wild fish causes a change in disease resistance.

This objective will be completed in the third and fourth year of the research.

Task 4.A: Produce F1 crossbred progeny between hatchery and wild fish:

Hatchery and wild adult fish will be mated and spawned in a 2x2 replicated factorial mating design (1 male and 1 female from each population) with approximately 5-10 replications of the spawning matrix. Each replicate will thus consist of four full-sib families: two within-population or control crosses (WxW, HxH) and two between-population or reciprocal crosses (HxW, WxH).

Eggs from each cross will be reared at the SDL in individual trays in stacking tray incubators supplied with flowing 12°C well water and treated with formalin to prevent fungal infection. Fry from each cross will be examined using the methods in task 1.B. to ensure they are pathogen-free.

Task 4.B.: Expose F1 progeny to pathogens:

Fish from each cross will be divided into 18 groups of 100 fish. Triplicate groups will be exposed to the three pathogens using the methods outlined under subtask 2.C.b and triplicate groups will act as negative controls. Fish mortality will be monitored for 35 days for IHNV and *F. psychrophilum*-exposed fish and for 60 days after exposure to *C. shasta*. The prevalence of infection and pathogen load will be measured as outlined under subtask 2.C.c.

Task 4.C.: Characterize genetic differences in the F1 progeny:

Each fish cross will be genetically analyzed as described in task 2.B. to determine if interbreeding caused genetic changes and if such changes can be correlated with disease resistance.

Expected Results of Objective 4:

It is expected that there will be a difference in mortality and infection due to the interbreeding of *C. shasta*-susceptible and resistant strains of salmonids. The effects of interbreeding on resistance to IHNV and *F. psychrophilum* are unknown.

Proposed Time Schedule:

	<u>Oct-Dec.</u> <u>1998</u>	<u>Jan.-Dec.</u> <u>1999</u>	<u>Jan.-Dec.</u> <u>2000</u>	<u>Jan.-Dec.</u> <u>2001</u>	<u>Jan.-Sept.</u> <u>2002</u>
Quarter:					
Task 1.a Literature Review	*****				
Task 1.b. Smolt survey	*****	*****			*****
Objective 2: Hatchery vs Wild Fish Disease Susceptibility		*****			
Objective 3 Survivor to Naive Fish Infection and Disease Transmission		*****	*****		
Objective 4: Effect of Interbreeding on Disease Resistance			*****		
Preparation of final report					*****

f. Facilities and equipment.

7.f. Facilities and Equipment:

Research will be conducted in the Department of Microbiology, OSU, at the Salmon Disease Research Laboratory (SDL) in Corvallis and at the fish facilities at Hatfield Marine Science Center, Newport, OR. The Department of Microbiology contains all the necessary equipment required for the proposed research: centrifuges, ultracentrifuges, ultracold freezers, transfer rooms for cell culture, incubators, a biohazard safety room, electrophoretic equipment, ELISA plate reader, computers, refrigerators, microscopes and histology equipment .

The SDL consists tanks of different sizes sufficient to hold a large number of experimental animals. There is a stable supply of specific pathogen-free 12°C water available for continuous culture of salmonid fish, and water temperature can be increased in individual tanks. All water discharged from the SDL is chlorinated by automatic chlorine drip pumps, run through a baffled holding tank to discharge the chlorine and held in holding ponds before the effluent water is released into the Willamette River.

Hatfield Marine Science Center (HMSC) has a fish rearing facility that contains tanks of various sizes and is supplied with fresh and salt water that is UV-irradiated. Effluent water is disinfected with chlorine prior to discharge.

All equipment at the SDL and HMSC is disinfected after use to eliminate the risk of spreading fish pathogens. Ancillary dry laboratory and office space is available at the SDL and HMSC for preparation and monitoring of experiments.

g. References.

7.g. References:

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Section 8. Relationships to other projects

8. Relationships to Other Projects:

The proposed research potentially includes a cooperative effort with BPA project #5503800 which is evaluating the stranding of chinook salmon on Hanford Reach since fish for the proposed research will be sampled at times when the water level is lowered for the other project. However, if the Hanford Reach Project is not funded, it will not negatively affect the proposed research. The research will complement the USFW Service's National Wild Fish Health survey and the research of Drs. J. Bartholomew and M. Engelking on a Fish Disease Risk Assessment Study supported by Portland General Electric Company by supplying their studies with our research results on the prevalence of fish pathogens found in wild and hatchery stocks. A collaborative agreement has been arranged with Dr. D. Campton, USFW Service for genetic analysis of fish stocks.

Section 9. Key personnel

9. Key Personnel:

The proposed research involves personnel university, state and federal agencies. The principal investigator on this project is Dr. Linda M. Bootland, assistant professor (0.75 FTE, curriculum vitae (c.v.) in appendix). Co-principal investigators are Dr. Jerri L. Bartholomew, assistant professor (0.33 FTE, c.v. in appendix) and Dr. Jo-Ann C. Leong, Distinguished Professor and chair, Department of Microbiology (0.05 FTE, c.v. in appendix). The project collaborator will be Dr. Don Campton, USFW Service (letter of collaboration enclosed, c.v. in appendix) and the project consultant will be Dr. Richard Holt, fish pathologist, ODFW (letter of cooperation enclosed). Drs. Bootland and Bartholomew will be responsible for the day to day management of the project and report writing and Dr. Bootland will also be closely involved in all other aspects of the proposed research, including experimental design, conducting experiments, and data analysis. Dr.

Leong will oversee overall project and accounting. Dr. Bootland's research is focused on the elucidation of virus-host interactions and vaccine development against fish pathogens. Dr. Leong's research program concentrates on the molecular biology and pathogenesis of salmonid viruses. Drs. Leong and Bootland have jointly been involved in studying all aspects of the life cycle of IHNV. Dr. Bartholomew's research program focuses on parasitic infections of salmonid fish and she has completed thorough studies on all aspects of the life cycle of the parasite *C. shasta*. All of their research programs involve the monitoring of infection and disease in salmonid fish and elucidation of parameters that affect the disease process, including pathogen and host factors. Dr. D. Campton will be completing the genetic analysis of the fish used in the proposed research under a subcontractual agreement. He is the regional geneticist for the USFW Service and an expert in production and quantitative genetics of fish. Dr. R. Holt will act as a consultant on research with *F. psychrophilum*. He has extensive experience working with *F. psychrophilum*, including studies on growth, diagnosis, factors affecting virulence and vaccine development.

Section 10. Information/technology transfer

10. Information/technology Transfer:

The information obtained from this project will be distributed to the public, the USFW Service and the NPPC by writing progress reports and publishing research results in peer-reviewed journals.

Appendix:

Preliminary Data:

IHNV: Duplicate groups of 100 fall chinook salmon fry belonging to two stocks (Salmon River and Upriver Brights) were exposed to four isolates of IHNV by a 5h immersion. Mortality was monitored for 40 days, then 3 months later the survivors were gradually acclimated to salt water and daily mortality was monitored for 12 days. The results indicated that the two fish stocks varied in their susceptibility to the IHNV isolates in fresh water and that fish survival in salt water was dependent on fish stock and was affected by previous exposure to IHNV (Fig. 1).

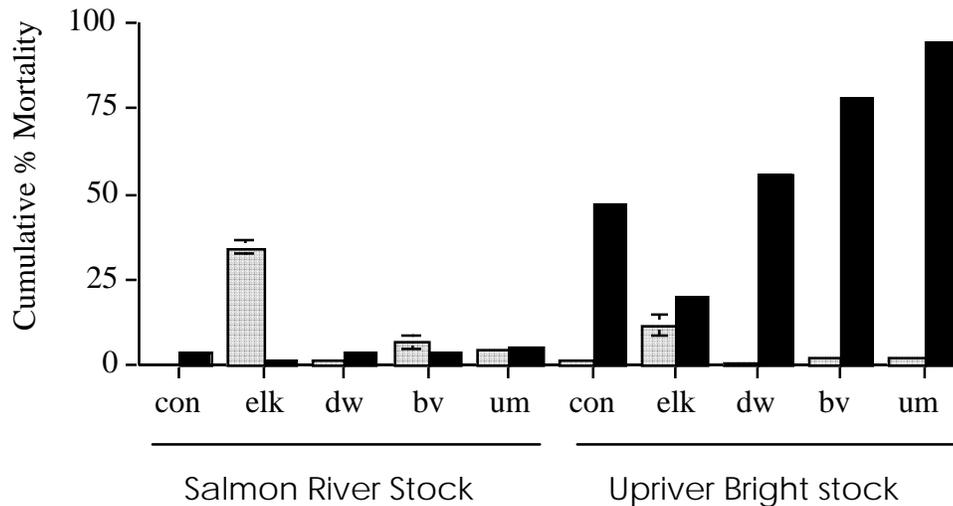


Figure 1. Cumulative percent mortality at 40 days after immersion of Salmon River or Upriver Bright stocks of fall chinook salmon in fresh water containing media (con), a Bonneville 1991 IHNV isolate (bv), a Umatilla 1986 IHNV isolate (um), a Dwarshak 1996 IHNV isolate (dw) or an Elk River 1987 IHNV isolate (elk) (▨). Fish mortality at 12 days after IHNV-survivors were transferred to salt water is also shown (■). Bars are standard error. (Bootland, Lorz and Leong, unpublished data)

C. shasta:

Table 1. Effects of salt water on steelhead trout and coho and chinook salmon exposed to the infectious stage of *Ceratomyxa shasta*.

Salmonid	Exposure length (days)	Fresh water			Salt water		
		# of fish recovered ^a	# of fish infected	% infected	# of fish recovered ^a	# fish infected	% infected
Alsea steelhead trout	3 ^b	21	21	100	6	3	50
	3 ^c	24	24	100	37	37	100
	control ^d	25	0	0	11	0	0
	5 ^b	23	23	100	13	7	54
	5 ^b	18	18	100	9	8	89
	control ^d	25	0	0	16	0	0
Big Creek coho salmon	5 ^b	25	1	4	25	0	0
	control ^d	25	0	0	25	0	0
Round Butte chinook salmon	3 ^c	27	0	0	30	0	0
	control ^d	25	0	0	27	0	0

a. Number of fish exposed minus number of fish that died before spores were detected.

b. Fifty fish exposed; 25 were transferred to fresh water and 25 to salt water.

c. One hundred fish exposed; 50 were transferred to fresh water and 50 to salt water.

d. Control fish were not exposed to the infectious stage of *C. shasta*

(adapted from Bartholomew et al. 1992)

CURRICULUM VITAE of Principal Investigator, Linda M. Bootland, Ph.D.

Education: B.Sc. Marine Biology 1982. Minors: Fisheries Science and Microbiology
University of Guelph, Guelph, Ontario, Canada
Ph.D. Microbiology 1990. University of Guelph, Guelph, Ontario, Canada

Professional Experience:

1994-present Assistant Professor, Senior Research, Dept. Microbiology, Oregon State
University (courtesy appointment 1994-1997), Corvallis, OR
1994-1997 Research Scientist, DiagXotics, Inc., Wilton, CT
1992-1993 Research Scientist, MariGenetics, Inc., Corvallis, OR (part time)
1990 - 1994 Postdoctoral Research Associate, Dept. Microbiology, OSU

Expertise:

Over the past fourteen years, I have gained extensive research expertise on the viral and bacterial pathogens of fish. Research emphasis is on pathogen-host interactions, vaccine development and development of diagnostic assays. During post-doctoral training, I developed a new diagnostic method for IHNV, studied IHNV infection and the resulting disease in rainbow trout, brook trout, chinook salmon and adult kokanee salmon and examined three non-salmonid species as potential reservoirs of IHNV infection. During employment with DiagXotics, I continued research initiated during my Ph.D. on the development of recombinant and genetic vaccines against infectious pancreatic necrosis virus. This research also included the development of a challenge protocol and a vaccine against *Yersinia ruckeri*, a serious bacterial pathogen of salmonid fish. I have extensive experience in fish cell line culture; growth and quantification of viruses and bacteria; diagnosis of salmonid viral and bacterial diseases; and the spawning, rearing and sampling of salmonid fish.

Relevant Publications:

Bootland, L.M. & J.C. Leong. 1998. Infectious haematopoietic necrosis virus. In: Woo, P.T.K. & D. Bruno (eds.). Fish Diseases and Disorders, Volume 3: Viral, Bacterial and Fungal Infections. CAB International, Oxon, UK. (in press)

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Leong, J.C., E. Anderson, L. Bootland, B. Drolet, L. Chen, C. Mason, D. Mourich, and G. Trobridge. 1993. Biotechnologic advances in fish disease research. Conference Proceedings of the International Marine Biotechnology Conference '91, Soc. Industrial Microbiology, Baltimore, Maryland, Oct. 13-15, Wm. Brown Communications, Iowa. pp. 573-586.

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Engelking, H.M., J. Kaufman and L. Bootland. 1992. Infectious hematopoietic necrosis virus (IHNV) in steelhead trout at spawning and during two epizootic outbreaks at Leaburg fish hatchery: detection and transmission. *AFS/FHS Newsletter* 20: 3-6.

CURRICULUM VITAE of Co-principal Investigator: Jerri L. Bartholomew

Education:

Ph.D. in Microbiology - Oregon State University, 1989
M.S. in Fisheries and Wildlife - Oregon State University, 1985
B.S. in Biology, minor in Marine Sciences - Pennsylvania State University, 1980

Professional Experience

1995-present Assistant Professor, Senior Research, Dept. Microbiology, Oregon State University
1994-1995 Instructor, Dept. Microbiology, Oregon State University
1992-1993 Microbiologist, USFWS, National Fishery Research Center, Seattle, WA
1990-1991 Research Associate, Department of Microbiology, OSU
1988-1990 Fishery Biologist, USFWS, National Fishery Research Center

Examples of Presently Funded Research

Use of DNA Probes to Characterize the Life Cycle of *Ceratomyxa shasta* USDA/NRICGP
Identification of the Intermediate Host and Actinosporean Life Stage of *Ceratomyxa shasta* USDA/Animal Health and Disease
Ecological Associations of *Tubifex tubifex* in Enzootic Waters in National Partnership on
Northeastern Oregon and Effects of Gas Supersaturation the Management of Wild
and Native Coldwater Fisheries

Expertise

My expertise is in parasitic and bacterial pathogens of salmonid fishes. I have been involved with research in the Columbia River Basin since the early 1980's and am familiar with the issues and research needs.

Publications

Bartholomew, J. L. 1998. Host resistance to infection by the myxosporean parasite *Ceratomyxa shasta*: a review. *Journal of Aquatic Animal Health*. Accepted for publication
Bartholomew, J. L., M. J. Whipple, D. G. Stevens and J. L. Fryer. 1997. Role of the freshwater polychaete, *Mayanukia speciosa*, in the life cycle of *Ceratomyxa shasta*, a myxosporean parasite of salmon and trout. *American Journal of Parasitology*. 83:859-868.
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Hoffmaster (Bartholomew), J. L., J. E. Sanders, J. S. Rohovec, J. L. Fryer and D. G. Stevens. 1988. Geographic distribution of the myxosporean parasite, *Ceratomyxa shasta* in the Columbia River basin. *Journal of Fish Diseases* 11, 97-100.
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Curriculum vitae of Co- Principal Investigator, JO-ANN C. LEONG, Ph. D.

Distinguished Professor and Chairperson
Department of Microbiology, Oregon State University

Education: B.A., Univ. Calif. Berkeley; Zoology, 1964.
Ph. D., Univ. Calif. S.F. Med. School; Microbiology, 1971

Professional Experience:

Chairperson, Dept. of Microbiology, 1996-
President, Fish Health Section, American Fisheries Society, 1996
Assist. Prof. to Distinguished Prof. of Microbiology, Oregon State Univ., 1975-
Assist. Res. Virologist, Cancer Res. Inst., Univ. Calif. S.F. 1975
Postgraduate Res. Biochem., Univ. Calif. S.F., 1971-74

Expertise:

Our laboratory has a strong commitment to developing strategies to control infectious diseases in fish, particularly salmonid fish. We have developed several vaccines for IHNV and IPNV as well as expression markers for determining the health status of these animals. Our current research funding is devoted to developing vectors for DNA vaccines for fish and providing methods to administer these vaccines in a cost effective manner. We are also looking at virus persistence in virus infected fish. Those efforts have led us to develop genetic markers for disease resistance and we are now looking to develop non-lethal sampling methods to use these markers in large population studies.

Relevant Publications:

- Mourich, D.V., J. Hansen, and J. C. Leong. 1995. Natural killer enhancement factor-like gene of rainbow trout (*O. mykiss*). Immunogenetics 42:438-439.
- Anderson, E., D.V. Mourich, and J. C. Leong. 1996. Gene expression in rainbow trout (*O. mykiss*) following intramuscular injection of DNA. Mol. Marine Biol. & Biotechnol. 5(2):105-113.
- Trobridge, G.D., P. P. Chiou, C. H. Kim, and J. C. Leong. 1997 Induction of the Mx protein of rainbow trout *Oncorhynchus mykiss* in vitro and in vivo with poly IC and IHNV. Dis. of Aq. Org. 30:91-98.
- J.C. Leong, E. Anderson, L.M. Bootland, P.-W. Chiou, M. Johnson, C. Kim, D. Mourich, and G. Trobridge. 1997. Fish Vaccine Antigens Produced or Delivered by Recombinant DNA Technologies. R. Gudding, A. Lillehaug, P.J. Midtlyng, F. Brown (eds.). Fish Vaccinology. Dev. Biol. Stand. Basel, Karger 90: 267-277.
- Trobridge, G.D., S. E. LaPatra, C. H. Kim, and J. C. Leong. 1997. Mx mRNA expression and RFLP analysis of rainbow trout (*Oncorhynchus mykiss*) genetic crosses selected for susceptibility or resistance to IHNV. Dis. Aq. Org., accepted.

Curriculum vitae of Collaborator: DONALD E. CAMPTON, Ph.D

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Education: Ph.D. Genetics, University of California, Davis, 1986.
M.S., Fisheries, University of Washington, Seattle, 1981.
B.S., Genetics, University of California, Berkeley, 1974.

Professional experience

1997-present: Fish Geneticist, U.S. Fish & Wildlife Service, Longview, Washington.
1992-1997: Assoc. Prof., Dept. of Fisheries & Aquatic Sciences, Univ. Fla, Gainesville.
1986-1992: Asst. Prof., Dept. of Fisheries & Aquatic Sciences, U.F.
1981-1986: Graduate Research/Teaching Assistant, University of California, Davis.
1978-1980: Fishery Research Biologist, Washington Dept. of Game, Olympia, WA

Current responsibilities

Serve as regional fish geneticist and program manager for the U.S. Fish & Wildlife Service on technical matters related to the conservation and management of indigenous fish species and associated fishery resources in the Pacific Northwest including California and Nevada. Genetically characterize hatchery and wild populations, develop regional policies and guidelines to protect genetic resources, establish and maintain information data bases on genetic variation, life history data, and population dynamics of hatchery and wild fish populations.

Expertise

Population and quantitative genetics of fish: molecular methods for studying population structures, evolutionary relationships, and introgressive hybridization; statistical/breeding methods for quantifying genetic variation for quantitative characters, and the effects of hatcheries and artificial propagation on the genetic constitution of hatchery and wild populations of salmonid fishes.

Relevant publications

- Campton, D.E. 1987. Natural hybridization and introgression in fishes: methods of detection and genetic interpretations, p.161-192. *IN: N. Ryman and F. Utter (eds.), Population Genetics and Fishery Management.* University of Washington Press, Seattle.
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