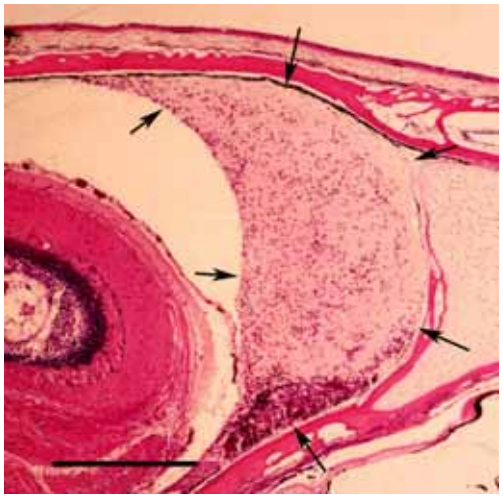
PNNL-14748

Prevention of Prespawning Mortality: Cause of Salmon Headburns and Cranial Lesions



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FINAL REPORT
June 2004

Prepared for the U.S. Army Corps of Engineers
Portland District, Portland, Oregon
Under a Related Services Agreement
With the U.S. Department of Energy
Contract DE-AC06-76RL01830

Pacific Northwest National Laboratory
Operated by Battelle for the
U.S. Department of Energy

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[(a)] AquaTechnics Inc., Carlsborg, Washington

Abstract

Head injuries on returning adult salmon and steelhead have been observed at Columbia Basin fish passage facilities since at least sometime in the 1970s (NMFS 1997) and were particularly noted by field biologists in Endangered Species Act (ESA) listed runs of fish beginning in 1996. The injuries commonly referred to as "headburns" or "scalping," are poorly defined (Elston 1996).

The Reasonable and Prudent Alternatives (RPA) listed in the 2000 Federal Columbia River Power System (FCRPS) BIOLOGICAL OPINION DECEMBER 21, 2000 include three actions calling for the enhancement of headburn investigations. Action 106 (9.6.1.6.1) asks that "investigations of adult passage problems…identify direct and indirect mortality-related problems and implement needed improvements." Action 107 identifies evaluations "to assess survival of adult salmonids migrating upstream and factors contributing to unaccounted losses…[with] specific investigations [to include] headburn." Action 108 calls for "a comprehensive evaluation to investigate causes of headburn in adult salmonids and shall implement corrective measures as warranted."

The U.S. Army Corps of Engineers (Corps of Engineers) funded this project, "Prevention of Prespawning Mortality: Cause of Salmon Headburns and Cranial Lesions," under the Anadromous Fish Evaluation Project. The project results reported here are offered to provide scientific information that follows the fundamental components of the RPA and allow the FCRPS to avoid jeopardizing the continued existence of listed salmon and steelhead.

"Headburn" is a descriptive clinical term used by fishery biologists to describe scalping or exfoliation of skin and ulceration of underlying connective tissue and muscle, primarily of the jaw and cranial region of salmonids observed at fish passage facilities on the Columbia and Snake Rivers. The overall goal of the project was to provide information about headburn that will enable Corps of Engineers managers to make adjustments in operational procedures or facilities to prevent loss of prespawning adult salmonids that migrate through the facilities.

When tested with juvenile Chinook salmon or rainbow trout, the simulated turbine passage used in this study did not cause the types of lesions that have been determined to be precursors to headburn lesions in adult ocean-return fish, although it did cause other potentially significant lesions in the juvenile fish.

Headburn lesions from adult spring Chinook salmon at downriver locations are typically fresh and recently initiated, while fish from upriver locations typically have old and chronically healing or re-injured lesions. The prevalence of headburn lesions increases in proportion to the distance upriver in the system. Fungal infections are initiated in fresh lesions in downriver fish, although the infections are subclinical and are not visible macroscopically. These fungal infections exacerbate the lesions and retard wound healing. Fall-run steelhead are apparently affected at a much lower prevalence of headburn lesions than spring Chinook salmon. Abrasions and ulcerations in both species are colonized by previously recognized and newly discovered bacteria and fungi.

Spring-run Chinook salmon with moderate to severe lesions reach upriver hatcheries in some cases, but their progressive physiological deterioration and chronic infection status suggest that many of these wounded hatchery and wild fish die in the river. There is a need to standardize the recording of headburn

fish at trapping facilities and fish hatcheries using the classification system provided in this report. Prespawning mortality loss to headburns may reach or exceed 22% of returning adult fish.

Headburn lesions are primarily caused when fish collide with concrete or other structures at dams and fish passage facilities, and may be exacerbated in some fish that "fallback" or pass over spillways or through turbine assemblies after having passed the dam through a fish ladder. Headburns in salmonids are apparently exacerbated in spring runs during high water years.

Prespawning mortality of headburned salmonids can be prevented or greatly reduced by therapeutic treatment of both hatchery and wild fish. Treatments would consist of topical application of an anti-fungal agent, injection of replacement plasma electrolytes into the peritoneal cavity, and injection of a broad-spectrum antibacterial agent at fish passage and trapping facilities or hatcheries. Conditions in raceways or ponds where mature fish are held to maturity for spawning should be improved to help reduce the severity of eruptive fungal infections and associated debilitating loss of physiological condition.

Executive Summary

The Reasonable and Prudent Alternatives (RPA) listed in the 2000 Federal Columbia River Power System (FCRPS) Biological Opinion December 21, 2000, include three actions calling for the enhancement of headburn investigations. Action 106 (9.6.1.6.1) asks that "investigations of adult passage problems…identify direct and indirect mortality-related problems and implement needed improvements." Action 107 identifies evaluations "…to assess survival of adult salmonids migrating upstream and factors contributing to unaccounted losses…[with] specific investigations [to include] headburn." Action 108 calls for "…a comprehensive evaluation to investigate causes of headburn in adult salmonids and shall implement corrective measures as warranted."

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U.S. Army Corps of Engineers funded this project, "Prevention of Prespawning Mortality: Cause of Salmon Headburns and Cranial Lesions," under the Anadromous Fish Evaluation Project. "Headburn" is a descriptive clinical term used by fishery biologists to describe scalping or exfoliation of skin and ulceration of underlying connective tissue and muscle, primarily of the jaw and cranial region of salmonids observed at fish passage facilities on the Columbia and Snake Rivers. The observations are usually made on upstream migrant adult salmon or steelhead. Past observations have suggested that the incidence of headburns may be associated with high river flow conditions or spillway discharges from dams and that passage of juvenile fish through turbines in dams might initiate damage that would later result in a headburn condition in adult ocean-return fish.

The overall goal of the project was to provide information about headburns that will enable Corps of Engineers managers to make adjustments in operational procedures or facilities to prevent loss of prespawning adult salmonids that migrate through the facilities. The specific objectives were to:

1. Conduct a laboratory study of exposure scenarios using a hyperbaric chamber to produce previously documented cranial lesions, including evaluation of additive effects of repeated exposures, chronicity of lesions, residual effects, and recovery.

2. Define the detailed nature and patho-physiology of the observed cranial lesions from the various exposure scenarios.

3. Determine long-term disposition and outcome of the lesions by growing exposed juveniles to maturity.
4. Collect adult salmonids from fish trapping facilities or hatcheries to define the pathological manifestations and development of headburns on prespawning adults, and make recommendations to manage and prevent adult prespawning losses to headburns.

5. Conduct a preliminary design analysis for an adult fish hyperbaric chamber.

The conclusions and recommendations of the study are briefly described below.

When tested with juvenile Chinook salmon or rainbow trout, the simulated turbine passage regime used in this study did not cause lesions in adult ocean-return fish, although it did cause a significant increase in mild and localized aneurysms in the brain of exposed juvenile rainbow trout but not in exposed juvenile Chinook salmon. The simulated turbine passage regime did result in a significant increase in hemorrhage in the olfactory organ of exposed juvenile Chinook salmon and rainbow trout. The lesions are likely to cause a short-term reduction in the ecological and survival fitness of the exposed fish and should be examined in river-caught fish to determine the extent of damage that may be caused by turbine passage exposure.

Headburn lesions from adult spring Chinook salmon at downriver locations are fresh and recently initiated while fish from upriver locations typically have old and chronically healing or re-injured lesions. The prevalence of headburn lesions increases in proportion to the distance upriver in the system. Fungal infections are initiated in fresh lesions in downriver fish, although the infections are subclinical and are not visible macroscopically. These fungal infections exacerbate the lesions and retard wound healing. Injured fish have a limited-to-nonexistent inflammatory response when bacteria infect the headburn lesions. Fall-run steelhead, although apparently affected at a much lower prevalence of headburns than spring-run Chinook salmon, have wounds that are colonized not only by the widely recognized *Saprolegnia sp*. but also by other fungi and a yeast (presumptively identified as *Aureobasidium pullans* and *Rhodotorula mucilaginosa*, respectively) not previously described from such lesions.

Spring-run Chinook salmon with mild lesions may heal and successfully reach hatcheries or spawning grounds. However, the healing process is incomplete and the fish are highly susceptible to wounding again. Spring-run Chinook salmon with moderate to severe lesions reach upriver hatcheries in some cases but their progressive physiological deterioration and chronic infection status suggest that many such wounded hatchery and wild fish die in the river.

There is a need to standardize the recording frequency and procedures for headburned fish at trapping facilities and fish hatcheries, and to consistently collect data to more accurately estimate the magnitude of this problem. A proposed classification system is described in this report. Data from Rapid River Hatchery suggests that recording of headburned fish when they arrive at the trapping facility may substantially underestimate the number of affected fish and that the loss of fish due to headburn lesions in upriver locations may be as high as 22%, even in moderate water years such as 2003.

Headburn lesions are primarily caused when fish collide with concrete or other structures at dams and fish passage facilities and may be exacerbated in some fish that “fallback” or pass over spillways or through turbine assemblies after having passed the dam through a fish ladder. Headburns in salmonids are apparently exacerbated in spring runs during high water years, perhaps because of increased water turbulence and high flow rates. Headburns could also be exacerbated by gas-supersaturation. The exacerbation and extent of headburns and the loss of injured fish needs to be studied in detail during a high water year.

Several species of opportunistic fish pathogenic bacteria and fungi, certain strains of which can also infect humans, were isolated.

Prespawning mortality of headburned salmonids can be prevented or greatly reduced by therapeutic treatment of selected populations of hatchery and wild fish. Treatments would consist of topical application of an anti-fungal agent, injection of replacement plasma electrolytes into the peritoneal cavity, and injection of a broad-spectrum antibacterial agent at fish passage and trapping facilities or hatcheries. An investigative program should be undertaken to evaluate the recommended therapeutic regimen for headburned fish, first using hatchery fish that are not needed for human consumption, to demonstrate that prespawning losses can be dramatically reduced and possibly eliminated.

A standardized system of classifying headburn lesions should be adopted, such as that developed in this report and used at fish trapping facilities and hatcheries.

Conditions in raceways or ponds where mature fish are held to maturity for spawning should be improved to help reduce the severity of eruptive fungal infections and associated debilitating loss of physiological condition.

A screening genetic assay should be conducted on the bacterial and fungal isolates, known to be related to zoonotic strains (i.e., strains affecting humans), obtained in this study, to verify whether or not these strains contain genes conferring human and/or fish pathogenicity and to better characterize the complex of fungi shown in this study to infect headburn lesions.

Contents

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Acronyms

Bm	basement membrane
ESA	Endangered Species Act
FCRPS	Federal Columbia River Power System
GMS	Gomori methenamine silver
H&E	hematoxylin and eosin (stain)
LSU	large subunit
NMFS	National Marine Fisheries Service
PNNL	Pacific Northwest National Laboratory
RPA	Reasonable and Prudent Alternatives
SC	stratum compactum
SS	stratum spongiosum
TDG	total dissolved gas
TSA	tryptic soy agar
TSB	tryptic soy broth

Acknowledgements

Many people made valuable contributions to this study and deserve acknowledgement. The authors of this report and Pacific Northwest National Laboratories thank the following individuals and organizations for their kind assistance and for allowing access to fish collecting facilities, terminal sampling of selected injured hatchery fish, and the collection and sharing of data.

David Clugston of the U.S. Army Corps of Engineers managed technical and contractual needs of the project and provided access to the fish trapping facility at Bonneville dam.

Mr. Steve Lee of the University of Idaho collected headburn lesion tissue, provided photographs of injured fish, and general information about the occurrence of headburned fish at Bonneville Dam. Dr. Chris Peery of the University of Idaho provided prevalence data regarding headburned fish at Bonneville and Lower Granite dams and shared information on headburned fish enumerated at Bonneville Dam.

Mr. Earl Prentice of the National Marine Fisheries Service facilitated initial access to the fish trapping facility at Bonneville Dam.

Mr. Preston Bronson of the Umatilla Tribe arranged for sampling fish at the Umatilla Tribe trapping facility on the Umatilla River. Initial contact and the cooperation of Tribal biologist Brian Zimmerman facilitated this arrangement. The Umatilla Tribe provided staff support, facility access, assistance in terminal sampling of hatchery fish, and data on headburn prevalence.

Mr. Ralph Steiner, Hatchery Manager of the Rapid River Hatchery, Idaho Department of Fish and Game at Riggins, Idaho kindly permitted the study team to terminally sample selected hatchery headburned fish in 2002 and 2003. Ms. Nicola Johnson assisted with the logistics of sampling trips, generously provided hatchery data regarding headburn prevalence and responded to multiple requests for information. Mr. Keith Johnson and Mr. Doug Munson of the Eagle Fish Health Laboratory, Idaho Department of Fish and Game, kindly provided approval for the sampling at Rapid River Hatchery and facilitated communications with hatchery staff.

Mr. Bill Miller, hatchery manager of Dworshak National Fish Hatchery, U.S. Fish and Wildlife Service, responded positively to our request to collect samples and submitted our study plan to the Hatchery Evaluation Team. Mr. Thomas Trock assisted with the approval for our sampling visits and gave final approval for sampling schedules and parameters. We are particularly indebted to Mr. Ralph Roseberg of the hatchery staff for assisting with sampling and ensuring that we were able to collect adequate samples and for providing, on several occasions, historical and current data regarding headburn prevalence.

Dr. Dennis Dauble, Pacific Northwest National Laboratory, reviewed the manuscript. Cary Counts was the technical editor and Debora Schulz provided the document design.

1.0 Background and Introduction

Head injuries on returning adult salmon and steelhead have been observed at Columbia Basin fish passage facilities since at least sometime in the 1970s (NMFS 1997) and were particularly noted by field biologists in Endangered Species Act (ESA) listed runs of fish beginning in 1996. The injuries commonly referred to as "headburns" or "scalping," are poorly defined (Elston 1996).

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"Headburn" is a descriptive clinical term used by fishery biologists to describe scalping or exfoliation of skin and underlying connective tissue primarily of the jaw and cranial region of salmonids observed at fish passage facilities on the Columbia and Snake Rivers. The observations are usually made on upstream migrant adult salmon or steelhead. Past observations have suggested that the incidence of headburns may be associated with high river flow conditions or spillway discharges from dams. This hypothesis was not tested during the sampling years of this study (2003 and 2004) primarily because these were low or moderate water discharge years. The etiology of headburns, its development as a clinical condition, the condition of affected fish, possible recovery of affected fish, and their ability to survive once reaching hatcheries or spawning grounds have not been previously investigated.

In a series of studies conducted prior to those reported here, Abernethy et al. (2000) determined that rainbow trout developed black cranial discolorations when exposed to various combinations of gas-supersaturation and pressure. Although the condition was found at low prevalence in fish that did not receive any gas-supersaturation or pressure treatment, such treatments markedly increased the prevalence of the condition to more than 23% in the most severe treatment conditions. These findings raised a concern about the possible relationship between gas and pressure exposures in juvenile fish and the subsequent development of headburns in adult fish.

Out-migrant smolts are potentially exposed to gas-supersaturation and multiple turbine passage pressurization scenarios. Resulting cranial lesions could sensitize the fish to trauma occurring during adult return. Alternatively, exposure of returning adult fish to gas-supersaturation or high-pressure spikes during fallback could directly result in the cranial lesions observed in the baseline study. Because headburns appear to be a potentially significant cause of prespawning mortality, particularly in low run fish and fish listed

under the Endangered Species Act (ESA), it was of utmost importance to define the cause(s) of headburns, to determine their development and exact consequences for affected fish, and to determine key biological and environmental factors that contribute to the lesion. Findings from these studies, reported here, enabled us to formulate and recommend rational mitigation and management strategies to prevent headburns and associated prespawning mortality. In addition, the studies reported here provide the first systematic description and classification of headburn or scalping lesions in adult salmonids. Biologists at fish collection and inspection facilities can use this information to better determine the magnitude and severity of the headburn problem.

There are only two known prior reports describing salmon headburns (Elston 1996; Groberg 1996). In June 1996, National Marine Fisheries Service (NMFS) biologists reported observations of headburns in adult salmonids at the Lower Granite Dam fish passage facility. Given the uncertain etiology of this condition, the question of their cause was once again highlighted. While there has been no additional evidence to link headburns with high dissolved gas saturation, the elevated total dissolved gas (TDG) levels in the Snake and Columbia Rivers during spring 1996 runoff at the time headburns were observed raised the question of a possible link between the lesions and high dissolved gas. Alternatively, headburns may represent primary abrasive lesions that proceed to a variety of outcomes.

1.1 Goals and Objectives of Study

The goals of the study were to 1) determine the detailed nature and cause of headburns, a condition that causes significant losses of prespawning adult salmonids in the Columbia Basin and 2) provide information about headburns that will enable the Corps of Engineers managers to make adjustments in operational procedures or facilities to prevent loss of prespawning adult salmonids migrating through the facilities (Figure 1). The specific objectives are described below.

Objective 1. Expose Juvenile Salmonid in a Hyperbaric Chamber. Conduct a laboratory study of exposure scenarios using a hyperbaric chamber to produce the previously documented cranial lesions, including evaluation of additive effects of repeated exposures, chronicity of lesions, residual effects, and recovery.

Objective 2. Define the Nature of Cranial Lesions in Juvenile Fish. Define the detailed nature and patho-physiology of the observed cranial lesions (darkening in the dorsal head region) from single and multiple exposure scenarios, including evaluation of residual tissue damage and predisposition to additional tissue damage as a result of the lesions and potential for healing.

Objective 3. Determine Long-Term Outcome of Juvenile Exposures. Determine long-term disposition and outcome of the lesions by growing exposed juveniles to maturity.

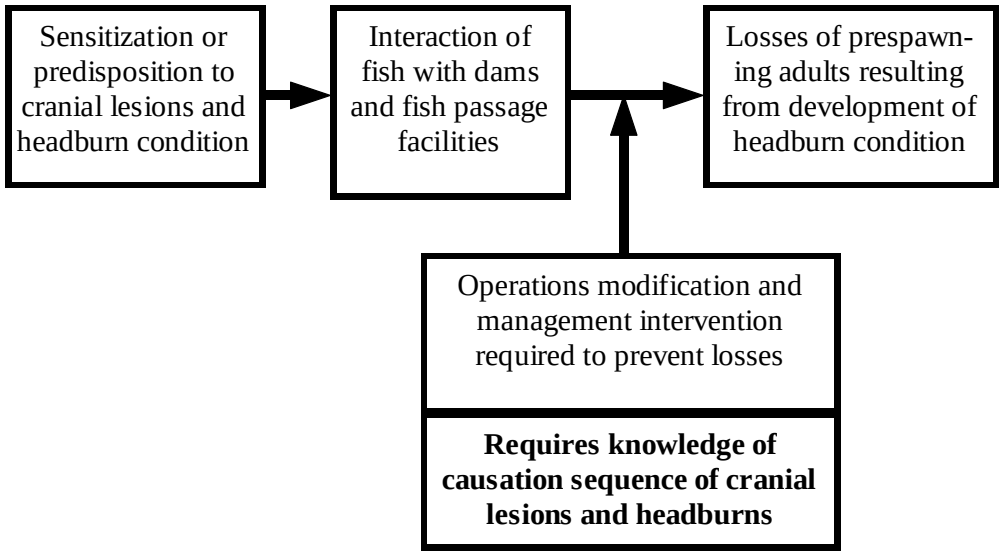

Figure 1.1. Pathway to developing and using information about headburns on salmonids to improve passage facilities at Columbia Basin dams.

Objective 4. Define Headburns and Their Cause in Adult Salmonids at Columbia and Snake River Collection Facilities. Collect adult salmonids from collection facilities (dams or hatcheries) to define in more detail the pathological manifestations and development of headburns on prespawning adults and make recommendations to manage and prevent adult prespawning losses to headburns.

Objective 5. Preliminary Design Analysis for Adult Hyperbaric Chamber. Complete a preliminary design analysis for a hyperbaric chamber in which adult salmonids can be subjected to the conditions that produce headburns.

2.0 Materials and Methods

2.1 Laboratory Studies of Juvenile Salmonids

2.1.1 Hyperbaric Exposures of Juvenile Salmonids

Juvenile fish exposures and analysis were conducted using the hyperbaric chamber at the Pacific Northwest National Laboratory (PNNL) (Figure 2.1). The objective was to examine the relative importance of pressure changes and gas saturation as a source of cranial lesions in juvenile salmonids. Based on preliminary data (Abernethy et el. 2000), the tests were designed to quantify the occurrence of lesions in response to rapid pressure changes typical of turbine passage, with and without the complication of the fish being acclimated to gas supersaturated water.

Two species (rainbow trout, *Oncorhynchus mykiss* and Chinook salmon, *O. tshawytscha*) were tested. Rainbow trout were obtained from Troutlodge, Inc. in Soap Lake, Washington. Eyed fall Chinook salmon eggs were obtained from the Washington Department of Fish and Wildlife's Priest Rapids Hatchery on the Columbia River near Mattawa, Washington. The eggs were hatched and reared at PNNL's aquatic laboratory until the juvenile fall Chinook salmon reached ~10 cm in length.

Tests were conducted at ~17°C). Forty fish (20 in each of two chambers) were acclimated to 135% gas saturation at the equivalent of 30 feet of depth (191 kPa, 27.7 psi) for a period of 16 to 22 hours. Each group was then subjected to a pressure spike simulating passage through a Kaplan turbine. During the computer-controlled program sequence, pressure increased to ~58 psi (~400 kPa) over 30 to 60 seconds to

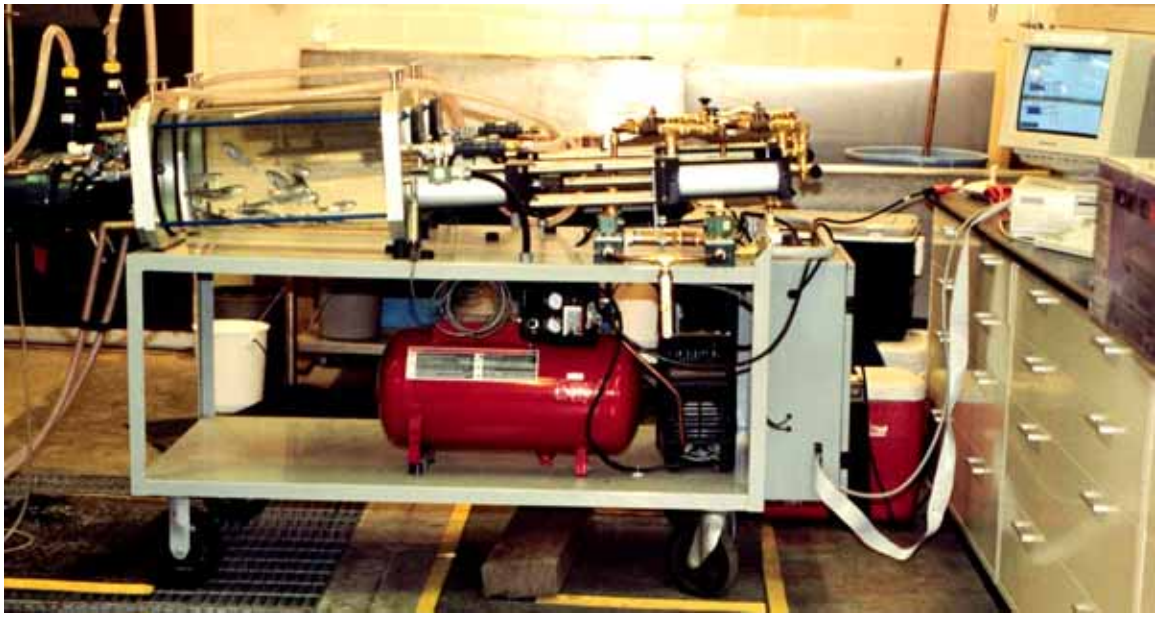

Figure 2.1. Turbine passage system with rainbow trout in hyperbaric chambers.

simulate fish entering the turbine intake and approaching the runner. Fish were then subjected to a sudden decrease in pressure to ~1 psi (210 kPa) to simulate the pressure downstream of the runner at a typical lower Columbia River dam. After the pressure spike, the pressure transition simulates a fish passing out of the draft tube and into the tailrace. After completion of the simulated turbine passage, fish were removed from the chambers and placed in a holding trough for observation.

2.1.2 Introduction of Fish into the Hyperbaric Chamber

The hyperbaric chambers were partially filled with water, and the delivery pump was turned off. Twenty fish were netted from the acclimation tank and placed in ~10 L of water in a bucket. The fish were then poured into a chamber through the 4-inch polyvinyl chloride (PVC) pipe and valve on the end of the hyperbaric chamber. After fish were added to each chamber, the valves were closed, the pump turned on, and all air evacuated from each chamber through a vent tube. During acclimation, both the vent tubes and the outlets remained wide open to prevent buildup of gas bubbles in the hyperbaric chambers. Pressure within the hyperbaric chambers was adjusted by making slight adjustments to the flow control valve at the outlet of the supply pump. When the desired pressure was achieved, a dark cover was placed over the chambers to calm the fish. An "acclimation" file was then initiated on the system's computer to record pressure at ~90-second intervals within a chamber during the acclimation period (16 to 22 hrs).

2.1.3 Simulation of Turbine Passage in the Hyperbaric Chamber

After the acclimation period, fish were subjected to the simulated turbine passage pressure spike shown in Figure 2.2. To initiate the sequence, the piston was first moved all the way in to purge any air that may have accumulated in the cylinder. Then, the piston was positioned in the middle of the stroke. When the sequence was started, the inlet, outlet, and vent tube valves were quickly closed, and the computer controlled the piston to maintain the appropriate pressure (191 kPa) within the hyperbaric chamber. After a brief period (~15 sec), the "dive" was initiated. The entire pressure sequence lasted about 90 seconds. At the end of the sequence, the chambers were taken to "surface" pressure (101 kPa), and water flow was restored to the chambers. Two minutes after completion of the pressure spike, the fish were removed from the chambers and placed in holding troughs.

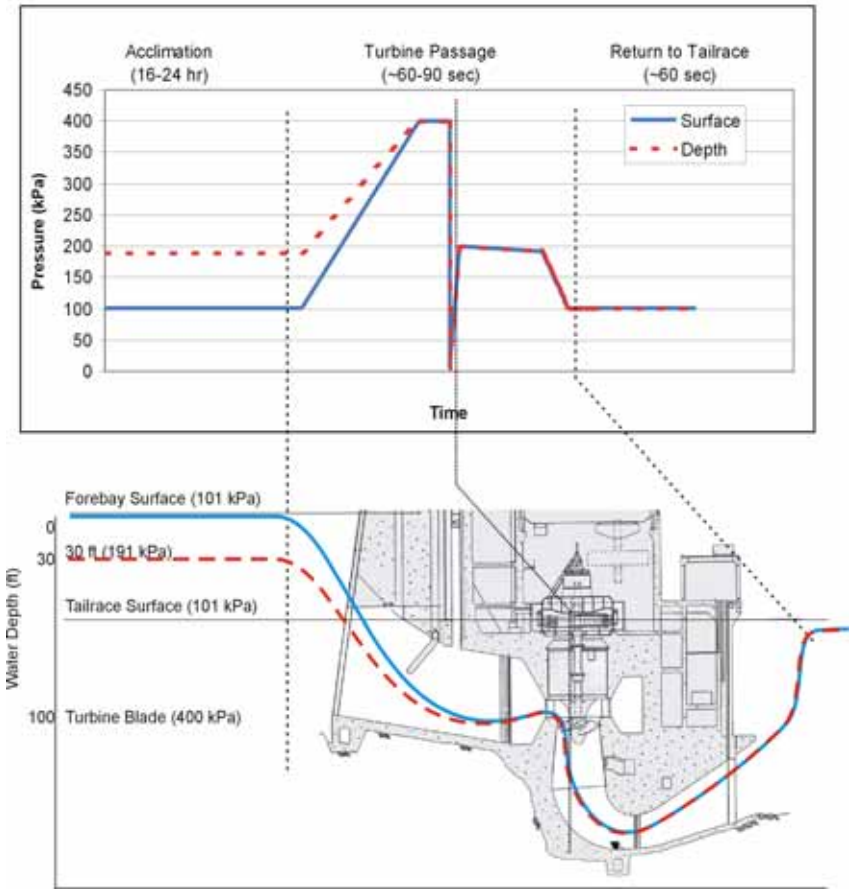

Figure 2.2. Surface (101 kPa) and 30-ft depth (19 kPa) acclimation and hyperbaric chamber pressure exposure simulation of turbine passage. Pressure increases as depth increases. The pressure spike occurs as fish pass the turbine blades. Pressures then return to surface pressure as fish pass through the draft tube and enter the tailrace.

2.1.4 Number of Fish Exposed

Enough fish were exposed to the pressure regime to provide sample populations sufficient to:

1. Provide a number of cranial lesions that could be examined to provide tissue and cellular-level evaluation of the lesions

2. Provide enough fish with cranial lesions to hold for rearing to maturity (this population included enough fish to allow sampling at 6-month intervals.

2.1.5 Clinical Evaluation and Preparation of Lesions From Exposed Juvenile Fish

Preparation of tissues and examination of affected fish was the basis for defining the nature and significance of cranial lesions. Fish were euthanized with an overdose of MS-222 (250 mg/L). Fish were first examined at necropsy and morphometric data recorded as described below.

The whole fish and all lesions were photographed. Each fish was inspected for external and internal lesions or abnormalities.

The heads of euthanized fish were severed posterior to the opercular flap and placed in neutral buffered formalin for fixation. The specimens were transported to the Sequim laboratory and processed for histological examination. For histological examination, the fixed heads of the fish were cut sagittally, splitting the head into two halves on a longitudinal plane. Each half of the head was trimmed to fit in a histological cassette and the histology laboratory instructed to cut three levels of section from each of the two cassettes from each fish. These sections were cut approximately at the surface: 30% through the tissue and 60% through the tissue. Each section was routinely prepared for paraffin-embedded histology and slides were stained with hematoxylin and eosin (Luna 1968).

2.1.6 Histological Evaluation of Lesions

Juvenile rainbow trout and Chinook salmon were clinically examined immediately after the simulated turbine passage exposure. The fish subjected to the simulated turbine passage regime were split into two groups based on this post-exposure examination, symptomatic and asymptomatic. Thirty fish from each of these two groups and 30 control fish were examined histologically.

The tissues fixed for histology were examined and selected lesions were examined for confirmation as part of a quality assurance procedure. Lesions were examined to determine the presence of any tissue damage, presence of any inflammatory response, and evidence of healing.

The multiple sections from each fish provided for observation of the following organs in the heads of most of the fish: nares, olfactory lobe, the forebrain, the pineal organ, the pituitary gland, the optic lobe, the cerebellar valvula, the metencephalon, and the medulla oblongata. Each slide was examined for lesions and anomalies that could reasonably be attributed to marked changes in pressure and other possible differences between the treated and control groups. These changes included but were not limited to:

- vascular hemorrhages, particularly in and around the brain
- vascular hemorrhages in the nares
- emboli in blood vessels, brain tissue and ventricles of the brain
- separation of blood cells from plasma in arteries and arterioles
- pigmentation distribution including contraction and extension of melanocytes.

2.1.7 Rearing Juveniles with Cranial Lesions to Adults and Examination of Lesions and Residual Tissue Damage

Fish that exhibited cranial lesions after the laboratory exposures, along with appropriate controls, were raised at the PNNL facilities in Richland, Washington. Sufficient numbers of individuals were initially produced in summer and fall 2002 to allow for the following examinations:

1. evaluation of lesion condition at 6-month intervals (terminal sampling)

2. retesting as adult fish, if indicated and approved, as a sequential project.

2.1.8 Design of Adult Hyperbaric Chamber

Because the examination of the cranial lesions in juveniles produced during the laboratory study and the headburns on adults did not exhibit similar or comparable tissue or cellular conditions, we did not proceed with the design of a chamber for adult salmonid exposure to gas saturations and pressure conditions.

2.2 Facilities and Equipment for Juvenile Exposures

2.2.1 Gas-Supersaturation System

A pressurized, packed-cell column was used to generate gas-supersaturated water. Point Four Systems of Port Moody, British Columbia, had designed the system (Figure 2.3) for use in previous studies at PNNL. Pressurized water and air was added to the column. The desired levels of TDS were achieved by controlling pressure within the column and by adjusting the position of a proximity switch on a sight glass.

For the tests undertaken in this project, 10 gpm of well water was pumped into the top of the column. Backpressure was created inside the column by restricting the discharge line at the bottom of the water column. As the water level increased within the column, a proximity switch sensor mounted on a sight glass controlled a valve that allowed pressurized air to enter the column and maintain the desired level

Figure 2.3. Gas-supersaturation system showing controls (lower right) and saturometer (upper right).

within the column. The combination of compressed air acting against the pressurized water source and the restricted outlet created gas-supersaturated water within the column. The water level inside the column affected the water/air interface. Therefore, adjusting water level and/or internal column pressure was used to change and control gas-supersaturation levels.

An internal pressure of ~11 psi at a flow rate of 10 gpm was used to produce TDG levels of ~135%. A pressure of ~6 psi resulted in a TDG level of ~120%. For "normal" saturated water, the outlet was completely opened so no backpressure was created. In this condition, the TDG level was <105%. Gas levels were monitored with a Sweeney Saturometer, Model DS-1B (Sweeney Aquamatic, Stony Creek, Connecticutt). The manufacturer calibrated the instrument before it was shipped to PNNL. In the laboratory, the instrument readings were compared to other instruments by the same manufacturer and found to be within tolerances required for the study (±1%).

2.2.2 Turbine Passage System

The turbine passage system (Figure 2.1) was designed and built by Reimers Engineering in 1994. The system was used to create a variety of pressure regimes and to simulate the pressure history that fish would experience in passing through a typical Kaplan turbine on the mid-Columbia River. The exposure chambers for the turbine passage system consisted of two 11-in. (27.5-cm) diameter acrylic tubes, 22-in. (55-cm) long. The volume of each cylinder is about 34 L.

The chambers were connected to hydraulic cylinders, which in turn were connected to pneumatic cylinders. Through a computer-controlled gas pressurization system attached to the pneumatic cylinders, the positions of the hydraulic cylinders were moved to either pressurize or depressurize the chambers. The maximum pressure of the chamber is 100 ft of head (3 atm, or ~400 kPa). The system can drop the pressure from 100 ft (~400 kPa) of head to close to the vapor pressure of water (~1 psi or 210 kPa) in 0.1 second.

A computer program, the Labtech Control Program (Labtech Control Version 4.2.0 for Microsoft® Windows™, Laboratory Technologies Corporation) controlled the gas cylinders used in the pressurization/depressurization sequence. Subprograms within the Labtech Control program were used for various chamber operations. Water was pumped from the gas-supersaturation system to the chambers at the desired TDG level (100, 120, or 135%), depending upon the test scenario.

2.2.3 Water Delivery System

Water from the gas-supersaturation system flowed into a trough. A second centrifugal pump withdrew water from the trough and pumped it to the hyperbaric chambers. A valve on the outlet side of the pump was used to precisely control the quantity of water delivered. Water from the supply line was split to enter both hyperbaric chambers. Flow was equalized through the two chambers by using a round orifice restrictor in the end of drain tubes leading from each chamber. During the acclimation period for fish, pressure within the hyperbaric chambers was set by adjusting the quantity of pressurized water delivered to the hyperbaric chambers, with backpressure determined by the size of the outlet orifice. For holding fish at 30 ft of depth (191 kPa), a 3/32-in. orifice was used. For holding fish at the surface (101 kPa) a 3/8-in. orifice was used. Flow through each hyperbaric chamber was ~10 and ~14 L/min, respectively, and turnover rate for each chamber was 3.4 and 2.5 minutes, respectively.

2.3 Collection and Examination of Adult Fish from Columbia and Snake River Sites

2.3.1 Scope of study

Adult Chinook salmon and steelhead of hatchery origin were examined at fish passage facilities or hatchery capture facilities on the Columbia and Snake river systems and tributaries to the river systems between June 2002 and February 2004 (Figure 2.4 and Table 2.1). Fish were examined and necropsied, and samples were taken onsite from the live fish by the project team, except at the Bonneville Dam fish trap, where Steve Lee, University of Idaho, collected the fish tissues and images.

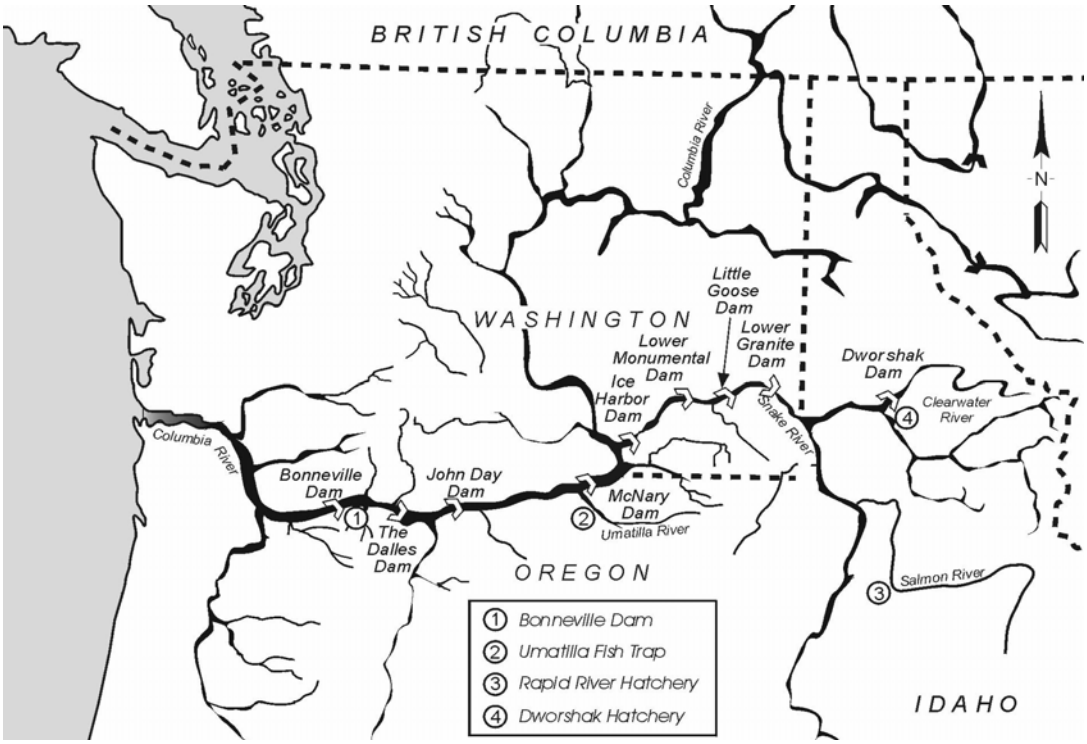

Figure 2.4. Location of the four sites sampled for adult fish in this study shown in the box on the map. In addition, data were provided from Lower Granite Dam (Chris Peery, University of Idaho, personal communication) and from Kooksia National Fish Hatchery on the Clearwater River in Idaho (Ralph Rosberg, personal communication).

Table 2.1. Location, collection date, species and number of hatchery origin adult fish examined or reported on for the headburn lesion study.

Sample Location	Collection Date	Species[1]	Number of Adult Ocean-Return Fish Examined[2]
Rapid River Hatchery, Riggins, Idaho[3]	June 24, 2002	Chinook salmon	6
Bonneville Dam, Fort Rains, Washington[4]	April 30, 2003	Chinook salmon	3
Bonneville Dam, fish trapping facility[5]	April 23, 2004, to July 7, 2004	Chinook salmon	11
Umatilla Fish trap, Umatilla River, Umatilla, Oregon[6]	May 8-10, 2003	Chinook salmon	8
Rapid River Hatchery, Riggins, Idaho[3]	June 18, 2003	Chinook salmon	5
Dworshak National Fish Hatchery, Orofino, Idaho[7]	November 12, 2003	Steelhead	6
Dworshak National Fish Hatchery, Orofino, Idaho[7]	November 25, 2003	Steelhead	4
Dworshak National Fish Hatchery, Orofino, Idaho[7]	February 24, 2004	Steelhead	15

[1] Chinook salmon—*Oncorhynchus tschawytscha*; Steelhead—*Oncorhynchus mykiss*.
[2] All fish samples contained fish with headburn lesions, except those from near Bonneville Dam, Fort Rains, Washington, that were purchased from Tribal fishers as control fish.
[3] Idaho Department of Fish and Game, Rapid River Hatchery on Rapid River, near Riggins, Idaho.
[4] Normal appearing gill net caught fish purchased fresh from Tribal fishers for control samples.
[5] Fish tissues and photographs of headburn fish collected and contributed to project by Steve Lee, University of Idaho. Prevalence data provided by Dr. Chris Peery, University of Idaho.
[6] Umatilla Tribal adult salmon ladder located on the north bank of the Umatilla River at the three mile mark, located about 4 km south of Umatilla, Oregon. The facility is operated by the staff from the Umatilla Tribal Fisheries Program (Confederated Tribes of the Umatilla Indian Reservation).
[7] U.S. Fish and Wildlife Service, Dworshak National Fish Hatchery on Clearwater River, near Orofino, Idaho.

2.3.2 Necropsy and Site Data Collection

The prevalence of headburn lesions at Bonneville Dam was believed to be extremely low based on discussions with biologists working at the fish trapping facility. Therefore, fixative and tissue collection materials were provided to personnel at the trapping facility. Photographs and tissues were collected and provided from the Bonneville Dam fish trapping facility by Mr. Steve Lee of the University of Idaho.

At the other sites, fish trapping personnel from the agency or Tribe operating the facility assisted PNNL personnel. Fish with visible head scalping or other significant lesions related to abrasions, scalping, or traumatic injury were selected during the usual process of sorting fish at the facility. Fish were measured

for standard length and/or weighed. The external features of selected fish (euthanized by an overdose of MS-222 [methane tricaine sulfonate]) were examined in detail and photographed. Only hatchery fish were sacrificed and examined for this study.

Lesions were scraped and tissue samples placed in sterile river water for subsequent fungal and yeast isolation procedures. In addition, blood was collected aseptically by heart puncture and retained in lithium heparin vacutainers for plasma sodium analysis and bacteriological culture.

Representative tissue sections were then cut from the lesions and adjacent normal tissue and placed in histological cassettes, which were then fixed in neutral buffered formalin (Figure 2.5). Internal organs were examined visually, and in selected cases, tissues were excised and preserved for histological analysis in neutral buffered formalin or saved for virological analysis in a chilled aseptic container and later archived at -70°C.

Fixed tissues in cassettes were dehydrated and embedded in paraffin using conventional techniques and stained with hematoxylin and eosin. Special stains were used as required for fungal hyphae confirmation (Gomori methenamine silver [GMS]) or bacterial confirmation (e.g., Brenn and Brown or Giemsa) (Luna 1968). Histological sections were interpreted by Dr. Ralph Elston, and selected sections were reread for confirmation and consultation by Dr. Paul Frelier.

Figure 2.5. Collection of headburn or scalping lesion tissue from adult Chinook salmon trapped at the Rapid River Hatchery for histological analysis in June 2002. The Idaho Department of Fish and Game operates the hatchery, which is near Riggins, Idaho.

2.3.3 Bacterial and Fungal Identification and Characterization

Bacterial isolates from the blood of affected and apparently normal fish were cultured and maintained on tryptic soy agar. Early passage isolates were archived at -70°C in tryptic soy broth containing 10% glycerol. Bacteria were characterized for whole cell fatty acid content (Microbial ID, Newark, Deleware) and compared in the Microbial Identification System for similarity index to known isolates.

Tissue for fungal and yeast isolation was scraped into tubes of sterile river water and into broth tubes of the medium described by Alderman and Polglase (1986). Cultures were then spread and picked on the same medium made in solid form (with 1.2% agar) and stored on slants of the same. Their identification was made by large subunit (LSU) gene sequence comparison (MIDI Labs, Newark, Deleware) using approximately 300 base pairs (bp) of the variable D2 region of the LSU rRNA gene and making sequence comparisons using the Applied Biosystems MicroSeqTM microbial analysis software and database.

2.3.4 Plasma Sodium Determination

The Veterinary Diagnostic Laboratory, College of Veterinary Medicine, at Oregon State University determined the plasma sodium concentrations using a Hitachi 717 Automated Chemistry Analyzer that uses an ion-specific electrode for quantifying sodium.

3.0 Results

3.1 Juvenile Studies

The summary results for baseline (i.e., the time at which no fish were subjected to simulated turbine passage) Chinook salmon and rainbow trout are shown in Tables 3.1 and 3.2. Only hemorrhages that were considered moderate or severe are reported in these tables. The histology summary sheets for individual fish are included in Appendix A.

3.1.1 Chinook salmon – Baseline Group (Examined Immediately After Exposure)

A total of 1000 fall Chinook salmon were exposed to the pressure sequence, resulting in 161 (16.1%) symptomatic fish. Thirty symptomatic fish were preserved and examined for baseline sampling, and the remainder was PIT-tagged and held for future sampling. Clinical observations showed a significant difference ($p < 0.01$) in the occurrence of head spots and superficial hemorrhage in the combined symptomatic and asymptomatic groups of Chinook salmon (n= 18 of 60) in comparison to the control group (n = 0 of 30) (Table 3.1). However, these alterations in external appearance were generally transient and mild (Figure 3.1).

Table 3.1. Summary clinical observations and histology results from examination of fall Chinook salmon baseline group exposed to turbine simulation pressurization-depressurization regime.

		Clinical Observations						**Histology Summary**			
	Total	**Head Spot**		**Hemorrhage**		**Popeye**		**Hemorrhage Brain**		**Hemorrhage Nares**	
	N	N	%	N	%	N	%	N	%	N	%
Symptomatic	30	18	60.0%	26	86.7%	1	3%	2	6.7%	4	13.3%
Asymptomatic	30	0	0.0%	0	0.0%	0	0%	0	0.0%	6	20.0%
Control	30	0	0.0%	0	0.0%	0	0%	1	3.3%	3	10.0%

Table 3.2. Summary clinical observations and histology results from examination of rainbow trout baseline group exposed to turbine simulation pressurization-depressurization regime.

		Clinical Observations						**Histology Summary**			
	Total	**Head Spot**		**Hemorrhage**		**Popeye**		**Hemorrhage Brain**		**Hemorrhage Nares**	
	N	N	%	N	%	N	%	N	%	N	%
Symptomatic	30	21	70.0%	28	93.3%	0	0%	6	20.0%	14	46.7%
Asymptomatic	30	0	0.0%	0	0.0%	0	0%	6	20.0%	8	26.7%
Control	30	0	0.0%	0	0.0%	0	0%	1	3.3%	6	20.0%

No statistically significant difference was found in the occurrence of hemorrhagic lesions, aneurysms, or other conditions in the histological examination of the Chinook salmon, although the symptomatic group had a higher prevalence of both hemorrhages in the brain and in the nares than the control group, and the asymptomatic group had the highest percentage of hemorrhages in the nares. In the two individuals with brain hemorrhages, these lesions occurred as aneurysms above the medulla oblongata and in the region of the optic lobe of the brain (Figure 3.2).

In addition, to moderate to severe hemorrhages in the exposed fish, mild and focal hemorrhage was found in various regions of the brain in all groups of fish, including controls. This finding is believed to be an artifact of the euthanasia or fixation process and was, therefore, excluded from the analysis of differences between groups.

Figure 3.1. Darkened area, or headspot, on dorsal interocular cranium on exposed Chinook salmon (arrow).

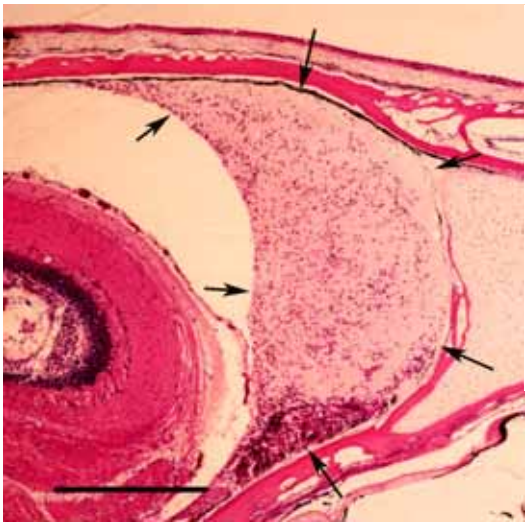

Figure 3.2. Hemorrhage in the optic lobe of the brain of an exposed juvenile Chinook salmon. The hemorrhage is outlined by the arrows. The optic lobe is on the left. The clear space between the hemorrhage and optic lobe is a fixation artifact. Cartilage is located to the right of the hemorrhage and bone is located above and below the hemorrhage. Hematoxylin and eosin stain. Bar, 1 mm.

3.1.2 Rainbow Trout – Baseline Group (Examined Immediately After Exposure)

A total of 840 rainbow trout were exposed to the pressure sequence, resulting in 152 (18.1%) symptomatic fish. Thirty symptomatic fish were preserved and examined for baseline sampling, and the remainder were PIT-tagged and held for future sampling. Clinical observations of rainbow trout showed a significantly ($p < 0.01$) higher proportion of darkened areas on the head and superficial hemorrhages in the combined symptomatic and asymptomatic exposed fish (n= 21 of 60 and 28 of 60) than in the control fish, n= 0 of 60) (Table 3.2). Like the Chinook salmon, these alterations tended to be transient in nature.

In addition, there was an increased prevalence of moderate to severe hemorrhages in the brains and in the nares of both clinically symptomatic and clinically asymptomatic exposed fish in comparison to control fish.

The hemorrhages in the brain of the exposed rainbow trout were found in various anatomical locations, including the surfaces of the frontal lobe, the optic lobe, and above the medulla oblongata. The most frequent sites of aneurysms or hemorrhages were in the small blood vessels near the surface of the optic lobe of the brain. Hemorrhages in the nares ranged from mild to severe and, when severe, the entire chamber of the nares was filled with blood.

3.1.3 Pigmentation Distribution in Trout and Salmon

The condition of melanocytes was recorded around various aspects of the brain. There were no differences observed between the control, asymptomatic, and symptomatic groups in either Chinook salmon or rainbow trout. Generally, melanocytes are concentrated above the pineal gland, which is in the center of the cranium between the eyes. The reaction of these prominent melanocytes to external stimuli could be responsible for occasionally observed darkening in this region of the cranium. The pineal gland is believed to be a sensory organ but its precise function is unknown. It lies below a markedly thinned area of cranial cartilage. However, the expansion and contraction of melanocytes, which would correspond with a macroscopically visible darkening or lightening of the skin, is a transient phenomenon and a reaction to the presence or absence of stimuli.

3.1.4 Vascular Emboli and Separation of Blood Cells From Plasma

No vascular emboli were observed in any of the fish. Fish from all groups and most individuals had some degree of blood separation, so this condition was most likely an artifact of fixation.

Due to the limited number of histological lesions found, the transient nature of the clinical changes in coloration, and the general lack of evidence of long-term damage at the microscopic anatomical level between the exposed and control fish, only the baseline fish (examined immediately after exposure) were examined histologically.

3.2 Adult studies

3.2.1 Headburn and Prespawning Prevalence and Related Mortality Loss Data

Headburn and scalping injury prevalence data were obtained from fishery managers and researchers at the study sites. These data for lesion prevalence are shown in Table 3.3.

The data in Table 3.3 demonstrate several key features of the significance of headburn in salmon and the difficulties in comparing data as it is presently collected. In both 2002 and 2003, the data from the Rapid River Hatchery trapping facility shows a lower prevalence of headburn lesions than the data from the holding raceways where the fish are kept until ready to spawn. According to the managers at the hatchery (Nicola Johnson, personal communication) this discrepancy is a result of undercounting the headburn lesions at the trap and inconsistencies in recording practices among different trapping crews. The data from the holding raceways or ponds are considered to be more accurate, because individual fish are counted and a careful examination is made of lesions on the fish. This difference in counting and examination procedures can lead to a very substantial difference, as shown in the trap data in 2003, which show a prevalence of only 1.4% while the raceway data show a headburn prevalence of 22.1%.

In addition, the data in Table 3.3 show a trend toward increasing prevalence of headburn lesions as the fish progress upriver from Bonneville Dam, to the Umatilla trap, to Lower Granite Dam, and finally at the Rapid River Hatchery (raceway data) and at the Dworshak National Fish hatchery in 2002.

The trap collected data from Rapid River Hatchery in 2003 are comparable to the trap data from Dworshak National Fish Hatchery, but the raceway data are substantially higher, in contrast, at Rapid

Table 3.3. Prevalence of headburn lesions at study sample locations and other locations for which comparable data were obtained.

Location[1]	Species[2]	Interval	Number of Fish with Headburn Injuries	Total Fish Inspected or Recorded from Facility	Percent of Fish with Headburn Injuries
Bonneville[3]	Chinook	2003	10	1184	0.8%
Umatilla	Chinook	March 27 to September 29, 2003	181	3742	4.8%
Lower Granite[4]	Chinook	2003	14	117	12.0%
Rapid River[5] Trap data	Chinook	2002	78	6887	1.1%
Rapid River[6] raceway mortality data	Chinook	2002	105	3143	3.3%
Rapid River[5] Trap data	Chinook	2003	48	3409	1.4%
Rapid River[6] raceway mortality data	Chinook	June 12 to August 8, 2003	542	2458	22.1%
Dworshak[7]	Chinook	June 25 to September 10, 2002	242	2110	11.5%
Dworshak[7]	Chinook	May 6 to July 17, 2003	25	931	2.7%
Dworshak[7]	Steelhead	Sept 17, 2002 to May 6, 2003	53	5176	1.0%

[1] Location details provided in Table 2.3 and Figure 2.3.
[2] Chinook are spring Chinook salmon—*Oncorhynchus tschawytscha*), and steelhead—*Oncorhynchus mykiss.*
[3] Data from Chris Peery, University of Idaho, personal communication.
[4] Data from Chris Peery, University of Idaho, personal communication.
[5] Data provided by Nicola Johnson, Idaho Department of Fish and Game, from Rapid River Hatchery 2002 and 2003 Brood Year Report. The numbers recorded at the time of fish capture are for data taken at the fish trap. Headburn lesions are recorded as "Nitrogen blister."
[6] Data provided by Nicola Johnson, Idaho Department of Fish and Game, personal communication, based on detailed examination of moribund and dead fish taken from holding ponds prior to spawning.
[7] Data provided by Ralph Roseberg, U.S. Fish and Wildlife Service, Dworshak National Fish Hatchery, personal communication.

River Hatchery than the trap data at either facility. It is not known if the prespawning losses at Dworshak Hatchery, after transferring spring Chinook salmon to holding raceways, is comparable to that at Rapid River Hatchery.

Examination of the data collected at the Umatilla fish trap showed that the prevalence of headburn fish appeared to have some correlation with the total number of fish passing through the facility (Figure 3.3), although the calculated correlation coefficient was only 0.485. Inspection of the data shows a cluster of increased numbers of headburn fish between about April 20, 2003, and May 18, 2003.

The loss of fish with headburn-related lesions at Rapid River Hatchery in 2003 is shown in Figure 3.4. In 2003, 2458 fish were trapped at the hatchery. Of these fish, 542, or 22%, died prior to spawning. Based on observations of hatchery managers (Nicola Johnson, personal communication), about 50% of the prespawning mortality was attributed to headburn lesions. Examinations undertaken during this study showed that the fish with headburn lesions arrived at the hatchery in poor condition and that this condition worsened during their residence time in the holding raceways. The total loss of prespawning spring

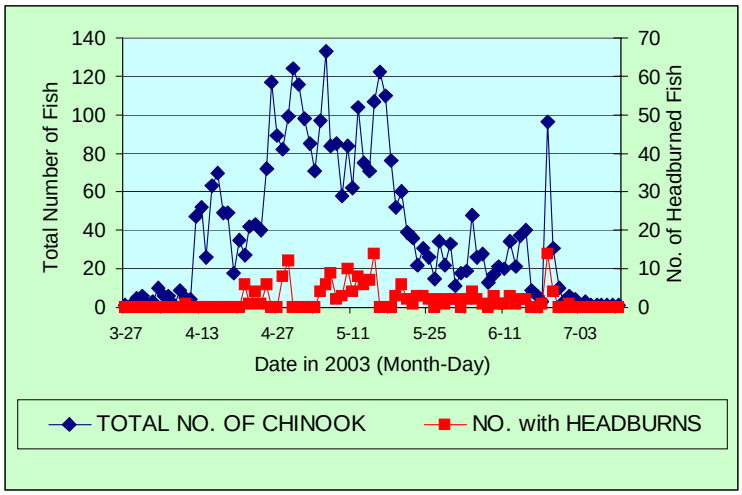

Figure 3.3. Spring Chinook salmon passing the Umatilla fish trap in 2003 (blue) and the number of fish with headburn or scalping lesions (red). Of the total, 4.8% of the fish had headburn lesions but there was only a correlation coefficient of 0.485 in terms of co-occurrence in time of the intact and wounded fish.

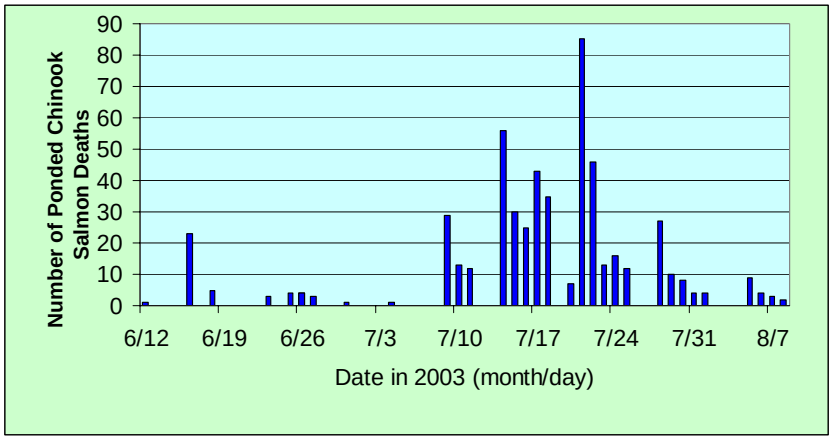

Figure 3.4. Mortality of spring Chinook salmon during prespawning holding period at Rapid River hatchery in 2003. Of a total of 2458 fish that were trapped, 542 died prior to spawning due to headburn injuries, accounting for about 50% of the prespawning mortality.

Chinook salmon over the prespawning holding period is shown in Figure 3.4. These loss data suggest that the fish may survive for up to several weeks in the raceway, finally succumbing primarily during the month of July in 2003.

The prevalence of headburn lesions in fall steelhead examined at Dworshak Hatchery was low, as indicated by the data in Table 3.3. According to personnel at the hatchery (Ralph Roseberg, personal communication), the prevalence of headburn lesions in steelhead is consistently lower than in spring Chinook salmon.

3.2.2 Classification of Headburn Lesions – Gross Lesion Types

The classification of headburn lesions and their differentiation from other types of wounds in adult ocean-return fish will provide a basis for consistent data collection. This will allow fishery biologists to accurately categorize headburn and other lesions with respect to potential cause, age of the lesion, and even prognosis for the affected fish. We have categorized headburn lesions in the following groups.

3.2.2.1 Shallow Epidermal Abrasions

These are mild lesions in which only the outer skin layer is abraded (Figure 3.5). They may be characterized by scratch marks and/or small patches of abraded skin revealing the underlying white connective tissue. These lesions typically occur in the cranial region and can be located dorsally, laterally, or on the vental aspect of the jaw. In addition, leading edges of the fins may be abraded, indicating, along with the preponderance of cranial lesions, that the fish is colliding more or less head-on with abrasive surfaces.

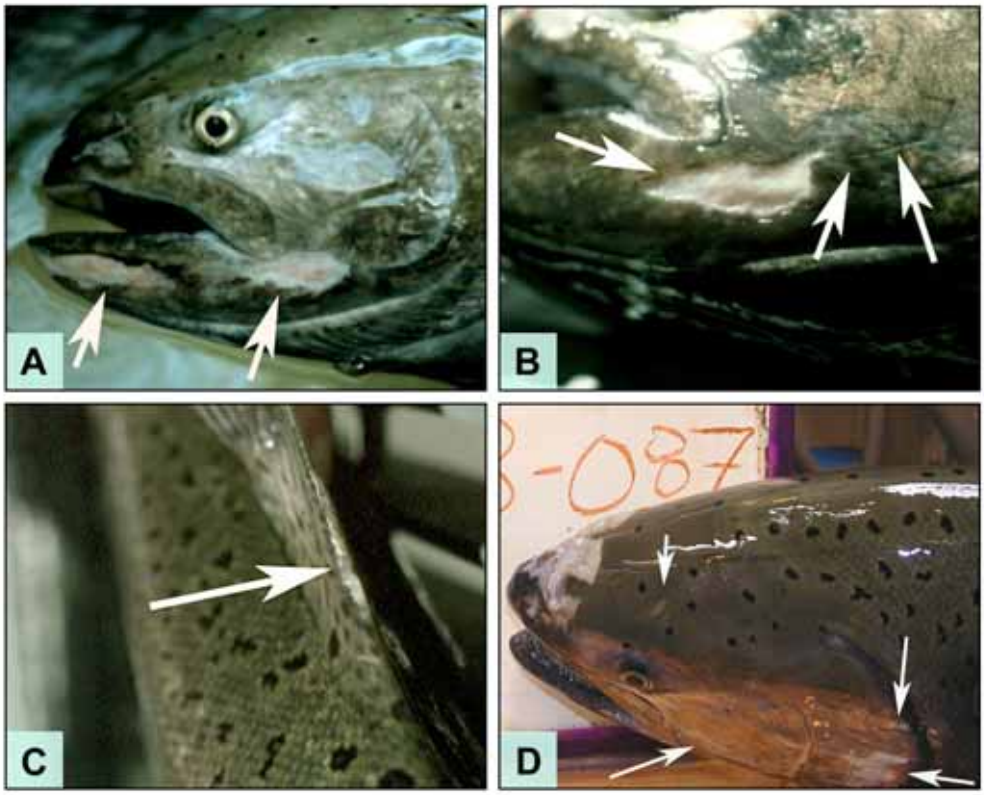

Figure 3.5. Shallow epidermal lesions. These lesions are most often on the head region or on the leading edges of fins. The epidermis may be eroded over a significant area (A, B, and D), but the white color of the lesion indicates that the dermis and underlying connective tissue of the SC are still intact. Often the abrasive scratches are oriented longitudinally to the body of the salmon (B and lower arrows in D) but occasionally, such lesions are lateral or oblique to the longitudinal plane of the fish (e.g., upper arrow in D). A, B, and C are spring Chinook salmon from Lower Granite Dam fish trap in 1996 (Elston 1996). D is a spring Chinook salmon from Bonneville Dam fish trap, courtesy of Mr. Steve Lee, University of Idaho.

3.2.2.2 Ulcerated Abrasions and Blunt Trauma

These are deeper, more severe abrasions in which the multiple layers of the epidermis and dermis have been abraded, exposing the underlying muscle tissue that appears bright red. In some cases, cranial bone and cartilage are exposed (Figure 3.6). The deep ulcerated lesions are usually accompanied by abrasion marks that can be oriented either in an anterior-posterior direction or laterally (photograph D in Figure 3.6), suggesting that the fish was injured in turbulent water during a lateral movement against an abrasive surface. In a few cases, depression of the cranium was noted without breakage of the skin, suggesting collision with a blunt object such as a turbine blade. Alternatively, such a lesion could be induced during an unsuccessful capture by a recreational fisher. However, the example shown in Figure 3.6, photograph C also shows abrasion marks at the anterior aspect of the upper jaw.

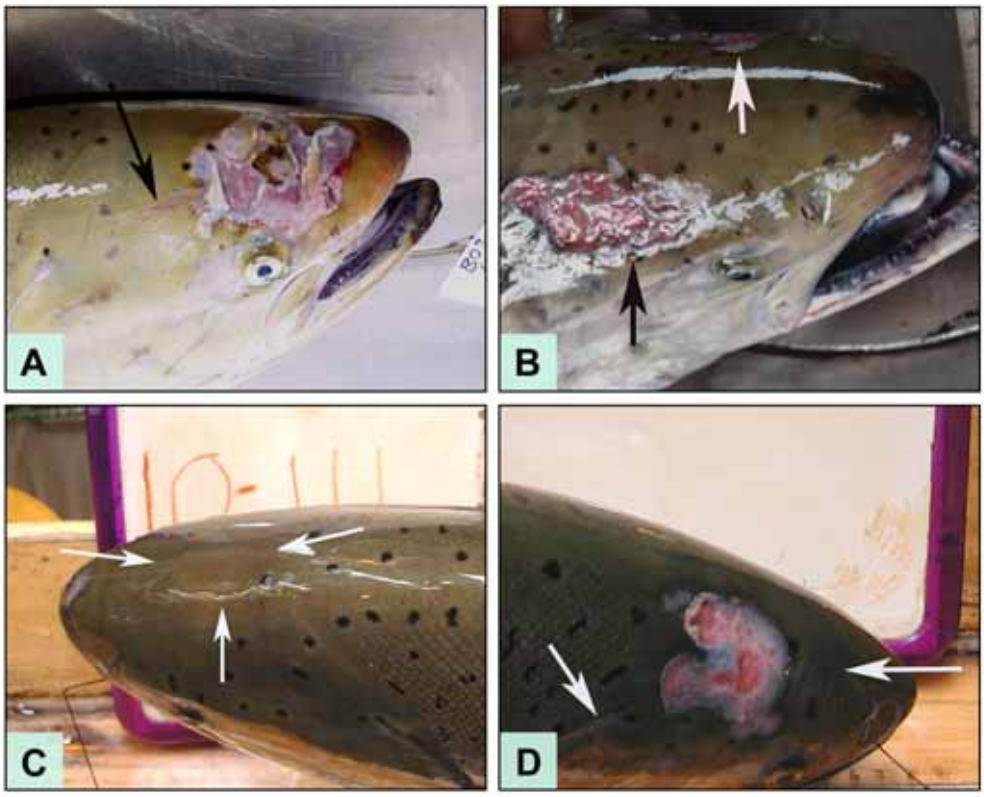

Figure 3.6. Fresh ulcerated abrasions and blunt trauma believed related to similar causation. Longitudinal abrasion marks are shown in A and C and lateral abrasion marks are shown in the right hand arrow in D. A, C and D show deep abrasions that expose the connective tissue, muscle and bone (A). The fresh nature of the lesions is demonstrated particularly in A and D, which show folds of connective tissue and skin at the periphery of the lesion. Figure C shows a depression in the center to right-hand side of the cranium, presumably from a blunt force collision, perpendicular to the surface of the head and therefore not causing a break in the skin. This lesion could result from various causes, including collision with a turbine blade or, alternatively, from a blow to the head from a fisher. These lesions demonstrate a more severe set of recently induced headburn lesions than those shown in Figure 3.5.

3.2.2.3 Extensive Ulcerated Abrasions

These are also deep, severe abrasions that cover a majority of the dorsal head region (Figure 3.7). Like those shown in Figure 3.6, these are ulcerated abrasions with areas of complete skin loss, exposing underlying muscle and occasionally cartilage and bone. Such lesions were typical of downriver locations but were occasionally observed at the furthest upriver location (Rapid River Hatchery) and, in the latter case, could represent an unhealed or completely re-injured lesion that would be difficult to discriminate macroscopically from a fresh lesion.

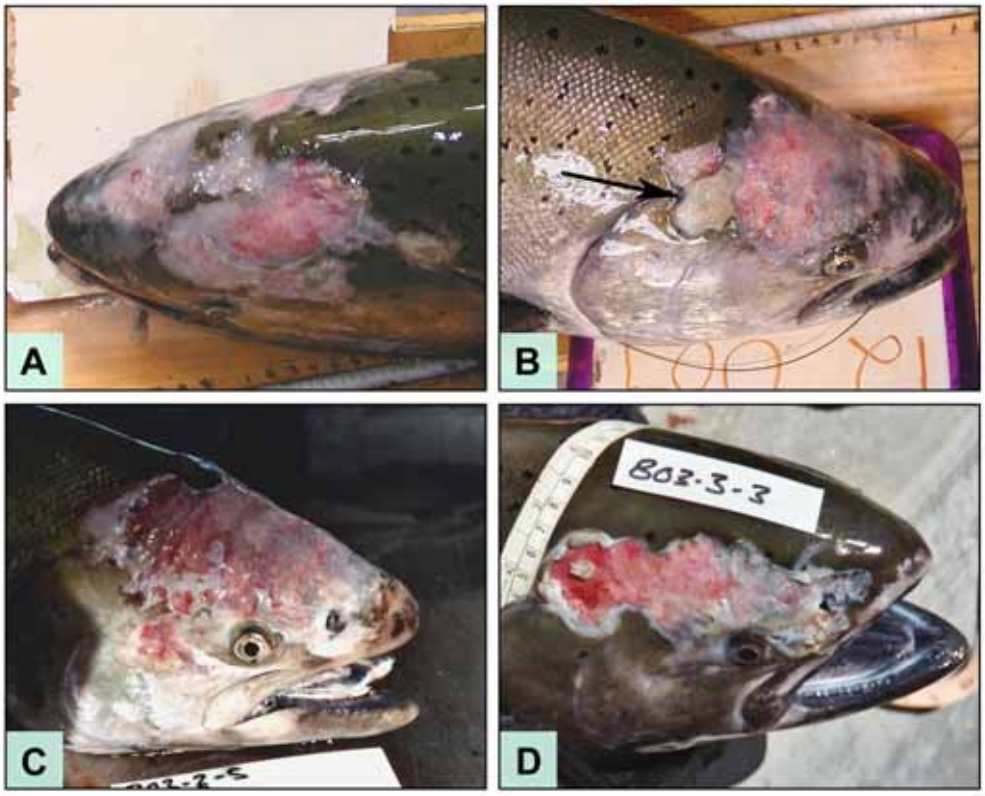

Figure 3.7. Extensive fresh abrasive lesions on Chinook salmon from Bonneville (A and B) Umatilla (C) and Rapid River Hatchery (D) fish trapping facilities. These lesions all are sufficiently deep to have removed the skin and underlying connective tissue layers in some areas, exposing muscle tissue. The lesion in C shows some aging due to the melanization at the anterior aspect. D demonstrates that fresh headburn lesions can occur as far upriver as Rapid River hatchery, although older lesions are more typical at upriver locations. Photographs A and B courtesy of Mr. Steve Lee, University of Idaho.

3.2.2.4 Skin and Scar Healing

Salmon in this condition show either re-growth of the epidermis and/or healing over of the ulcerated lesions with scar tissue (Figure 3.8). New skin growth tends to be a very dark olive green color against the lighter colored normal skin. Scar tissue has a black appearance. In the case of skin re-growth, the new skin is very fragile as it consists primarily of metaplastic epithelial cells without the formation of underlying supporting dermal tissue, as shown in histological studies.

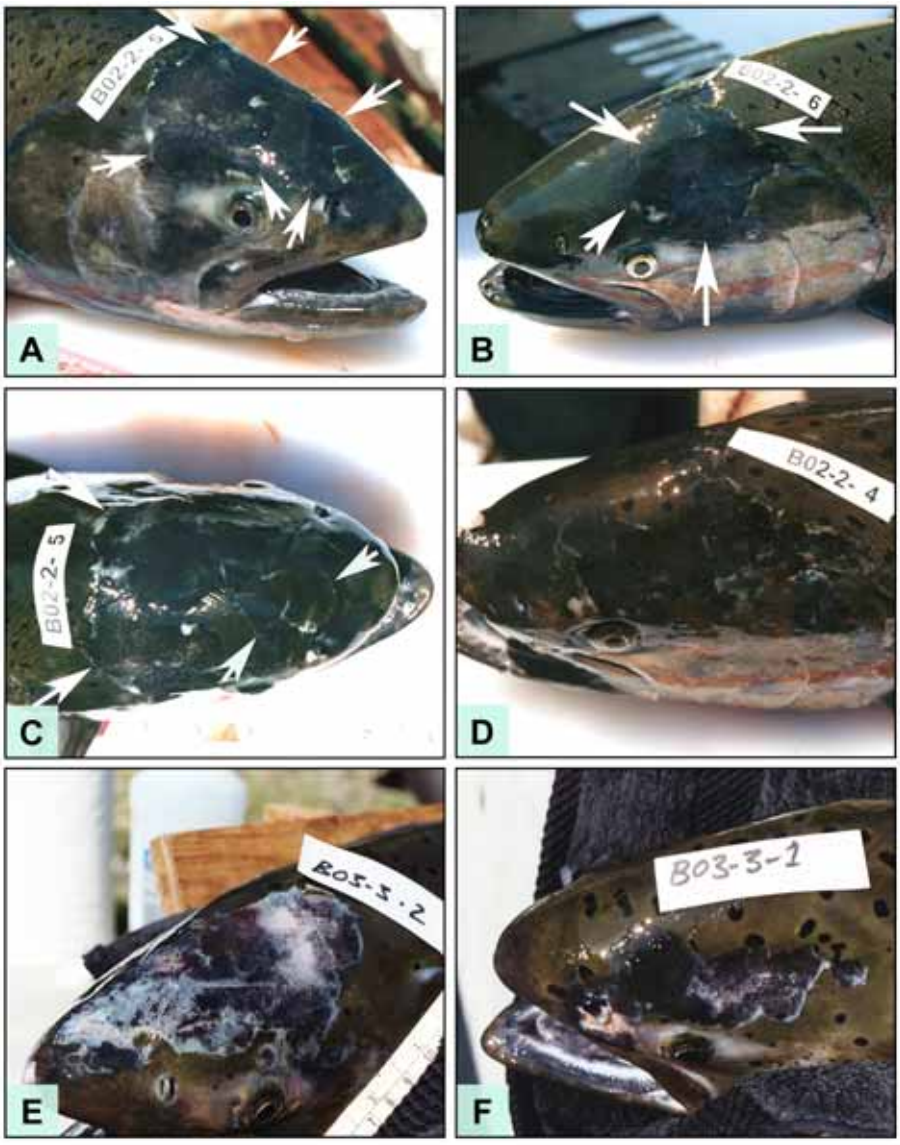

Figure 3.8. Healed headburn or scalping lesions from Rapid River Hatchery, Idaho Department of Fish and Game. The dark character of new skin re-growth in wound healing is evident in photographs A through D against the background of lighter olive-toned skin with black pigment spots. Photograph C (same fish as shown in photograph A) shows the posterior margins of a large healed wound (two left-hand arrows) and the disorganized plates of new skin and scar tissue covering the wound area (two right-hand arrows) suggesting a history of re-wounding. Photograph E shows extensive scar tissue formation over a large dorsal cranial scalping lesion and photograph F shows a more limited area of scar tissue formation over a headburn lesion. The white areas in E suggest rewounding.

3.2.2.5 Re-injury of Healed Lesions

A number of fish show a patchwork of healed new skin, which is much darker than old skin and new abrasive lesions that remove the healed skin or scar tissue (Figure 3.9). In this case, the appearance of the dorsal aspect of the head may be multiple shades of olive green to black. These colors result from a patchwork of plates of old skin, new skin, scar tissue, re-injuries, and re-healing of new injuries.

Figure 3.9. Healed and re-injured headburn lesions in spring Chinook salmon. Photograph A shows a healed injury on the dorsal aspect of the head with the posterior margin outlined by white arrows. An area of re-injury is shown at the black arrow. Photograph B shows the characteristic dark rehealing skin on top of the head with a new abrasion evident above and behind the right eye. Photograph C shows the dorsal aspect of the head of a Spring Chinook salmon with various areas of re-injury of the darkened healing skin.

3.2.2.6 Eruption of Fungal Infections

Histological studies show that even freshly abraded lesions, common in downriver locations such as Bonneville Dam and the Umatilla fish trap, are infected with fungal hyphae. However, these infections are considered subclinical because they cannot be seen with the naked eye. However, once hatchery fish are "ponded" or placed in holding raceways to mature for spawning, the fungal infections erupt into grossly visible masses of white fungal hyphae growth on the surface of the abraded and ulcerated lesions (Figure 3.10). Fish with visible fungi were rarely observed at fish trapping facilities.

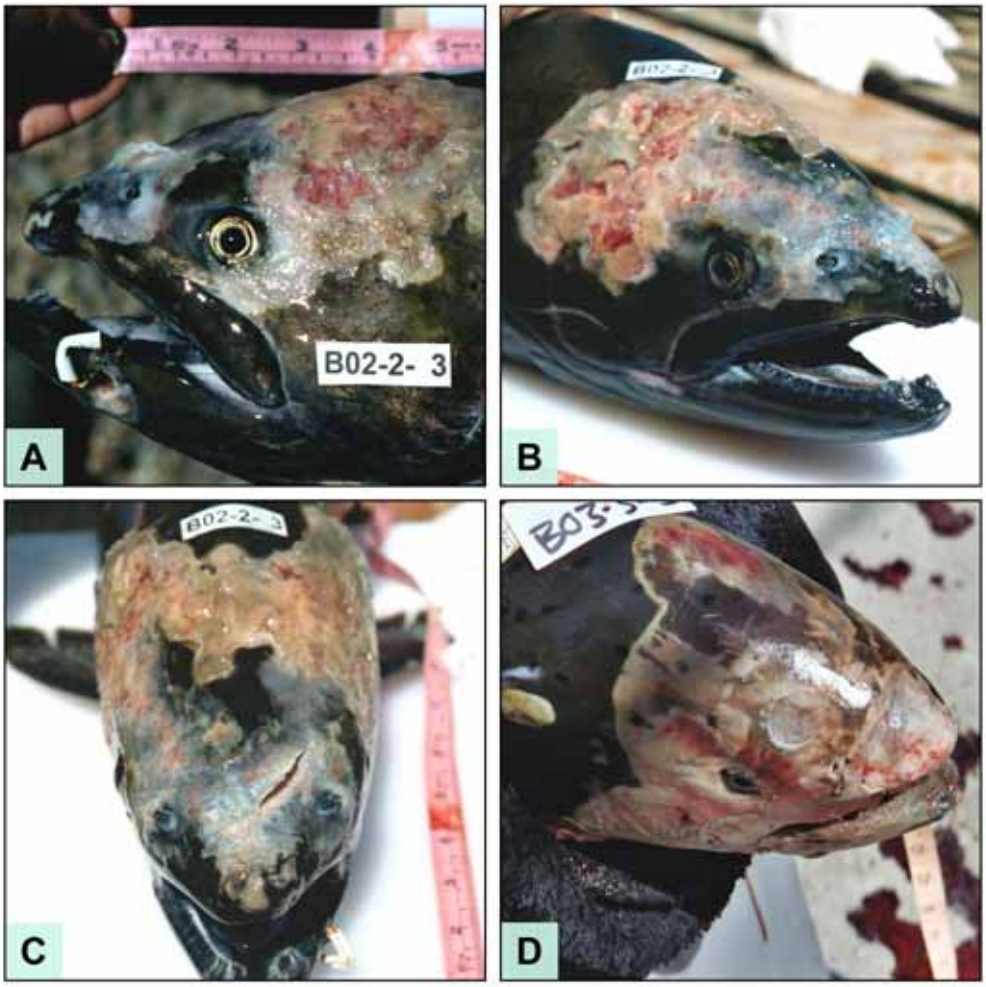

Figure 3.10. Headburn lesions infected with fungi in spring Chinook salmon after holding in prespawning pond at Rapid River Hatchery, Idaho. Histological studies show that even fresh lesions are infected with fungal hyphae while the fish are in the river but that the fungal mats do not usually become visible to the naked eye until the fish are ponded and held for spawning. The fungal infections then aggressively invade any breaks in the integument of the fish.

3.2.2.7 Mixed Headburn and Traumatic Lesions

It is assumed that the primary cause of headburn in salmon is the collision of fish with concrete or other hard structures either as they pass upriver through fish ladders, but more probably, when the fish "fall back" downstream over spillways or through turbines. Occasionally fish with abrasions will be observed with traumatic lesions in the cranial area and these may co-occur with deep wounds that are consistent with gaff or hooking wounds (Figure 3.11).

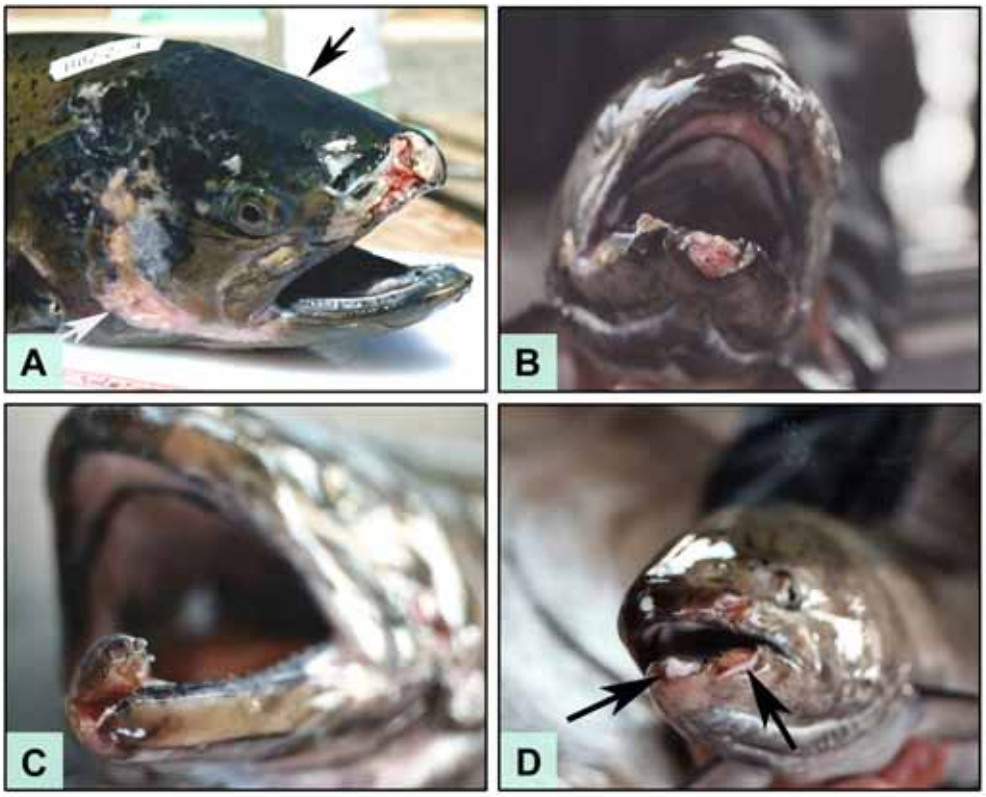

Figure 3.11. Photograph A shows a spring Chinook salmon with extensive healing of a headburn lesion (black arrow and a re-injured lesion (white arrow) along with a deep traumatic lesion in the anterior portion of the upper jaw. Photographs B to D show various traumatic lesions to the lower jaw, including those that appear to be related to abrasions in photograph D (arrows).

3.2.2.8 Gill Net Marks

Fish were obtained from Tribal fishers at Fort Rains, Washington, in 2003 to provide samples of normal, relatively fresh skin. These fish are illustrated to show the appearance of successfully gill-netted fish (Figure 3.12). Presumably some fish escape gill nets, and the lesions resulting from gill-netting should be differentiated from headburn lesions, although reports of such fish at trapping facilities were rare.

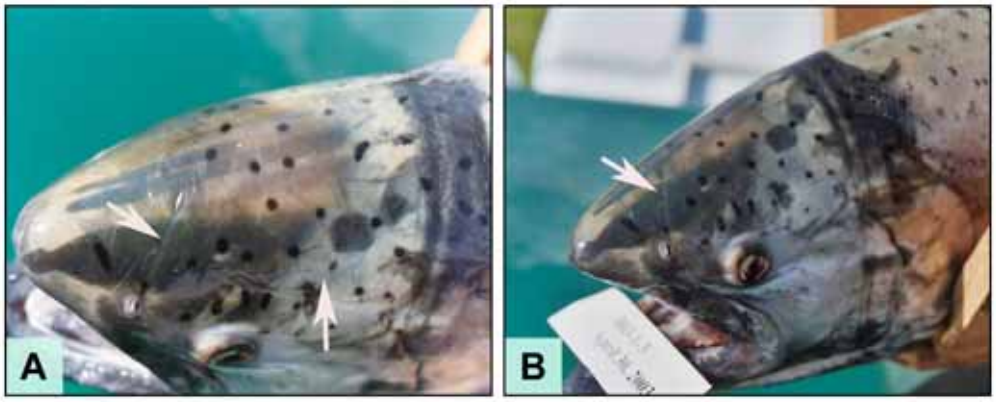

Figure 3.12. Photographs A and B. Normal spring Chinook salmon caught by gill net. The gill net marks are shown at the posterior aspect of the head. The white arrows show minor scratches and marks from handling or from the gill net. These are distinguished from headburn injuries in which the abrasion marks are typically in a uniform direction and often but not always parallel, and often along the longitudinal axis of the fish, rather than in mixed or other directions.

3.2.2.9 Headburns on Fall Steelhead

The prevalence of headburn lesions on fall steelhead observed at Dworshak National Fish Hatchery was very low. However, lesions in these fish are similar to those observed in spring Chinook salmon. In addition, steelhead with breaks in the integument appear to be subject to a variety of fungal and yeast infections that were not found in Chinook salmon (Figure 3.13). These fungi are identified and discussed in more detail below. They impart a yellowish cast to the infected tissue. Once steelhead are ponded, like salmon, those with breaks in the integument show an eruptive hyphal fungal infection within a matter of days.

Figure 3.13. Steelhead with headburn injuries. Photograph A shows a relatively minor injury on top of the head that has been colonized by a combination of yeast and fungi, described in the text. Photograph B shows a more severe lesion colonized by fungal hyphae after a period of ponding. Photograph C shows a minor abrasion on the lower jaw of a steelhead (black arrow).

3.2.3 Consequences of and Associations with Headburn Lesions

At each project site, samples were collected to assist in characterizing the consequences and potentially the causes of headburn lesions. Through an understanding of the cause and consequences of the lesions, it may be possible to formulate strategies to reduce the frequency of the lesions, reduce their severity in individual fish, and to provide therapeutic intervention at hatcheries or in wild fish inspected at fish traps that will moderate the effects of the condition and enable the fish to survive to spawning.

3.2.3.1 Histological Characterization of Lesions

A detailed histological assessment of representative adult fish is provided in Appendix B. Histological results with representative photographs are provided in the following text.

Headburn Histology of Chinook Salmon from Downriver Locations

The Chinook salmon from downriver locations (Bonneville Dam and Umatilla fish trap) were generally affected by fresh headburn lesions. Deeper tissue damage consisted of collagen and hypodermal degeneration (Figure 3.14). Mild lesions consisted of loss of epidermis (Figure 3.15, photograph A) only while more severe lesions consisted of ulceration that could affect the stratum compactum (SC) of the dermis or consist of its removal and exposure of underlying tissues. Fibrosis was evident in the connective tissue of ulcerated areas (Figure 3.15, photograph A). Healing was evident in some cases, and was characterized by a thickened basement membrane and neovascularization (Figure 3.15, photograph B).

Ulcerative dermatitis with early (bacterial) abscess formation, coagulative necrosis, hypodermal mucoid degeneration, and thrombosis were found in these lesions (Figure 3.14 and Figure 3.15, photographs A and B). The lesions were characterized as multifocal erosive dermatitis with intra-lesional fungal hyphae. Chronic degenerative dermatitis and osteitis with intra-lesional fungal hyphae, chronic degenerative dermatitis, and myositis were typical. Multifocal vascular necrosis was found in some fish while in others myo-regeneration was evident. The occurrence of fungal hyphae, visible microscopically but not macroscopically in the lesions, was universal (Figure 3.16, photograph C).

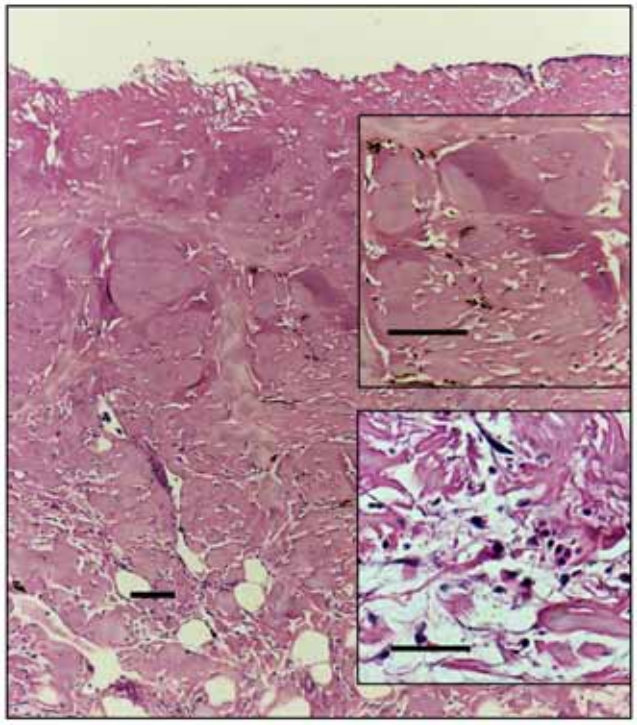

Figure 3.14. Collagen and hypodermal degeneration in freshly ulcerated fish from Umatilla fish trap. Upper inset shows collagen degeneration. Lower inset shows hypodermis with myxomatous degeneration. All scale bars, 100 um, all hematoxylin eosin (H&E) stain.

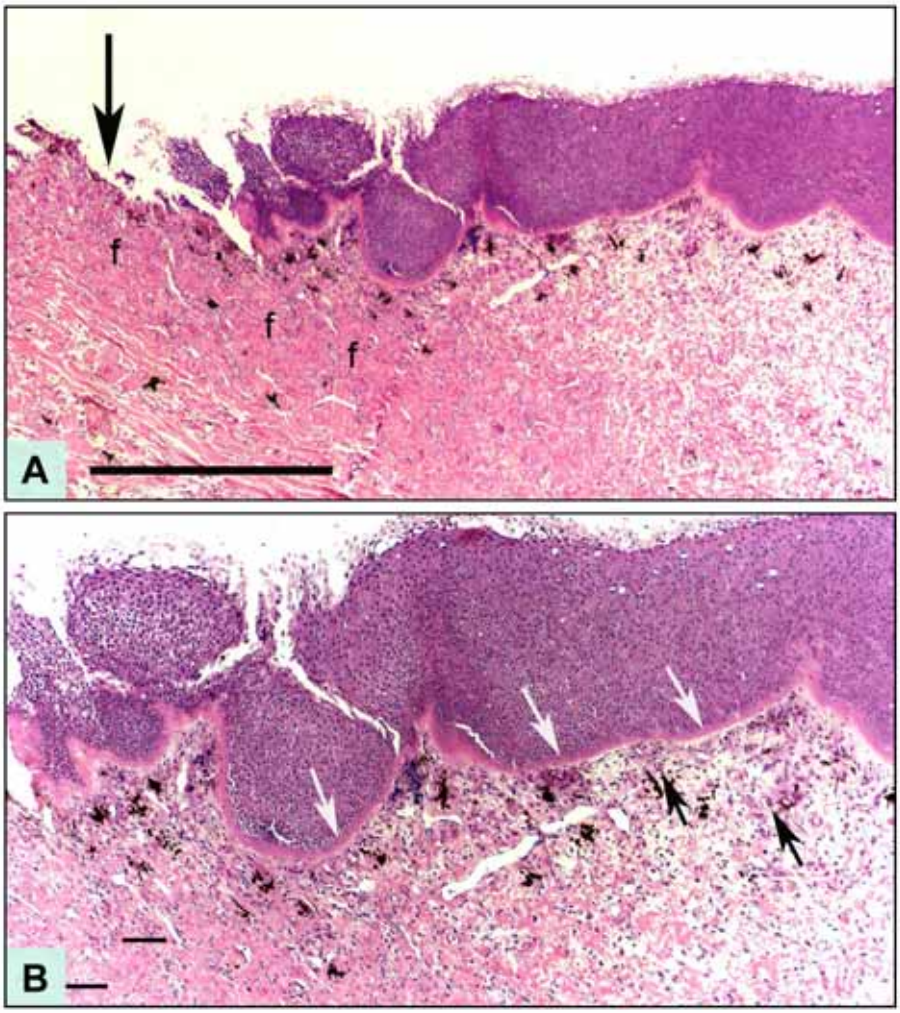

Figure 3.15. Photograph A. Ulcer (arrow) with healed epithelium to right of arrow. Note the fibrosis in the SS underneath the ulcer (f), bar- 1 mm, H&E stain. Photograph B. Healed skin growth showing neovascularization (black arrows) and thickened basement membrane (white arrows), both characteristic of healing, bar 100 um, H&E stain.

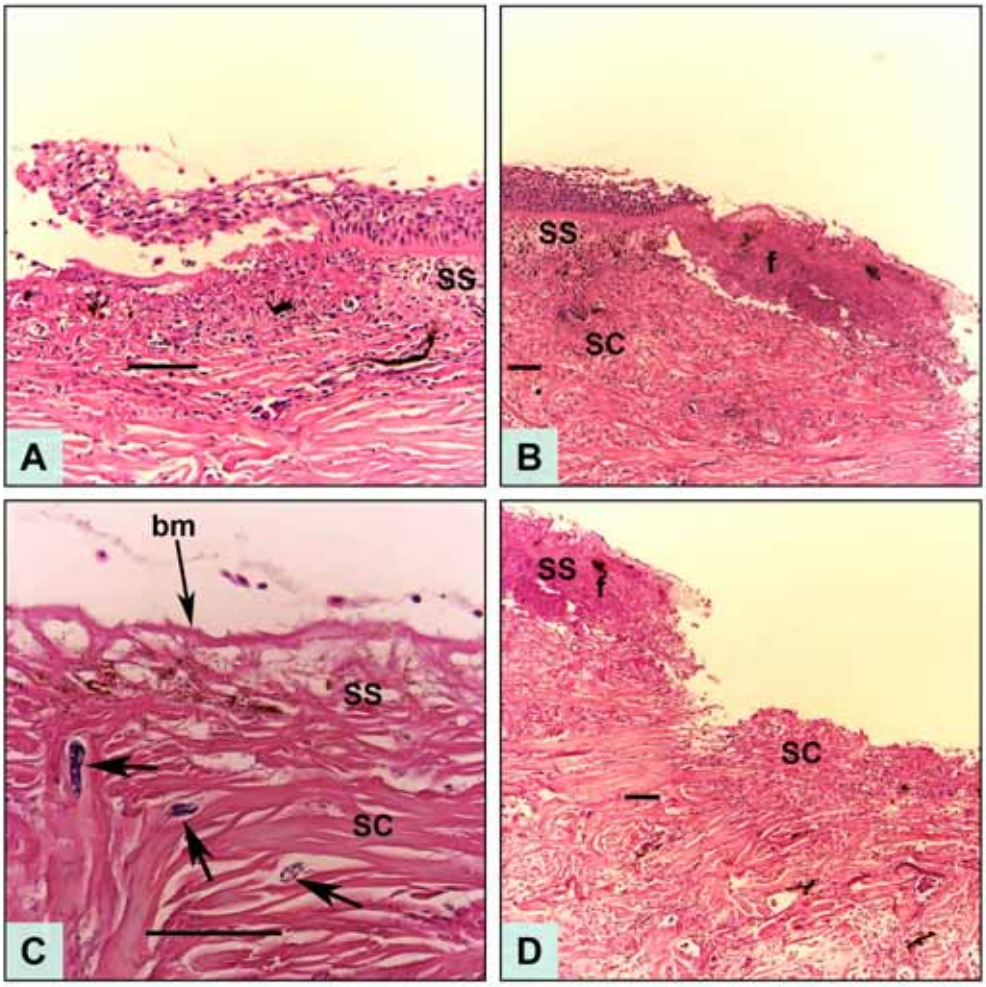

Figure 3.16. Ulcerated lesions in fish from Umatilla trap. Photograph A. Normal skin on right with normal underlying SS, with skin lifted and disrupted basement membrane on left. Photographs B and D. Normal skin on left showing SS and SC with ulcer on the right and fibrosis (f) of the SS. Photograph C. Loss of epithelium only exposing the basement membrane (bm) and showing intact SS and SC. Arrows show invading fungal hyphae. All scale bars, 100 um, H&E stain.

Figure 3.16, photographs A through D show focal erosion of the epidermis, accompanied by loss of the upper layer of stratum spongiosum (SS) that normally is found just beneath the basement membrane, fibrosis (early formation of scar tissue), and the infection of the upper layers of the dermis with fungal hyphae.

Figure 3.17 shows damage to deeper layers of the supporting connective tissue (hypodermal mucoid degeneration).

Headburn Histology of Chinook Salmon from Upriver Location

The Chinook salmon from the upriver location (Rapid River Hatchery) were characterized by chronic dermal scarring, healed dermal scars with mild melanosis, and active mild hypodermal degeneration. The healing lesions were characterized by multifocal squamous hyperplasia and overgrowth of this fragile layer of skin without underlying supporting dermal tissue (Figure 3.18).

In addition, many of the lesions were characterized by deep bacterial colonies in the dermal layers with a complete absence of an inflammatory response. In addition, the surface of the lesions contained a mat of fungal hyphae that were simple fungi, lacking zoospores and evidence of concurrent yeast infection (Figure 3.19).

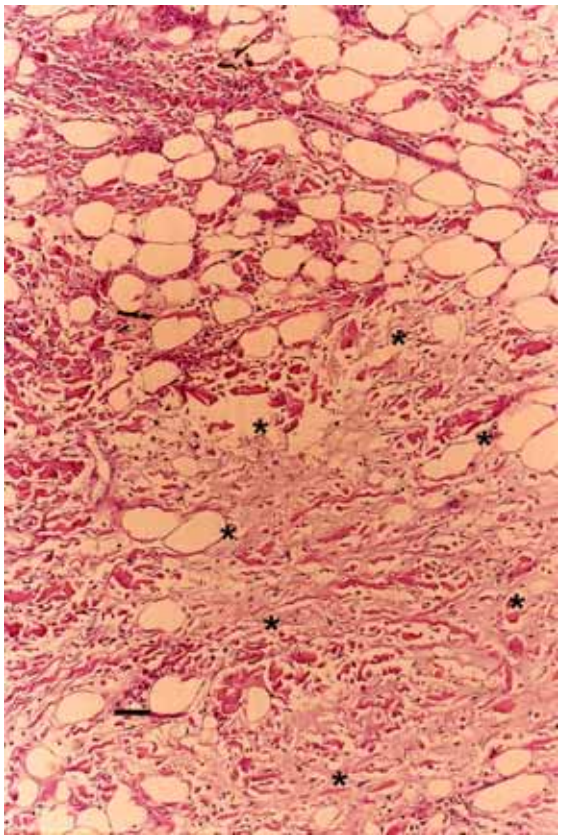

Figure 3.17. Dermis of fish with ulcerated headburn showing are of normal tissue (upper left) and hypodermal degeneration (outlined by *). Bar 100 um, H&E stain.

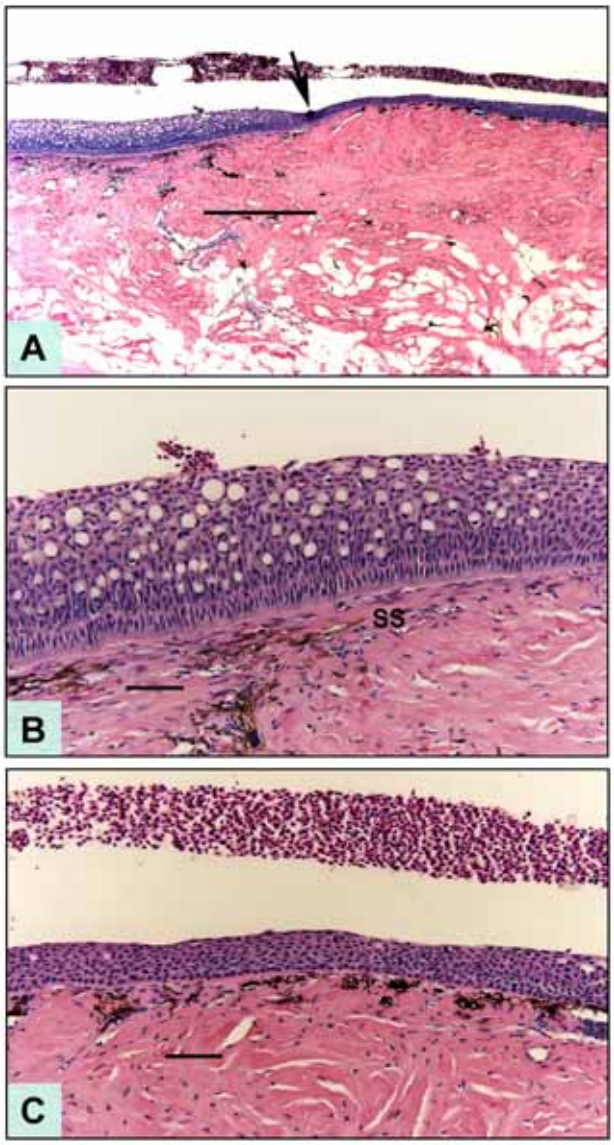

Figure 3.18. Healing and normal skin from fish captured at upriver location, Rapid River Hatchery. Photograph A. Normal skin to left of black arrow and healed skin to right of black arrow. Bar 1 mm, H&E stain. Photograph B. Normal skin showing the typical underlying SS. Bar 100 um, H&E stain. Photograph C. Squamous metaplasia, characteristic of healed, new growth skin. There is no SS supporting this healed skin as is found in the normal skin in photograph B. Bar, 100 um, H&E stain.

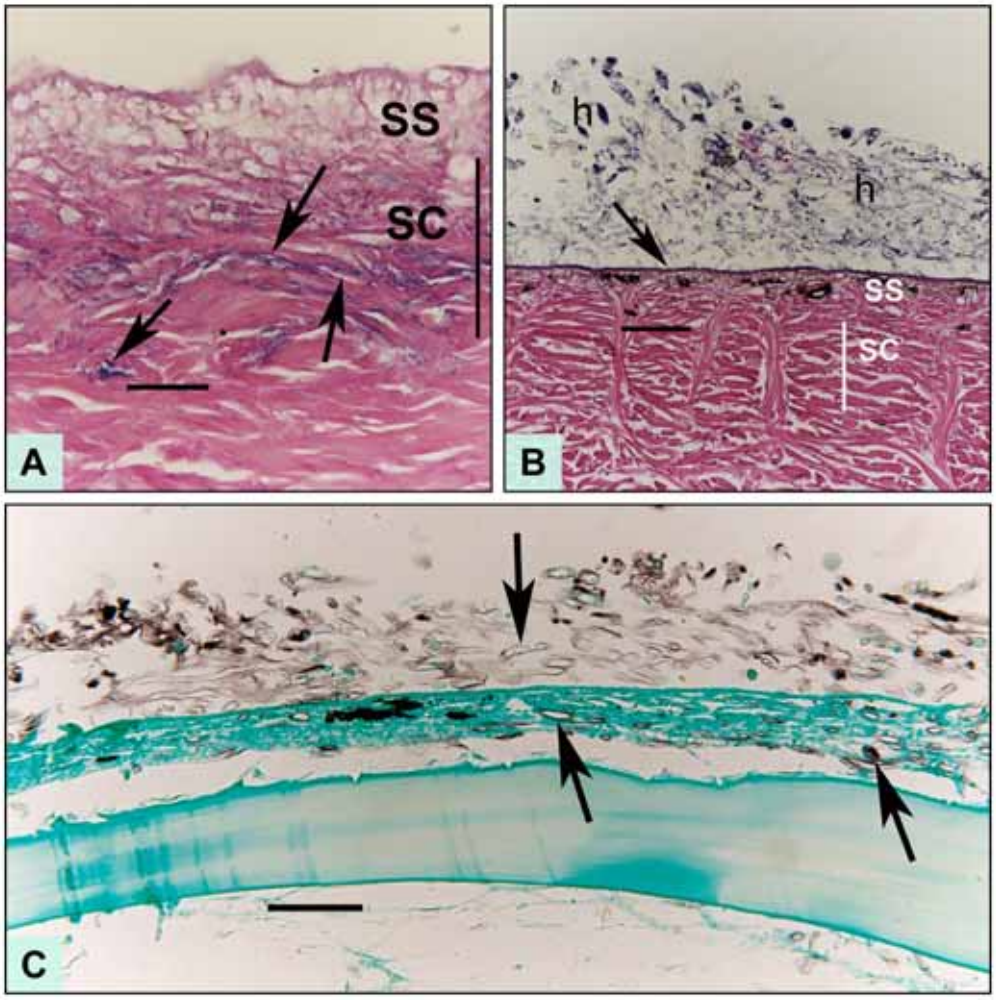

Figure 3.19. Headburn lesion from upriver location. Photograph A. Infection with colonies and clumps of bacterial rods with no inflammatory response. The SS is shown along with the SC (indicated along the vertical line). Photograph B. Another area of epidermal loss in which the damaged area is infected with fungal hyphae (h). Again the SS and SC (indicated along the vertical line) are shown. The basement membrane is shown at the arrow. H&E stain both A and B. Photograph C. Fungal hyphae (examples at arrows) above the basement membrane and invading the exposed connective tissue around a scale. GMS stain. All scale bars 100 um.

Headburn Histology of Steelhead

Steelhead headburn lesions were characterized by subacute mucoid degeneration of the SC and hypodermal degeneration on the head. The lesions were commonly associated with subacute-to-chronic active and erosive ulcerative mycotic dermatitis, superficial collagen degeneration of the SC, myositis, and occasional focal epithelial regeneration.

Lesions in steelhead were characterized by a more diverse population of fungi and yeast than the lesions in Chinook salmon, as shown in Figure 3.20.

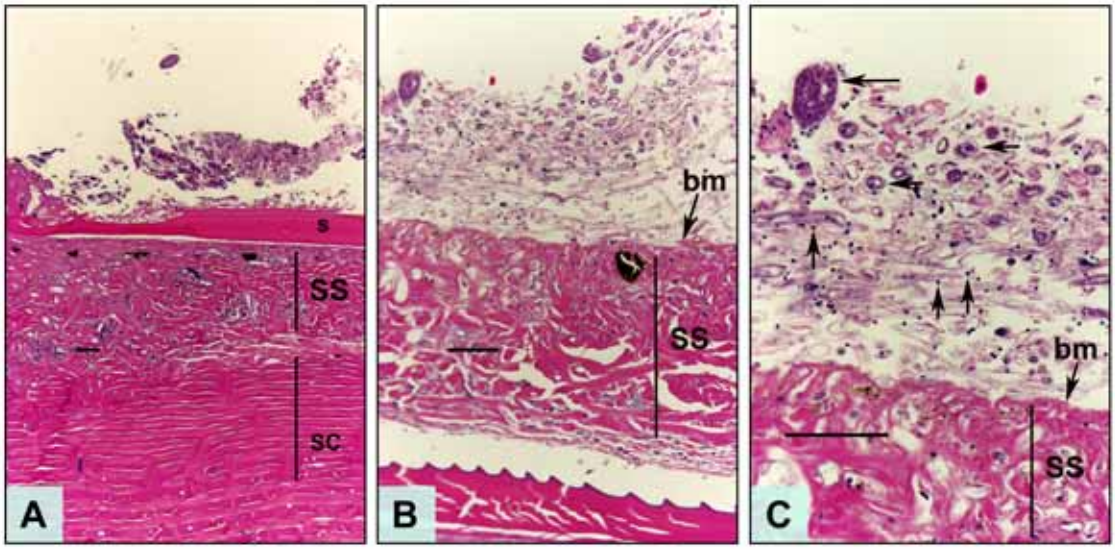

Figure 3.20. Headburn lesions in steelhead from upriver location. Photograph A. Mat of fungi and yeast above surface of scale (s) with erosion of scale. Underlying SS and SC shown along vertical lines. Photograph B. Erosion of epidermis associated with fungal and yeast infection. Basement membrane remnants and location (bm) shown above underlying SS represented by depth of vertical line. Photograph C. Similar location to B, showing fungal hyphae (horizontal arrows), with hyphae containing zoospores at topmost arrow and yeast cells (examples at vertical arrows). Location of basement membrane (bm) and vertical bar showing depth of SS. All scale bars 100 um, all panels H&E stain.

3.2.3.2 Systemic Bacterial Infections in Affected Fish

Salmon and steelhead sampled in 2003 were tested for systemic bacterial infections by culturing blood taken aseptically from a heart puncture on tryptic soy agar (TSA). Of all the fish tested, only two of the Chinook salmon sampled from Rapid River Hatchery holding raceway clearly had systemic bacterial infections. The fish had been held an estimated 7 to 10 days. Both fish had significant headburn lesions colonized by grossly visible fungi. One fish yielded a single uniform set of colonies, and the second fish yielded two types of uniform colonies. Low passage number isolates were archived at -70°C in tryptic soy broth (TSB) with 10% glycerol. The cultures were characterized by fatty acid analysis and yielded the following similarities to known isolates:

Fish 1. *Aeromonas ichthiosmia* (=*Aeromonas hydrophila*) (similarity index 0.819)

Fish 2. *Aeromonas veroni* – GC subgroup B (similarity index 0.888)
Bacillus megaterium – GC subgroup B (similarity index 0.690)

Aeromonas ichthiosmia and *Aeromonas hydrophila* are considered identical based on 16S rRNA gene sequences (Collins et al. 1993).

Isolates with a similarity index of at least 0.600 are considered good species matches.

Sensitivity of Bacterial Isolates to Various Antibiotics

These bacterial strains were screened for antibiotic sensitivity to a variety of antibiotics, including erythromycin, which is routinely injected into hatchery fish at capture facilities to treat possible infection with *Renibacterium salmoninarum*, a causative agent of bacterial kidney disease. Only screening tests using sensitivity discs were conducted for this study. The results are shown in Table 3.4.

Table 3.4. Sensitivity of bacterial isolates from Chinook salmon blood to antiobiotics using a preliminary antibiotic disc sensitivity screening method.

	Isolate Name Based on Fatty Acid Analysis		
Antibiotic disc identification	***Aeromonas hydrophila***	***Aermonas veroni***	***Bacillus megaterium***
	Diameter of Inhibition Zone (mm)[1]		
Florfenicol 30 ug	42	45	35
Erythromycin 15 ug	42	27	0
Sulfamethoxazol 23.75 ug Trimetoprim 1.25 ug	10	9	19
Oxytetracylcine 30 ug	40	0	17
Tetracycline 30 ug	40	0	0
Azithromycin 15 ug	28	12	25
Norfloxacin 10 ug	20	36	40
Penicillin G 10 units	16	0	0
Streptomycin 10 ug	25	9	11

[1] Disc diameter approximately 6.5 mm. Inhibition zone is total diameter of zone including disc in center of zone. The minimum measurable zone was considered to be 8 mm. For tests with no inhibition, the zone is recorded as 0.

The results show best inhibition of the three isolates by Florfenicol, a proprietary antibiotic owned by Schering-Plough, which is being tested for possible application for aquatic animal diseases. Erythromycin and norfloxacin showed the next best overall sensitivity.

3.2.3.3 Loss of Plasma Fluids by Plasma Sodium Indicator

Plasma sodium was measured in selected samples of salmon and steelhead during the 2003 study year. Results of these measurements are shown in Tables 3.5 and 3.6.

Table 3.5. Plasma sodium concentrations from adult Chinook salmon collected in 2003.

Animal ID	Plasma Sodium mEq/L	Source and condition
B03-2-2	168	Umatilla - headburn directly from trap
B03-2-4	171	Umatilla - headburn directly from trap
B02-2-5	166	Umatilla - headburn directly from trap
B03-2-6	162	Umatilla - headburn directly from trap
B03-2-7	162	Umatilla - headburn directly from trap
B03-2-8	170	Umatilla - headburn directly from trap
Average:	**166.5**	
B03-3-1	171	Rapid River Hatchery - headburn directly from trap
B03-3-2	148	Rapid River Hatchery - headburn directly from trap
B03-3-3	141	Rapid River Hatchery - headburn directly from trap
Average:	**153.3**	
B03-3-4	126	Rapid River Hatchery - from pond, with fungus on headburn lesions
B03-3-5	96	Rapid River Hatchery - from pond, with fungus on headburn lesions
Average:	**111.0**	

For reference, normal values for migrating adult Chinook salmon in freshwater are cited as 160 mEq/L (no range given)(Robertson et al. 1961) and 159 and 161 mEq/L for spawning male and female Chinook salmon, respectively (Urist and Van de Putte 1967). Therefore, these results show that fish at the Umatilla fish trap were not osmotically compromised, at least by using plasma sodium as an indicator, even though some of these fish had severe ulcerative lesions (e.g. Figure 3.5, photograph C). However, in the upriver location at Rapid River Hatchery, some of the fish examined directly at the trapping facility with headburn lesions had marginally low plasma sodium concentrations while those fish held in the raceway, prior to spawning, had markedly depressed plasma sodium concentrations, suggesting that they were losing electrolytes in an uncontrolled manner and were thus debilitated not only by fungal and bacterial infection but by loss or dilution of plasma electrolytes, as indicated by sodium concentration.

In addition, we measured plasma sodium concentrations from steelhead at Dworshak National Fish Hatchery. Results are shown in Table 3.6.

Table 3.6. Plasma sodium concentrations from adult steelhead salmon collected in 2003 and 2004 from Dworshak National Fish Hatchery trap holding facility. The fish are grouped by severity of lesions with the most severe group at the top of the table.

Animal ID	Plasma Sodium mEq/L	Condition
B03-5-2	121	very large head wound
B03-5-3	136	large head wound
B03-5-4	126	large head wound
B04-1-2	148	jaw and head lesion
Average:	**133**	
B04-1-3	154	jaw lesions
B04-1-4	142	epidermal scrape only
B04-1-5	148	epidermal fungal infection only
Average:	**148**	
B03-4-1	154	superficial skin lesion
B03-4-2	145	superficial skin lesion
B03-5-1	151	superficial skin lesion
B04-1-11	152	superficial skin lesion
B04-1-12	156	superficial skin lesion
Average:	**152**	
B04-1-6	151	normal appearing
B04-1-7	144	normal appearing
B04-1-8	156	normal appearing
B04-1-9	164	normal appearing
B04-1-13	154	normal appearing
B04-1-14	158	normal appearing
B04-1-15	163	normal appearing
Average:	**156**	

For comparison, normal values for rainbow trout in freshwater are given as 155.8+/- 3.5 mEq/L (mean +/- SD) and 158.9 +/- 3.3 by Řehulka (2003) in two groups of experimental fish. These values suggest that fish with significant lesions are moderately stressed by loss of plasma electrolytes, although the range of values for normal appearing fish includes one value as low as 144 mEq/L.

3.2.3.4 Infection of Lesions by Fungi and Yeast

Following the detailed histological examination of fresh Chinook salmon headburn lesions in May 2003 from Umatilla trap samples, which showed histologically visible but not grossly visible fungal infections, isolation of fungal species from lesion scrapings of fish with similar fresh lesions was attempted. These isolations were made from steelhead collected from Dworshak National Fish Hatchery, in Orofino, Idaho. Table 3.7 shows the frequency of three isolate types from the steelhead lesions and the identification of the isolates by rRNA base pair analysis and comparison with type species.

Table 3.7. Characterization of fungi and yeasts from headburn lesions from steelhead based on LSU rRNA gene sequence similarity.

		Saprolegnia ferax	**Black Mold (*Aureobasidium pullans*)**	**Pink Yeast (*Rhodotorula mucilaginosa)***	**Other**
Gene sequence similarity (% difference):[1]		1.61%	0.31%	0.31%	
Fish Number	Date isolated	Positive for isolation (++) or no isolates made (-)			
B03-4-1	11/12/2003	++	-	-	-
B03-4-2	11/13/2003	-	++	++	-
B03-4-5	11/14/2003	++	++	-	-
B03-4-6	11/15/2003	++	-	-	-
					-
B03-5-1	11/25/2003	++	-	++	-
B03-5-2	11/26/2003	-	-	++	-
B03-5-3	11/27/2003	++	-	-	-
B03-5-4	11/28/2003	++	-	-	-
B04-1-1	2/24/2004	++	-	-	Brown mold
B04-1-2	2/25/2004	-	-	-	Greenish-gray mold
B04-1-4	2/26/2004	++	-	-	-
B04-1-5	2/27/2004	++	-	++	-
B04-1-7	2/28/2004	++	-	-	-
B04-1-9	2/29/2004	-	-	++	-
B04-1-10	3/1/2004	-	-	++	-
B04-1-12	3/2/2004	++	-	++	-
B04-1-13	3/3/2004	-	-	++	White yeast
Proportion and (percent) of positives by column:		11/17 (65%)	2/17 (12%)	8/17 (47%)	3/17 (18%)

[1] Several studies have indicated that separate fungal species generally have genetic sequence similarities (% difference) greater than 1%, according to MIDI Labs (Kurtzman and Robnett 1997; Fell et al. 2000). Therefore, the identification of *Saprolegnia ferax* may be interpreted as an artifact of the classification library, which may not contain the expected species, *Saprolegnia parasitica*.

4.0 Discussion, Conclusions and Recommendations

4.1 Juvenile Studies

4.1.1 Discussion – Juvenile Salmon and Trout Study

We were able to show in preliminary studies that a low percentage (up to 15%) of Chinook salmon juveniles exposed to simulated turbine passage pressures had internal lesions (Figure 3.2) that most likely corresponded to the external lesions characterized as "head spots" and hemorrhage. Head spots are transient darkening of the skin (Figure 3.1) that occur on the dorsal cranium in the inter-orbital region. However, in the definitive exposures, we were able to find only minor hemorrhages in various areas of the brain. In Chinook salmon juveniles, the number of such hemorrhages was not significantly different in control and exposed fish, but was significantly higher in exposed rainbow trout juveniles, compared to control fish. These aneurysms were in capillaries and appeared to be very localized and contained, with an anticipated high likelihood of healing, in both the rainbow trout and Chinook salmon baseline studies.

Up to 13.3% and 20% of the two exposed Chinook salmon groups had hemorrhage in the nares, while only 10% of the control fish had similar bleeding. However, in rainbow trout, the exposed groups had about 47% and 27% of individuals with blood in the nares compared to 20% in the control fish. This lesion is clearly a sensitive indicator of handling and other traumatic stress events, such as the pressurization regime the fish received.

While in definitive studies, none of the lesions observed in the baseline exposed juvenile Chinook salmon or rainbow trout appeared to be precursors to headburn lesions in adult fish or life-threatening lesions, the presence of blood in the nares could reduce the olfactory sensitivity of the fish until the lesion was cleared from the olfactory organ. It is expected that such clearance might take several days to a week or two, during which time the survival and ecological fitness of the affected fish would be reduced.

4.1.2 Conclusions – Juvenile Salmon and Trout Study

4.1.2.1 Simulated Turbine Passage and Headburns

The simulated turbine passage regime used in this study did not cause lesions in juvenile Chinook salmon or rainbow trout that are precursors to headburn lesions in adult ocean-return fish.

4.1.2.2 Simulated Turbine Passage Causes Aneurysms

The simulated turbine passage regime used in this study caused a significant increase in mild and localized aneurysms in the brains of exposed juvenile rainbow trout, but not in exposed juvenile Chinook salmon.

4.1.2.3 Simulated Turbine Passage Causes Transient Cranial Darkening

The simulated turbine passage regime used in this study caused a significant increase in grossly visible darkening and presumed hemorrhage in both juvenile Chinook salmon and rainbow trout but these changes were transient and disappeared within two days of exposure.

4.1.2.4 Simulated Turbine Passage Causes Olfactory Organ Bleeding

The simulated turbine passage regime used in this study resulted in a significant increase in the number of hemorrhages in the olfactory organ (nares) of exposed juvenile Chinook salmon and rainbow trout. The lesions are likely to result in a short-term reduction in the ecological and survival fitness of the exposed fish.

4.1.3 Recommendations—Juvenile Salmon and Trout Study

4.1.3.1 Examine River Captured Fish for Turbine Passage Lesions

The extent of the lesions observed in juvenile Chinook salmon and steelhead should be examined in river-caught fish that may have passed through turbines to determine the extent of damage that may be caused by such exposure. These examinations can be based on terminal sampling of hatchery fish for histological evaluation.

4.2 Adult Studies

4.2.1 Discussion

4.2.1.1 Fish Health and Characterization of Headburns by Location

Chinook Salmon

The studies showed that the headburn lesions found on adult spring Chinook salmon are recently initiated lesions when examined at downriver locations such as Bonneville Dam and Umatilla fish trap. Lesions at these locations consisted of superficial and localized abrasions to deep ulcerative abrasions over extensive areas of the head with little evidence of healing. The lesions, even in this fresh and recently initiated state, were colonized by hyphal filaments of fungi, although the fungi was not visible macroscopically on the lesions. This is the first verification that fish with headburn and skin lesions become infected with fungi in the river, rather than after being placed in hatchery holding ponds or raceways. The lesions may be severe and affect the entire dorsal area of the cranium or they may be localized. In a few cases, fish with blunt traumatic lesions but without breakage in the skin were observed.

Adult spring Chinook salmon examined at an upriver location (Rapid River Fish Hatchery) in 2002 and 2003 had primarily, in contrast, lesions that were old and either chronically infected with fungi, superficially healed with skin or a combination of skin and scarring (connective) tissue, or re-injured lesions. The lesions on these fish ranged from localized to extensive but the majority of the fish examined had extensive lesions over the dorsal aspect of the cranium. In addition, localized lesions were observed on the side of the head or on the lower jaw, as they were in fish from downriver locations.

Healed headburn lesions found in upriver fish are only superficially healed, as shown by histological studies. The healing process includes the overgrowth of a new epithelial layer of skin that is a darker olive color in contrast to normal skin, but without the re-growth of the underlying and supporting layers of connective tissue including the SC. Such healed injuries are more easily re-injured than normal skin, and re-injured areas of headburn lesion were observed in fish from the upriver location.

The studies also showed that, at least in some individual Chinook salmon, there is a limited to non-existent inflammatory response in the headburn injuries in that bacterial colonies were found in the ulcerated muscle and connective tissue of headburn lesions but without the presence of responsive inflammatory cells.

Steelhead

Headburn lesions studied in fall steelhead from Dworshak National Fish Hatchery were similar to those found in Chinook salmon but appeared to occur at a much lower frequency. In addition, affected steelhead lesions were infected by a variety of fungi and yeast species. This microbiological evaluation of steelhead lesions was more extensive than that conducted for Chinook salmon, so this investigation does not preclude the infection of Chinook salmon headburn lesions with a similar mix of fungi and yeast. However, a specific yeast, *Rhodotorula mucilaginosa*, was isolated from steelhead, and yeast cells were observed histologically in the headburn lesions from steelhead. No yeast cells were observed histologically in the headburn lesions of spring Chinook salmon. In addition, a hyphal fungal infection was found in steelhead that was differentiated from that in Chinook salmon and presumptively identified as being caused by *Aureobasidium pullans*.

4.2.1.2 Classification and Consequences of Salmonid Headburns

A classification system based on the characterization of lesions and the condition of the fish was presented in the results (Figure 4.1). This showed that severe headburn lesions can be initiated at Bonneville Dam but that the prevalence of such lesions increases as the fish move upriver. Lesions at downriver locations are characterized as fresh abrasions to deep ulcerations and may be limited in extent or involve most of the head area of the fish. Fish with ulcerated lesions are compromised by fungal infections that are not visible to the naked eye and the fish begin to loose plasma electrolytes. Upriver fish are characterized by headburn lesions that have healed superficially but are fragile and easily re-injured. The fish that survive to migrate to upriver locations are likely to have greater dilution of plasma electrolytes by dilution through the wound. Hatchery fish with headburn lesions, including those that are partially healed are likely to succumb to the consequences of headburn lesions prior to spawning, once placed in holding areas, due to rapidly progressive loss of plasma electrolytes, eruptive fungal and yeast infections, and opportunistic systemic bacterial infections.

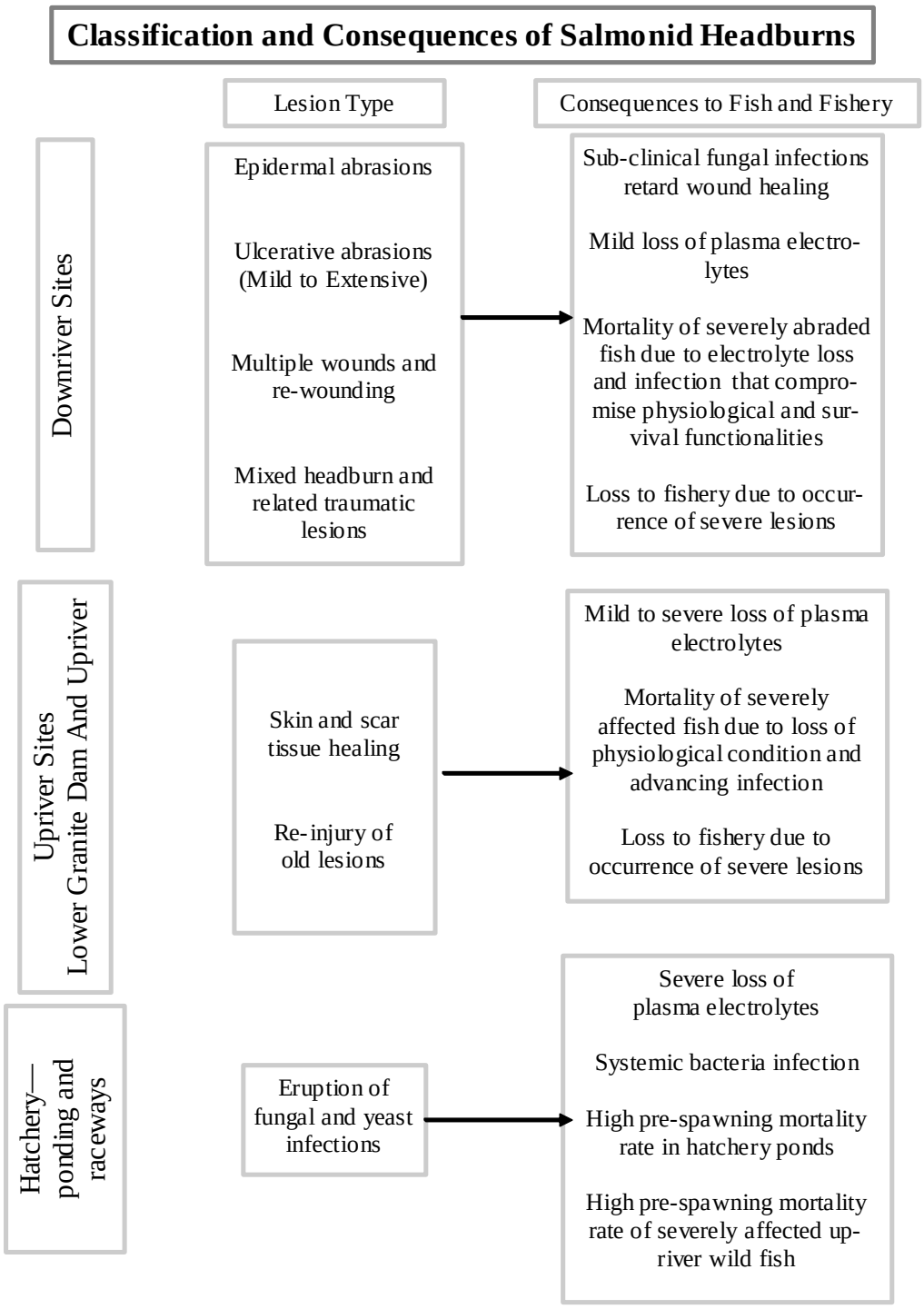

Figure 4.1. Classification and consequences of salmonid headburn lesions. The consequences of headburns for wild fish are essentially unknown but are likely to be similar to those of hatchery fish.

4.2.1.3 Specific Health Consequences for Fish Affected with Headburns

The health consequences for fish with headburn lesions are progressive. It appears likely that while some fish survive in a compromised condition to upriver locations, others succumb to the combined effects of plasma electrolyte loss, chronic fungal infections that retard wound healing, and finally to systemic bacterial infections.

Fish That May Successfully Heal

The pathological and histological characterization of fish from the upriver location showed that healing does occur in some fish, while in other fish, healing is retarded or prevented due to fungal and bacterial infection of the wound. We did observe some fish at the upriver location with healed lesions that appeared to have maintained plasma sodium levels within the normal range. The wound healing is superficial and consists of epidermal healing without the development of supporting underlying layers of dermis and connective tissue and with limited repair to damaged muscle. Therefore, healing will enable fish to maintain physiologic homeostasis, but they are more susceptible to re-wounding than unwounded fish due to the fragility of the healed cranial skin.

Chronic Debilitation of Lesions, Rewounding, Loss of Electrolytes, and Secondary Infections

The lack of an inflammatory response in some fish with bacterial infections indicates that the infections can proceed in an uncontrolled manner, debilitating the fish progressively. As the ulcerations remain exposed, plasma fluids continuously seep out of the fish and are replaced by an influx of water, as is reflected in the reduced concentration of plasma sodium found in the fish. This study provided a snapshot of the condition of fish with chronic lesions and suggests that, while an undetermined proportion of wounded fish reach the hatchery (or spawning grounds, in the case of wild fish), the severity of the lesions is so sufficient that many may die in the river.

Effect of Ponding or Raceway Holding of Prespawn Headburned Fish

Holding fish with skin lesions in ponds appears to universally result in the eruption of already established subclinical fungal and yeast infections. Simultaneously, these fish proceed on a rapid downhill course in terms of measured health parameters. Plasma electrolytes are rapidly lost through wounds, the eruptive fungal infections cause the epidermal lesions and ulcers to enlarge, and the fish become infected with systemic bacterial infections with which they would normally most likely not be infected.

This study observed this phenomenon of eruptive fungal infections after only a short period of ponding or raceway holding at Rapid River Hatchery for spring Chinook salmon and at Dworshak National Fish Hatchery for steelhead. A prior report on salmon headburns (Groberg 1996) noted similar conditions at Looking Glass Hatchery in Oregon for fish moved from the Lower Granite trapping facility to Looking Glass Hatchery. Therefore, it seems likely that this phenomenon is widespread, if not universal, at salmonid hatcheries on the river system, and may also affect wild fish with headburn lesions that migrate into lower flow tributaries of the main river system where they would spawn.

4.2.1.4 Quantification and Known Magnitude of Headburn Lesion Problem

The quantification and known magnitude of headburn lesions in salmonids in the Columbia and Snake river systems is somewhat problematic due to the heretofore lack of a standardized system for classifying headburn lesions and inconsistent recording of such lesions. There is no reason to doubt the validity of the data obtained from participants and collaborators on the project; however, standardization and consistency of recording throughout the system does not appear to exist.

The condition of headburn fish is recorded in detail at Rapid River Hatchery. However, it appears that the prevalence of headburn fish at the trapping facility is underestimated at Rapid River Hatchery when the fish first arrive at the hatchery, and managers at the facility expressed this belief. Fish are also examined after placement in a raceway to mature for spawning, and fish lost to head lesions and eruptive fungal infections are recorded. The percentage of headburn fish recorded from the raceway environment was substantially higher than that recorded when the fish were trapped. While this indicates a need to conduct a more thorough enumeration of lesions when the fish arrive at the trapping facility, it does not change the fact that a high percentage of "ponded" fish placed in raceways are lost to headburn-related injuries prior to spawning, according to observations of hatchery personnel, and this loss rate was estimated to be as high as 22% at Rapid River Hatchery in 2003, a moderate water flow year.

A significant part of the problem in recording headburn injuries accurately is that it requires dedicated effort and must be incorporated into the hatchery operating plan. However, most hatcheries do not have the financial resources necessary to adequately monitor and classify these injuries. In addition, fish health professionals expressed that there was a need for a standard classification system of headburn injuries, such as that provided in this study.

In addition to the problem of quantifying the number of hatchery fish with headburn lesions, there is even less data on the prevalence of this problem in wild fish. Headburn lesions could be a significant problem in low number runs of wild fish, if headburns occur at the higher percentage levels indicated for hatchery fish.

4.2.1.5 Probable Causes of Headburns

The proximal cause of headburns in Columbia and Snake river salmonids is presumed to be collision with concrete or other structures at Columbia and Snake river dams. In addition, based on the capture of such fish at Bonneville Dam, a portion of the headburn problem may result from fish that "fallback"; that is, pass back downstream over spillways or through turbine assemblies after upstream passage of the dam on a fish ladder. The scalping nature of some of the lesions and the blunt trauma seen in a few fish is consistent with the phenomenon of "fallback." In addition, the increasing prevalence of headburn lesions as fish travel upriver is consistent with the belief that the abrasive and ulcerative lesions result from collision of fish with concrete and other hard structures associated with the dams.

This occurred during two moderate water flow years on the Columbia and Snake river systems. However, the two previous reports on the subject of adult salmonid headburns (Elston 1996; Groberg 1996) were conducted during a high water year. Groberg (1996) reported a very high incidence of headburns in fish moved from Lower Granite Dam fish trap to Looking Glass Hatchery. Groberg reported that many of the fish held in ponds at Looking Glass Hatchery appeared to have signs of gas bubble disease that

exacerbated the headburn lesions. To more systematically investigate whether there is a relationship between headburn lesions and high nitrogen supersaturation in the river, fish will have to be examined in a detailed study during a high water year.

4.2.1.6 Microorganisms Infecting Headburn Lesions

Bacteria

The fatty acid analysis showed that two species of *Aeromonas* and one species of *Bacillus* were isolated in near-pure cultures from the blood of Chinook salmon with headburns that had been placed in holding ponds. *Aeromonas veroni* can be a human pathogen affecting compromised patients (Wang et al. 2000; Mencacci et al. 2003). Certain strains are also fish-pathogenic, at least in tropical environments (Rahman et al. 2002), but we have no information to suggest that the strain isolated from the hatchery fish is a human pathogen. Similarly, *Aeromonas hydrophila* is considered an opportunistic fish pathogen and may be more aggressive in tropical environments, but certain strains are also regarded as human pathogens (Janda and Abbott 1998), and in fact, the strain is regarded as a zoonotic bacteria (an animal-infecting strain that also infects humans) (Gonzalez et al. 2001; Lehane and Rawlin 2000). Virulence markers have been reported for both species of *Aeromonas* isolated in this study from freshwater fish and from a case of human diarrhea (Gonzalez-Serrano et al. 2002), and hemolytic and proteolytic activities are reported from both strains (Gonzalez-Rodriguez et al. 2004). Again, there is no evidence to suggest that the isolates in this study are human pathogens, although they were not tested for the presence of pathogenicity genes as a part of this study. Human and animal pathogenicity are conveyed by one mechanism, at least, in *Aeromonas hydrophila* by the presence of hemolytic toxin genes in the pathogenic strains (Heuzenroeder et al. 1999). Certain strains of *A. veroni* produce an enterotoxin (Trower et al. 2000; Vila et al. 2003) and *bacteremia* has been reported in humans (Thomsen and Kristianse 2001).

Fungi and Yeast

The most commonly isolated fungal species from steelhead was identified as *Saprolegnia ferax* by gene sequence analysis, although these more likely represent isolates of *Saprolegnia parasitica*. *S. parasitica* is the species generally associated with infection of wounds and eggs in salmonids. We are not aware of previous reports of the isolation of the fungus *Aureobasidium pullans* or the yeast *Rhodotorula mucilaginosa* from wounds in salmonid fishes but the latter yeast species has been reported isolated from codfish (Schmidt-Lorenz and Farkas 1961). Without further investigation, the role of these microorganisms in lesion development or retardation of wound healing is presumptive. However, we did observe yeast cells, as well as abundant fungal hyphae in the histological sections of headburn wounds of steelhead and fungal hyphae from headburn wounds in Chinook salmon.

Rarely, some strains of *Aureobasidium pullans* can infect humans and usually affect immuno-compromised individuals (Bolignano and Criseo 2003). *Rhodotorula mucilaginosa* is a commonly occurring yeast from aquatic environments, at least in some regions of the world (Libkind et al. 2002), but it can also infect immuno-compromised humans (Maeder et al. 2003). There is no evidence to suggest that the isolates from this study are human pathogen, but no tests were conducted in this study to verify or reject this possibility.

4.2.1.7 Treatment Regime to Prevent Mortality of Headburned Fish

Hatchery Fish

Therapeutic treatment could be applied to reduce the losses of prespawning mortalities in hatchery fish. Such treatment would require the application of antibiotics and would need to be conducted under an investigational permit. Furthermore, such treatments would most likely preclude the treated fish from being used for human consumption after spawning. Nonetheless, therapeutic treatment would be useful and effective on a certain subpopulation of hatchery fish that are reserved for spawning purposes and are not needed for human consumption and for wild fish with extremely low return numbers. The three features of compromised health that need to be treated are 1) loss of plasma electrolytes, 2) superficial bacterial infections, and 3) systemic bacterial infections. These could potentially be treated in the following manner but a systematic evaluation of the treatments needs to be conducted at an affected hatchery where treatable fish are available.

- Therapeutic treatment of loss of plasma electrolytes. Intra-peritoneal injection of an electrolyte concentrate that mimics normal concentrations of plasma electrolytes in Chinook salmon or steelhead. The volume and concentration of injected therapeutic electrolyte would be based on body weight of the fish and assumed level of dilution of plasma electrolytes. For hatchery fish, this therapy would be applied when the headburn injured fish are handled at the trapping facility.

- Therapeutic treatment of fungal and yeast infections. Topical application of an antifungal agent could reverse and prevent the eruptive fungal infections that occur once fish are placed in raceways. Again, this could be easily applied when the fish are trapped. An appropriate therapeutic compound may be bronopol (2-bromo-2-nitropropane-1,3-diol) that currently is being evaluated as a potential treatment under the brand name Pyceze as an antifungal agent to treat salmonid eggs and fish (Pottinger and Day 1999).

- Therapeutic treatment of systemic bacterial infections. Systemic bacterial infections could be treated with an effective broad-spectrum antibiotic. One such possibility is Florfenicol, which is currently undergoing consideration registration for use on fish. This is a proprietary drug manufactured by Schering-Plough pharmaceutical company. The preliminary screening of antibiotics against isolated opportunistic systemic bacterial infections suggested that this drug has a broad spectrum of activity and could be highly effective. Prior to the general registration of Florfenicol, it could be tested in a controlled program on hatchery fish, if an investigational permit were put in place.

  However, injection of erythromycin should also be effective for many opportunistic bacterial infections. Although it inhibited only two of the three isolated strains (Table 3.4), these are likely the two most significant strains. Because a form of erythromycin is injected into all fish entering Rapid River Hatchery, the isolation of these strains from presumably injected fish indicates that the dosage or duration of effect may not be adequate and should be confirmed and evaluated.

Wild Fish

If a program of evaluation on hatchery fish demonstrated that the proposed treatment regime was effective in preventing prespawning mortality, a similar treatment regime could be applied to severely

headburned wild fish when they pass through fish trapping facilities. Such fish are likely not to survive to spawning and application of an effective treatment regime would increase their chance of survival.

4.2.1.8 Need for Improvement of Ponding Conditions for Holding Prespawning Hatchery Fish

The eruptive fungal and yeast infections common to hatchery holding facilities result when previously injured headburned fish enter the facilities with subclinical fungal and/or yeast infections. The eruption of fungal and yeast infections within a few days of placement of fish in the raceways suggests that flows in holding raceways are too low and possibly that the raceways need better sanitation to reduce the number of fungal zoospores or infective yeast elements.

4.2.2 Conclusions Regarding Adult Salmon and Steelhead

- Headburn lesions from spring Chinook salmon at downriver locations are fresh and recently initiated while fish from upriver locations typically have old and chronically healing or re-injured lesions.

- The prevalence of headburn lesions increases in proportion to the distance upriver in the system from Bonneville Dam.

- Fungal infections are initiated in fresh lesions in downriver fish, although the fungal infection is subclinical and is not visible macrocopically in most cases. These fungal infections exacerbate the lesions and retard wound healing.

- Injured fish have a limited-to-non-existent inflammatory response when headburn lesions are infected by bacteria.

- Fall steelhead, although apparently affected at a much lower prevalence of headburns than spring Chinook salmon, have wounds that are colonized not only by the widely recognized *Saprolegnia sp*. but also by other hyphal fungi and a yeast (presumptively identified as *Aureobasidium pullans* and *Rhodotorula mucilaginosa*, respectively) not previously described from such lesions.

- Spring Chinook salmon with mild lesions may heal and successfully reach hatcheries or spawning grounds. However, the healing process is incomplete and the fish are highly susceptible to re-wounding.

- Spring Chinook salmon with moderate to severe lesions reach upriver hatcheries in some cases but their progressive physiological deterioration and chronic infection status suggest that many such wounded hatchery and wild fish may die in the river.

- There is a need to standardize the recording of headburn fish at trapping facilities and fish hatcheries for headburn lesions and to consistently collect data to accurately estimate the magnitude of this problem. The classification system provided in this report can be used to help standardize the recording of headburn lesions. It is recommended that the lesions be photographed at the trapping and hatchery facilities for later evaluation.

- Data from Rapid River Hatchery suggests that recording of headburn fish when they arrive at the trapping facility may substantially underestimate the number of affected fish and that the loss of fish due to headburn lesions in upriver locations may be as high as 22% even in moderate water years such as 2003.

- Headburn lesions are primarily caused by collision of fish with concrete or other structures at dams and fish passage facilities and may be exacerbated in some fish that "fallback" or pass over spillways or through turbine assemblies after having passed the dam through a fish ladder.

- Headburns in salmonids are apparently exacerbated in spring runs during high water years. This may be due to increased water turbulence and high flow rates and could also be exacerbated by gas-supersaturation. The exacerbation and extent of headburns and loss of injured fish needs to be studied in detail during a high water year.

- Two species of bacteria and two species of fungi or yeast (*Aeromona veroni* and *Aeromonas hydrophila* and *Aureobasidium pullans* and *Rhodotorula mucilaginosa*, respectively) were isolated from headburn lesions in salmon and steelhead, or from the blood of affected fish, in addition to the more commonly isolated *Saprolegnia sp*. All of these species are opportunistic fish pathogens and certain strains of each species can also infect humans. We are not aware of prior reports of these isolates from diseased Chinook salmon or steelhead.

- Prespawning mortality of headburned salmonids can be prevented or greatly reduced by therapeutic treatment of selected populations of hatchery and potentially wild fish. Therapy would slow, reverse or prevent the debilitating physiological condition and associated infections that cause prespawning mortality, particularly in spring Chinook salmon. Treatment would consist of topical application of an anti-fungal agent, injection of replacement plasma electrolytes into the peritoneal cavity, and injection of a broad-spectrum antibacterial, such as Florfenicol.

- The dose and application consistency or duration of protection of erythromycin injections at Rapid River Hatchery may not be effective because some isolated strains of opportunistic pathogenic bacteria from fish at that facility showed high sensitivity to this antibiotic.

- Prespawning ponding or raceway holding of salmon or steelhead results in eruptive fungal infections in headburned fish. This probably occurs in all or most facilities. The result is that the affected fish undergo a rapid decline in physiological condition and often die prior to spawning. These fungal infections are acquired in the river but sub-optimal holding conditions allow their rapid proliferation to the eruptive visible fungal infection commonly seen in hatchery holding raceways or ponds.

4.2.3 Recommendations Regarding Adult Spring Chinook Salmon and Adult Steelhead

- A standardized system of classifying headburn lesions at fish trapping facilities and hatcheries should be adopted, such as that developed in this report.

- Where staff are available, headburn lesions should be systematically and consistently enumerated throughout each fish run each year. Because of the apparent magnitude of this problem, funding should be made available for hatchery and trapping facility staff to implement these recommendations.

- An investigative program should be undertaken to evaluate the recommended therapeutic regimen for headburned fish, first using hatchery fish that are not needed for human consumption purposes, to demonstrate that prespawning losses in both hatchery and wild fish can be dramatically reduced and possibly eliminated.

- The dose and efficacy of erythromycin injections of fish at Rapid River Hatchery should be confirmed and evaluated to determine why sensitive strains of opportunistic pathogens are not being controlled by injections of the antibiotic.

- Raceway or pond conditions where mature fish are held to condition for spawning should be improved to help reduce the severity of eruptive fungal infections and associated debilitating loss of physiological condition.

- A screening genetic assay should be conducted on the bacterial and fungal isolates obtained in this study, known to be related to zoonotic strains (affecting humans) to verify whether or not these strains contain human and/or fish pathogen genes and to better characterize the complex of fungi shown in this study to infect headburn lesions.

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Appendix A

Clinical and Histological Description of Juvenile Fish Lesions

Appendix A

Clinical and Histological Description of Juvenile Fish Lesions

Table A.1. Summary of occurrence of clinical and histological findings for symptomatic fall Chinook salmon. Each line indicates one fish, and includes the summary findings from six histological sections examined for that fish. The presence of a plus symbol (+) indicates a positive finding for the indicated lesion or observations.

Count	SYMPTOMATIC Chinook salmon			Clinical Observations			Histology Summary	
	Date	Fish ID		Head Spot	Hemorrhage	Popeye	Moderate or severe brain hemorrhage	Hemorrhage in nares
1	08/14/02	B	21-1-1			+		
2	08/14/02	B	21-1-2	+	+			
3	08/14/02	B	21-1-3		+			+
4	08/14/02	B	21-2-1	+	+			
5	08/14/02	B	21-2-2		+			
6	08/14/02	B	21-2-3	+				
7	08/14/02	B	21-2-4	+	+			+
8	08/15/02	B	22-1-1	+	+			+
9	08/15/02	B	22-1-2	+	+			
10	08/15/02	B	22-2-1	+	+			
11	08/15/02	B	22-2-2	+	+			+
12	08/16/02	B	23-1-1		+			
13	08/16/02	B	23-1-2	+	+			
14	08/16/02	B	23-2-1	+				
15	08/16/02	B	23-2-2	+				
16	08/16/02	B	23-2-3		+			
17	08/21/02	B	24-1-1		+			
18	08/21/02	B	24-1-2		+			
19	08/21/02	B	24-1-3	+	+			
20	08/21/02	B	24-1-4	+	+			
21	08/21/02	B	24-2-1	+	+		+	
22	08/21/02	B	24-2-2	+	+		+	
23	08/22/02	B	25-1-1	+	+			
24	08/22/02	B	25-1-2	+	+			
25	08/22/02	B	25-1-3		+			
26	08/22/02	B	25-2-1		+			
27	08/22/02	B	25-2-2	+	+			
28	08/23/02	B	26-1-1		+			
29	08/23/02	B	26-1-2		+			
30	08/23/02	B	26-2-1		+			
			Totals:	**18**	**26**	**1**	**2**	**4**

Table A.2. Summary of occurrence of clinical and histological findings for asymptomatic fall Chinook salmon. Each line indicates one fish and includes, the summary findings from six histological sections examined for that fish. The presence of a plus symbol (+) indicates a positive finding for the indicated lesion or observations.

Count	ASYMPTOMATIC Chinook salmon			Clinical Observations			Histology Summary	
	Date	Fish ID (Cassette No.)		Head Spot	Hemorrhage	Popeye	Moderate or severe brain hemorrhage	Hemorrhage in nares
1	8-21-02	B02-3-	31					+
2	8-21-02	B02-3-	32					
3	8-21-02	B02-3-	33					
4	8-21-02	B02-3-	34					
5	8-21-02	B02-3-	35					
6	8-21-02	B02-3-	36					
7	8-21-02	B02-3-	37					
8	8-21-02	B02-3-	38					
9	8-21-02	B02-3-	39					
10	8-21-02	B02-3-	40					
11	8-21-02	B02-3-	41					+
12	8-21-02	B02-3-	42					
13	8-21-02	B02-3-	43					+
14	8-21-02	B02-3-	44					
15	8-21-02	B02-3-	45					
16	8-21-02	B02-3-	46					
17	8-21-02	B02-3-	47					
18	8-21-02	B02-3-	48					+
19	8-21-02	B02-3-	49					
20	8-21-02	B02-3-	50					
21	8-21-02	B02-3-	51					+
22	8-21-02	B02-3-	52					+
23	8-21-02	B02-3-	53					
24	8-21-02	B02-3-	54					
25	8-21-02	B02-3-	55					
26	8-21-02	B02-3-	56					
27	8-21-02	B02-3-	57					
28	8-21-02	B02-3-	58					
29	8-21-02	B02-3-	59					
30	8-21-02	B02-3-	60					
		Totals:		**0**	**0**	**0**	**0**	**6**

Table A.3. Summary of occurrence of clinical and histological findings for control Chinook salmon. Each line indicates one fish, and includes the summary findings from six histological sections examined for that fish. The presence of a plus symbol (+) indicates a positive finding for the indicated lesion or observations.

Count	CONTROL Chinook salmon			Clinical Observations			Histology Summary	
	Date	Fish ID (Cassette No.)		Head Spot	Hemor-rhage	Popeye	Moderate or severe brain hemorrhage	Hemorrhage in nares
1		B02-3-	1					
2		B02-3-	2					
3		B02-3-	3					
4		B02-3-	4					
5		B02-3-	5					+
6		B02-3-	6					
7		B02-3-	7					
8		B02-3-	8					
9		B02-3-	9					
10		B02-3-	10					
11		B02-3-	11					
12		B02-3-	12					
13		B02-3-	13					
14		B02-3-	14					
15		B02-3-	15					
16		B02-3-	16					+
17		B02-3-	17					
18		B02-3-	18					+
19		B02-3-	19					
20		B02-3-	20					
21		B02-3-	21					
22		B02-3-	22					
23		B02-3-	23				+	
24		B02-3-	24					
25		B02-3-	25					
26		B02-3-	26					
27		B02-3-	27					
28		B02-3-	28					
29		B02-3-	29					
30		B02-3-	30					
		Totals:		**0**	**0**		**1**	**3**

Table A.4. Summary of occurrence of clinical and histological findings for symptomatic rainbow trout. Each line indicates one fish, and includes the summary findings from six histological sections examined for that fish. The presence of a plus symbol (+) indicates a positive finding for the indicated lesion or observations.

Count	SYMPTOMATIC Rainbow Trout			Clinical Observations			Histology Summary	
	Date	Fish ID		Head Spot	Hemorrhage	Popeye	Moderate or severe brain hemorrhage	Hemorrhage in Nares
1	12/3/03	B-	17-1-1		+		+	
2	12/3/03	B-	17-1-2	+	+			+
3	12/3/03	B-	17-1-3		+			
4	12/3/03	B-	17-1-4	+	+		+	+
5	12/3/03	B-	17-1-5	+				
6	12/3/03	B-	17-2-1	+	+			+
7	12/3/03	B-	17-2-2	+	+		+	+
8	12/3/03	B-	17-2-3	+	+			+
9	12/3/03	B-	17-2-4		+			
10	12/4/03	B-	18-1-1	+	+			+
11	12/4/03	B-	18-1-2		+			+
12	12/4/03	B-	18-1-3	+	+			
13	12/4/03	B-	18-2-1	+	+		+	
14	12/4/03	B-	18-2-2	+	+		+	+
15	12/4/03	B-	18-2-3		+			
16	12/5/03	B-	19-1-1	+	+			+
17	12/5/03	B-	19-1-2	+				
18	12/5/03	B-	19-1-3	+	+		+	
19	12/5/03	B-	19-2-1	+	+			
20	12/5/03	B-	19-2-2		+			
21	12/5/03	B-	19-2-3	+	+			+
22	12/10/03	B-	20-1-1		+			
23	12/10/03	B-	20-1-2	+	+			
24	12/10/03	B-	20-1-3		+			+
25	12/10/03	B-	20-2-1	+	+			
26	12/10/03	B-	20-2-2	+	+			+
27	12/11/03	B-	21-1-1	+	+			
28	12/11/03	B-	21-1-2		+			
29	12/11/03	B-	21-2-1	+	+			+
30	12/11/03	B-	21-2-2	+	+			+
			Totals:	**21**	**28**	**0**	**6**	**14**

Table A.5. Summary of occurrence of clinical and histological findings for asymptomatic rainbow trout. Each line indicates one fish, and includes the summary findings from six histological sections examined for that fish. The presence of a plus symbol (+) indicates a positive finding for the indicated lesion or observations.

Count	AYMPTOMATIC Rainbow Trout			Clinical Observations			Histology Summary	
	Date	Fish ID		Head Spot	Hemorrhage	Popeye	Moderate or severe brain hemorrhage	Hemorrhage in nares
1	12/3/03	B-	17-1-6				+	
2	12/3/03	B-	17-1-7					
3	12/3/03	B-	17-1-8				+	+
4	12/3/03	B-	17-1-9					
5	12/3/03	B-	17-1-10				+	
6	12/3/03	B-	17-2-5					
7	12/3/03	B-	17-2-6					
8	12/3/03	B-	17-2-7					
9	12/3/03	B-	17-2-8					
10	12/4/03	B-	18-1-4					
11	12/4/03	B-	18-1-5					
12	12/4/03	B-	18-1-6					
13	12/4/03	B-	18-2-4				+	
14	12/4/03	B-	18-2-5					
15	12/4/03	B-	18-2-6					
16	12/10/03	B-	20-1-4					+
17	12/10/03	B-	20-1-5					+
18	12/10/03	B-	20-1-6					
19	12/10/03	B-	20-1-7					+
20	12/10/03	B-	20-1-8				+	+
21	12/10/03	B-	20-1-9					
22	12/10/03	B-	20-2-3					
23	12/10/03	B-	20-2-4					
24	12/10/03	B-	20-2-5					
25	12/10/03	B-	20-2-6					+
26	12/10/03	B-	20-2-7					
27	12/11/03	B-	21-1-3					
28	12/11/03	B-	21-1-4					
29	12/11/03	B-	21-2-3					+
30	12/11/03	B-	21-2-4				+	+
			Totals:	**0**	**0**	**0**	**6**	**8**

Table A.6. Summary of occurrence of clinical and histological findings for control rainbow trout. Each line indicates one fish, and includes the summary findings from six histological sections examined for that fish. The presence of a plus symbol (+) indicates a positive finding for the indicated lesion or observations.

	CONTROL Rainbow Trout			Clinical Observations			Histology Summary	
Count	Date		Fish ID	Head Spot	Hemorrhage	Popeye	Moderate or severe brain hemorrhage	Hemorrhage in nares
1	12/3/03	B-	17-1-11					
2	12/3/03	B-	17-1-12					+
3	12/3/03	B-	17-1-13					
4	12/3/03	B-	17-1-14					
5	12/3/03	B-	17-1-15					
6	12/3/03	B-	17-2-9					
7	12/3/03	B-	17-2-10					
8	12/3/03	B-	17-2-11					
9	12/3/03	B-	17-2-12					
10	12/4/03	B-	18-1-7					+
11	12/4/03	B-	18-1-8					
12	12/4/03	B-	18-1-9					
13	12/4/03	B-	18-2-7					
14	12/4/03	B-	18-2-8					
15	12/4/03	B-	18-2-9					
16	12/16/03	B-	16					+
17	12/16/03	B-	17				+	+
18	12/16/03	B-	18					
19	12/16/03	B-	19					
20	12/16/03	B-	20					+
21	12/16/03	B-	21					
22	12/16/03	B-	22					
23	12/16/03	B-	23					
24	12/16/03	B-	24					
25	12/16/03	B-	25					
26	12/16/03	B-	26					
27	12/16/03	B-	27					
28	12/16/03	B-	28					+
29	12/16/03	B-	29					
30	12/16/03	B-	30					
			TOTALS:	**0**	**0**	**0**	**1**	**6**

Appendix B

Detailed Histological Description of Adult Fish Headburn Lesions

Appendix B

Detailed Histological Description of Adult Fish Headburn Lesions

Abbreviation key
MDX morphologic diagnosis
NSL no significant lesion

B.1 Salmon skin slides case numbers B03-2; 1,2,3,4,5,6 (Umatilla Fish Trap, May 2003)

Fish # 1:

B03-2-1; slide 1, skin of head:

A large ulcer is present extending to the acellular stratum spongiosum (SS). The lower SS and hypodermis contain small to moderate numbers of inflammatory cells consisting predominantly of polymorphonuclear leukocytes (neutrophils) and lesser numbers of mononuclear cells. There is mild edema with separation of collagen fibers in the area. A minute focus lacks collagen fibers and contains moderate numbers of neutrophils suggesting the formation of an early abscess. The hypodermis contains moderate amounts of pale blue mucinous material separating cells and collagen fibers. A small number of small vessels in the hypodermis contain aggregates or plugs of inflammatory cells.

The epithelium is present at one margin of the section. Adjacent to the ulcer, the epithelium is one cell thick and slowly increases to the normal epithelial thickness. Adjacent to the ulcer there is a thin zone of subepithelial proteinaceous material entrapping inflammatory cells, many undergoing degeneration in the SS. There is also inter-epithelial edema with unicellular necrosis and unicellular neutrophil infiltration overlying this discrete zone. Distant from the ulcer the epithelium and dermis are normal.

MDX: Subacute ulcerative dermatitis with early abscess formation and hypodermal mucoid degeneration

B03-2-1; slide 2, skin of head:

A large ulcer is present extending to the dense collagen of the lower SS. The base of the ulcer and the adjacent hypodermis contain small numbers of scattered inflammatory cells consisting of mononuclear cells and neutrophils. Overlying the center of the ulcer, small numbers of inflammatory cells are entrapped in proteinaceous material. A few small vessels in the hypodermis contain dense aggregates of leukocytes. Scattered small basophilic areas of mucinous degeneration are present in the hypodermis. A few scattered melanocytes are dendritic with penetration into the hypodermis. One margin of the ulcer contains a small focus of epithelium with moderate thinning; this area is directly adjacent to the ulcer. There is very mild trans-epithelial neutrophilic infiltration in this area with slight edema. There is a small linear zone in the SS of neutrophilic infiltration and edema underlying the basement membrane (bm) of the area. The remaining epithelium is normal. There is erosion of the contra-lateral margin with only the

bm remaining. There is edema with moderate inflammatory cell infiltration of a thin zone underlying the bm in the SS. Many small vessels in the region contain plugs of inflammatory cells.

MDX: Subacute ulcerative dermatitis with hypodermal mucoid degeneration

B03-2-1; slide 3, skin of head:

A large area of ulceration is present extending to the stratum compactum (SC)-hypodermal junction. There is mild to moderate scattered inflammatory cell infiltration in the surface and adjacent underlying stroma. Occasionally, a thin linear zone of superficial collagen lysis is present within the ulcerated area. The inflammatory cell population consists of neutrophils and mononuclear cells. A few small vessels contain inflammatory cell aggregates; thrombosis is present in a single vessel. The upper adjacent hypodermis contains a few scattered inflammatory cells and a few small areas of pale blue mucinous degeneration. At one margin of the ulcer, there is overlying proteinaceous material admixed with inflammatory cells and areas of collagen lysis. The other margin contains an area of epithelial erosion extending to a thin layer of epithelium, which then extends to a normal area of epithelium with normal underlying areas of dermis and hypodermis.

MDX: Subacute ulcerative dermatitis with thrombosis and hypodermal mucoid degeneration

B03-2-1; slide 4, skin of head:

Three distinct erosions are present, two with epithelial cell loss extending to the bm. The third erosion contains the basal cell layer and a few overlying epithelial cells except in one minute focus where the denuded bm is exposed. In the largest erosion the upper SS is acellular except for a few scattered individual non-septate fungal hyphae (presumptive, *Saprolegnia* sp.) There is mild to moderate inflammatory cell infiltration and perivascular cuffing of the underlying mid-SC zone to the upper hypodermis. In the small erosion, there is only mild inflammation in the upper SS. The epithelial cell margins of the erosions contain moderate numbers of inflammatory cells underlying the bm. There is edema and unicellular necrosis only in the epithelium adjacent to the margin of the erosions.

GMS stain: positive for fungi consistent with *Saprolegnia* sp.

MDX: Multifocal subacute erosive dermatitis with intra-lesional fungal hyphae

B03-2-1; slide 5, skin of head:

A large area of ulceration is present extending to the upper SC. Very mild, patchy inflammatory cell infiltration is present in the remaining underlying SC. A few scattered small vessels contain aggregates of inflammatory cells. An early thrombus is present in one small to medium vessel. At one margin adjacent to the ulcer, a few scattered basal cells are present with mild edema in the SS underlying the bm.

MDX: Subacute ulcerative dermatitis with early thrombosis

B03-2-1; slide 6, skin of head, two irregular sections:

The sections consist of bone and surrounding hypodermis, and a small island of hyperplastic epithelium with an irregular bm and thickened SC.

Scattered severe inflammatory cell infiltration is present in the hypodermis with irregular areas of fibrosis obliterating lipid vacuoles. A single thrombus is present with early re-canalization in one hypodermal island. An irregular area of bone lysis is present adjacent to an area of collagen hyalin degeneration, hemorrhage, and inflammatory cell infiltration. Adjacent to one island of hypodermis are two isolated very pale areas that contain large numbers of fungal hyphae (*Saprolegnia* sp.).

MDX: Chronic dermatitis and osteitis with intra-lesional fungal hyphae

Third small irregular island of skin with SC: no significant lesions (NSL)

B03-2-1; slide 8, liver: NSL:

Fish # 2:

B03-2-2; slide 1, skin of head:

A large ulcer is present extending to one margin of the section. The ulcer extends to the mid-SS. There is mild inflammatory cell infiltration in the remaining underlying SS and hypodermis. The cells consist of neutrophils and mononuclear cells. A few small thrombi are within the area, as well as inflammatory cell aggregates in scattered vessels. Mild scattered edema, mucinous degeneration, and collagen lysis are present in the hypodermis. The underlying bone and periosteum are normal. The surface of the ulcer contains fragmented collagen fibrils; no bacteria are discernible. The contra-lateral margin consists of a small erosive zone with the remaining bm that extends to a progressively thickened epithelium. There is individual cell separation and desquamation of the superficial epithelial layer. The hypodermis in this area contains slightly basophilic, degenerating swollen collagen fibers. No inflammation is associated with the basophilic fibers.

MDX: Subacute ulcerative dermatitis with thrombus and hypodermal collagen degeneration

B03-2-2; slide 2, skin of head:

As slide 1 above. The ulcer extends to one margin of the section, epithelium extends to the contra-lateral margin. Adjacent to the ulcer, the epithelium is undergoing degeneration to the epithelial mid-zone. The cells in this degenerating area are pale pink, markedly elongated, and perpendicular to the bm. The underlying basal layer is basophilic but is also markedly elongated.

As in slide #1, the SS underlying the epithelium adjacent to the ulcer contains swollen basophilic degenerating collagen fibers. The area is acellular. Distant from this area the SC contains normal-sized pink collagen fibers. The ventral bone is normal. No bacteria are associated with the ulcer that extends to the SC. Moderate numbers of fungal hyphae (*Saprolegnia* sp.) are associated with the adjacent erosive area, overlying the bm. A rare hyphae may be present just underlying the bm in the area. No organisms are associated with the epithelial surface.

MDX: Subacute ulcerative dermatitis with intra-lesional fungal hyphae and hypodermal mucoid degeneration

B03-2-2; slide 3, skin of head:

As slide # 2 above. An area of the ulcer contains a thin overlying zone of fungal hyphae (*Saprolegnia* sp.). A rare hyphae is invading the remaining SS. A few hyphae are in the upper SC in the erosive area that contains a bm.

MDX: Subacute ulcerative dermatitis with intra-lesional fungal hyphae and hypodermal mucoid degeneration

B03-2-2; slide 4, posterior kidney:

There is moderate hyperplasia of hematopoietic cells. There is a small discrete focus of hemorrhage into an area of hematopoietic tissue.

B03-2-2; slide 5, gills:

Multiple cross sections of a copepod are present between two filaments. There is slight epithelial hyperplasia of adjacent filaments.

MDX: Multifocal hyperplasia of filaments, intra-lesional copepods

Fish # 3

B03-2-3; slide 1, spleen: There is moderate lymphoid hyperplasia.

B03-2-3; slide 2, posterior kidney: A very small focus of loosely arranged macrophages is present.

MDX: Focal chronic interstitial nephritis

B03-2-3; slide 3, skin of head:

The normal dermal cytoarchitecture is markedly altered. In one discrete depressed area, the SS is absent and has been replaced with proteinaceous material, hemorrhage and pink homogeneous degenerating collagen. A few neutrophils are associated with the degenerating collagen. The hypodermis contains multiple coalescing foci of regenerating muscle fibers isolating small nests of lipid vacuoles. The muscle layer contains multiple areas of basophilic regenerating myofibers; a few multi-nucleated myocytes are present. A few remaining degenerating myofibers are present within the regenerating area. They are characterized by loss of striations, dissolution, and swelling. A dense, deeply basophilic focus of myo-regeneration is present adjacent to normal muscle bundles. The overlying epithelium is slightly thinned with slight intercellular edema partially isolating individual cells.

At one margin adjacent to the depressed area the SC is thickened and consists of disorganized interlacing collagen fibers.

A minute area of epithelial loss is present with normal SS (artifactual loss).

Trichrome and muscle PTAH: demonstrate fibrous tissue and muscle fibers.

MDX: Chronic degenerative dermatitis and myositis with epithelial and myo-regeneration

B03-2-3; slide 4, skin of head:

A large erosion is present with epithelial loss and slight edema underlying the bm. In the center, the bm is absent [artifactual loss?]. One margin contains a thin layer of amorphous material entrapping fungal hyphae (*Saprolegnia* sp.). A rare hyphae is invading the adjacent acellular upper SS. The upper SC contains a moderate number of swollen slightly basophilic degenerating collagen fibers. A small number of inflammatory cells are focally scattered at the SC-hypodermal junction. The hypodermal contains a few small pale blue mucinous areas. One margin of the erosion contains a thin epithelial layer that in a short distance expands to normal thickness. The contra-lateral epithelium contains a very small superficial to mid-zonal area with pink fusiform degenerating epithelial cells.

MDX: Subacute erosive dermatitis with intra-lesional fungal hyphae and hypodermal mucoid degeneration

B03-2-3; slide 5, skin of head:

A small discrete ulcer is present with epithelial cell and loss of the bm. The surface contains a thin zone of fragmented collagen fibers. A few vessels in the upper SC contain plugs of cells and plump endothelial cells. Very few scattered inflammatory cells are present in the underlying SC. The upper underlying hypodermis contains a few small pale blue degenerating foci.

One margin of the ulcer consists of a slightly thickened epithelium with an irregular undulating bm. The underlying SS is absent, and replaced with a distinct zone of interlacing, loosely arranged fibrocytes with neo-vascularization (healed ulcer). Underlying this zone is a thick zone of large individual loosely arranged collagen fibers with mild to moderate inflammatory cell infiltration. The underlying hypodermis is normal.

MDX: 1. Subacute ulcerative dermatitis with hypodermal mucoid degeneration

2. Chronic epithelium regeneration with dermal fibrosis

B03-2-3; slide 7, posterior kidney: NSL

Fish # 4

B03-2-4; slide 1, skin of head:

Two areas of ulceration are present. Adjacent to the larger ulcer, one margin consists of an erosive area and an adjacent epithelium with an undulating irregular bm. The underlying acellular SS is widened and contains some irregular, interlacing bundles of collagen (chronic healed skin lesion). The ulcer extends to the upper SC. Multiple small, ocassionally coalescing, hyalin-pink, coagulative necrotic foci are in the SC underlying the ulcer, many extend to the surface. The areas contain pink-hyalin fibrillar to a homogenous material entrapping cells with pyknotic nuclei and nuclear debris. Two necrotic areas each contain a single necrotic vessel surrounded with karyorrhectic debris (vascular necrosis). A few other necrotic foci contain small vessels with plugs of leukocytes and segmental necrosis of vascular walls. Small numbers of neutrophils and mononuclear cells are scattered in the SC underlying the ulcer. The

underlying bone and SC are normal. The smaller ulcer is separated from the larger ulcer by a thin linear segment of epithelium. There is separation and desquamation of a thin zone in the superficial epithelium. There is a thin patchy zone of hyalin degeneration of the exposed SC. At one margin this hyalinized degenerating collagen extends underneath the adjacent epithelium in the SS and is admixed with small amounts of proteinaceous material. There is epithelial edema with separation of cells and individual epithelial cell necrosis and degeneration. Mild trans-epithelial neutrophilic infiltration is present. Areas of the ulcer and underlying SC contain scattered inflammatory cells consisting of neutrophils and mononuclear cells.

GMS stain: negative for fungi; PAS stain is negative; Gram stain is negative.

MDX: 1. Acute to subacute ulcerative dermatitis with multifocal necrosis, vascular necrosis, and hypodermal mucoid degeneration

2. Focal epithelial and dermal regeneration

B03-2-4; slide 2, skin of head:

Two very small ulcers are present. One contains segmental areas of remaining bm. At one epithelial margin, the SS is very thin with a few infiltrating inflammatory cells. At this margin, there is mild vacuolar degeneration of the epithelium with individual cell necrosis. This epithelial layer extends to the other ulcer. Between the ulcers the bm is thickened and irregular and contains a disorganized basal cell layer lacking their normal perpendicular arrangement to the bm. A small linear zone of interlacing vacuolated fusiform cells with collagen degeneration and fragmentation are present underlying the bm in the SS. Unicellular necrosis is ocassionally present. The normal SS is absent underlying this epithelium, and there is only the hypodermis with a few scattered pale blue collagen fibers.

The other adjacent ulcer extends to the hypodermis. Very mild underlying inflammatory cell infiltration is present consisting of neutrophils and mononuclear cells. Occasional vessels contain aggregates of leukocytes. The contra-lateral epithelium to the ulcer is normal and contains a normal dermis and hypodermis.

MDX: Chronic ulcerative dermatitis with focal epidermitis and focal epithelial degeneration

B03-2-4; slide 3, skin of head:

A large depressed ulcer is present extending to the lower SC. A small thin 5-cell-thick segment of epithelium is present in the central area. The cells are predominantly squamous; the basal layer is disorganized and cuboidal. A normal bm is not discernible. Small areas of the bm are irregular, fragmented, hyalinized, or granular. Scattered inflammatory cells are underlying and infiltrating the degenerating bm. There is a paucity of inflammatory cells in the adjacent ulcerated SC. The remaining SC contains a few scattered pale blue swollen collagen fibers, and a few small vessels contain leukocytic aggregates. At the margin of the slide, a small segment of the distal epithelium is thickened and contains an irregular slightly thickened bm. An excessive number of melanocytes underlay the bm (epithelial regeneration). No inflammatory cells are present.

MDX: Chronic active ulcerative dermatitis with focal epithelial regeneration

B03-2-4; slide 4, skin of head:

A large ulcer is present extending to the hypodermis. Scattered mild inflammatory cell infiltration is present in the hypodermis. A few small vessels contain cellular leukocytic plugs and lumenal margination of leukocytes. The surface of the ulcer consists of fragmented collagen. At one margin of the ulcer the adjacent epithelium is composed of one to three squamous cells. The underlying bm is not discernible, and the area consists of a thin linear zone of degenerating fragmented and globular collagen with a few inflammatory cells of neutrophils and mononuclear cells in the ss. Distant from this area the epithelium increases in thickness to 5 to 10 cells and contains a slightly thickened bm with an underlying excessive number of melanocytes and a few inflammatory cells and fusiform cells. The SC is normal. The deep hypodermis contains an epithelialized cleft, partially surrounding an area of mucinous connective tissue. The deep bone is normal.

MDX: Subacute ulcerative dermatitis with hypodermal mucoid degeneration and adjacent epithelial regeneration

B03-2-4; slide 5, anterior kidney: There are a few, very small macrophage foci in the interstitium.

Fish # 5

B03-2-5; slide 1, skin of head:

A large ulcer extends to one margin of the slide and the ulcer extends from the upper to the lower SS. Very few scattered inflammatory cells underlie the ulcer. The remaining SS and hypodermis contain small to moderate numbers of scattered small vessels with leukocytic plugs or lumenal leukocytic margination, many vessels also contain inflammatory cellular cuffs. The upper SC contains a few small pink necrotic foci, some with surrounding or segmental collagen necrosis and endothelial cell necrosis (vascular necrosis). The upper SC contains many swollen pale blue degenerating collagen fibers. A segment of the adjacent remaining epithelium is thin and edematous with individual cellular separation. A very small amount of scattered karyorrhectic nuclear debris is present in the edematous epithelial segment. There is a slight excess of mononuclear cells in a thin linear zone underlying the bm in the ss. The hypodermis is degenerating and consists of pale blue mucinous cells. The underlying cartilage is normal. The Gram stain is negative for bacteria.

MDX: 1. Subacute ulcerative dermatitis with dermal necrosis, vascular necrosis, and dermal and hypodermal mucoid degeneration.

2. Acute epidermitis

B03-2-5; slide 2, skin of head:

The section consists of a large ulcer extending to the upper SC and upper hypodermis, no epithelium is present. There is mild scattered inflammatory cell infiltration of the underlying tissues with scattered leukocytic vascular plugging, margination, and perivascular cuffing. The remaining SC contains swollen and slightly basophilic collagen fibers. No degeneration is present in the hypodermis.

MDX: Subacute ulcerative dermatitis with dermal mucoid degeneration

B03-2-5; slide 3, anterior kidney: NSL

Fish # 6

B03-2-6; slide 1, skin of head:

Two ulcers are present; the larger of the two extends to one of the margins of the slide. The ulcer extends to the hypodermis. A discrete, thin underlying zone of basophilic degenerating collagen bundles with moderate numbers of inflammatory cells underlies the ulcer. A few vessels in the area contain thrombi, one contains a re-canalized thrombus, and several contain plugs of leukocytes, occasionally with perivascular cuffing. The underlying muscle contains three small irregular necrotic foci consisting of swollen to shrunken basophilic myofibers with loss of striations. One margin of the ulcer contains a small segment of adjacent epithelium that is only eight cells thick. This epithelium abuts the smaller ulcer. Underlying the epithelial bm is a thin zone of proteinaceous material entrapping large to moderate numbers of scattered neutrophils and mononuclear cells in the ss. Underlying this zone is a single longitudinal section of a thrombus with segmental inflammatory cell infiltration of the wall. A hypodermis underlies this epithelium; no SS is present. The SC contains a few pale basophilic swollen collagen fibers. There is edema of the epithelium with epithelial cell individualization and trans-epithelial neutrophilic cell infiltration. The basal cell layer is intact. This epithelium extends to the small ulcer that contains the SC with a few basophilic degenerating collagen fibers. There is a paucity of inflammatory cells underlying this ulcer. The contra-lateral epithelial margin is approximately 10 cells thick and rapidly progresses to a normal epithelium. A rare karyorrhectic cell is in the epithelium. A large segment underlying the epithelium adjacent to the ulcer contains amorphous pink hyaline material with moderate numbers of neutrophils, mononuclear cells, and dendritic melanocytes. A small to moderate number of basophilic collagen fibers is in the underlying SC and hypodermis extending to the margin of the section. Gram stain: negative for bacteria.

MDX: Chronic active ulcerative dermatitis and epidermitis with thrombosis, multifocal myodegeneration, and mucoid degeneration of the dermis and hypodermis

B03-2-6; slide 2, skin of head:

A large ulcer is present extending to the hypodermis and to one margin of the slide. Mild scattered inflammatory cell infiltration and vascular leukocytic plugging is present in the area. The adjacent hypodermis contains scattered pale blue degenerating collagen fibers. Adjacent to the remaining epithelium the ulcer contains a small area of hyalinized degenerating collagen with inflammatory cells. Adjacent to the ulcer a short segment of thin epithelium is composed of squamous cells. The remaining epithelium is thickened and hyperplastic with down-growth of epithelial rete plugs into the SS. The bm is irregular and undulating with a thin underlying zone of fibrosis, inflammatory cell infiltration, and excessive numbers of dendritic melanocytes in the ss.

MDX: Chronic ulcerative dermatitis with hypodermal mucoid degeneration and adjacent epithelial regeneration

B03-2-6; slide 3, skin of head:

A large ulcer is present extending to the mid-zone of the SS. Large to moderate numbers of inflammatory cells are present in the SS and underlying hypodermis consisting of neutrophils and mononuclear cells. Large numbers of swollen basophilic collagen fibers are present in the SC and hypodermis. Occasional small areas of fragmented basophilic fibers are associated with inflammatory cells. At the junction of the ulcer and one epithelial margin is a superficial mass of fungal hyphae (*Saprolegnia* sp.) with absence of adjacent inflammatory cells. Adjacent to this margin, the sub-epithelium contains a linear zone of moderate to severe inflammatory cell infiltration, the lesion progressively lessens to the margin of the section. The SC contains basophilic degenerating fibers. The contra-lateral epithelial margin contains a small area adjacent to the ulcer of proteinaceous material entrapping moderate numbers of inflammatory cells. This epithelium is slightly edematous with slight epithelial separation and mild trans-epithelial neutrophilic infiltration. A small number of shrunken cells with pyknotic nuclei are at the epithelial margin.

MDX: 1. Chronic ulcerative dermatitis with dermal mucoid degeneration and intra-lesional fungal hyphae

2. Focal epithelial hyperplasia

BO3-2-6; slide 4, skin of head:

No ulcer is present. A large area of epithelium in the center of the section consists of degenerating and necrotic cells. The region is depressed from the adjacent epithelial margins, lacks a SS and contains only a hypodermis (old resolved scar). The area is characterized by separation, individualization, loss, and necrosis of epithelial cells. Some individualized cells contain a vacuolated cytoplasm. A few shrunken cells with pyknotic nuclei are throughout the area. Moderate numbers of migrating trans-epithelial inflammatory cells are present; consisting of neutrophils and mononuclear cells. The basal cell layer is cuboidal and slightly disorganized. Underlying the bm there is a thin linear zone of pink fragmented collagen admixed with moderate numbers of neutrophils and mononuclear cells in the ss. The adjacent upper hypodermis contains many swollen basophilic degenerating collagen fibers. The lower hypodermis contains scattered pale blue collagen fibers. The epithelial margins contain a SS and hypodermis. One margin of the section contains basophilic collagen fibers in the SS and hypodermis.

MDX: Chronic resolved ulcerative dermatitis with epithelization and an acute degenerative necrotizing epidermitis and dermal mucoid degeneration

BO3-2-6; slide 5, anterior kidney: NSL

Fish # 7

B03-2-7; slide 1, skin of head:

A large central ulcer is present that extends to the hypodermis. Large numbers of degenerating, variable-sized basophilic hypodermal collagen fibers underlie the ulcer. Inflammatory cells consisting of mononuclear cells and neutrophils surround many shrunken to fragmented collagen fibers. Many fibers are undergoing peripheral phagocytosis. A small island of cartilage deep in the hypodermis contains a

small degenerating focus with a few inflammatory cells. A few small normal hypodermal foci are present containing pink collagen fibers. One margin of the ulcer contains adjacent erosion with only the bm remaining except for a small segment of eroded epithelium one to five cells thick extending to the end of the slide. The contra-lateral ulcer margin contains a small segment of epithelium five cells thick, which extends to a denuded bm that extends to the end of the section. Underlying the eroded areas there is scattered hyalinization and thickening of the bm with collagen degeneration, fragmentation, edema, and mild inflammatory cell infiltration of the SS. The SC contains basophilic degenerating collagen fibers with mild scattered inflammatory cell infiltration.

MDX: Subacute ulcerative dermatitis with a multifocal degenerative hypodermatitis and focal chondritis

B03-2-7; slide 2, skin of head:

A large ulcer is present extending to the mid-SC. A large area of the underlying and adjacent SC and hypodermis contain basophilic swollen to shrunken to fragmented collagen fibers. Scattered phagocytosis of degenerating fibers is present. A few inflammatory cells are present directly underlying the ulcer in the SC and adjacent hypodermis. The hypodermis contains scattered large areas of myxomatous degeneration consisting of pale blue amorphous material entrapping fibrillar material and swollen to shrunken to fragmenting basophilic collagen fibers. The adjacent margins of the ulcer contain an eroded epithelium that progresses from a thickness of 1 cell to normal thickness. The SS and hypodermis are normal at one margin of the section. The underlying muscle contains a small number of individual myofibers undergoing degeneration and phagocytosis by a small number of neutrophils. No bacteria or fungal elements are discernible in the section.

MDX: Subacute ulcerative dermatitis with degeneration of the hypodermis, SC, and deep muscle

B03-2-7; slide 3, skin of head:

A large ulcer is present extending to the lower SS and to one margin of the section. The remaining SS and upper hypodermis contain basophilic degenerating collagen fibers with very mild scattered inflammatory cell infiltration. A few scattered pink collagen fibers in the dermis are fragmented with a few adjacent neutrophils and are undergoing phagocytosis. The hypodermis contains a few scattered small pale blue mxyomatous degenerating foci as described above for B03-2-7, slide 2. One margin of the ulcer is adjacent to denuded bm that progresses to an epithelium that is one cell thick, and then progresses to an epithelium of normal thickness containing only a few mucous cells. There is mild epithelial cell separation adjacent to the ulcer with a few migrating transepithelial neutrophils. Underlying the bm a minute focus of fragmented collagen contains moderate numbers of neutrophils. The SC is normal underlying the intact epithelium, although there are multiple myxomatous degenerative foci in the underlying hypodermis. The SC and hypodermis adjacent to the margin of the section are basophilic and degenerating as described above for B03-2-7, slide 2.

MDX: Subacute ulcerative dermatitis with dermal degeneration

B03-2-7; slide 4, skin of head:

An ulcer is present extending to the mid-SC with basophilic degeneration of the remaining collagen in the SC. Pink collagen is undergoing degeneration in the lower SC with mild inflammatory cell infiltration and phagocytosis as slide 2 above. One margin of the section contains an epithelium that is one to eight cells thick with slight cellular separation. The contra-lateral epithelium is three cells thick and rapidly progresses to normal thickness.

MDX: Subacute ulcerative dermatitis with mild SC degeneration

B03-2-7; slide 5, posterior kidney: NSL

Fish # 8

B03-2-8; slide 2, skin of head:

A large erosive area is present interspersed with a few minute to small ulcerated foci extending to the upper SC. In the erosive area, several areas of bm degeneration and fragmentation are present. Overlying the eroded area is a pale blue mucinous linear zone entrapping large numbers of fungal hyphae. The SC underlying the ulcer is undergoing basophilic degeneration with swelling of collagen fibers. Small amounts of nuclear debris are within the degenerating SC. The hypodermis contains large areas of myxomatous degeneration entrapping basophilic collagen fibers. Pink and basophilic fibers are undergoing degeneration characterized by fragmentation, dissolution, and phagocytosis. Mild inflammatory cell infiltration is present throughout the hypodermis and is prominent at the SS-hypodermal junction. One margin of the erosion abruptly progresses to an epithelium that is 10 to 12 cells thick with slight cellular separation adjacent to the erosion. The adjacent SS is thickened with pink fibrillar material and a few inflammatory cells. The contra-lateral margin of the erosion abruptly and rapidly progresses to an epithelium of normal thickness. Both margins of the section contain a basophilic degenerating SS and hypodermis with an overlying normal epithelium.

MDX: Subacute erosive dermatitis with intra-lesional fungal hyphae and dermal degeneration

B.2 Salmon skin slides case numbers BO3-3; 1,2,3,4,5; collected June 18, 2003, Rapid River Hatchery, Riggins, Idaho.

Fish # 1

B03-3-1; slide 1, skin of head: (skin lesion and normal skin)

An area of a distinct SC is absent. The hypodermis extends to a slightly thickened SS. There is very slight proliferation of melanophages underlying the bm. At the point of SC loss, the bm is distinctively elevated, protruding into the epithelium. Only a thin five-cell layer of stratified epithelial cells overlies this protrusion. The epithelium on both sides of the protrusion is normal. The hypodermis contains scattered pale blue degenerative areas.

Morphologic Diagnosis (MDX): Healed dermal scar with mild melanosis and active mild hypodermal degeneration

B03-3-1; slide 2, skin of head: (skin lesion and normal skin)

At one margin of the slide, a distinct normal SS is present with an underlying hypodermis. The remaining section lacks a SS with the hypodermis extending to the SC. The hypodermis contains a few irregular fronds of collagen obliterating adipocytes. A few small foci of the SC are thickened with fibrocytes and contain small numbers of inflammatory cells with neovascularization. There is a single small focus of mild epithelial hyperplasia in this area. A very small area of the epithelium contains five to six cells of stratified squamous cells. The underlying muscle is normal.

MDX: Chronic healed dermal scar with focal epithelial hyperplasia

B03-3-1; slide 3, skin of head: (skin lesion and normal skin)

The section consists of a segment of normal epithelium with a normal underlying SS. At mid-section, there is epithelial thinning, loss of mucous cells, and an increase in epithelial squamous cells. The dermis is slightly elevated in this area. Extending to the other margin of the section there is loss of the normal SS and slight fibrosis of the SC with mild inflammatory cell infiltration with occasional peri-vascular cuffing. The hypodermis extends to the SC with slight fibrosis at the junction of the SS and hypodermis. There is mild melanosis of the SS and the overlying epithelium contains an excessive number of mucous cells.

MDX: Chronic dermal scarring with mild melanosis

B03-3-1; slide 4, kidney:

The section consists of a large blood clot with a minute amount of renal tissue: NSL. Gram stain: NSL.

B03-3-1; slide 5, gill

A very small number of lamellae contain distal tips that are hypercellular. One gill arch is hypercellular, containing neutrophils and mononuclear cells. Multiple cross sections of copepods surround the gill arch.

MDX: Chronic focal parasitic branchitis

Fish # 2

B03-3-2; slide 1, skin of head: (skin lesion)

As described above for B03-3-1, slide 3, at mid-section there is a small focus of epithelial thinning associated with slight dermal elevation. From this focus to one margin of the section, there is a normal epithelium with a normal underlying SS and dense SC and hypodermis. From this focus to the contra-lateral margin of the section there is loss of the SC and replacement by the hypodermis. The SS is slightly thickened with linear fibrosis. There is mild inflammatory cell infiltration by neutrophils and mononuclear cells with mild melanosis of the SS and adjacent upper hypodermis. Large dendritic melanocytes are preset in the SS and upper hypodermis. The underlying muscle is normal.

MDX: Chronic dermal scarring with mild melanosis

B03-3-1; slide 2, skin of head 2 sections (A andB): (skin lesion)

Section A: (shorter of the two). The SC is absent and is replaced by the hypodermis. A few small scattered foci of the SS are slightly thickened with fibrocytes and are infiltrated with a few mixed inflammatory cells with occasional peri-vascular cuffing. A few very small irregular pale basophilic degenerative foci are in the hypodermis. A few scattered dendritic melanocytes are in the SS and upper hypodermis. There is scattered mucous cell loss in the epithelium with occasional very mild 5-8 cell thick squamous hyperplasia.

MDX: Chronic scarring with multifocal mild squamous hyperplasia

Section B: (longer of the two sections). As fish 2, slide 1 above. There is a discrete focus of dermal elevation with mild to moderate fibrosis and thickening of the SS and overlying epithelium. From this focus to one margin of the section, the epithelium, SS, SC, and hypodermis are normal. From the focus to the contra-lateral margin of the section a normal SC is absent and the hypodermis extends to the SS. There are collagen bundles separated by adipocytes extending to the SS. The bm is slightly undulating, and there are a few small foci of mild fibrosis and inflammatory cell infiltration in the SS. A few dendritic melanocytes are also present in these areas. There is very superficial erosion of one to three cells of the epithelium [artifactual presumptive?]. The muscle layer is normal.

MDX: Chronic scarring with multifocal mild squamous hyperplasia

B03-3-2; slide 3, skin of head: (normal skin only): NSL

B03-3-2; slide 4, skin of head, 2 sections (A and B): (blackish scarred skin)

Section A: The SC is absent and replaced by the hypodermis containing collagen bundles interspersed with adipocytes. A few small foci in the SS are slightly thickened with fibrocytes, and excessive number of melanocytes. Dendritic melanocytes are in the SS and upper hypodermis. The upper hypodermis contains a few scattered inflammatory cells, some associated with individual to nests of proliferating interlacing pale blue fibrocytes. There is slight loss of mucous cells in the epithelium and erosion of the epithelial surface to a one-to-three cell thickness [artifactual or traumatic?].

MDX: Chronic dermal scarring

Section B: As section A above. The SC is replaced by hypodermis containing collagen bundles, scattered individual to nests of lipid cells and scattered interlacing proliferating pale blue fibrocytes. There is scattered mild fibrosis of the SS. An excessive number of dendritic melanocytes are present in the SS and upper hypodermis. The epithelial bm slightly undulates in a few areas. There is mucous cell loss in the densely cellular epithelium.

MDX: Chronic dermal scarring

B03-3-2; slide 5, skin of head: (whitish scarred skin)

The entire section is ulcerated except for a minute focus of remaining epithelium at one margin of the section. The epithelium is one to five cells thick with mild fibrosis in the underlying SS. In the remaining section, the ulcer extends to the upper to deep SC and to the upper hypodermis. There is a

paucity of inflammatory cells with only a rare peri-vascular mononuclear cell cuff present. A few, small, scattered pale blue degenerative foci are present in the hypodermis.

MDX: Acute erosive to ulcerative dermatopathy with mild hypodermal degeneration

B03-3-2; slide 6, skin of head, 2 sections (A and B): (blackish scarred lesion)

Section A: The epithelium consists of a dense population of epithelial cells with occasional mucous cell loss. A few scattered areas of the SS contain an excessive number of fibrocytes. Areas of the dermis lack a normal SC, and only a rare focus of deep SC is present underlying the bm. The remaining dermis is composed of the hypodermis that underlies the bm. An excessive number of dendritic melanocytes are in the SS and upper hypodermis. A few small scattered inflammatory cell foci consisting of neutrophils and mononuclear cells are in the upper hypodermis.

MDX: Chronic dermal scarring with melanosis

Section B: See "A" above. There is mild epithelial erosion at one margin of the section [artifactual loss?].

MDX: Chronic dermal scarring with melanosis

B03-3-2; slide 7, gill

Two gill arches are hypercellular and contain an excessive number of neutrophils and mononuclear cells. In one arch, a small amount of spiraled foreign debris is deep in the dermis with surrounding inflammatory cells. Multiple cross sections of a copepod are adjacent to the gill arch.

MDX: Chronic parasitic branchitis with endogenous foreign body material

B03-3-2; slide , kidney: Only a rare tubule and glomerulus are present in a blood clot—NSL.
Gram stain: NSL

Fish # 3

B03-3-3; slide 1, skin of head, 2 sections (A and B): (skin lesion and normal skin)

Section A: A large ulcer extends from the lower SC to upper hypodermis. The surface of the ulcer contains scattered areas of collagen degeneration consisting of pink-hyalin homogeneous amorphous confluent fibers. Moderate numbers of inflammatory cells consisting predominantly of mononuclear cells and lesser numbers of neutrophils are present underlying the ulcer. At one margin of the section, the epithelium adjacent to the ulcer is eroded for a short segment. There is edema with cell separation of this epithelium and of the adjacent full thickness epithelium. A very small number of neutrophils are migrating through the edematous epithelium. The SS is markedly thickened with collagen degeneration underlying the eroded and edematous epithelium. The SC is absent in this area with the hypodermis extending to the degenerating SC. The remaining epithelium, dermis and underlying muscle layer are normal.

MDX: Subacute ulcerative dermatitis and epithelial erosion and degeneration of the SS

Section B: At one margin of the section there is a deep ulcer extending to the hypodermis with a few scattered mononuclear cells. Adjacent to the ulcer, the SS is markedly thickened and contains degenerating pink swollen homogenous amorphous confluent collagen fibers with moderate numbers of neutrophils. In a few areas small segments of detached edematous epithelial cell separation are present. The SC is absent in this area with the hypodermis extending to the degenerating SS. At the margin of the degenerating SS there is an abrupt focus where the SC is then present with normal overlying epithelium and dermis.

MDX: Subacute ulcerative dermatitis with degeneration of the SS

B03-3-3; slide 2, skin of head: (skin lesion and normal skin)

There is a discrete depressed area of the epithelial surface at one margin of the section. At the beginning of the depression, the SC is absent with the hypodermis extending to the SS. Underlying the depressed area, there is degeneration and thickening of the SS. The SS consist of pink amorphous collagen fibers with moderate numbers of neutrophils, many degenerating. There is mild edema of the overlying epithelium with mild cell separation and transepithelial neutrophilic infiltration. The contra-lateral margin of the section contains a prominent epithelium with basophilic cells and a slightly thickened bm. There is an excessive number of dendritic melanophages in the SS. Melanophages are not present in the SS of the depressed area.

MDX: Chronic healed ulcerative dermatitis with re-epithelization and degeneration of the SS

B03-3-3; slide 3, skin of head: (skin lesion and normal skin)

A large ulcer is present extending to the hypodermis. Overlying the area is a mass of fungal elements are present. At one margin of the ulcer, there is mucinous degeneration of the deep dermis with overlying fungi almost extending to the bone. At the contra-lateral margin of the ulcer, there is an area of degeneration of the SC with a mass of overlying fungal elements. A rim of inflammatory cells is adjacent to the degenerating SC. There is a small area of epithelial erosion of the adjacent epithelium ranging from 1 to 10 cells thick. A small area only contains the bm.

MDX: Chronic active necrotizing ulcerative dermatitis with intra-lesional fungi

B03-3-3; slide 4, skin of head: (normal skin only): NSL

B03-3-3; slide 5, skin of head, 2 sections (A and B): (deep skin lesion)

Section A: A large ulcer is present extending to the hypodermis. There is mild, scattered mononuclear cell infiltration underlying the ulcer. The adjacent epithelial cells are deeply basophilic; the SS contains an excessive number of dendritic melanophages. The underlying hypodermis at the margin of the section that contains epithelium contains a few areas of pale blue foci of degeneration.

MDX: Chronic ulcerative dermatitis with hypodermal degeneration

Section B: See description of section A above.

MDX: Chronic ulcerative dermatitis with hypodermal degeneration

B03-3-3; slide 6, 2 sections (A and B), skin of head: (deep skin lesion from snout)

Section A: A large ulcer is present extending to the upper SC. Underlying the ulcer the SC contains degenerating swollen basophilic collagen fibers. The underlying hypodermis contains pale blue to pink fibrillar degenerative material mixed with inflammatory cells.

MDX: Chronic ulcerative dermatitis with dermal degeneration

Section B: Larger of the two sections. See description of section A above. Large areas of basophilic to acidophilic collagen degeneration are present. Many degenerating collagen fibers are swollen to fragmented.

MDX: Subacute ulcerative dermatitis with collagen degeneration

B03-3-3; slide 7, kidney: only 2 small islands of renal tissue are present: NSL. Gram stain: NSL.

B03-3-3; slide 8, gill:

A single copepod is present between two filaments. A small area of adjacent lamellae is hypercellular. A small focus of fused lamellae is present with moderate inflammatory cell infiltration. Within the area are multiple round to oval cyst-like structures filled with red minute granules. A pale clear wall surrounds the granules. A few gill arches contain moderate numbers of the cyst-like structures that are intra-cytoplasmic.

MDX: Chronic focal branchitis with possible parasites

Fish # 4

B03-3-4; slide 1, skin of head: (skin lesion and normal skin)

A large erosive focus is present extending to one margin of the section. The focus lacks an epithelium and contains a very pale pink bm and adjacent SS. There is an excessive number of minute free melanosomes scattered in the SS. No inflammatory cells are present. The epithelium adjacent to the erosion is pale with slight cellular separation and mild epithelial cell loss. The hypodermis is normal.

MDX: Focal erosive dermatopathy

B03-3-4; slide 2, skin of head: (skin lesion)

There is a large area of erosion extending from one margin to the contra-lateral margin with a few minute superficial ulcers extending to the superficial SC. Areas of the superficial SC are pale and undergoing dissolution. Moderate numbers of scattered individual bacterial rods are in the SC with no inflammation. There is a linear, wide-zone of the lower hypodermis that contains large numbers of neutrophils, occasionally containing a few bacterial rods. At one margin of the section, the adjacent muscle bundles contain scattered degenerating myofibers, lacking any inflammation.

MDX: Acute erosive dermatitis with subacute deep septic dermatitis and acute myodegeneration

B03-3-4; slide 3, skin of head, 2 sections A and B): (skin lesion)

Section A: See description of slide 2 above. Linear area of deep hypodermis with neutrophils and underlying myodegeneration

MDX: Acute erosive dermatitis with focal linear suppurative hypodermatitis and myodegeneration

Section B: The epithelium is absent with diffuse erosion of the surface. The bm, SS and superficial SC are pale pink and undergoing dissolution. Large numbers of bacterial rods are in the SC with no inflammation. The SC contains pale and dark pink collagen fibers. The hypodermis contains multiple small irregular degenerative foci of fragmented collagen with surrounding mononuclear cells and neutrophils.

MDX: Erosive dermatitis and multifocal hyodermatitis with intra-lesional bacteria and dermal degeneration

B03-3-4; slide 4, skin of head: (deep lesion on nose area)

The surface is composed of erosions and ulcers to the mid-SC. The SC contains pale to dark pink collagen fibers and small foci of collagen lysis and fragmentation. The hypodermis contains multiple small inflammatory cell foci associated with collagen lysis and fragmentation. A few of the foci contain adjacent pale lipid vacuoles. Moderate to large numbers of individual bacterial rods are in the SC, a few are in the hypodermis.

MDX: Subacute to ulcerative dermatopathy with a degenerative hypodermatitis and intra-lesional bacteria

B03-3-4; slide 5, skin of head: (deep lesion in skin)

There is erosion and superficial ulceration of the surface with dissolution and fragmentation of the SS and superficial SC. A basophilic focus of collage degeneration underlies a small ulcer. There is a large area of the upper SC with individual scattered fungal hyphae with no associated inflammation. The surface contains a few scattered fungal hyphae. The superficial SC contains a large number of individually scattered bacterial rods with no inflammation. The hypodermis contains a few small basophilic degenerative foci, some with adjacent mononuclear cells. The muscle layer contains an area with a few swollen to fragmented to lytic myofibers, many containing a single fungal hyphae.

MDX: Chronic erosive to ulcerative dermatopathy and hypodermal degeneration and myopathy with intra-lesional fungi hyphae

B03-3-4; slide 6, skin of head: (normal skin only)

The bm is irregular with a scalloped pattern. The SS contains a thin continuous row of melanophages. Mucous cells are only in the upper quarter of the epithelium. The SC is composed of pink collagen fibers. At one margin, they are interlacing; at the contra-lateral margin, there are single layers of fibers, each layer perpendicular to the other.

MDX: Possible resolved erosion

B03-3-4; slide 7, posterior kidney: Glomerular capillary membranes are prominent: NSL. Gram stain: NSL.

B03-3-4; slide 8, gill

A rare gill arch is hypercellular with fusion of a few adjacent lamellae. A rare very small focus of fusion of lamellae is present. Within the focus are a few cyst-like structures as in slide BO3-3-3-6 (arrow). A single copepod is adjacent to a filament. There is mild clubbing of lamellae with fusion of a few adjacent lamellae.

MDX: Focal mild branchitis with intra-lesional copepod

B03-3-4; slide 8 split: As above. Cross sections of copepods are present with mild focal adjacent fusion of a few lamellae.

Fish # 5

B03-3-5; slide 1, skin: (normal scaled skin): NSL

B03-3-5; slide 2, skin of head: (fungus infected skin, head)

There is complete erosion of the surface with total epithelial loss. Overlying the bm is an amorphous basophilic zone of fungal hyphae with a few invading the upper SC. The SS and superficial SC are composed of very pale pink fragmented collagen fibers.

Areas of the hypodermis are undergoing degeneration that consists of pale basophilic areas with large amounts of fibrillar interlacing collagen fibers. Small numbers of neutrophils and a few individual bacterial rods are in the hypodermis.

MDX: Subacute erosive dermatitis with intra-lesional fungal hyphae and subacute to chronic degenerative hypodermatitis with intra-lesional bacteria

B03-3-5; slide 3, skin of head: (fungus infected skin, head)

There is diffuse erosion of the epithelial surface extending to the bm. Scattered areas of the surface contain overlying amorphous mucinous material entrapping fungal hyphae. The SS is pale and edematous. The hypodermis contains a few scattered individual bacterial rods and a very small number of degenerating individual lipid vacuoles with surrounding mononuclear cells and neutrophils. The lumenal margin of two large vessels in the hypodermis contains moderate numbers of individual bacterial rods and neutrophils. A very small number of individual myofibers are undergoing dissolution.

MDX: Diffuse subacute erosive dermatopathy with intra-lesional fungal hyphae and mild hypodermatitis with bacteria and mild myodegeneration

B03-3-5; slide 4, skin of head: (deep lesion on nose area)

There is diffuse ulceration of the section extending to the hypodermis. There is an absence of inflammatory cells. Large numbers of short and long bacterial rods are scattered throughout the

hypodermis. Many bacteria are within fragmenting and degenerating collagen fibers. A few, slightly pale, blue lipid vacuoles are surrounded by bacteria. Scattered aggregates of mononuclear cells and neutrophils are adjacent to the underlying cartilage, occasionally with cartilage lysis. A linear area of collagen overlying the cartilage is undergoing mucinous degeneration and fragmentation with mild inflammatory cell infiltration.

MDX: Subacute ulcerative dermatopathy with septic hypodermatitis and chondritis

B03-3-5; slide 5, skin of head, 2 sections (A and B): (fungus infected skin, head)

Section A: There is diffuse erosion of the epithelium to the bm with overlying bacterial rods and fungal hyphae. There is edema and collagen fragmentation in the SS and superficial SC. Large numbers of bacterial rods are in the SS and upper SC with an absence of inflammatory cells. A rare fungal hyphae is in the SC. The hypodermis contains scattered aggregates of bacterial rods associated with basophilic degenerating collagen and lipid vacuoles with surrounding inflammatory cells and nuclear debris.

MDX: 1. Diffuse subacute erosive dermatopathy with intra-lesional fungal hyphae and bacterial rods

2. Subacute multifocal degenerative septic hypodermatitis

Section B: As A above.

B03-3-5; slide 6, skin of dorsum: (fungus lesion on back)

Only a small segment of epithelium is present at one margin of the section. The epithelium decreases to 1 to 4 cells thick before progressing to a large eroded area. The bm is present extending to the margin of the section with overlying mucinous amorphous material entrapping large numbers of fungal hyphae and occasionally bacterial rods. The SS and upper SC are pale pink, edematous and contain large numbers of infiltrating fungal hyphae and occasional small foci of bacterial rods.

The hypodermis overlying the muscle contains a few hypercellular foci containing mononuclear cells and a rare fungal hyphae. A very small number of individual red and white myofibers are undergoing degeneration and necrosis. The fibers are swollen to shrunken with dissolution or condensation of the sarcoplasm. Some of the fibers are surrounded by mononuclear cells and neutrophils. A rare degenerating fiber contains a fungal hyphae.

MDX: 1. Subacute extensive erosive dermatopathy with intra-lesional fungal hyphae

2. Acute multifocal necrotizing myositis with intra-lesional fungal hyphae

B03-3-5; slide 7, posterior kidney: NSL. Gram stain: NSL.

B03-3-5; slide 8, gill:

A lamella contains five red cyst-like structures, one with five red bodies within a cyst-like structure. A rare small focus of lamellar fusion is present. A large area of filaments is necrotic with large masses of adjacent and invading fungal hyphae. Scattered bacterial colonies are also present within the necrotic area. Inflammatory cells and nuclear debris are adjacent to the necrotic lamelae.

MDX: Subacute necrotizing branchitis with intra-lesional fungal hyphae and bacteria

B.3 Detailed histological interpretation: Steelhead skin slides case numbers B03-5, B04-4

B03-5-2-1: skin of head:

Four small irregular masses of dense interlacing collagen of the SC are present. No epithelium or SS in the section. Scattered collagen fibers are undergoing basophilic degeneration.

MDX: Subacute mucoid degeneration of the SC

B03-5-3-1; skin of head:

Multiple denuded islands of collagen are present lacking any epithelium or SS. There is basophilic collagen degeneration and hyaline swelling of fibers. Many surfaces contain a thick zone of debris and fungal hyphae. Many hyphae are filled with zoospores. Scattered small oval darkly basophilic yeast is present mixed with the fungal hyphae. The margin of one mass of SC contains thick fronds of epithelium with down-growth. There is mononuclear cell infiltration underlying the basal lamina of one scale.

MDX: Subacute ulcerative mycotic dermatitis with focal epithelial regeneration

B03-5-4-1; slide 3, skin of head:

Scattered islands of SC are present lacking any epithelium or SS. Overlying the surface are masses of debris and fungal hyphae. One area of dense mononuclear cell infiltration mixed with a few polymorphonuclear leukocytes (neutrophils) is present with an empty central core. The surrounding internal edges contain infiltrating hyphae.

MDX: Chronic active ulcerative mycotic dermatitis

B04-4-1; skin of head, 3 sections (A, B, and C):

Section A: Scaled skin. A large ulcer is present with epithelial loss. Overlying the ulcer is a mat of fungal hyphae, many of which are swollen and filled with zoospores. Scattered yeast is present within the mat. Scattered hyphae are invading the SC, occasionally present and associated with mononuclear cell infiltration.

MDX: Subacute mycotic ulcerative dermatitis

Section B: As section A above. One margin of the section contains epithelium. There is dissolution, erosion, and fungal invasion adjacent to the ulcer. Distant from the ulcer, the epithelium contains a small focus of erosion with epithelial ballooning degeneration. A rare possible fungal hyphae and a very small number of bacterial rods are at the epithelial basal layer junction.

MDX: Subacute mycotic ulcerative dermatitis

Section C: As section A above. Hyphae are invading the SC with mild scattered inflammatory cell infiltration.

MDX: Subacute mycotic ulcerative dermatitis

B04-4-2; skin of head, 3 sections (A, B, and C):

Section A: One margin contains an erosion with epithelial loss and ballooning degeneration with invading fungal hyphae. Overlying the erosion is a mat of fungal hyphae, many containing zoospores. A few basophilic scattered yeast are present within the mat. A few basophilic lipid ells are in the hypodermis (mucoid degeneration).

MDX: Acute erosive mycotic epidermatitis

Section B: As section A above. A large area of erosion and epithelial degeneration is present with a large mat of fungal hyphae and a few yeast. A rare hyphae is invading the dermal-epithelial junction. The hypodermis contains a few inflammatory cells.

MDX: Acute erosive mycotic epidermatitis

Section C: As section A above. At the margin of the section, there is invasion of the upper dermis with hyphae and a few associated inflammatory cells.

MDX: Subacute erosive mycotic dermatitis

B04-4-3: skin, 3sections (A, B, and C):

Section B: A large erosion is present with epithelial cell degeneration and loss. Only a very small number of hyphae and yeast are present on the surface with a few hyphae invading the epithelium. The SS is expanded with dilated vessels containing inflammatory cells that extend into the upper SC. Mild mucoid degeneration of the upper SC is present.

MDX: Acute erosive mycotic dermatitis

Section A: As section B above. The center contains a small ulcer extending to the upper SC. No organisms are discernible.

MDX: Acute erosive dermatitis

Section C: As section B above. Definitive hyphae are not present, but a few empty vacuolar spaces are present in the upper SC, which are highly suggestive of hyphae. A GMS would be necessary for confirmation. The hypodermis contains a few basophilic lipid vacuoles.

MDX: Acute erosive mycotic dermatitis (presumptive)

B04-4-4; skin, 3 sections (A, B, and C);

Section A: The entire section is eroded with total epithelial cell loss. At one margin, there is ulceration to the mid SC. One area contains a large mat of overlying fungal hyphae, many containing zoospores.

Small numbers of scattered yeast cells are also in the mat. Scattered invading hyphae are present in the upper SC. At one margin with no inflammation, the sc underlying the ulcer contains scattered inflammatory cells consisting of mononuclear cells and polymorphonuclear cells that occasionally extend into the adjacent hypodermis. There is hyalin degeneration of collagen with the area. A small cyst-like structure lined by epithelium is present deep in the SC. A few epithelial cells contain pink round small bodies.

MDX: Subacute erosive to ulcerative mycotic dermatitis

Section B: As section A above. A large erosion is present with an overlying mat of fungal elements, many filled with zoospores. A few yeasts are also present. There is mild to moderate inflammatory cell infiltration of the SC and upper hypodermis with occasional deep fungal invasion.

MDX: Subacute erosive mycotic dermatitis

Section C: As section B above. The SC contains a small linear area of collagen degeneration. The area is characterized by shrunken to fragmented fibers. There is scattered basophilic mucoid degeneration of the deep hypodermis.

MDX: Subacute erosive mycotic dermatitis with hypodermal degeneration

BO4-4-5, skin 4 sections (A, B, C, and D):

Section A: Scaled skin. Erosion is present with an overlying mat of fungi and a few yeast with superficial fungal invasion of the adjacent epithelium and superficial SC. There is mild inflammatory cell infiltration of the SC.

MDX: Subacute erosive mycotic dermatitis

Section B: As section A above. A large long linear mat of fungi is present overlying the erosion. There is basophilic degeneration of the upper SC with occasional fungal invasion. The lipid containing hypodermis, separating the SC from the muscle layer contains scattered inflammatory cells and deep invading fungal elements. Within the area there is fragmentation of collagen fibers. One margin of the section contains a small focus of remaining epithelium.

MDX: Subacute erosive mycotic dermatitis with mild superficial collagen degeneration of the SC

Section C: As section A above with fungal invasion and inflammation into the superficial SC.

Section D: As sections A and B above. There is erosion with an overlying mat of fungal elements and a few yeasts are present. Areas of the SC are undergoing hyalin to basophilic degeneration with a few invading fungi. The hypodermis contains inflammatory cells with degeneration and fragmentation of the adjacent SC. This SC also contains scattered fungal hyphae.

MDX: Subacute erosive mycotic dermatitis and hypodermatitis

B04-4-7: skin, 4 sections (A, B, C, and D):

Section A: Scaled skin. There is marked erosion and ulceration extending to the deep SC and adjacent scale. There is a thick mat of fungi and moderate numbers of yeast overlying the lesion. There is dissolution of the SC with massive fungal invasion in one area. There is a paucity of inflammatory cells in the region.

MDX: Subacute erosive to ulcerative mycotic dermatitis

Section B: **S**mall section of scaled skin with isolated adjacent section of muscle. Skin: As section A above. Muscle: One area of connective tissue contains moderate numbers of inflammatory cells. A large number of myofibers are undergoing degeneration characterized by hyalinization, condensation and fragmentation.

MDX: Subacute erosive to ulcerative mycotic dermatitis and myositis

Section C: Skin. There is ulceration and erosion extending to the SC with an absence of epithelium and a thick overlying layer of fungal hyphae and yeast. There is an absence of inflammatory cells.

MDX: Subacute erosive to ulcerative mycotic dermatitis

Section D: Skin. There is diffuse erosion of the surface with epithelial loss and with a thick overlying mat of fungi and moderate numbers of yeast. At one margin of the section, there is early ulceration with separation and dissolution of the upper SC. Scattered fungal hyphae are invading the SC with no inflammation. The underlying hypodermis contains large numbers of infiltrating mononuclear cells and neutrophils with scattered collagen degeneration. A rare fungal hyphae is within the area.

The underlying muscle layer contains multiple degenerating myofibers characterized by hyalinization, loss of striations, and fragmentation. Only a few inflammatory cells are in the upper adjacent connective tissue.

MDX: Diffuse subacute to chronic mycotic erosive to early ulcerative dermatitis and myositis

B04-4-10: skin, 3 sections (A, B, and C):

Section A: The surface lacks any epithelium with scattered overlying fungi and yeast. A few invading hyphae are present in the upper SC. There are moderate numbers of inflammatory cells in the lower SC and hypodermis with collagen degeneration and fragmentation. Many degeneration fibers are basophilic to hyalinized.

MDX: Diffuse erosive mycotic dermatitis

Section B: Skin. As shown in Section A above. The hypodermis is undergoing mucoid basophilic degeneration.

MDX: Diffuse erosive mycotic dermatitis and hypodermal degeneration

Section C: As shown in Section A above.

B04-4-11: skin, 5 sections (A, B, C, D, and E)

Section A: Erosion of surface with epithelial loss, only a few fungi are on the surface. There is mucoid degeneration of the hypodermis.

MDX: Acute erosive mycotic dermatitis with mild hypodermal degeneration

Section B: As shown in Section A above. A few fungi are invading the superficial SC. One margin contains epithelium.

Section C: As shown in section B above. Two erosions are present. Two areas of the SC contain mild to moderate numbers of inflammatory cells. Rare, isolated fungal hyphae is present within the inflammatory area.

MDX: Subacute mycotic erosive dermatitis and focal hypodermal degeneration

Section D: As shown in section C above. A large area of the hypodermis is undergoing mucoid degeneration with scattered hyalin degeneration of collagen. No inflammatory cells present.

Section E: As shown in section D above.

B04-4-12: 7 skin sections (A, B, C, D, E, E, and G)

Section A: There is diffuse erosion with total epithelial loss and overlying fungi and yeast. The SC contains a linear zone of moderate inflammatory cell infiltration consisting of mononuclear cells and neutrophils. In one area the cells extend to the hypodermis. Within the area are hyalinized and fragmented collagen fibers. Rare fungal hyphae is associated with the inflammatory cells in the SC. Focal mucoid degeneration is present in the hypodermis.

MDX: Diffuse subacute to chronic erosive mycotic dermatitis with focal hypodermal degeneration

B, C, D, E, F: As shown in section A above.

G: As shown in section A above. At one margin an ulcer is present with only a thin zone of SC remaining. A few deep invading hyphae are in the area. The surface of the ulcer contains a thick mat of fungi and a few yeasts. Many hyalinized degenerating collagen fibers are in the SC and hypodermis.

MDX: Chronic ulcerative mycotic dermatitis

B04-4-13: skin; 3 sections (A, B, and C):

Section A: A small focus of partial to complete erosion to the bm is present; no fungi are present. The SS contains a few inflammatory cells. There is slight expansion of the hypodermis.

MDX: Acute erosive dermatitis

Section B: Large erosion is present with a focal ulcer. A mat of fungi and a few yeast are on the surface. The SS is hypercellular. The surface of the ulcer contains degenerating fragmented collagen. The SC contains moderate numbers of scattered inflammatory cells and a few fragmented hyalinized degenerating

collagen fibers. There is mucoid degeneration of the hypodermis characterized by scattered areas of finely granular basophilic material.

MDX: Subacute erosive to ulcerative mycotic dermatitis with hypodermal degeneration

Section C: As shown in section A above.