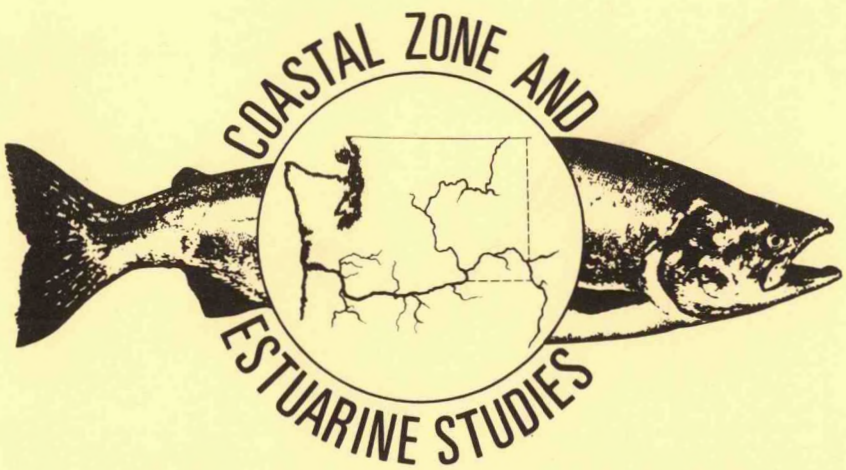
Snake River Fall Chinook Salmon Brood-Stock Program, 1984

by
Lee W. Harrell
Thomas A. Flagg
Thomas M. Scott
and
F. William Waknitz

February 1985



SNAKE RIVER FALL CHINOOK SALMON BROOD-STOCK PROGRAM, 1984

by
Lee W. Harrell
Thomas A. Flagg
Thomas M. Scott
and
F. William Waknitz

Annual Report of Research
Financed by
Bonneville Power Administration
(Contract DE-A179-83BP39642 Project 82-7)

and

Coastal Zone and Estuarine Studies Division
Northwest and Alaska Fisheries Center
National Marine Fisheries Center
National Oceanic and Atmospheric Administration
2725 Montlake Boulevard East
Seattle, Washington 98112

February 1985

ABSTRACT

The objective of the Snake River Fall Chinook Salmon Brood-stock Program is the enhancement of upriver stocks through research and development of an eggbank source. Viable gametes, produced from fish held to maturity in sea pens at the National Marine Fisheries Service's (NMFS) Manchester Marine Experimental Station, Manchester, Washington, will be made available for restoration purposes on the Snake River. Perhaps equally as important is the fact that captive brood-stock rearing also offers the unique opportunity of documenting factors affecting seawater growth and survival.

Seawater entry trials with 0+-age and 1+-age fish have shown that 0+-age Snake River fall chinook salmon are not amenable to seawater entry and will either die or require up to 6 months to fully adapt to seawater. However, 1+-age smolts experience little problem at seawater entry; it is therefore suggested that Snake River fall chinook salmon be released as 1+ smolting fish in hatchery situations.

NMFS researchers have documented important marine mortalities occurring from osmoregulatory dysfunction, Bacterial Kidney Disease, and precocity at various life stages. Also, a previously unreported marine fungal pathogen has been identified. Mortality from this pathogen occurs from 3-years of age to maturity and can exceed 0.5% per day (resulting in losses to 90+%). Because of the seriousness of this fungal pathogen, and its implications to overall marine survival, the NMFS has begun an intensive investigation concerning its pathogenesis and control.

At the end of December 1984, NMFS had Snake River fall chinook salmon from 1980 (n=67), 1981 (n=876), 1982 (n=4,809), and 1983 (n=7,100) broods under production. Because of the extensive mortality due to the marine fungal

pathogen, only seven spawners were obtained from the 1980 stock in fall 1984. The 1980-brood spawners produced only minimal eggs and these will be used to investigate possible vertical transmission of the fungal pathogen.

TABLE OF CONTENTS

INTRODUCTION

The purpose of the Snake River Fall Salmon Brood-stock Program is the enhancement of upriver stocks through the development of alternatives to traditional rear/release strategies. The National Marine Fisheries Service (NMFS) program utilizes captive brood-stock rearing concepts with fish being reared to maturity in seawater net-pens. The end product of the program will be the production of viable gametes for restoration purposes on the Snake River. Throughout the production program, research is conducted in several areas of concern, e.g., disease diagnosis and control, nutrition, acclimation to seawater, and spawning strategies. During 1984, NMFS had Snake River fall chinook salmon from 1980, 1981, 1982, and 1983 broods under production.

A parallel program goal is an understanding of the seawater phase of the life-cycle of chinook salmon. Captive rearing in seawater offers the unique opportunity to document factors affecting growth and survival. Currently, management models consider the seawater period of the chinook salmon life-cycle to be a "black box" with perhaps the greatest mortality occurring as predation on young fish. Once the fish reach size (3-4 lbs), it is assumed that survival to the adult is good. NMFS research suggests that this may not be true. Our research has discovered previously unreported adult diseases which are killing up to 90%+ of our brood stock at between 3 years of age and maturity. NMFS researchers, in conjunction with the Battelle Marine Laboratories at Sequim, Washington, are describing these diseases and investigating methods of control.

DESCRIPTION OF STUDY AREA

Brood-stock Program

Snake River fall chinook salmon have historically made significant contributions to ocean and lower Columbia River fisheries and are uniquely adapted to upper Snake River environmental conditions. In recent years, these stocks have become severely depressed, requiring an extensive restoration effort by state and federal agencies. The NMFS brood-stock program is intended to provide a stable eggbank supply from the Snake River stock. Eyed eggs or fry from Snake River fall chinook salmon stocks are provided to NMFS, fish are reared through their freshwater cycle, and subsequently transferred to seawater net-pens. These fish are reared to maturity in captivity in seawater and resultant eggs are made available for enhancement or research.

Special Disease Investigations

Experiments since August 1983 aim at understanding the source, occurrence, and pathogenesis of an infectious marine fungus that killed most of the 1980-brood Snake River chinook salmon during their final year before maturation. This previously undocumented fungus was also responsible for substantial losses in the 1981-brood fish during fall 1984.

MATERIALS AND METHODS

Freshwater Husbandry

Approximately 15,000 eggs or swim-up fry of the Snake River fall chinook salmon stock were received from eggbank facilities on the Snake and Columbia Rivers during early winter 1981, 1982, 1983, and 1984. Freshwater rearing is conducted at the Northwest and Alaska Fisheries Center (NWAFC), Seattle, Washington, or the NMFS experimental hatchery at the University of

Washington's Big Beef Creek Fish Research Station, Seabeck, Washington. All water for incubation and rearing purposes is either dechlorinated City of Seattle water or groundwater at Big Beef Creek. Fish that reach sufficient size and smoltification status are transferred to the NMFS Marine Experimental Station at Manchester, Washington.

Marine Husbandry

Fish transferred to Manchester are acclimated to full-strength seawater (28 ppt) using intermediate salinities over several days. Brood stocks are held in 24- x 24- x 10-ft deep net-pens at a density of 0.5 lb/ft^3. Seawater temperatures range from 7° to 13° C during the year, and mean salinity is 28 ppt. Fish are fed pelleted rations from several commercial manufacturers supplemented with fresh frozen herring, Clupea harengus, and whole krill, Euphausia pacifica. All fish are injected intraperitoneally with a vibrio bacterin/oxytetracycline mixture at 6- to 8-month intervals during their seawater residence. The salmon are also fed antibacterial drugs during epizootics of bacterial disease. Dead and moribund fish are removed from the population daily, weighed and measured, and necropsies performed.

Spawning Strategies

Maturing 4-year-old 1980 Snake River Chinook salmon stock spawned in fall 1984. These fish were sorted for maturity in September and October 1984 and, under Washington Department of Fisheries (WDF) provisions, moved to quarantine facilities at the Battelle Marine Laboratory. This facility is supplied with constant-temperature (11.3° C) pathogen-free groundwater, and the effluent is chlorinated. Mature fish were subsequently spawned, and each female's eggs were placed in isolated incubation systems.

Special Disease Investigations

The first 1980 Snake River chinook salmon smolts (approximately 6,500) were successfully transferred to full-strength seawater in April 1982, During the following 15 months of seawater residence, losses to vibriosis (Vibrio anguillarum) was prevented by vaccination, and mortality due to bacterial kidney disease (BKD) (Renibacterium salmoninarum) was moderated with chemotherapeutics. In August 1983, however, a sudden increase in mortality could not be attributed to typical pathogens. During the following 10 months, over 4,500 3-year-old brood stock (1,200-1,500 g) succumbed to a previously-unreported systemic infection (Fig. 1). Because of the seriousness of this pathogen and its implication to overall marine survival, a cooperative between NMFS' Manchester Marine Experimental Station and the Battelle Marine Laboratory was established. Study of the pathogenesis of this organism included:

Pathology

Samples of kidney and spleen tissue were aseptically streaked on typicase soy or Sabouraud's agar with 1% fetal calf serum for determination of typical bacterial or fungal pathogens. These tissues were also used to prepare wet mounts and gram-stained smears.

Moribund fish were dissected and major organ systems fixed in Bouin's solution and either paraffin-embedded or processed in a plastic histological embedding medium. Tissue sections were stained with Harris hematoxylin and eosin (H+E), periodic acid Schiff (PAS), Grocott's methenamine silver nitrate (GMS), or Lugol's iodine.

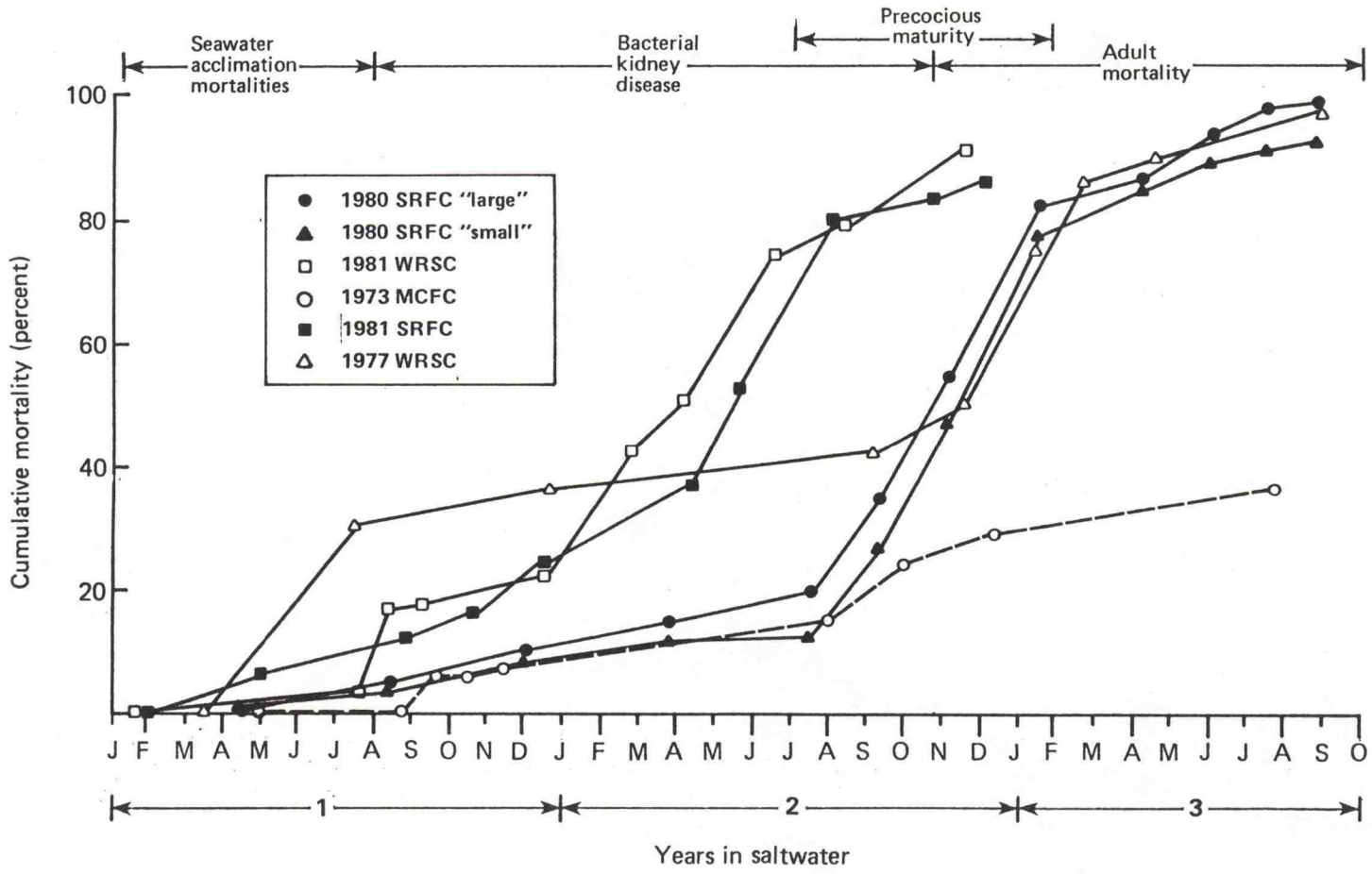


Figure 1.--Cumulative mortality in seawater for chinook salmon reared at NMFS Marine Experimental Station. Brackets indicate major causes of mortalities. Mortality profiles for "large" 1980 Snake River fall chinook salmon (●), which entered seawater averaging 29 g; "small" 1980 Snake River fall chinook salmon (▲), which entered seawater averaging 15 g; 1981 White River spring chinook salmon (□); 1973 Minter Creek fall chinook salmon (o); 1981 Snake River fall chinook salmon (■); and 1977 White River spring chinook salmon (△).

Hematology

Blood was sampled from caudal vessels with heparinized 3-ml glass syringes, and whole blood smears were prepared on microscope slides and stained with DIFF-Quik.[1/] Samples were centrifuged in microhematocrit tubes at 10,000 rpm for 3 min, and the percent packed cell volume was recorded. Hemoglobin values were obtained with an American Optical hemoglobinometer.

Electron Microscopy

Tissues for electron microscopy were fixed in 4% gluteraldehyde in 0.1 M sodium cacodylate buffer adjusted to pH 7.4. Tissues were post-fixed with 1.0% OsO_4 in the same buffer for 1 h. Tissues were dehydrated through an ethanol series and embedded in Medcast. The thin sections were routinely stained with uranyl acetate and lead citrate and examined with a Phillips EM 300 at an accelerating voltage of 60 kV.

RESULTS AND DISCUSSION

Stock History

The NMFS has 4 brood years of Snake River fall chinook salmon in culture (1980, 1981, 1982, and 1983 stocks). Freshwater growth and survival were variable (1980, 1981, and 1982 broods), however, now that the Big Beef Creek facility is operational (late 1982) both growth and survival have been excellent (Figs. 2 and 3). At the hatchery at Big Beef Creek, the fish are reared in large (13-ft diameter) Fiberglass tanks supplied with constant-temperature (10° C) pathogen-free groundwater and fed via automatic feeder, allowing high growth to be maintained throughout the year.

[1/] Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

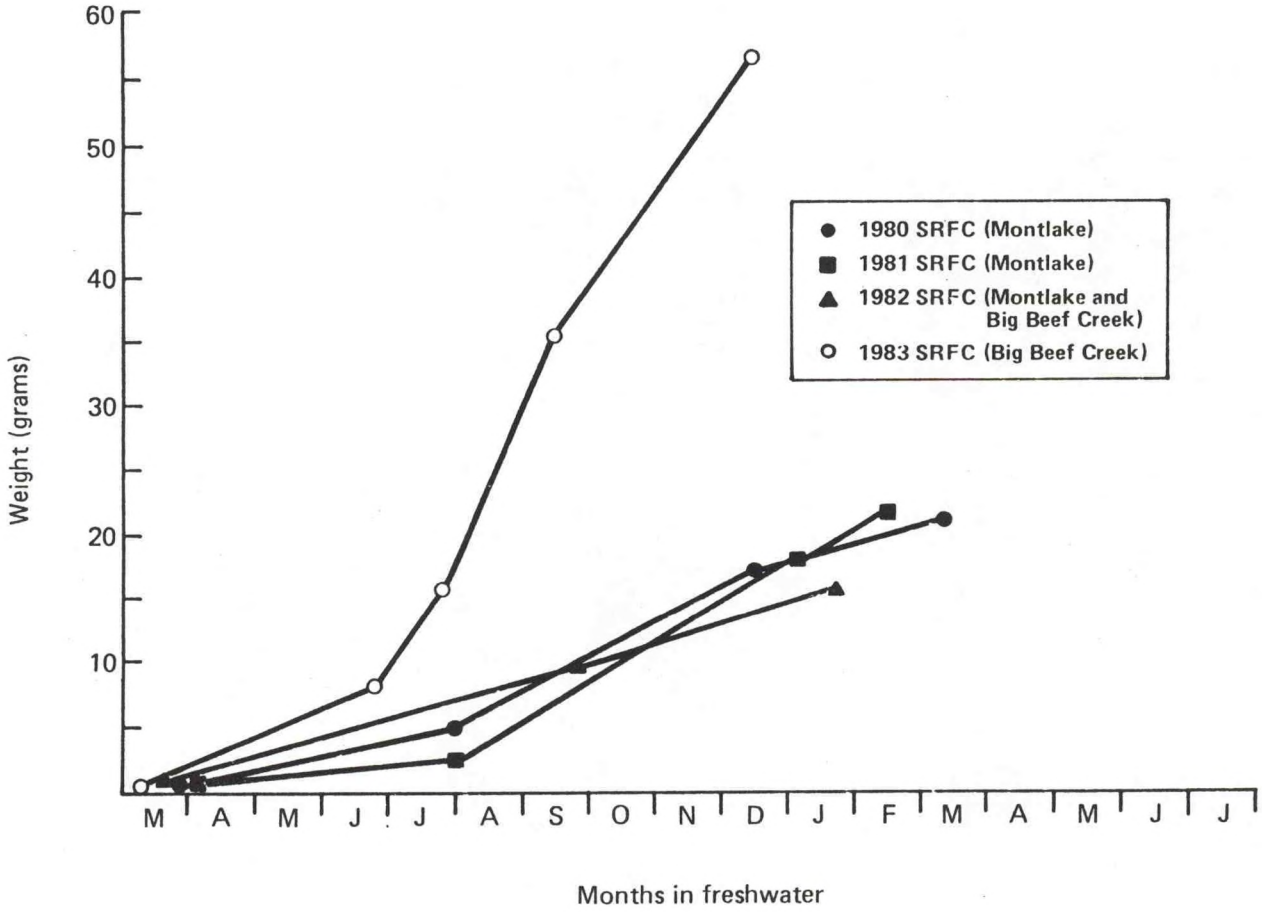


Figure 2.--Freshwater growth for Snake River fall chinook salmon (SRFC) broods. Freshwater rearing was conducted at NMFS Montlake laboratory or NMFS Big Beef Creek hatchery as indicated.

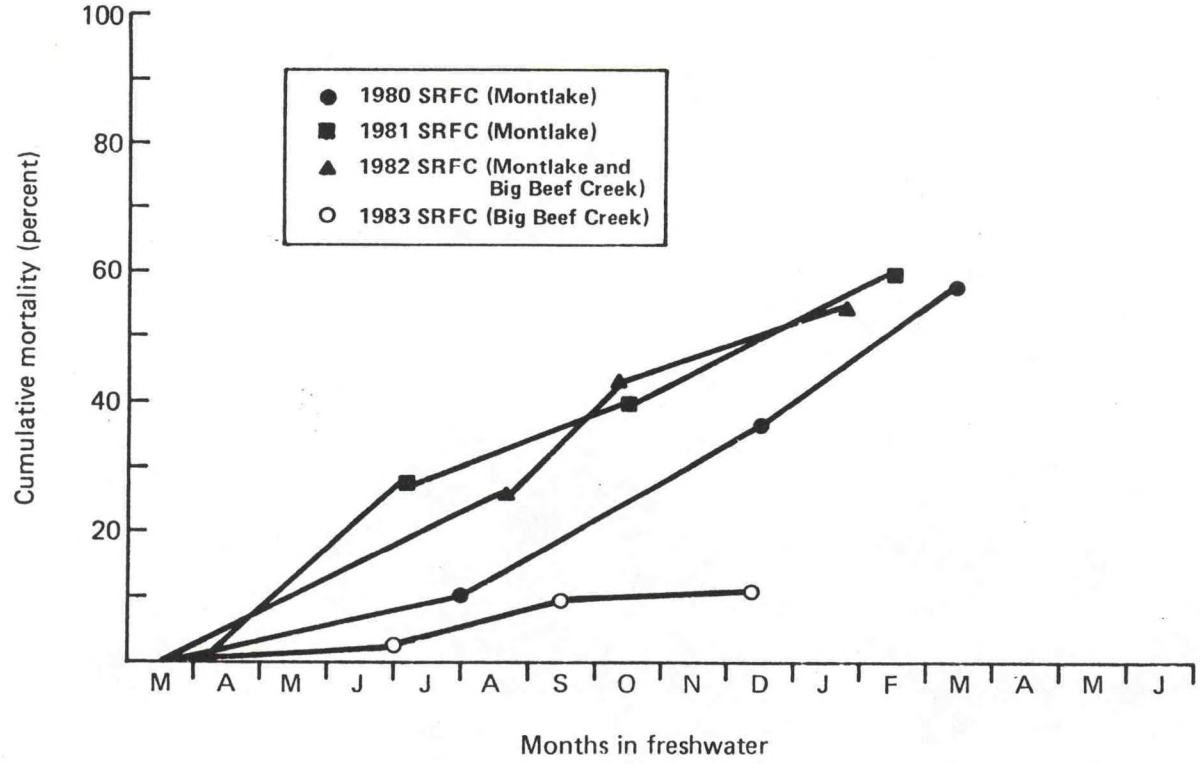


Figure 3.--Cumulative freshwater mortality for Snake River fall chinook salmon (SRFC) broods. Freshwater rearing was conducted at NMFS Montlake laboratory or NMFS Big Beef Creek hatchery as indicated.

Portions of all brood years were introduced into seawater as 0+-age and 1+-age fish. The 0+-age entries were not entirely successful; fish experienced osmoregulatory-related mortalities and survivors required up to 6 months to fully adapt to seawater. However, 1+-age fish experienced few problems at seawater entry; in the future, all fish will be reared to 1+-age before entry to seawater. After fish fully adapted to seawater, growth was excellent (Fig. 4).

1980-Brood

The surviving 1980-brood Snake River fall chinook salmon reared in seawater net-pens completed their life-cycle this year after 4 years of captive rearing. These fish (n=6,454) were tranferred to seawater at the Manchester Marine Experimental Station on 5 April 1982, at an average weight of 30 g. In late summer 1983, the 1980-brood suffered a severe increase in mortality due to an unknown fungal pathogen (Fig. 1). Attempts to learn more about the infectious nature of the pathogen continue at both Battelle and NMFS. Twenty-four fish were transferred to fresh water at Battelle Marine Laboratories in Sequim, Washington, for final maturation. Sixty-seven adults that failed to mature were left in seawater pens at Manchester. Mortality continued in fresh water until seven females survived to spawn. Only two of these females (average 51 cm and 2,174 g), both infected with the fungal pathogen, produced viable eggs (52% viability). Their average fecundity of 2,488 eggs therefore yielded 2,600 viable eggs. These eggs will be utilized in investigations to determine if vertical transmission of the fungal pathogen is possible.

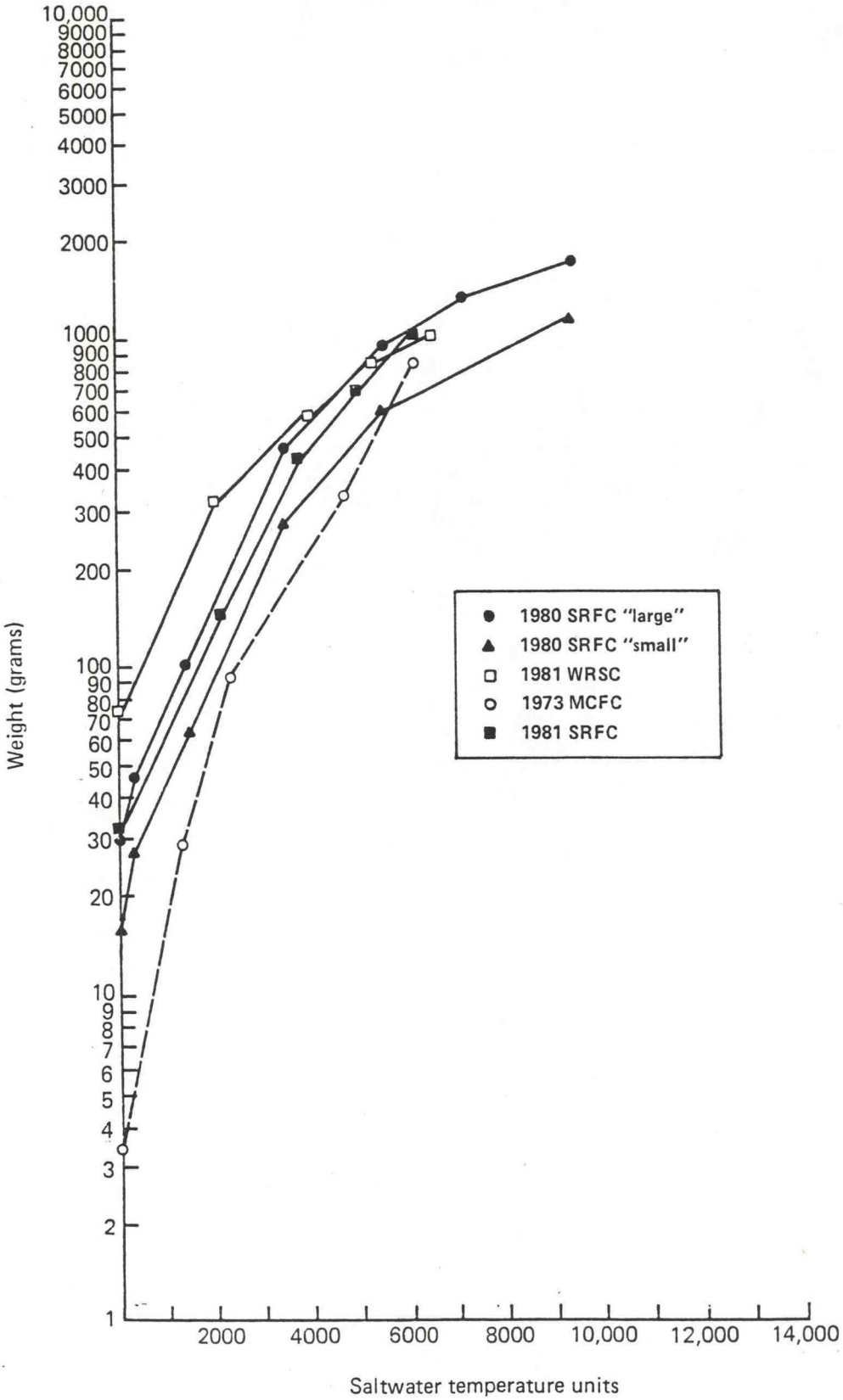


Figure 4.--Seawater growth for chinook salmon reared at NMFS Manchester Marine Experimental Station. Growth profiles for "large" 1980 Snake River fall chinook salmon (●), which entered seawater averaging 29 g; "small" 1980 Snake River fall chinook salmon (▲), which entered seawater averaging 15 g; 1981 White River spring chinook salmon (□); 1973 Minter Creek fall chinook salmon (o); and 1981 Snake River fall chinook salmon (■).

1981-Brood

All 1981-brood fall chinook salmon were acclimated to marine net-pens by the end of spring 1983. These fish were inoculated with a Vibrio anguillarum/antibiotic preparation as they entered seawater. Mortality from December 1983 through late July 1984 was primarily due to BKD. The outbreak of BKD this year initially caused less mortality than in the previous year. However, the disease lasted nearly twice as long as in the previous year and resulted in near-identical mortality (approximately 20%). The development of precocious males resulted in an additional loss of 25% of the fish originally transferred to seawater. Survival is now at 5.8% (876) of the original egg take, and the new fungal disease is currently responsible for mortality exceeding 0.5% per day in these fish. Although "in vitro" trials suggested oxytetracycline would inhibit the development of the pathogen, injections of this drug in the 1981-brood fish appears ineffective.

1982-Brood

The 1982-brood juveniles were reared at the University of Washington's Big Beef Creek Fish Research Station using groundwater and at NWAFC using dechlorinated City of Seattle water. The smolts were acclimated in mid-February 1984 to seawater net-pens at Manchester. Initial losses due to seawater entry were low and the survival is at 37% (4,809) of the total number of fish started (13,000). In anticipation of outbreaks of both the new fungal pathogen and BKD, three prophylactic chemotherapeutics are on trial in a controlled experiment. The treatments used are: intraperitoneal injection (IP) of oxytetracycline, IP injection of oxytetracycline/furacin/vibrio bacterin, and oral feedings of erythromycin phosphate. A control group of 1982-brood fish is being given no treatments. Mortalities are monitored daily and examined for both pathogens.

1983-Brood

Prior to the 1984 summer solstice (20 June), 6,300 1983-brood Snake River fall chinook salmon averaging 8.2 g were transferred to Manchester's seawater net-pens using intermediate salinities over several days. The remaining fish are being reared at Big Beef Creek (3,100). Shortly after transfer to seawater, the 1983-brood fish developed signs of "bloating," caused by intake of excessive amounts of seawater, followed with an increase in mortality. Elevated levels of divalent cations were found in blood samples of all bloated fish. These findings suggest that the fish lack complete osmoregulatory control. Previous occasions of bloating had been observed in the 1980-brood fish but without substantial losses. Herring and other dietary supplements were used in attempts to alleviate the distress. These efforts were generally successful, and the bloating was not evident through the winter months. To further investigate this problem, a diet study was initiated with three treatment groups and one control. The three diets, with one replicate each, were: Oregon Moist Pellets (OMP), a control; krill, herring, and OMP; and herring alone. At the start of the study, the OMP-fed group showed the highest incidence of bloating and mortality. After 3 months the bloating has moderated, and the highest mortality occurred in the herring-fed group. Mortality by December was within acceptable limits.

In September, after the fish retained at Big Beef Creek had reached 30-40 g, a second seawater entry was attempted, during which 100% of the fish died from osmoregulatory dysfunction.

Considering the smolting time (i.e., 1+ years) of these fish, husbandry similar to that used for spring chinook salmon is indicated. Therefore, the remaining fish held at Big Beef Creek will enter seawater as 1-year-plus smolts in 1985. Survival of the 1983-brood is at 59% (7,100).

Special Disease Investigations

Necropsy of dead and moribund 1980- and 1981-brood chinook salmon revealed swollen kidneys and spleens. Considerable losses were attributed to early-maturing males. However, these fish, as well as later mortalities, exhibited signs of the pathological conditions. Fish were severely anemic with packed cell volumes averaging 12% and hemoglobin values of 4.9 mg/dl. Whole blood smears stained with DIFF-Quik indicated a pronounced lymphocytosis. Kidney and spleen tissue streaked on typicase soy and Sabouraud's agar plates were negative for bacterial or fungal growth. Gram-stained smears of these tissues were negative for R. salmoninarum, the causative bacterium of BKD. However, clusters of gram-positive staining, variable sized (3-7 diameter), spherical organisms were observed. The organisms were intensely birefringent in wet-mount preparations observed with Nomarski interference contrast microscopy. The organisms appeared to initially accumulate and replicate within fixed macrophages of the spleen and kidney. In more severe infections, the pathogen was seen in peripheral blood and the vascular spaces of liver, gonad, heart, brain, and intestinal mucosae. Variable-sized free organisms were formed within the interstitium of spleen and kidney parenchyma and were associated with areas of edema and focal necrosis. There was little evidence of inflammatory change or fibroblastic proliferation associated with this disease. Staining reactions of the organism in tissue sections indicated that they were positive to both PAS and GMS, and brown after Lugol's solution.

Transmission electron microscopy of the spherical organisms demonstrated the existence of intracellular clusters. Detailed ultrastructural examination of the causative organisms revealed a cell wall composed of a single-layered

outer membranous structure (possibly of host cell origin), a second moderately electrondense layer, and a thin, inner electronlucent zone that separated the second layer from the plasma membrane. The organisms contained peripherally-oriented mitochondria within a ribosomal matrix and both membrane-bound and non-membrane-bound vacuoles of varying density. Nuclei were relatively indistinct.

The cause of mortality in the 1980 Snake River chinook salmon brood stock was unquestionably a result of the invasive nature of the pathogen and the associated anemia. Except for limited macrophage activity and edema, there was negligible tissue reaction in the form of inflammation or granulation. This minimal host response could be attributed to the immunologically inert cellulose cell wall of the pathogen or may be an indication of the lack of development of a phylogenic relationship between host and pathogen. The lymphocytosis was probably a result of the limited inflammatory response to the new pathogen, although the infection appeared to overwhelm the salmon before an effective defense mechanism was established. The cell wall of the causative organism appears to be composed of cellulose based on its positive staining with PAS, GMS, and Lugol's and its marked degree of birefringence. Organisms considered to have fungal affinities are known to have cell walls composed of chitin, cellulose, or a combination thereof.

Other systemic fungal infections of marine-reared salmonids have been attributed to the feeding of raw marine fish. Both 1980- and 1981-brood Snake River fall chinook salmon were fed supplements of raw herring and krill, however no evidence of the chinook salmon pathogen was observed after microscopic examination of either supplement.

A feeding trial was conducted during early 1984 in an attempt to induce the disease in naive chinook salmon smolts. Approximately 200 fish each were

fed either chopped herring or OMP as a sole ration for 60 days. Although not conclusive, the new fungal disease was observed in both experimental groups in marine net-pens.

It is inconceivable that this is a freshwater pathogen. Routine gram-staining of kidney and spleen tissues is a common practice of fishery biologists, and it would be difficult to fail to recognize the organism in gram-stained smears. We have also examined more than 300 pre-smolt Snake River fall chinook salmon and have seen no evidence of the pathogen.

Tissue from fish infected with the new organism was inoculated on chinook salmon embryo cell lines (CHSE cells). After 30 days incubation, the cells were infected with the obligate intracellular parasite. The fungi were subsequently harvested from the cell culture and used in attempts to infect chinook salmon. Naive fish were either injected intraperitoneally or force-fed the tissue culture isolate. The disease and mortality was reproduced using the tissue culture isolate, and, in addition, the organisms were reisolated in CHSE cells 25 days after inoculation with tissue from moribund fish. Identification of the reisolated organism was confirmed with the tissue culture isolate by morphological and antigenic methods. Antigenic identification was confirmed between tissue culture isolate and field isolated organisms. These laboratory results demonstrate that the causative organism of the marine (fungal) mortality has been identified. The developed capability to culture the causative organism will facilitate both further investigations of pathogenesis and control, and studies to place the organism in proper taxonomic classification.

SUMMARY AND CONCLUSIONS

1. Results of NMFS seawater acclimation trials indicate that Snake River fall chinook salmon should be released as 1+ smolting fish in hatchery situations.

2. Maintaining chinook salmon to maturity in net-pens affords a unique opportunity to observe marine growth and survival during an otherwise inaccessible phase of their life cycle.

3. Our investigations have shown that there are many factors affecting marine survival of chinook salmon. Marine mortalities first occur during the osmoregulatory adaptation to seawater. During the first winter of seawater residence, Snake River fall chinook salmon mortality increases markedly due to BKD. Losses to this disease may continue for up to 6 months and mortality can exceed 25%. During the fall of the following year, chinook salmon are infected with previously undocumented diseases. We have recently identified a probable fungal pathogen that is responsible for catastrophic losses (95+ %) in fish at 3-4 years of age in seawater. Other serious adult diseases (e.g., an infectious anemia) have been observed at the Manchester Marine Experimental Station in captive spring chinook salmon. A better understanding of these diseases may provide insight on problems of high-seas survival.

4. NMFS currently has Snake River fall chinook salmon from 1980 (n=67), 1981 (n=876), 1982 (n=4,809), and 1983 (n=7,100) broods under production. Until the problems of adult mortality in marine net-pens can be controlled, a viable brood-stock program cannot be assured.

ACKNOWLEDGMENTS

Support for this research came from the region's electrical ratepayers through the Bonneville Power Administration.

APPENDIX

Budget Information

A. Summary of expenditures

1.	Labor	$51,700
2.	Travel	3,700
3.	Transportation of things	100
4.	Rents	3,300
5.	Printing	100
6.	Contract services	18,100
7.	Supplies	30,900
8.	NOAA and DOC overhead	18,900
	Total	$126,800

B. Major property items

1. None