
PART I - ADMINISTRATIVE

Section 1. General administrative information

Title of project

Sources Of Myxobacterial Pathogens In Propagated Salmonids

BPA project number: 20104

Contract renewal date (mm/yyyy): **Multiple actions?**

Business name of agency, institution or organization requesting funding

Abernathy Salmon Culture Technology Center/U.S. Fish & Wildlife Service

Business acronym (if appropriate) SCTC/USFWS

Proposal contact person or principal investigator:

Name Dr. Peter W. Taylor

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NPPC Program Measure Number(s) which this project addresses

7.2A.6, 7.2D.1, .3, .4 and .6

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

N/A

Other planning document references

N/A

Short description

Determine the sources and progression of Bacterial Cold Water Disease in propagated salmon. Develop methods to diagnose and treat this disease prior to clinical outbreaks.

Target species

coho salmon, chinook salmon and steelhead

Section 2. Sorting and evaluation

Subbasin
systemwide

Evaluation Process Sort

CBFWA caucus	Special evaluation process	ISRP project type
Mark one or more caucus	If your project fits either of these processes, mark one or both	Mark one or more categories
<input checked="" type="checkbox"/> Anadromous fish <input checked="" type="checkbox"/> Resident fish <input type="checkbox"/> Wildlife	<input 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

Section 4. Objectives, tasks and schedules

Past accomplishments

Year	Accomplishment	Met biological objectives?

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Objectives and tasks

Obj 1,2,3	Objective	Task a,b,c	Task
1	methods development	a	evaluate and select testing protocol
2	sampling	a	collect samples, test samples
2		b	data analysis to determine control points
3	treatment selection	a	test treatments

Objective schedules and costs

Obj #	Start date mm/yyyy	End date mm/yyyy	Measureable biological objective(s)	Milestone	FY2000 Cost %
1	1/2000	6/2000	complete protocols		35.00%
2	8/2000	4/2002	collect samples		65.00%
3	3/2002	1/2003	test treatments		0.00%
4	1/2003	5/2003	final analysis		0.00%
				Total	100.00%

Schedule constraints

Sample collection needs to begin with a spawning season; if funds are available by 08/1999, it would begin then. If not it would begin in 08/2000.

Completion date

05/2003

Section 5. Budget

FY99 project budget (BPA obligated):

FY2000 budget by line item

Item	Note	% of total	FY2000
Personnel		% 29	26,000
Fringe benefits		% 9	7,800
Supplies, materials, non- expendable property		% 11	10,000
Operations & maintenance		% 6	5,000
Capital acquisitions or improvements (e.g. land,		% 17	15,000

buildings, major equip.)			
NEPA costs		%0	0
Construction-related support		%0	0
PIT tags	# of tags:	%0	0
Travel		%6	5,000
Indirect costs		%22	19,800
Subcontractor		%0	0
Other	publications	%2	1,500
TOTAL BPA FY2000 BUDGET REQUEST			\$90,100

Cost sharing

Organization	Item or service provided	% total project cost (incl. BPA)	Amount (\$)
USFWS	0.5FTE salary	%26	32,000
		%0	
		%0	
		%0	
Total project cost (including BPA portion)			\$122,100

Outyear costs

	FY2001	FY02	FY03	FY04
Total budget	\$69,000	\$69,000		

Section 6. References

Watershed?	Reference
<input type="checkbox"/>	Bader, J.A., 1995. Identification and Classification of Three Bacteria Pathogenic to Fish in the Genus Flexibacter Using the Polymerase-Chain-Reaction and Sequence Analysis on the Small Subunit (16S) Ribosomal RNA Gene. Doctoral Dissertation, Univ. of GA
<input type="checkbox"/>	Brown, L.A., Cox, W.T., Levine, R.P. 1997. Evidence that the Causal Agent of Bacterial coldwater disease Flavobacterium psychrophilum is transmitted within salmonid eggs. Dis. Aquatic Organisms 29: 213-218
<input type="checkbox"/>	Chase, D.M. and Pasco, R.J., 1997. Nested PCR for Amplification of a Sequence of the p57 Gene of Renibacterium salmoninarum (abstract only). 39th Annual Western Fish Disease Workshop, Parkville, B.C.
<input type="checkbox"/>	Cipriano, R.C., Ford, L.A., Teska, J.D., Hale, L.E., 1992. Detection of Aeromonas salmonicida in the Mucus of Salmonid Fish. J. Aquat. Animal Health 4:114-118
<input type="checkbox"/>	Cipriano, R.C., Schill, W.B., Teska, J.D., Ford, L.A., 1996. Epizootological

	Study of Bacterial Cold-water Disease in Pacific Salmon and Further Characterization of the Etiologic Agent, <i>Flexibacter psychrophilum</i> . <i>J. Aquat. Animal Health</i> 8: 28-36
<input type="checkbox"/>	FDA, 1984. Bacteriological Analytical Manual, 6th edition. AOAC Publications, Arlington, VA
<input type="checkbox"/>	Ford, L.A. 1993. Detection of <i>Aeromonas salmonicida</i> from Water Using a Filtration Method. <i>Aquaculture</i> 122: 1-7
<input type="checkbox"/>	PNFHPC. 1997. 28th Meeting Report. Portland, OR.
<input type="checkbox"/>	Toyama, T., Kita-Tsukamoto, K., Wakabayashi, H. 1994. Identification of <i>Cytophaga psychrophila</i> by PCR Targeted 16S Ribosomal RNA. <i>Fish Pathology</i> 29:271-275
<input type="checkbox"/>	Wood, J.W. 1974. Diseases of Pacific Salmon: their prevention and treatment, 2nd Edition. State of Washington, Department of Fisheries, Hatchery Division. Olympia, WA

PART II - NARRATIVE

Section 7. Abstract

Bacterial Cold Water Disease (BCWD) and Columnaris Disease (CD) are two of the most detrimental fish health problems at hatcheries in the Pacific Northwest. Their cost, measured in numbers of fish lost and overall production dollar value, makes them the most expensive fish health problem in the region. This project proposes to look at the sources of these diseases and examine disease progression through the production cycle in hatcheries. By better defining the diseases' source and progress during the production cycle, critical control points can be determined to reduce or eliminate the disease. Once critical control points are established, treatment options to reduce disease impact will be tested. Goals of this project address FWP goals in areas of fish health, production cost, smolt quality improvement and hatchery-wild fish interactions. The polymerase chain reaction (PCR) is a highly sensitive, species-specific method to target pathogen DNA. This test will be used to screen water, wild fish, returning spawners and eggs for the presence of BCWD. Water-hardened eggs, sac fry, alevins and fingerlings also will be screened to determine the progress of the disease throughout the hatchery production cycle. By examining a bacterial presence profile, critical control points can be selected for treatment methods to minimize the disease impact.

Section 8. Project description

a. Technical and/or scientific background

Myxobacterial infections caused by *Flavobacterium psychrophilum* (Bacterial Cold Water Disease), *F. columnare* (Columnaris Disease) and other yellow-pigmented bacteria (YPB) have recently begun to severely affect salmon production in the Pacific

Northwest. Of this group, BCWD is by far the most prevalent disease. Coho salmon (*Oncorhynchus kisutch*), chinook salmon (*O. tshawytscha*) and steelhead (*O. mykiss*) are the most susceptible salmonid species. Federal, State, Tribal and Private hatcheries have all reported severe fish health problems due to these pathogens (28th Meeting, PNFHPC, Feb., 1997). Current control methods for these diseases consist of maintaining good management practices to prevent disease or the use of medicated feed once clinical signs appear and appropriated diagnosis has been made. Unfortunately, good management practices are no guarantee that disease will not occur and medicated feeds are only useful when the disease is caught prior to the fish going off feed.

Myxobacterial species are common, water-born bacteria found in almost all natural waters. The sources of these bacteria are not fully understood; Holt (1993) states that the natural reservoirs for *F. psychrophilum* are uncertain while Wood (1968) felt that wild populations and adult spawners were possible sources of BCWD. Wild fish would explain the disease at facilities utilizing natural waters (ie. river or lake), but theoretically could rule them out at hatcheries using well or spring water. Holt (1993) showed that *F. psychrophilum* could be isolated from ovarian fluids and milt of returning spawners thus providing a source of infection in eggs and fry. Brown et al. (1997) showed that *F. psychrophilum* was present inter-ova (in ovarian fluid) and intra-ova (within the egg), further re-enforcing vertical transmission of the disease from parent to offspring. Actual source of the disease is probably a combination of water, wild fish and returning spawners.

These bacterial diseases affect fish production in a number of ways. The most obvious is actual mortality due to bacterial infection. Loss of fish affects required hatchery production goals, stocking or release goals and, ultimately, return goals. However, mortality is only the ultimate manifestation of a disease problem. There are a number of other areas within a hatchery program that are affected by a fish health problem. Energy expenditure of an animal during and after an infection (recovery time) will ultimately influence its general health and survivability. Energy spent on overcoming and recovering from a disease means less energy available for normal growth. This “down time” is reflected in overall hatchery production and fish survival. Fish health problems affect hatchery economics with increased labor, treatment costs, inefficient feed conversion and decreased survival potential. Good fish management practices do a great deal to prevent the outbreak of fish health problems. Nonetheless, fish health problems still occur, even under the most conscientious management programs. Rarely do such problems give the manager any degree of warning. Once the onset of a fish health problem has started, early diagnosis and treatment are essential. However, even if mortality or clinical signs of a disease are arrested, the long-term damage to that fish stock has already occurred.

Increased knowledge of the disease source and routes of infection are needed before solutions to hatchery disease problems can be initiated. The impact of infected wild fish, returning spawners and other sources of hatchery disease outbreaks needs to be studied and evaluated. A monitoring system needs to be developed that would detect and minimize these diseases prior to reaching a full-blown clinical stage in hatchery fish.

Critical control points need to be established within the production cycle. Two basic criteria are needed to define a critical control point: that time within the production cycle where the problem is increasing to harmful levels and a point that provides a window for possible remedy prior to reaching critical levels. Establishment of valid control points would be invaluable to the hatchery manager in reducing fish mortality and improving economic cost of hatchery operation.

b. Rationale and significance to Regional Programs

This proposal addresses needs and measures cited in sections 7.2A.6 and 7.2D.1, .3, .4 and .6 of the Columbia River Basin Fish and Wildlife Program (Northwest Power Planning Council, Dec. 14, 1994).

1. Section 7.2A.6: Integrated Hatchery Operations, Fish Health Policy.

This proposal addresses concerns of introduction and spread of disease in hatchery situations. Its ultimate goal is to improve the health of fish destined for release. This proposal will better define possible sources and routes of Bacterial Cold Water Disease and other myxobacterial infections. It will define and establish critical control points within hatchery operations that could be used to better control disease outbreaks. Reducing incidence of disease will result in healthier fish for release and lower production costs.

2. Section 7.2D.1, .3, .4 and .6: Improved Propagation at Existing Facilities.

This proposal addresses the improvement of health and survival of artificially propagated fish. It will improve fish health protection at hatcheries. Methods developed in this proposal will improve detection, diagnosis and control of disease. This proposal will add to knowledge of disease potential between hatchery reared and naturally reared populations.

c. Relationships to other projects

(Replace this text with your response in paragraph form)

d. Project history (for ongoing projects)

(Replace this text with your response in paragraph form)

e. Proposal objectives

1. Methods development:

A number of techniques and protocols exist for bacterial sampling and identification of a target species. This objective will determine which methods are the most appropriate and develop a standardized protocol for the isolation and

identification of BCWD. Criteria for the protocols will include sensitivity level, ease of use, time expenditure and cost. The selected protocol will be made available to all Fish Health laboratories in the region for incorporation into diagnostic and sampling endeavors.

2. Sampling:

Initiate a sampling program that will provide information on the sources of BCWD and a profile of the progression of BCWD throughout the production cycle. Sampling will include hatchery waters, wild fish, returning spawners and life stages of propagated fish within the system. Incidence profiles of BCWD will be used to define potential critical control points in the production system. A report on critical control points will be presented to fish production managers in the region.

3. Treatments:

Fish disease control is limited by availability of legal therapeutants; the U.S. Food and Drug Administration has licensed very few compounds for aquacultural use. Treatments fall into two basic areas: chemical compounds added to the water and antibiotics added to fish feed. Evaluation of critical control points will dictate which legal therapeutants can be used in any given situation. The objective is to select a number of treatment regimes using FDA cleared compounds to test for their disease reduction potential.

f. Methods

1. Methods development:

Highly sensitive technologies are available for species specific identification of bacteria. The polymerase chain reaction (PCR) is based on DNA recognition of a target species using specific nucleic acid primers. Appropriate primers have been developed for both BCWD (Toyama et al., 1994 and Cipriano et al., 1996) and CD (Bader, 1995). Originally these primers were used under stringent laboratory conditions using pure bacterial cultures as source material; samples from clinical sources were not run. However, PCR protocols for clinical samples do exist. Chase and Pasco (1998) have developed a PCR protocol for the identification of *Renibacterium salmoninarum* (Bacterial Kidney Disease) directly from infected fish. Preliminary studies in our laboratory using the two different sets of BCWD primers indicate that similar results can be derived directly from clinical samples.

Fish will be experimentally infected with BCWD in our laboratory. Samples of kidney, liver, spleen, gills, mucus and tank water will be taken and evaluated by PCR. Different DNA extraction methods will be tested in order to select the best protocol based on test sensitivity, ease of use and cost effectiveness. PCR results on the different samples will be evaluated to determine the best type of sample for future testing.

2. Field Sampling:

There is some precedence for bacterial pathogen monitoring in hatchery situations. Cipriano et al. (1992) developed a method for monitoring the incidence of *Aeromonas salmonicida* (furunculosis) using a selective bacteriological media to isolate the pathogen from external mucus of fish. Ford (1993) used the same media to isolate *A. salmonicida* from hatchery water samples. Our proposal to use PCR instead of a selective media should provide a more sensitive method of detection while reducing the testing time to hours instead of days.

At least two hatcheries will be selected as sampling sites; one using natural waters and one using well or spring water for rearing. Samples will be screened for BCWD by PCR to establish the presence of the disease agent in the hatchery system. In conjunction with PCR, samples will also be plated on media (FDA, 1984) to obtain quantitative numbers. Samples will be taken from the following sources:

- a. Wild fish from adjacent waters.
- b. Incoming water (natural water source or source of well/spring).
- c. Ovarian fluid, milts and green eggs from returning spawners.
- d. Water-hardened eggs and sac fry.
- e. Fingerlings
- f. Effluent water from fish holding units.

Samples from a, b and c will be evaluated to determine possible sources of BCWD and to determine incidence levels entering the hatchery system. Samples from d, e and f will be taken weekly to trace the progression of potential infection through the hatchery production cycle.

3. Treatments:

Once the sources and incidence levels of BCWD have been determined, different treatment regimes will be examined to reduce the impact of the disease. Should returning spawners be a critical source, antibiotic treatments of adult fish would be tested. Should BCWD build-up occur in incubators or raceways, chemical treatments will be used to reduce bacterial numbers. Treatment compounds might include Terramycin, erythromycin, sodium chloride, formalin, potassium permanganate and hydrogen peroxide.

g. Facilities and equipment

The Abernathy Salmon Culture Technology Center, Longview, WA, has complete laboratory capabilities for conducting research in areas of fish health, nutrition, genetics and fish production. The fish health laboratory has capabilities in bacteriology, virology and immunology. The only equipment needed for this project that is not already in place

would be an extra cold temperature incubator, PCR thermocycler and UV/vis spectrophotometer.

h. Budget

The project would require salary and fringe benefits for a laboratory technician able to process the samples and collect appropriate data. Supplies and materials (expendable) will be needed for laboratory testing and sample preparation. Operations and maintenance funds will be needed for operating the wet lab facilities for BCWD challenges. An incubator, thermocycler and spectrophotometer will be needed to handle increased sampling. Travel funds will be needed to collect samples. Indirect costs were calculated at 22% as per USFWS regulations. Publications cost will be needed to disseminate information.

Section 9. Key personnel

Principal Investigator: Dr. Peter W. Taylor, Research Fish Pathologist, 0.5 FTE. Will oversee all aspects of the project. Will train field personnel in sample collection at participating hatcheries. Will conduct all laboratory testing of samples and analysis of data collected.

Resume

Peter W. Taylor
U.S. Fish and Wildlife Service
Abernathy Salmon Culture Technology Center
Longview, WA 98632

Education: BS, Wildlife and Fisheries Science, New Mexico State Univ., 1973
MS, Aquaculture and Fisheries, Auburn Univ., 1975
PhD, Fish Pathology, Auburn Univ., 1977

Certification: Certified Fisheries Scientist, AFS, 1988
Certified Fish Pathologist, FHS/AFS, 1989

Employment:

1996 to present USFWS, Abernathy Salmon Culture Technology Center,
Longview, WA 9863
1992-1995 National Biological Survey, Southeastern Fish Culture Lab.,
Marion, AL 3675
1986-1991 MS Coop. Extension Service, Miss. State Univ., Belzoni, MS 3903

Current Responsibilities: Conduct research in areas of fish health impacting Region 1 of the USFWS. Supply expertise in fish health to hatcheries operating in Region 1.

Expertise: Over ten years experience as a clinical diagnostician in fish health working with warm water and cold water Aquaculture. Over 20 years experience conducting research in parasitology, bacteriology, virology and immunology of fish. Have served as a fish health specialist for projects sponsored by USAID, USDA and FDA both nationally and overseas.

Publications:

Taylor, P.W., J.E. Crawford and E.B. Shotts. 1995. Comparison of Two Biochemical Test Systems With Conventional Methods for the Identification of Bacteria Pathogenic to Warmwater Fish. *Journal of Aquatic Animal Health*, 7: 312-317

Jenkins, J.A., and P.W. Taylor. 1995. An Alternative Bacteriological Medium for the Isolation of *Aeromonas*. *Journal of Wildlife Diseases*, 31(2): 272-275

Taylor, P.W. 1992. Fish-eating Birds as Vectors for *Edwardsiella ictaluri* on Commercial Catfish Ponds in Mississippi. *Journal of Aquatic Animal Health*, 4: 240-243

Section 10. Information/technology transfer

Information will be published in peer-reviewed journals, reports to hatchery managers, direct meetings with hatchery managers and workshops.

Congratulations!