



Assessment of Smolt Condition for Travel Time Analysis Project Review 1987-1997



**U. S. Department of Energy
Bonneville Power Administration
Division of Fish and Wildlife**

**U. S. Department of the Interior
U. S. Geological Survey
Biological Resources Division
Columbia River Research Laboratory**

**ASSESSMENT OF SMOLT CONDITION
FOR TRAVEL TIME ANALYSIS**

PROJECT REVIEW 1987-1997

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Project No. 87-401
Contract No. DE-A179-87BP35245

ABSTRACT

The Assessment of Smolt Condition for Travel Time Analysis Project (Bonneville Power Administration Project 87-401) monitored attributes of salmonid smolt physiology in the Columbia and Snake River basins from 1987 to 1997, under the Northwest Power Planning Council Fish and Wildlife Program, in cooperation with the Smolt Monitoring Program of the Fish Passage Center. The primary goal of the project was to investigate the physiological development of juvenile salmonids related to migration rates. The assumption was made that the level of smolt development, interacting with environmental factors such as flow, would be reflected in travel times. The Fish Passage Center applied the physiological measurements of smolt condition to Water Budget management, to regulate flows so as to decrease travel time and increase survival.

Significant findings were that in-river migration played an important part in the development of smoltification, as measured by gill sodium, potassium ATPase levels and condition factor. A characteristic profile of low ATPase activities in the hatchery with increasing ATPase activities during the migration was found for Columbia basin salmonid stocks. Differences in predictors of travel times were found between chinook salmon and steelhead. Riverflow was the only significant predictor of travel time in steelhead, while river flow and changes in flow, ATPase activity, condition factor, and water temperature are significant predictors in spring chinook salmon. Bacterial kidney disease prevalence in juvenile migrants was evaluated and differences in prevalence and changes during the migration were found between the Columbia and Snake Rivers. Prevalence in Snake River fish that were exposed to higher water temperatures and longer migration distances, increased after release.

Other indices of smolt condition that correlate with gill ATPase activity were developed by the project: a morphometric index describing changes in body shape, a reflectance method that quantifies silvering and guanine deposition, and a microassay for gill ATPase. Technical assistance was provided to regional fishery agencies on cooperative projects to evaluate the physiological effects of adaptive management strategies, including modified hatchery rearing protocols, natural rearing methods, and release and acclimation programs. Understanding the interaction of fish physiology with environmental conditions, and its effect on survival during the juvenile migration, remains the continuing objective of the ASCTTA project.

EXECUTIVE SUMMARY

The Assessment of Smolt Condition for Travel Time Analysis (ASCTTA), a project of the Columbia River Research Laboratory, United States Geological Survey, Biological Resources Division, has monitored the physiological condition of juvenile salmonids in the Columbia and Snake River basins from 1987 to the present. Juvenile salmonids (*Oncorhynchus* spp.), including chinook, coho, sockeye salmon and steelhead, were examined to determine how smolt condition and health respond to river flow, and affect migration rates and survival.

This report includes both a review of the ASCTTA project and information on current project activities. The project review (Section One) provides a chronological overview of project activities and accomplishments, as well as modifications made to project objectives in response to innovations in the adaptive management of salmonid stocks in the Columbia River basin. Results of travel time analysis, monitoring of smolt development (using gill sodium, potassium-activated adenosine triphosphatase activity), and the prevalence of bacterial kidney disease in Columbia River stocks are reviewed for the ten-year period. The project status summary (Section Two) describes current ASCTTA technical assistance and cooperative research activities; progress in the development, validation, and application of non-lethal methods for assessing smolt condition and health; the comprehensive smolt physiology database; and the survey of hatchery rearing conditions. New information on the relation between thyroid activity and water temperature on the smolt physiology of chinook salmon is also presented.

Integration of the smolt monitoring activities of the project with those of regional fish management agencies involved in Northwest Power Planning Council Fish and Wildlife Program research has expanded in recent years as requests for technical assistance have increased. Project support of Columbia basin fish management projects for mitigation, supplementation, and production developed through a close association with hatcheries and fish passage facilities. The Columbia River Research Laboratory provided research assistance to fifteen associated projects in 1997 and 1998, including monitoring and evaluation projects of federal, state, utility, and tribal agencies. Technical assistance was restricted to projects investigating smoltification and its effects on juvenile migration rates and survival. Research protocols were reviewed, and assistance was provided if the projects addressed goals, objectives, and tasks of the ASCTTA. Priority was given to projects managing ASCTTA reference stocks. In 1997, technical assistance was limited to research design review, sample analysis, and data release activities that could be provided at present staff and funding levels. All cooperative activities directly addressed objectives of the 1997 Statement of Work and are reviewed in the following report. Our primary focus in 1997 was the development and distribution of a hatchery survey designed to evaluate how rearing conditions at ASCTTA reference hatcheries may have influenced annual changes in smoltification.

Changes in physiological profiles of migrating smolts observed during the ten-year period suggest that flow was not the only factor affecting migration rates. The level of smoltification, although a contributor to migration rate, was not uniform or of the same magnitude in all species and stocks. Annual variations in both smoltification indices and migration rates not related to flows indicated factors prior to release could account for these differences. Consultations with hatchery production staff, especially to verify rearing information pertinent to our interpretation of patterns of smoltification discovered during the past ten years, resulted in the present survey. A major activity of 1998 will be to combine hatchery rearing data with the ASCTTA comprehensive database to determine which rearing factors make the greatest contribution to juvenile migration success.

The Assessment of Smolt Condition for Travel Time Analysis Project continues to monitor and document smoltification in salmonid species of the Columbia River basin. Acceptance of the need for flow augmentation to enhance juvenile salmon emigration and survival has resulted in the redirection of our smolt monitoring program to the evaluation of other management practices to improve smolt quality.

ACKNOWLEDGEMENTS

We thank the many people and organizations who made the Assessment of Smolt Condition for Travel Time Analysis project possible, including the Bonneville Power Administration, Fish Passage Center, U.S. Fish and Wildlife Service, National Marine Fisheries Service, U.S. Army Corps of Engineers, Idaho Department of Fish and Game, Oregon Department of Fish and Wildlife, Washington Department of Fish and Wildlife, the Smolt Monitoring Program of the Chelan County Public Utility District, and the Nez Perce Tribe. The cooperation of managers and staff of federal and state hatcheries in the Columbia and Snake River basins was instrumental to this project. We are grateful to the past and present staff of the Columbia River Research Laboratory for their efforts on the project, including Derrek Faber, Joyce Faler, Mary Free, Rodney Garland, Diane Liljegren, Robert Reagan, David Venditti, Eric Wagner, and especially Dennis Rondorf.

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LIST OF ACRONYMS, UNITS, AND NOMENCLATURE

Organizations/Offices/Programs

ASCTTA = Assessment of Smolt Condition for Travel Time Analysis

BPA = Bonneville Power Administration

BRD = Biological Resources Division of the USGS

COE = United States Army Corps of Engineers

CRRL = Columbia River Research Laboratory

DFO = Canada Department of Fisheries and Oceans

ESA = Endangered Species Act

FPC = Fish Passage Center

FWP = Fish and Wildlife Program

IDFG = Idaho Department of Fish and Game

NFH = National Fish Hatchery

NPPC = Northwest Power Planning Council

NMFS = National Marine Fisheries Service

ODFW = Oregon Department of Fish and Wildlife

SFH = State Fish Hatchery

SMP = Smolt Monitoring Program

USFWS = United States Fish and Wildlife Service

USGS = United States Geological Survey

WDFW = Washington Department of Fish and Wildlife

Scientific Terminology/Units

ATPase units = $\mu\text{mol P}_i \cdot \text{mg protein}^{-1} \cdot \text{h}^{-1}$

BKD = bacterial kidney disease

Cl⁻ = plasma chloride

ELISA = enzyme-linked immunosorbent assay

FAT = fluorescent antibody test

GBT = gas bubble trauma

K = condition factor

MS-222 = tricaine methane sulfonate, used to anaesthetize fish for handling

Na⁺, K⁺-ATPase = gill sodium, potassium-activated adenosine triphosphatase

OD = ELISA optical density

PIT tag = passive integrated transponder tag

RIA = radioimmunoassay

rkm = river kilometers

RS = *Renibacterium salmoninarum*

T₄ = thyroxine

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INTRODUCTION

The Assessment of Smolt Condition for Travel Time Analysis Project (ASCTTA) has evaluated the effects of physiology and fish condition on smoltification and seaward migration of juvenile salmonids in the Columbia River basin for more than ten years. The project has provided the Smolt Monitoring Program (SMP) of the Fish Passage Center (FPC) with biological and migration data for travel time analysis for juvenile salmonids (*Oncorhynchus spp.*) in the Columbia and Snake Rivers for Water Budget management. The Water Budget was used to allocate water to enhance flows during salmon migration. The measurements of smolt condition generated by the ASCTTA project were used by the FPC to guide flow management to improve juvenile survival during emigration. The emphasis of the project was to determine what effect smolt condition had on travel time of juvenile salmonids of the Columbia and Snake Rivers under different flow regimes. As Water Budget and fish passage management has changed in the region, the project has adapted by reorganizing monitoring efforts to fulfill the needs of regional fishery managers, and by developing new non-lethal methods to assess smolt condition of fish listed under the Endangered Species Act. The project now provides technical support to fish managers in the Columbia River basin by providing physiological information for a wide range of regional juvenile salmonid and smolt monitoring projects.

The ASCTTA project was initiated in 1987 to investigate the relation between biological characteristics of juvenile salmon and steelhead, and successful emigration. The project developed from the need to determine the effect of fish condition on migration rates and survival. The ASCTTA project provided the FPC with information on fish condition and health, under a program of physiological and health monitoring, for Water Budget management of flows to enhance juvenile emigration. Central to a characterization of the project is an understanding of smoltification, and how measures of smolt assessment have been modified throughout the project history to meet management objectives in the region. The extensive record of smolt monitoring activities and information for the ASCTTA project is available in annual reports to the Bonneville Power Administration. A review of annual project activities is provided in Section One, Part One, of this report.

The Fish Passage Advisory Committee (FPAC) determined that daily monitoring of smolt condition and health was no longer required for in-season river flow management during 1997. The committee acknowledged that the data are important to an understanding of smoltification, migration behavior, and survival in the Columbia River basin. We were directed to analyze existing data and determine in what capacity the project should perform in the future. Although in-river monitoring activities of the ASCTTA project ceased, we continued to provide the physiological monitoring capacity to a number of regional projects funded directly or indirectly under the mandate of the Fish and Wildlife Program. We have provided a summary of smolt monitoring assistance activities that were requested in 1997 and 1998, in Section Two, Part One.

Travel time analysis to estimate the effects of smolt condition and river flow on emigration times of juvenile salmonids was the core objective of the project since 1988. A summary of travel time analysis for 1988 to the present (Section One, Part Two) describes the relation of smolt condition and flow to travel time for our reference stocks. The measurement most commonly used to determine the physiological status of juvenile migrants is gill sodium, potassium-activated adenosine triphosphatase (Na^+ , K^+ -ATPase). We provide a brief ten-year review of pre- and post-release ATPase profiles of our reference stocks to accompany the travel time analysis information (Section One, Part Three). Results of monitoring for bacterial kidney disease (BKD) prevalence are summarized for 1987 through 1996 (Section One, Part Four).

Project history may be divided into two major parts. During the first five years, the emphasis of the project was on survival and travel time estimates for management of the Water Budget. Marking, particularly freeze branding, was used to provide stock-specific information on migration characteristics. Physiological monitoring to assess handling stress sought to determine how hatchery rearing and transport practices affected survival estimates. Standard assays for plasma cortisol, glucose, and thyroxine were used to compare groups of fish under different release and transport schemes, with gill Na^+ , K^+ -ATPase being the standard reference measure of smoltification. During the subsequent five years, we continued to provide information on smoltification and health to the FPC, while requests from other fish management agencies for monitoring of smolt indices for associated projects increased. Based on the performance of hatchery stocks in the region, fishery managers developed new rearing and release techniques to enhance smolt development before release and survival during migration. Those entities familiar with our monitoring project have requested advice for project design and technical assistance in the form of training, sampling and analysis, and interpretation of results. Fish from cooperative projects are usually stocks that have served as ASCTTA reference stocks for the FPC Smolt Monitoring Program. A long-term project database, described in this report (Section Two, Part Three), exists for baseline comparison in interpreting the results of new management techniques for promoting growth, smoltification, emigration, and smolt-to-adult survival. Combining the hatchery survey data with this database is our primary objective for 1998.

The review of ASCTTA data for the past decade revealed changes in the seasonal profiles of smoltification for our reference stocks that could not be explained entirely by river flows. Hatchery release data indicated that many changes in rearing practices had occurred during the ten-year period. An important objective of the 1997 work statement was to develop a consistent database of the health and physiology of fish prior to release from the hatcheries. Following an extensive literature search, we developed a hatchery survey based on our physiological database, expanded to include hatchery rearing variables that might contribute to differences in smolt development and survival. A description of the survey is included in this report (Section Two, Part Four). During 1998, the combined databases will be used to determine what factors during hatchery rearing influence indices of smoltification used in monitoring programs.

SECTION ONE
PART ONE

Assessment of Smolt Condition for Travel Time Analysis
Project Review 1987 to 1996

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Development of the Smolt Monitoring Program (SMP) was initiated by the Northwest Power Planning Council (NPPC) Fish and Wildlife Program (FWP). The SMP called for the Bonneville Power Administration (BPA) to fund a tribal and multiple-agency program to monitor migration characteristics of anadromous salmonids in the Columbia River basin. Smolt monitoring research was implemented by the National Marine Fisheries Service (NMFS) in the early 1980's, and included annual estimates of survival and travel time of marked groups of migrating salmonids through river reaches. In 1984, administration of the Smolt Monitoring Program was transferred from NMFS to the Fish Passage Center (FPC) (formerly the Water Budget Center). The FPC was responsible for management of the Water Budget, a volume of water in the hydropower system set aside to enhance river flows during the spring and summer emigration of juvenile salmonids. This strategy was based on the hypothesis that decreased travel time and increased survival of migrants could be achieved with higher seasonal flows. Emphasis of the SMP shifted from research under NMFS to adaptive management under the FPC, which was mandated to oversee and evaluate the annual water budget and other hydropower systems operation. The FPC compiles information about annual physical river conditions and migrational characteristics of juvenile salmonids, including: flow; water temperature; timing, duration and magnitude of the smolt migration; and the travel time of marked groups of migrating fish from upriver locations on the Snake and mid-Columbia rivers to a collection site on the lower Columbia River. Figure 1 shows the locations of sampling sites used during the ASCTTA project's ten-year history.

Early in the program, studies on marked groups of fish indicated significant differences in survival estimates between paired test (released at hatcheries) and control (truck-transported to downstream release sites) groups of fish. Survival estimates of over 100% suggested that statistical assumptions of the monitoring design had been violated. The assumptions were that test and control groups of fish were a) identical at the time of release, and b) identical at the recovery site, McNary Dam, and therefore equally susceptible to collection (Rondorf et al. 1988). By 1986, Water Budget managers recognized that fish health and physiological status might account for apparent errors in survival estimates. The 1986 Smolt Monitoring Program

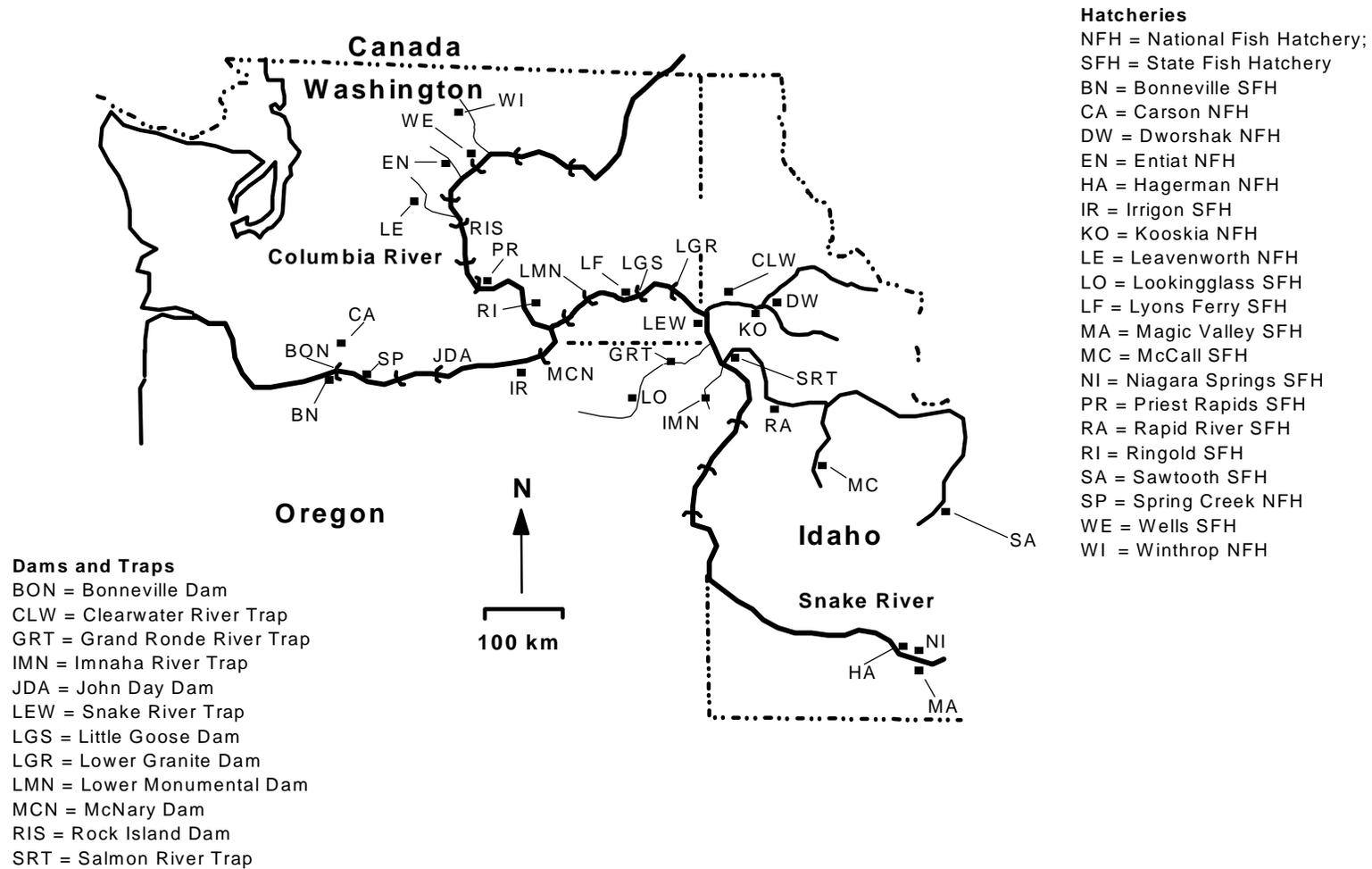


Figure 1. Location of sampling sites in the Columbia and Snake River basins for the Assessment of Smolt Condition for Travel Time Analysis Project, 1987-1997 (BPA Project No. 87-401).

incorporated subjective observations of fish health and mean length measurements of replicate groups of marked fish, and initiated in-river sampling and marking of fish.

1987

In 1987, the SMP increased its emphasis on fish health and physiological status, including prevalence of disease (bacterial kidney disease), transport-induced stress, and selected physiological measurements of smoltification of marked and in-river groups of fish. The work was contracted out to the Columbia River Research Laboratory (CRRL), U.S. Geological Survey, Biological Resources Division, formerly the Columbia River Field Station, U.S. Fish and Wildlife Service, under the ASCTTA project.

The objective of smolt monitoring work at the CRRL in 1987 was to determine if transport-induced stress could account for differences between test and control groups of steelhead (*Oncorhynchus mykiss*) used for survival and travel time estimates by the FPC. We also investigated the response of yearling spring chinook salmon (*O. tshawytscha*) to handling stress. The approach was to subsample groups of steelhead before and after long- and short-term truck transport and compare them to control groups released directly from the hatchery. To evaluate the acute stress response of steelhead to transport situations, we measured blood plasma levels of cortisol and glucose of fish prior to release, at release, and at McNary Dam, the downstream recapture site. Plasma cortisol and glucose levels from groups of fish sampled pre-release and at release were compared to samples collected from fish challenged with a standardized handling stress to evaluate stress response. Changes in blood cortisol and glucose levels are reliable indices for measuring the stress response of fish--both increase in response to acute and chronic stress (Wedemeyer and McLeay 1981).

We also began investigating the degree of smoltification in groups of marked transport fish in 1987. There was concern that longer in-river travel time of test groups compared to control groups of fish might enhance smolt development and bias survival estimates that assumed test and control groups were identical. The objective was to determine if different stages of smolt development influenced fish guidance efficiency at downriver collection sites. To investigate the degree of smolt development in juvenile salmonids, we utilized biochemical analysis of gill sodium, potassium-activated adenosine triphosphatase (Na^+ , K^+ -ATPase) levels, and plasma levels of the thyroid hormone, thyroxine (T_4). Prior research and development of a biochemical assay for gill Na^+ , K^+ -ATPase levels in smolting salmonids was accomplished by Zaugg (1982), and provided an excellent analytical tool for monitoring smolt development. Zaugg and McLain (1972) found that gill Na^+ , K^+ -ATPase levels were low in juvenile salmonids prior to smoltification and that levels of the enzyme increased as fish underwent smoltification. Increases in gill Na^+ , K^+ -ATPase levels of anadromous salmonids occur with the proliferation of chloride (Cl^-) cells in gill tissue and prepare juvenile fish for osmoregulation in seawater (see Hoar 1988). Dickhoff et al. (1978) followed blood levels of T_4 during the parr-to-smolt transformation, and identified a significant surge in T_4 prior to increases in gill Na^+ , K^+ -ATPase. The

radioimmunoassay (RIA) for measuring blood levels of thyroid hormones in salmonids, adapted by Dickhoff et al. (1978) and modified by Specker and Schreck (1982), made investigation of T_4 measurements feasible in our smoltification assessment.

A morphometrics method was also employed to assess the degree of smoltification. Using an apparatus with a fixed camera, background, and light level, individual fish were photographed from test and control groups as they were collected at McNary Dam. Subsequently, photographs of fish were digitized and the lengths of line segments between specific morphological landmarks on the fish were measured. This was similar to a method developed by Winans (1984), and was based on well-documented changes in body shape during the parr-to-smolt transformation (see Hoar 1988).

As part of the effort to understand the influence of smolt development on survival estimates of marked groups of fish, 24-hr seawater challenge performance tests were conducted at McNary Dam on test and control groups of steelhead from Wells State Fish Hatchery. Fish were collected at McNary Dam and held for 24 hr in an artificial stream tank supplied with fresh ambient temperature Columbia River water. Fish were then transferred to a similar tank containing 30 parts per thousand (ppt) salinity for 24 hrs. After the 24-hr seawater challenge, samples were collected to determine gill Na^+ , K^+ -ATPase levels, plasma osmolarity, as well as plasma Na^+ , K^+ , and Cl^- levels. Measurements of gill Na^+ , K^+ -ATPase levels in test and control groups of steelhead provided information on whether differences in the in-river travel time between the groups resulted in significantly different enzyme levels. The seawater challenge trials allowed us to determine whether differences in gill Na^+ , K^+ -ATPase levels translated into differences in osmoregulatory competence as quantified by Na^+ , K^+ , and Cl^- ion concentrations in the plasma of fish. Osmoregulatory competence in seawater is the ultimate consequence of smolt development, wherein a fully functional smolt is physiologically capable of maintaining plasma osmolarity and blood ion homeostasis when exposed to a hyperosmotic seawater environment. Less-smolted fish will show increases in plasma ion concentrations and osmolality due to osmotic influx of these ions from the seawater environment. Plasma osmolarity was measured using a vapor pressure osmometer, plasma Na^+ and K^+ levels were measured with a flame photometer, and plasma Cl^- levels were determined using a chloridometer.

Prevalence of bacterial kidney disease (BKD) in hatchery fish was hypothesized to influence the in-river survival of fish. BKD levels were determined in pre-release and recovery groups of spring chinook salmon from Winthrop National Fish Hatchery in 1987, employing two methods for determining prevalence of BKD in fish. A fluorescent antibody test (FAT) was available for detecting the causative agent of BKD, *Renibacterium salmoninarum*, in smears of kidney or spleen tissue. An enzyme-linked immunosorbent assay (ELISA) method for BKD, developed by Pascho and Mulcahy (1987), which detects soluble antigens of *R. salmoninarum*, was also used. The research on the prevalence of BKD in Winthrop spring chinook salmon found the ELISA method sensitive enough to detect differences between hatchery-released test and transported control groups of fish. We also found that the prevalence of BKD was higher in

hatchery populations at upriver release sites than at McNary Dam, suggesting that fish with higher levels of BKD were disappearing from the population during migration. In addition, later migrating spring chinook salmon from Winthrop showed a greater percentage of individuals with acute BKD infections. The pattern of higher BKD levels in spring chinook salmon at hatcheries and upstream collection sites compared to downstream collection sites, and greater prevalence of acute BKD infections later in the migration, has also been identified between Lower Granite Dam on the Snake River and McNary Dam on the Columbia River (CRRL, unpublished data).

The results of research in 1987 provided compelling evidence that differences did exist in the physiological status of test and control groups of steelhead and spring chinook salmon. The results indicated that the experimental design used to calculate survival estimates of migrating juvenile salmonids did not satisfy assumptions about the similarity of test and transported control fish at release and upon recapture. Based on plasma cortisol levels, we found control steelhead less stressed than hatchery released test fish. Hatchery released spring chinook salmon showed low levels of plasma cortisol, while transported groups of spring chinook salmon showed a normal stress response.

A complete description of 1987 ASCTTA results can be found in Rondorf et al. (1988).

1988

In 1988, the FPC dropped survival estimates, relying on travel time estimates of marked groups of fish through index reaches to provide information for in-season management of the Water Budget and post-season analysis of the migration. Our directed research for the SMP in 1988 was to measure stress, smoltification, prevalence of BKD, and to investigate a potential smolt index for selected groups of steelhead and spring chinook salmon used by the FPC in their travel time estimates. Stress assessment in 1988 followed two strategies: 1) monitor the stress level of groups of spring chinook and steelhead before and after truck transport to release sites and 2) expose selected groups of marked fish to a handling stress, then monitor stress levels at the hatcheries, at release, and when collected at McNary Dam. As in 1987, the stress assessments were based on plasma cortisol and glucose levels. Plasma Cl^- values were added to the 1988 stress assessment as a sensitive indicator of stress-induced osmoregulatory compromise.

Other smolt monitoring activities from 1987 continued in 1988, accompanied by several new developments. Monitoring of the prevalence and severity of BKD continued, with the comparison of results of the ELISA and FAT techniques for BKD. Development of the technique to measure changes in body morphology of fish during smoltification also continued. We investigated the degree of smoltification in selected groups of marked fish, but dropped plasma osmolality and Na^+ and K^+ determinations from the analysis. Skin purine analysis was added to our investigation to provide a quantitative measurement of body silvering, another developmental characteristic of smolting salmonids. Two purines were measured in the skin of fish, guanine and

hypoxanthine, using the enzyme method of Staley (1984). Although guanine and hypoxanthine are present in the skin layers of parr, deposition of these purines increases in smolts, a byproduct of changes in purine nitrogen metabolism during the smolt transformation (Hoar 1988). Body silvering in juvenile anadromous salmonids is thought to preadapt these fish for the pelagic seawater stage of their life cycle.

In 1988, we investigated the use of smolt condition indices as tools for judging the overall condition of fish and probability of survival after hatchery release. Such information could be used to assist in management and evaluation of the Water Budget. Preliminary research identified several parts of the organosomatic examination method developed by Goede (1988) as having potential application in monitoring fish condition at hatcheries and dams, particularly if a numerical rating was employed. Our research goal was to develop indices that would provide a relative evaluation of fish health and smolt quality from set criteria. Development of the indices required establishing strong correlations between physiological measurements of smoltification and fish health. For example, our field and laboratory data showed a strong positive correlation between guanine levels in the skin of fish and gill Na^+ , K^+ -ATPase activity. Findings such as this raised the potential for the eventual development of a reliable, non-lethal set of criteria for establishing overall health and degree of smoltification in fish.

Research on stress in 1988 suggested that degree of smoltification, and differences in water temperatures experienced by juvenile salmonid stocks from diverse locations in the Columbia River basin, may have influenced the ability of different hatchery groups to respond to stress. Multiple stressors, such as low oxygen (O_2) levels in the water and stressful hatchery operations (raceway cleaning, fish marking), may have contributed to the observed stress response pattern of various groups of fish.

Results of the ASCTTA project in 1988 are summarized in Rondorf et al. (1989).

1989

Investigations into the effects of stress, smoltification level, and BKD on travel time and survival of smolts continued in 1989. We began sampling the juvenile salmonid migration-at-large in the spring, and the juvenile fall chinook migration during the summer. The approach to assessing stress and incidence of BKD in spring chinook salmon and steelhead continued from 1988.

Assessment of smolt development, as indicated by gill Na^+ , K^+ -ATPase and plasma T_4 levels in marked groups of fish released from the hatcheries and recaptured at downstream collection sites, continued in 1989. Sampling expanded to include groups of yearling and subyearling fall chinook salmon in addition to spring chinook and steelhead. Gill Na^+ , K^+ -ATPase levels and in-river travel times of marked fish through index reaches were compared with flow

levels. The general pattern that emerged from the analysis was that gill Na^+ , K^+ -ATPase levels increased and migrant travel time decreased with increasing flow. However, disposition to migrate and increased gill Na^+ , K^+ -ATPase activity are two separate aspects of smolt development (see Hoar 1988). Increases in gill Na^+ , K^+ -ATPase activity and migration rate can be achieved in yearling spring chinook salmon by advancing photoperiod before hatchery release (Muir et al. 1994). During the parr-to-smolt transformation, increased gill Na^+ , K^+ -ATPase activity and development of migratory tendency occur despite river flow. Increased river flow accelerates downstream movement of fish, thereby decreasing travel time of migrating juvenile salmon (Berggren and Filardo 1993; Jonsson 1991; Raymond 1968, 1979; Smith et al. 1998). Increased flows may also displace pre-smolts not physiologically ready to migrate downstream (Ewing et al. 1980; Jonsson 1991). River flow can hasten or lengthen migrant travel time, depending on seasonal runoff conditions. Interestingly, Smith et al. (1998) did not find significant within-year correlations between river flow and travel time, or river flow and survival for passive integrated transponder (PIT)-tagged spring chinook salmon and steelhead for index reaches in the Snake and lower Columbia rivers. However, when the authors combined all points from several study years, highly significant correlations emerged between river flow and travel time, as well as river flow and survival through index reaches.

We continued to investigate the feasibility of an index of smoltification in 1989. We measured changes in condition factor (K or K factor), body silvering as indicated by guanine content in the skin, and body morphology as fish underwent the parr-to-smolt transformation. Condition factor was correlated with gill Na^+ , K^+ -ATPase levels, although the two measures are not functionally related. The health and nutrition of fish were found to affect the condition factor as well. Mean guanine content of skin samples was significantly correlated ($r = 0.52$, $P < 0.05$) with mean plasma T_4 , but not mean gill Na^+ , K^+ -ATPase levels, in spring chinook salmon. For steelhead, mean guanine correlated significantly with mean gill Na^+ , K^+ -ATPase levels ($r = 0.90$, $P < 0.05$) and date of sample. Some overlap in the components of body morphometric determinations at the hatcheries and corresponding migrants at McNary Dam was noted. Overall, development of a non-lethal index of smoltification appeared promising from our 1989 research.

Upstream sampling of the smolt migration occurred at Rock Island Dam on the mid-Columbia River, the Snake River Trap at Lewiston, and Lower Granite Dam on the Snake River. Downstream sampling was conducted at McNary Dam. At upstream locations, gill Na^+ , K^+ -ATPase levels were significantly higher in wild steelhead than in hatchery-reared steelhead. However, at McNary Dam, the difference in gill Na^+ , K^+ -ATPase activity was not significant. Spring chinook salmon showed the highest gill Na^+ , K^+ -ATPase activity late in the migration at upstream locations, while maximum enzyme activity at McNary Dam occurred during the middle of the migration. It is not surprising that late-migrating fish showed higher gill Na^+ , K^+ -ATPase levels upstream than at McNary Dam; increasing water temperature to about 12°C has been shown to stimulate gill Na^+ , K^+ -ATPase activity in steelhead, coho, and Atlantic salmon (*Salmo salar*) (Adams et al. 1973; Johnston and Eales 1970; Zaugg and McLain 1972). Zaugg (1981a) showed that gill Na^+ , K^+ -ATPase activity was inhibited by water temperatures above 12°C in

steelhead, and over 15°C in coho, chinook, and Atlantic salmon (Clarke and Blackburn 1977; Donaldson and Brannon 1975; Komourdjian et al. 1976; Saunders and Henderson 1970; Zaugg and McLain 1976).

The measurement of stress in control and transported groups of juvenile salmonids was discontinued after 1989, since most groups of fish displayed what was considered a characteristic “normal” physiological response to the stress of transport, including elevated plasma glucose and cortisol levels, and decreased plasma chloride concentrations. Exceptions occurred when there were deviations in transport protocol or when stressful hatchery operations such as marking of fish or raceway cleaning occurred a short time before transport. We encouraged agencies to develop strict guidelines for transport procedures to minimize stress on fish.

Beeman et al. (1990) describes results of ASCTTA work conducted in 1989.

1990

Our research initiatives in 1990 were to continue collecting physiological information on juvenile salmonids used for travel time estimates, analyze this data, and continue research and development of an index of smolt condition. Investigation of smolt development and incidence of BKD in marked groups of hatchery fish remained unchanged from 1989, although we dropped the FAT assay for BKD, relying solely on the more sensitive ELISA technique. In 1990, we began synthesis of the effects of flow and smoltification on travel time, pooling data from 1989 and 1990. We considered travel time of spring chinook salmon from Rock Island Dam to McNary Dam, Snake River Trap to Lower Granite Dam, and Lower Granite to McNary Dam; and steelhead travel time from Lyon’s Ferry to McNary Dam. In addition, travel times of juvenile spring chinook salmon from the Clearwater Trap to Lower Granite Dam were determined for the first time. The results from this limited data set varied between index reaches and species. Generally, we found that as flow and/or gill Na^+ , K^+ -ATPase levels increased, travel time of migrants decreased. Migratory readiness, as indicated by gill Na^+ , K^+ -ATPase activity, influenced travel times to a greater extent during periods of low river flow than during high-flow periods. Analysis of travel time databases from 1989 and 1990 underscored the idea that a multi-year database would be required to thoroughly analyze the relationship of flow and gill Na^+ , K^+ -ATPase to fish travel time. Due to the great variation in flow across years, these relationships could not be fully represented by two years of data.

Development of a non-lethal smolt condition index continued in 1990 with further research on the relationship between gill Na^+ , K^+ -ATPase activity, condition factor, and skin reflectance. Skin guanine analysis was used to check the validity of skin reflectance measurements. Skin reflectance was negatively correlated with condition factor, and both variables were highly correlated with gill Na^+ - K^+ ATPase activity in steelhead. However, results were less encouraging for spring chinook salmon. High correlations were found between skin reflectance and gill Na^+ , K^+ -ATPase activity, yet a much weaker correlation was found between

skin reflectance and guanine levels in spring chinook salmon. Synthesis of smolt index data collected in 1988, 1989, and 1990, though promising, indicated continued development, testing, and preliminary application were necessary to examine inter-specific differences and the influence of factors other than smoltification on fish condition.

Seawater tolerance trials were included in smolt index development in 1990, as a method of evaluating smolt condition. These trials involved transport of selected groups of fish to the Marrowstone Field Station (USGS-BRD, formerly USFWS), Nordland, Washington, acclimation for several days in freshwater following transport, increasing seawater exposure to full seawater (28 ppt) over a four-day period, then rearing fish in seawater for 180 days. Mortality was monitored for three hatchery rearing treatments, fish taken directly from the hatchery versus in-river migrants, and migrant fish classified into several groups based on visual characteristics. Kidney and spleen samples were collected to determine and compare prevalence and severity of BKD in the different treatment groups of fish. Though hatchery and migrant groups had similar incidence of BKD, migrant fish had higher mortality in seawater, and higher prevalence of BKD than hatchery fish after seawater residence. We suggested that increased sensitivity to stress due to smoltification, in combination with stress associated with transportation to the research facility, may have negatively influenced the capacity of the more smolted migrants to fight infection (Beeman et al. 1990). The results showed similar mortality rates between hatchery treatment groups, but higher mortality in migrant groups than in hatchery groups. Migrant groups collected at Bonneville Dam had higher mortality than migrants collected upstream at McNary Dam. Higher mortality in migrant fish in seawater may have been directly related to their developing higher incidence of BKD after transfer to seawater. Sanders et al. (1992) found evidence that fish with BKD may die upon entering saltwater.

Results of the ASCTTA project in 1990 are given in Beeman et al. 1991.

1991 and 1992

Sampling efforts in 1991 and 1992 were similar to 1990. The combined 1991-92 biennial report to BPA emphasized synthesis of multi-year data into research publications. Analysis of travel time of spring chinook salmon and steelhead in index reaches in the Columbia and Snake rivers in relation to physiological and environmental variables for the years 1989 through 1992 is summarized in the 1991-92 report (Maule et al. 1994). Prevalence and severity of BKD in spring chinook salmon monitored from 1988 through 1992 was reported by Maule et al. (1996); a summary of data on BKD for the years 1987 through 1996 is included in this report (Section One, Part Four). Completed research products for a non-lethal index of smoltification were also given in the 1991-92 report. Three non-lethal methodologies were developed: a micro-assay for gill Na^+ , K^+ -ATPase activity (Schrock et al. 1994), a photo reflectance video analysis system for skin reflectance (Haner et al. 1995), and a morphometric measurement which employed digitized anatomical landmarks from photographs of fish (Beeman et al. 1994, 1995).

1993

Significant changes were made in the ASCTTA project in 1993. The FPC discontinued routine freeze-branding of hatchery fish, and initiated use of PIT-tagged individuals to determine travel times. In 1993, a single freeze-branded group (Priest Rapids fall chinook) was monitored, out of a total of 12 releases from reference hatcheries. Several new reference groups were added to the project. Subyearling chinook and sockeye salmon from the run-at-large, as well as wild steelhead, were sampled at Rock Island Dam on the mid-Columbia River. We began monitoring physiology of wild fish for a comparison with hatchery stocks. In the Snake River, comparison of wild and hatchery spring chinook and steelhead was made possible by the identification of all hatchery fish in Idaho with an adipose fin clip. Monitoring was expanded to include the Salmon River Trap, and sampling continued at the Snake River Trap at Lewiston, and the Clearwater Trap.

During 1993, a microassay for the determination of gill sodium, potassium-activated adenosine triphosphatase activity (Na^+ , K^+ -ATPase) was developed (Schrock et al. 1994) to replace the method of Zaugg (1982a) that had required sacrificing fish. The microassay allowed fish to be released and resampled further in the migration, but the smaller tissue pieces required more careful handling and extraction. Tagging of individuals with PIT tags allowed for correlation analysis of smolt indices including ATPase, condition factor, and flow with travel times for individuals rather than means of release groups.

The development of other non-lethal methods as indices of smolt condition progressed in 1993, with completion of analysis of morphometric measurements from juvenile spring chinook from 1988 to 1990 (Beeman et al. 1994). The 34 morphometric characters used were measurements of distances between 15 anatomical landmarks from digitized photographs. A single canonical variate, which described variation in several measurements, correlated significantly with ATPase activity in both spring chinook salmon (Beeman et al. 1994) and steelhead (Beeman et al. 1995).

Accompanying progress with non-lethal monitoring methods for ATPase, reflectance, and morphometrics, were investigations into a measurement to assess health. A component of non-specific immune response, lysozyme, was monitored in conjunction with other ongoing laboratory and hatchery experiments. The approach was to determine baseline levels for ASCTTA reference stocks, and to document the lysozyme profile during the period associated with smoltification. Sampling with established research projects and hatchery activities allowed us to complete a comprehensive survey of lysozyme activities in Pacific salmon and steelhead without establishing a separate program. Results for 1993 and 1994 include juvenile and adult coho salmon, chinook salmon, and steelhead. Preliminary results have been analyzed and are reported in the 1993-94 annual report.

Because fish were no longer sacrificed for gill ATPase tissue collection, BKD monitoring was discontinued at the dams. Health monitoring continued with assessment of BKD severity in hatchery fish and fish in reservoirs, using an ELISA method. Monitoring continued, to determine if the decrease in prevalence of BKD in a majority of the reference hatcheries since 1992 was continuing. A review of BKD monitoring efforts from 1987 to 1996 is included in this report (Section One, Part Four).

In the spring of 1993, we assessed the health and condition of fish captured, PIT- tagged, and released as part of the National Marine Fisheries Service's (NMFS) Lower Granite Survival Study (Iwamoto et al. 1994). Hatchery spring chinook salmon were collected in Lower Granite Reservoir, Lower Granite Dam, and Little Goose Dam, PIT-tagged, and released after a 24-hr recovery period. The purpose was to determine whether physiological or morphological differences between groups related to differences in fish survival. Analysis of plasma cortisol did show that fish sampled at the dams were more stressed than the fish collected in the reservoir, but other physiological data suggested no biological differences when comparing releases within a site. The experience of being collected, held in tanks, and released at the dams was more stressful for the fish than being purse seined, held in net pens, and released in the reservoir. Smolt development progressed as the fish travelled downstream, as evidenced by the increase in gill ATPase and decrease in condition factor from upstream to downstream sites. The data supported the findings of Iwamoto et al. (1994) who found no differences in travel time or survival between multiple releases of fish from an individual site.

Results of monitoring activities in 1993 are summarized in Section One, Parts Three and Four.

1994

Changes in project sampling continued in 1994. Hatchery release groups were increased to 15 in 1994, and two additional traps on Snake River tributaries were added for hatchery and wild spring chinook and steelhead: the Imnaha and Grande Ronde Traps. We also sampled freeze-branded spring chinook salmon from Dworshak National Fish Hatchery, part of an Army Corp of Engineers (COE) funded project, to field test the computer system for reflectance and morphometric measurements. We determined that, because high flows reduced trap efficiency and altered expected patterns of fish passage, our daily sample size should increase during high flow periods.

Sampling with the NMFS Lower Granite Survival Study (Muir et al. 1995) continued from 1993. Yearling hatchery steelhead and hatchery spring chinook salmon were collected at Lower Granite Reservoir, Lower Granite Dam, Little Goose Dam, and Lower Monumental Dam, and were PIT-tagged and released as part of the survival study. Physiological samples were again collected to explain any observed differences in survival estimates between groups of PIT-tagged

fish released at the different sites. The data showed that gill ATPase increased over time in hatchery spring chinook and hatchery steelhead when sampled at one site. Gill ATPase also tended to increase as the fish traveled downstream from one site to the next. Neither of these conclusions were unexpected, as gill ATPase has been shown by numerous researchers to increase over time at a particular site, and as the fish travel downstream (Beeman et al. 1994; Zaugg and McLain 1972). Although several differences in gill ATPase within a release site were statistically significant, Muir et al. (1995) reported no significant differences in survival between any of the release groups from a particular site. This suggests that the statistically significant differences in gill ATPase were not biologically significant for the survival of the fish.

Routine smolt assessment continued to document smoltification in run-at-large fish, to determine changes in smoltification status in migrants over time, and to correlate smoltification and flow with travel time in individual fish. Hatchery fish were screened for BKD prevalence before release, and ATPase values of migrants were provided to the FPC on a daily basis for in-season management of the Water Budget.

Development of non-lethal methods continued with the screening of hatchery release groups for nare (nasal) mucus lysozyme levels. Nare mucus was collected because contamination of skin mucus from the water, or vent mucus samples with feces, appeared to increase variability among samples. Baseline levels of nare mucus lysozyme activity were found to be uniform among hatchery stocks just prior to release. Individuals of a single stock of spring chinook salmon reared at Rapid River SFH in Idaho and Lookingglass SFH in Washington had the highest mean nare mucus lysozyme levels among ten hatcheries. Continued monitoring is needed to determine what genetic and environmental factors contribute to baseline levels. Long-term sampling of coho and chinook salmon at two other hatcheries (Willard and Little White Salmon NFH) documented a decline in mucus lysozyme levels during the time associated with smoltification, but found little difference between the two species at the same time of year.

A summary of 1994 results is given in Section One, Parts Three and Four, of this report.

1995

A major change in both the objective and organization of the project came in 1995, when the FPC requested that the ASCTTA project assume the responsibility for monitoring fish for signs of gas bubble trauma (GBT) at six dams on the Snake and Columbia Rivers. Experienced project staff were dedicated to the task of developing a protocol for assessing GBT in salmon and steelhead.

Routine sampling for the SMP continued at 13 hatcheries, five traps and four dams. Sampling of two hatcheries not included in the 1995 SMP was continued, in order to provide the long-term monitoring of BKD prevalence initiated at these hatcheries in 1988. Comparison of correlations of ATPase, flow, and travel time using individual PIT-tagged fish in 1993 with

correlations from means or medians of groups of fish made prior to 1993 indicated that no additional information was gained by using individual fish. The number of fish sampled at dams was therefore reduced. Power analysis of the variability of gill ATPase levels in 1990 from McNary Dam, Rock Island Dam, and the Snake River Trap revealed that sample sizes could be reduced to 30 for a detectable difference of 6 to 8 ATPase units. Reduction of sample sizes came at a critical time when declining fish numbers and Endangered Species Act listings called for a more conservative sampling approach.

We investigated the influence of disease and environment on mucus lysozyme levels. In cooperation with the USFWS Abernathy Salmon Culture Technology Center, plasma or serum lysozyme and mucus lysozyme were determined in fish fed experimental diets containing glucans. Glucans, which are polysaccharides found in the cell walls of yeast and fungi, have been shown to increase lysozyme levels in fish during bath, injection, and feeding trials. Interest at hatcheries in the region focuses on the possible use of glucans in production feeds to enhance growth, improve immunity, and promote rapid seawater entry of juvenile salmonids. We found increased growth in fish fed the glucans, but interpretation of results was confounded by the seasonal decline in lysozyme that accompanied smoltification during the experiment.

In a cooperative study with the Canada Department of Fisheries and Oceans, we tested the effects of pollution, specifically bleached kraft mill effluent, on lysozyme levels. Preliminary results determined a slight rise in lysozyme levels in fish held in more concentrated effluent, but temperature control in the experiment may have influenced lysozyme levels. This experiment was also conducted during the period associated with smoltification, which may have confounded results.

Results of physiological monitoring at hatcheries and dams in 1995 is reviewed in Section One, Parts Three and Four.

1996

The major change to the ASCTTA project in 1996 was the establishment of gas bubble monitoring as a separate project. Skilled staff from the projects were shared. Smolt monitoring was continued at 13 hatcheries and two traps, but only two dams, Lower Granite and Rock Island. PIT-tagged fish sampled for gill ATPase at the Salmon and Snake River Traps were detected and resampled at Lower Granite Dam. We continued to collaborate with Abernathy and Canada DFO to characterize our non-lethal methods under different environmental conditions, including the effects of pollutants and disease.

See Section One, Parts Three and Four, for summaries of 1996 results.

Summary

Monitoring activities of the ASCTTA project have provided an unbroken record of smolt physiology in the Columbia basin for over a decade. The measurements of gill ATPase, condition factor, and BKD prevalence describe the condition and status of emigrating juvenile salmon under a variety of seasonal temperature and flow regimes. The project expanded from two reference hatcheries in 1987, to a maximum of 15 in 1992. Emphasis changed from tracking freeze-branded hatchery releases, to run-at-large monitoring of PIT-tagged individuals. A major turning point in the project was a restriction on the numbers of fish that could be lethally sampled, prompting the development of non-lethal and non-invasive methods for assessing smoltification.

The major function of the project was to describe smoltification in reference stocks of the Columbia River. The measurements were assumed to represent the migration as a whole, and were used to modify flows to increase migration speed and survival. Although it is now accepted that augmented flows enhance migration, the need to determine smolt condition in Columbia River salmon stocks has not diminished. Current adaptive management measures, including changes in rearing practices and redesign of fish passage facilities, have created a demand for smolt assessment beyond the Smolt Monitoring Program. A review of ASCTTA technical assistance activities related to smolt evaluation are included in this report (Section Two, Part One).

SECTION ONE PART TWO

Travel Time Analysis A Review 1988 to Present

John Beeman

Columbia basin fish managers are interested in the time required for juvenile salmonids to emigrate from natal streams to the Columbia River estuary. This interest is based on a belief that impoundment of the Columbia and Snake rivers has increased travel times and decreased survival of juvenile salmonids. Evidence of these trends came largely from Raymond (1968, 1979) indicating a positive relation between river flow and travel time, and Raymond (1979) and Sims and Ossiander (1981) reporting a positive correlation between river flow and survival. Thus, the Water Budget, a plan to use a specific volume of water to augment the migration speed of juvenile salmonids, was created with the expectation that reductions in travel times would result in increases in survival (Northwest Power Planning Council 1987).

This project was funded in 1987 to monitor several measures of smolt physiology to help explain variation in survival and travel times of juvenile salmonids. This was prompted by published studies indicating relations between smoltification and the disposition to migrate (Rodgers et al. 1987), though not all data supported this relation (Hart et al. 1981). Smoltification is the process of physiological, morphological and behavioral changes juvenile salmonids undergo preparatory to the osmoregulatory demands of life in seawater. We assessed smoltification through measures of gill Na^+ , K^+ -ATPase activity (ATPase) and condition factor, and attempted to explain variability in travel time estimates based on these variables and physical variables such as river flow and water temperature using multiple regression techniques.

Our analyses of travel time were structured by the fish marking operations of the Smolt Monitoring Program (SMP). The SMP marking program was designed to evaluate the effectiveness of the Water Budget using mark and recapture studies to determine survival and travel time. Survival estimates from the program were discontinued after 1987 due to violations of key assumptions identified by this project. The assumptions were that test and control groups of fish were identical at the time of release, and identical at the recovery site, McNary Dam, and therefore equally susceptible to collection (Rondorf et al. 1988). Survival and travel time estimates prior to 1988 were based on mark and recapture of freeze-branded fish. This method provided travel time estimates of groups rather than individuals. Beginning in 1988, travel time

estimates were based on releases and recaptures of fish implanted with passive integrated transponder (PIT) tags, allowing an estimate of travel time for each individual tagged and released. Data from this method was more precise than the earlier method, and could allow population- and individual-level analyses. However, travel time estimates were still largely based on pooled data from fish released in daily groups, due to the similarity of fish released at the same time.

Separate analyses of data from 1988 to 1992, and 1993 to 1996, were needed due to differences in ATPase assay methods. Prior to 1993, we used an ATPase assay method requiring a large sample of gill tissue resulting in lethal sample methods (Zaugg 1982a). Fish were sacrificed for ATPase analysis, with the results assumed to be representative of other fish released on that day; the ATPase activities of the migrating individuals were not known. We adapted the method described by Zaugg (1982a) for use with smaller tissue amounts enabling non-lethal sampling from 1993 to 1996 (Schrock et al. 1994). The result was the ability to take samples from PIT-tagged fish that were subsequently released, allowing analyses on an individual level.

Several travel time analyses were performed under this project based on PIT-tagged fish. The main difference between the analyses was the number of years of data used. The last analysis, based on data from 1988 through 1992 (Maule et al. 1994), indicated that river flow, ATPase activity, condition factor, water temperature, and change in flow were significant predictors of the travel times of juvenile spring chinook salmon (*Oncorhynchus tshawytscha*) (Figure 1), and that river flow was the only significant predictor of travel times of steelhead (*O. mykiss*) (Figure 2). We found that neither flow-related nor smoltification-related variables were consistently more important predictors than the other. These results were generally similar to those of Berggren and Filardo (1993). They found that flow and, for one reach, water temperature were significant predictors of steelhead travel time and that surrogate variables for smoltification (i.e., previous time in river and water temperature) were additional predictor variables of chinook salmon travel time. However, flow-related variables were consistently more important predictors than other variables. Berggren and Filardo (1993) concluded that multiple-regression techniques using flow-related and smoltification-related variables were required to best explain travel times of juvenile salmonids. We reached a similar conclusion, based on our analyses, using specific measures of smoltification (Maule et al. 1994).

Recent work at the University of Washington has taken a different approach to predicting the migration of juvenile salmonids. Previous work by the ASCTTA project and Berggren and Filardo (1993) was based on multiple regression techniques using many variables as potential predictors of travel time. The analysis by Zabel and Anderson (1997) was based on models describing the mechanisms thought to drive the migration of juvenile salmonids. The basis of these mechanisms was research published in peer-reviewed journals and grey literature describing the distribution and migration of juvenile salmonids. Their approach was to create

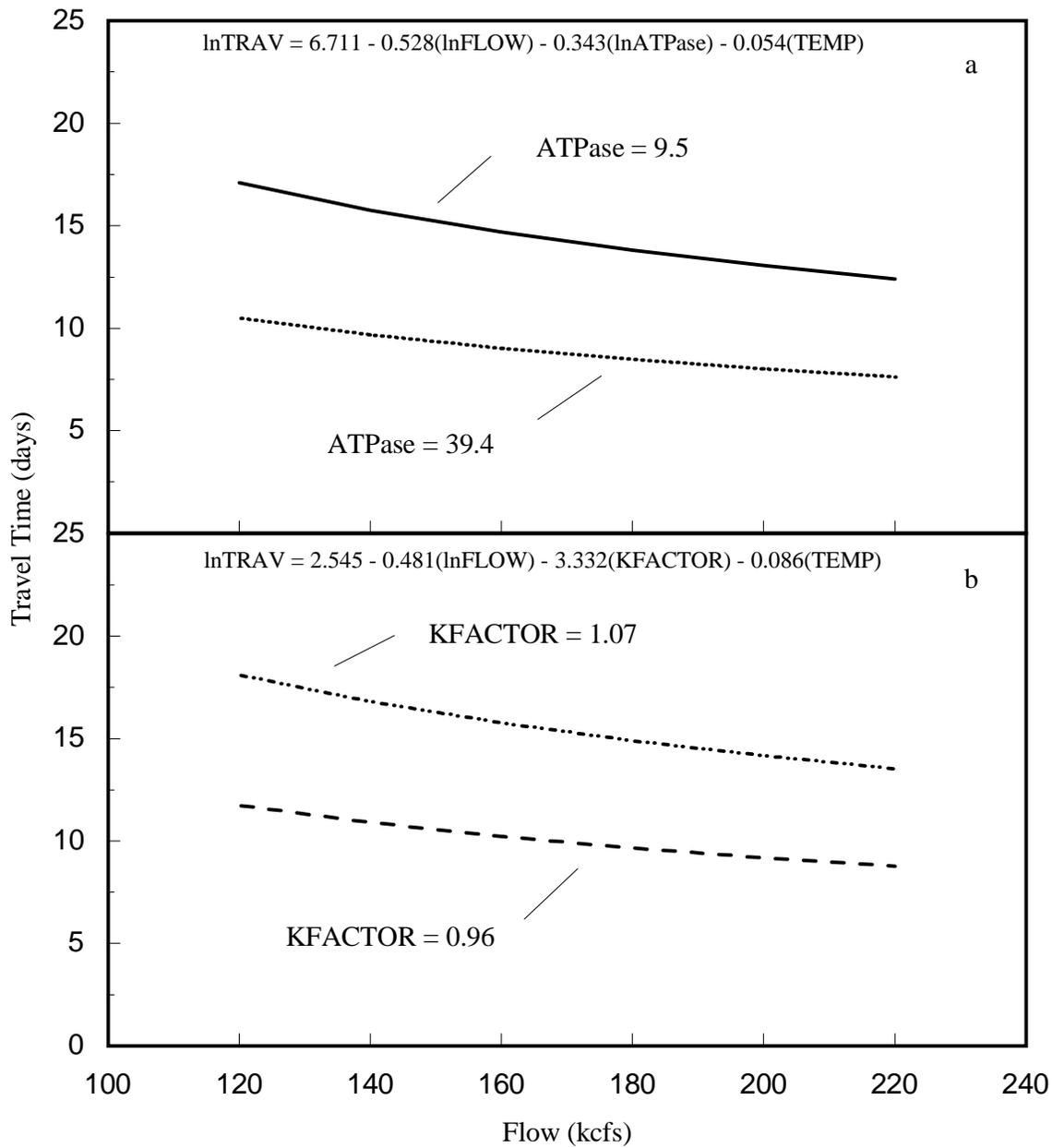


Figure 1. Predicted flow-travel time relations of juvenile spring chinook salmon migrating between Rock Island Dam and McNary Dam based on a multiple regression including (a) gill sodium, potassium activated-ATPase activity (ATPase) or (b) condition factor (KFACTOR). Levels of ATPase and condition factor represent minimums and maximums from data collected in 1989-1992. For the purpose of these plots, the water temperature variable (TEMP) was held constant at its mean.

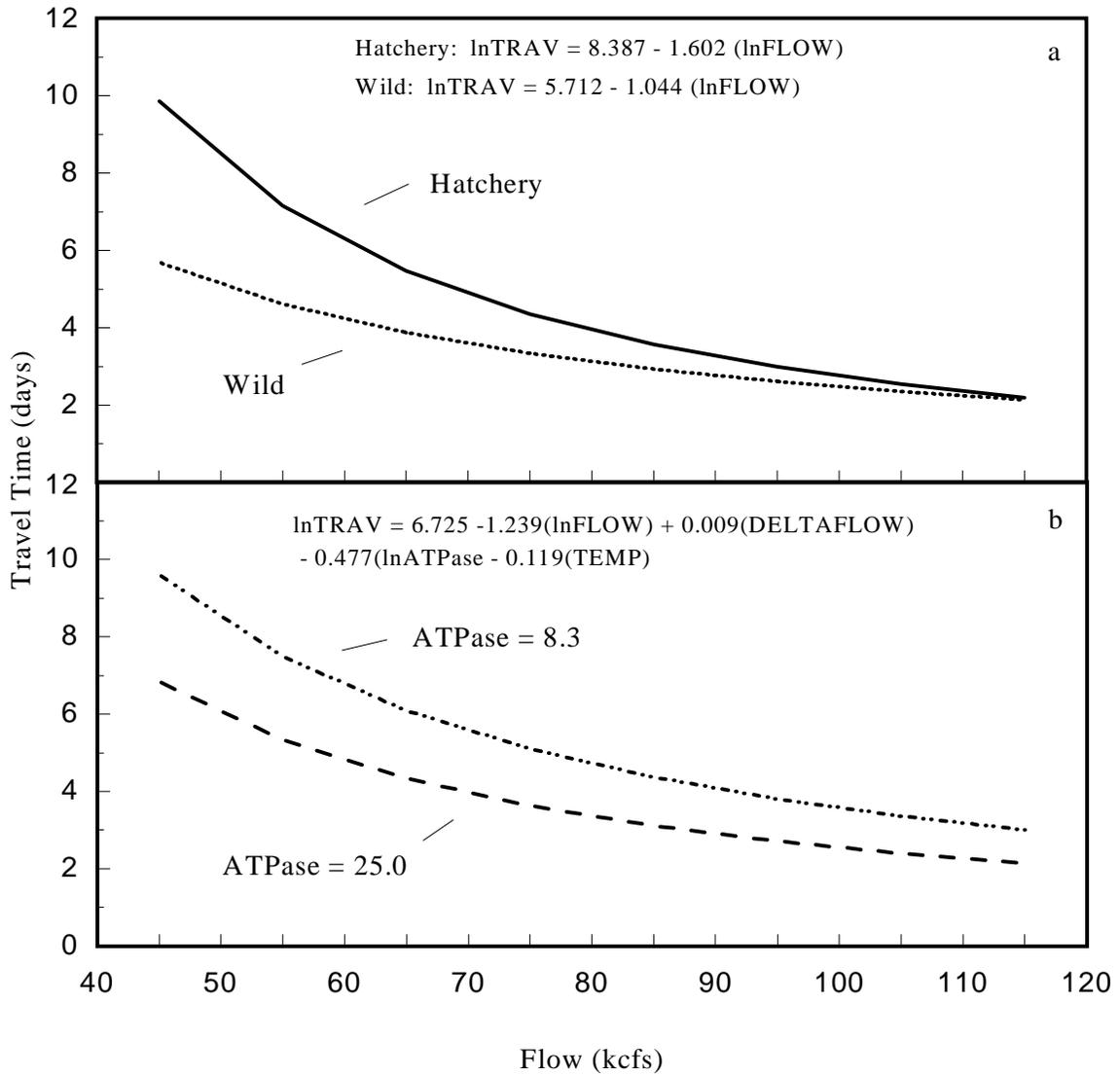


Figure 2. Predicted flow-travel time relations of juvenile steelhead of hatchery and wild origin migrating between the Snake River Trap and Lower Granite Dam: (a) shows results of separate simple regressions for hatchery and wild steelhead, (b) shows results of multiple regression analysis of pooled hatchery and wild steelhead data. Levels of gill sodium, potassium-activated ATPase (ATPase) represent minimums and maximums from data collected 1989-1992. The DELTAFLOW variable was held constant at its mean.

mathematical expressions to mimic the current theory of smolt migrations. The result is a multi-reach model explaining 87% of the variability in migration rates of juvenile spring chinook salmon from the Snake River. Their final model contains factors to account for river flow, population spreading, a flow-independent change in the effect of flow (i.e., the fish's contribution to migration), and migration experience. The changes in flow effects and migration experience are used to describe changes in fish behavior over time.

The model of Zabel and Anderson (1997) is based on specific assumptions chosen to provide a good fit to existing migration data, but the assumptions also agree with biological data from the ASCTTA project. In particular, they assume that 95% of the fish's contribution to downstream migration develops over the first 25 days of migration, which is similar to the rise and plateau of ATPase activities (Rondorf et al. 1989; Beeman et al. 1990) and morphological indices of smoltification (Beeman et al. 1994, 1995). However, a causal relation between these indices of smoltification and the disposition to migrate has yet to be clearly proven (Ewing et al. 1980; Hart et al. 1981). In 1994, we stated our belief that increases in these indices are part of a suite of physiological changes occurring during smoltification, and probably have a correlative, rather than a causative, relation to migration (Maule et al. 1994). The accuracy of the model in Zabel and Anderson (1997) supports the theory that there is some change in behavior affecting the fish's contribution to migration (e.g., the flow-independent fish migration rate increases substantially over the first 25 days). This type of migratory behavior is described generally in Northcote (1992) and Jonsson (1991).

There should be a difference in smoltification indices of individuals with low and high migration rates if changes in migration behavior are related to smoltification. The objective of current analyses of smoltification and travel time within the ASCTTA project is to test this hypothesis. The goal of the analysis is not to develop a new equation to predict travel times of juvenile salmonids, but rather to determine if the cause of the increased migration tendency described by Zabel and Anderson (1997) can be attributed to measured indices of smoltification. The analysis is currently in progress, with completion planned by mid-1998.

SECTION ONE PART THREE

Smoltification and Gill Sodium, Potassium-Activated Adenosine Triphosphatase Activity 1987 to 1996

Karen M. Hans
Robin M. Schrock

Introduction

Smoltification, the process of physiological change in anadromous juvenile salmonids whereby they develop the ability to osmoregulate in seawater, is associated with a variety of biochemical phenomena. Endocrine control of smoltification in juvenile salmonids (*Oncorhynchus* spp.) (Barron 1986) guides and accompanies hormonal (Rand-Weaver and Swanson 1992), immunological (Maule et al. 1993), and metabolic (Sheridan 1988; Staley and Ewing 1992) changes that occur under environmental influence (Wedemeyer et al. 1980; Zaugg 1982b; Clarke et al. 1988; Thorarensen et al. 1988; Muir et al. 1992). The range and magnitude of changes that occur as anadromous fish develop have yet to be fully characterized; however, some physiological measurements exhibit characteristic profiles during smoltification. Both condition factor and gill sodium, potassium-activated adenosine triphosphatase (Na^+ , K^+ -ATPase) (Zaugg and McLain 1972; Folmar and Dickhoff 1981a; Borgatti et al. 1992) are routinely used as references to determine the level of smolt development in salmonids. Regional fish managers apply measures of smoltification provided by the Assessment of Smolt Condition for Travel Time Analysis (ASCTTA) project to production-based research and fisheries management.

ATPase is an enzyme that facilitates ionic transport across membranes by hydrolyzing adenosine triphosphate as an energy source. The enzyme is involved with the absorption of sodium chloride (NaCl) across gill epithelium of freshwater teleosts and the excretion of NaCl in marine species (Hoar and Randall 1984; Borgatti et al. 1992). Anadromous juvenile salmonids must therefore reverse the flow of salts as they enter saltwater. The measurement of gill Na^+ , K^+ -ATPase activity is an established method of determining the level of smoltification in juvenile salmon (Folmar and Dickhoff 1981a; Zaugg 1982a; Dickhoff et al. 1985). It is used in combination with other morphological, physiological, and environmental variables as an indicator of smoltification (Wedemeyer et al. 1980; Folmar and Dickhoff 1981a; Zaugg 1982a; Dickhoff et al. 1985; Sower and Fawcett 1991). Reporting gill ATPase activity on a weekly basis has been a routine part of smolt monitoring programs in the Columbia River basin (Beeman et al. 1991). Gill Na^+ , K^+ -ATPase activity varies in absolute concentration among species during different developmental stages, but displays a characteristic profile among Columbia basin salmonids

during rearing and seaward migration. In hatcheries, ATPase activities stay low, showing only a gradual increase, then accelerate considerably after the fish are released into the river. The increase in ATPase activity continues until late in the migration (Beeman et al. 1991). If release is delayed, fish at hatcheries may experience a decrease in ATPase activity followed by a rapid increase upon release and during migration (Zaugg 1982b).

Characterization of stock profiles of Na^+ , K^+ -ATPase as an indicator of smolt development for use as a management tool depends on the establishment of a continuing record for individual stocks with careful attention to environmental variables during rearing and migration. The limitation on using measurements of gill ATPase to determine the level of smoltification is that there are no baseline ranges known to be consistent from year to year; measurements differ among stocks and species, and are determined by environmental factors (Wedemeyer et al. 1980; Zaugg 1981b, 1982b). Because smoltification is influenced by many environmental variables (photoperiod, water temperature, water quality, stocking densities, feed, time of release) the life history of the fish must be carefully documented to allow interpretation of ATPase levels on particular dates.

Several methods of measuring gill Na^+ , K^+ -ATPase exist, and the detectable range of ATPase activity depends on the method used (Zaugg 1982a; Johnson et al. 1991; McCormick 1993; Schrock et al. 1984). Of great significance is the determination of when measurements are made during the annual ATPase profile for a particular stock. Our research suggests that several measurements should be made before release, and during migration, to adequately interpret differences in ATPase levels between groups. As the time of migration approaches, ATPase levels of fish within the hatchery may increase, then decline if the fish are not released. Therefore, levels from a single pre-release or in-river sample would not indicate where fish are in the smoltification cycle, if other ATPase measurements are not referenced.

Other measurements to identify smolt condition have been investigated, and each offers a different level of information about the complex process of smoltification. An example of how gill ATPase levels may be misleading when determining the level of smoltification in different species is the comparison of steelhead with chinook salmon. Steelhead have lower levels of ATPase while still in the hatchery than chinook. Early reports interpreted these steelhead as being "less smolted," while results at the dams suggest that fish may have already experienced increased ATPase levels in the hatchery, that declined when fish were not released, only to increase again upon release into the river. Interspecific and stock differences, as well as time of year and developmental stage, must be considered when comparing profiles of ATPase activity among different species.

Many agencies with biological and economic interests in water resource distribution in the Columbia River basin cooperate to ensure the successful seaward emigration of juvenile salmonids in the system. Delays in smolt downstream migration, often attributed to river impoundment, may be influenced by the physiological condition and health of the fish. Gill ATPase activity levels in

juvenile Pacific salmonids prior to release from hatcheries and during migration have been used to make water management recommendations in the Columbia River basin.

ASCTTA Assessment Fish 1987-1996

Methods

Chinook salmon and steelhead at 21 Columbia River (Table 1) and Snake River (Table 2) fish hatcheries were sampled from 1988 to 1996. Snake River basin hatcheries where chinook salmon were sampled included Sawtooth, McCall, and Rapid River State Fish Hatcheries (SFH), and Dworshak and Kooskia National Fish Hatcheries (NFH) in Idaho, and Lookingglass SFH in Oregon. Columbia River basin hatcheries where chinook salmon were sampled included Entiat, Leavenworth, and Winthrop NFH, and Ringold, Priest Rapids, and Turtle Rock SFH in Washington, and Bonneville SFH in Oregon. Steelhead were sampled at Dworshak NFH, Hagerman NFH, Magic Valley SFH and Niagara Springs SFH in Idaho, Lyons Ferry SFH and Wells SFH in Washington, and Irrigon SFH in Oregon.

Fish were collected at juvenile bypass facilities at Rock Island, McNary, John Day and Bonneville dams on the Columbia River, and at Lower Granite and Little Goose dams on the Snake River. We also sampled fish at traps operated by Idaho Department of Fish and Game on the Salmon, Clearwater, and Snake Rivers. Sockeye of hatchery and wild origin were sampled at Rock Island Dam. Hatchery sockeye may have originated from a Lake Wenatchee net pen operated by the Washington Department of Fish and Wildlife (WDFW) for the Chelan County Public Utility District. In 1994 and 1995, fish were sampled at a trap operated by the Nez Perce Indian Nation on the Imnaha River, and a trap operated by the Oregon Department of Fish and Wildlife (ODFW) on the Grande Ronde River.

From 1988 to 1992, two sampling strategies were used to assess smoltification of juvenile salmonids during downstream migration. The first strategy involved sampling and freeze branding groups of chinook salmon and steelhead prior to release, enabling us to identify and sample the fish at downstream smolt monitoring sites. These fish were considered representative of corresponding unmarked hatchery release groups. The second strategy involved subsampling fish from migrating populations. These subsamples were considered representative of the general population of migrating salmonids. The hatchery groups were sampled three times: at about one month before release, two weeks before release, and shortly before release. When marked fish were recaptured at monitoring sites, a subsample of 20 fish was collected from the early (25%), middle (50%), and late (75%) segments of the migration. These sampling strategies provided a broad-based assessment of the smoltification profile of migrating hatchery salmonids (see Figure 1 for an example of a characteristic profile).

Hatchery release groups were sampled once before release, from 1993 to 1996. Past results showed that ATPase levels consistently remained low until the fish were released. The

exceptions were at Dworshak NFH (1993) and Priest Rapids SFH (1993, 1994, 1996) when multiple pre-release samples were taken as part of release group studies with other agencies.

Table 1. Chinook salmon and steelhead sampled at Columbia River hatcheries by the Assessment of Smolt Condition for Travel Time Analysis project. Table lists national fish hatcheries (NFH) and state fish hatcheries (SFH), species sampled, and calendar years when at least one sample was taken by the project.

Hatchery, State	Species/Stock	Years Sampled
Entiat NFH (WA)	spring chinook salmon	1988 to 1996
Leavenworth NFH (WA)	spring chinook salmon	1988 to 1996
Ringold SFH (WA)	spring chinook salmon	1988, 1990 to 1996
Winthrop NFH (WA)	spring chinook salmon	1988 to 1996
Wells SFH (WA)	summer chinook salmon	1995, 1996
Bonneville SFH (OR)	fall chinook salmon	1992
Lyons Ferry SFH (WA)	fall chinook salmon	1988 to 1990
Priest Rapids SFH (WA)	fall chinook salmon	1988 to 1996
Turtle Rock SFH (WA)	fall chinook salmon	1993
Lyons Ferry SFH (WA)	steelhead	1988 to 1992
Wells SFH (WA)	steelhead	1988 to 1991

Table 2. Chinook salmon and steelhead sampled at Snake River hatcheries by the Assessment of Smolt Condition for Travel Time Analysis project. Table lists national fish hatcheries (NFH) and state fish hatcheries (SFH), species sampled, and calendar years when at least one sample was taken by the project.

Hatchery, State	Species/Stock	Years Sampled
Dworshak NFH (ID)	spring chinook salmon	1988 to 1996
Kooskia NFH (ID)	spring chinook salmon	1992 and 1996
Lookingglass SFH (OR)	spring chinook salmon	1994 to 1996
Rapid River SFH (ID)	spring chinook salmon	1988 to 1996
Sawtooth SFH (ID)	spring chinook salmon	1988 to 1996
McCall SFH (ID)	summer chinook salmon	1988 to 1996
Dworshak NFH (ID)	steelhead	1988 to 1993
Hagerman NFH (ID)	steelhead	1988
Irrigon SFH (OR)	steelhead	1988 to 1993
Magic Valley SFH (ID)	steelhead	19889
Niagara Springs SFH (ID)	steelhead	1988 and 1989

Sampling of migrants at upriver monitoring sites and lower river dams allowed us to track the smoltification profile of migrating fish. Sampling at traps and dams was coordinated with passive integrated transponder (PIT) tagging, with our sample sizes determined by the number of PIT-tagged fish we expected at the downstream PIT-tag detection site. Fish collected at traps were held up to 24 h, anaesthetized and PIT-tagged before a non-lethal gill sample was taken. At smolt traps and dam collection facilities, we sampled adipose fin-clipped chinook salmon and steelhead, as well as fish with intact adipose fins. All hatchery steelhead in the Columbia River and Snake River basins have their adipose fins removed to distinguish them from wild steelhead. All hatchery chinook salmon in the Snake River basin have been marked with a fin clip since 1993, but chinook with intact adipose fins in the Columbia River could have been of either wild or hatchery origin.

Data from chinook salmon and steelhead sampled at traps and dams were reported weekly during the migration season (April through August) from 1993 through 1996. Project personnel collected gill clips from migrants and transported the samples to the Columbia River Research

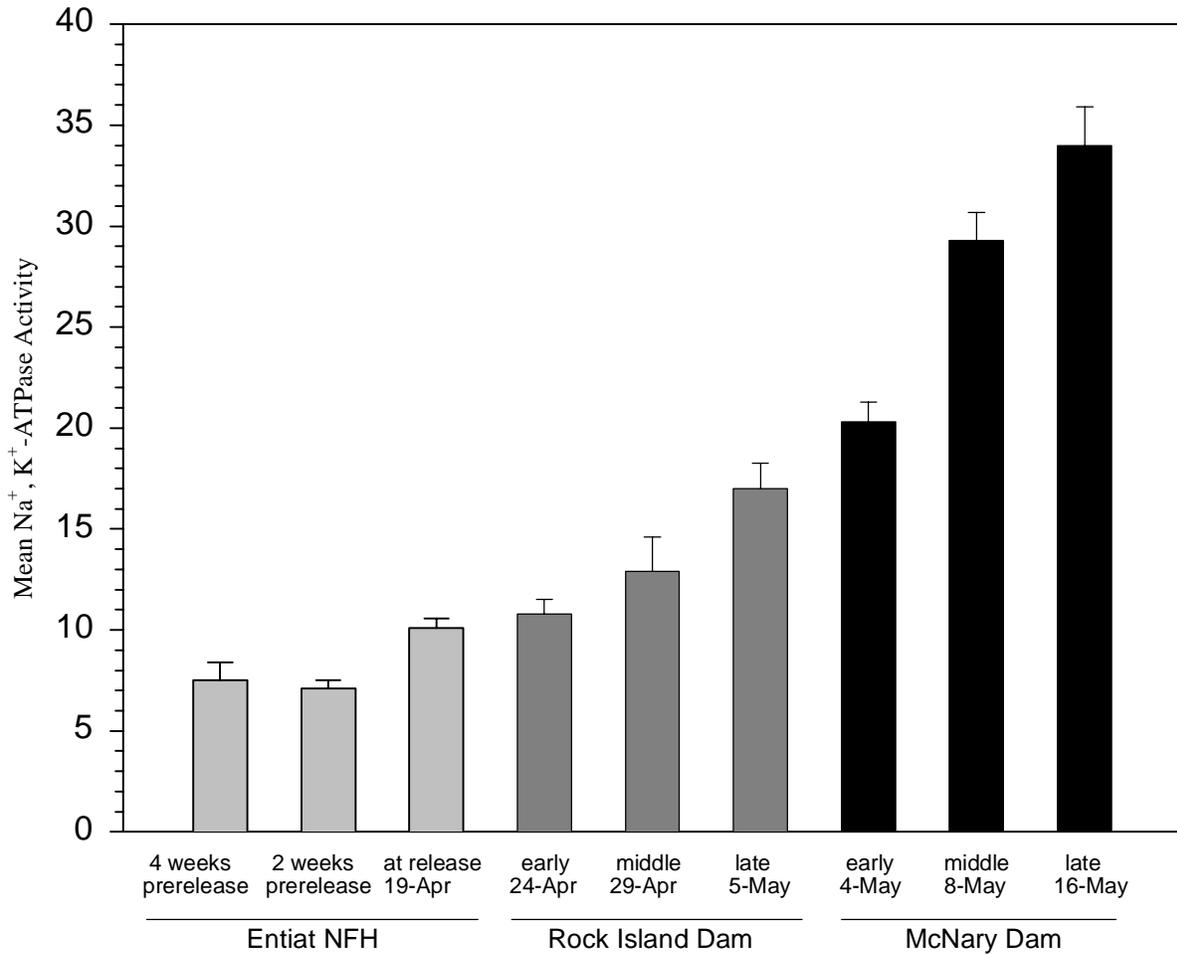


Figure 1. Mean (\pm SE) Na⁺, K⁺-ATPase activities ($\mu\text{mol P}_i \cdot \text{mg protein}^{-1} \cdot \text{h}^{-1}$) for spring chinook salmon brand release groups sampled at Entiat National Fish Hatchery, Rock Island Dam and McNary Dam during emigration in 1989. Mean values at dams represent the 25th percentile (early), 50th percentile (middle), and 75th percentile (late) of the run as it passed the dam.

Laboratory (CRRL), Cook, Washington, at the end of each week. The laboratory technician selected a random sample from the total number of gill clips for each day's samples from each site.

A report was transmitted to the Fish Passage Center (FPC) with information on mean ATPase level, mean condition factor, smolt index, and a visual assessment of BKD. Data for ATPase and condition factor included the mean value, percent maximum change, and sample size. Reports included data from the current week and year to date for all monitored species and sites. In addition to the weekly reports to the FPC, all gill ATPase samples were analyzed at the close of the migration season, and the data for the entire migration year was summarized.

Results and Discussion

Data from all sample years shows gill ATPase was low (5.0 to 7.0 $\mu\text{mol P}_i \cdot \text{mg protein}^{-1} \cdot \text{h}^{-1}$) in fish sampled at hatcheries (Figure 2). The exception was spring chinook salmon sampled at Ringold SFH, which typically had higher mean ATPase levels than fish from other hatcheries (1991-1994, 1996). Water at Ringold SFH was warmer (12.5°C) than at other hatcheries where fish were monitored (5 to 7°C). The higher mean ATPase level of fish at Ringold SFH supports studies that found water temperature was an environmental stimulant to smoltification (Zaugg et al. 1985; Jonsson 1991). Mean ATPase levels of hatchery and wild spring chinook salmon and steelhead, were lower at upper Snake River basin traps (Salmon River Trap, Imnaha River Trap, Grande Ronde River Trap, Snake River Trap) than at the dams (Lower Granite Dam, McNary Dam). This would be expected, because past research indicated ATPase levels remained low in the hatcheries and increased once fish were released and began migrating (Beeman et al. 1991).

The mean ATPase levels in migrating wild fish were consistently higher than those of migrating hatchery fish (Figure 3). This was true for yearling chinook salmon sampled at the Salmon, Clearwater, and Snake River traps, and Lower Granite Dam. Differences between wild and hatchery fish sampled at Columbia River dams could not be determined because not all hatchery fish were marked in the mid-Columbia reach. Wild steelhead had higher ATPase levels than hatchery steelhead sampled at the Snake, Salmon, Imnaha, and Grande Ronde River traps, and at Lower Granite, Little Goose, McNary, and Rock Island dams. There were a number of published reports which suggested that hatchery fish did not migrate immediately upon release and that river residence was an important factor promoting smoltification (Wedemeyer et al. 1980; Folmar and Dickhoff 1981a; Zaugg et al. 1985). Other variables, such as constant water temperature, artificial diets, high raceway densities, and artificial photoperiod have been shown to slow the parr-to-smolt transformation (Folmar and Dickhoff 1981a; Nishioka et al 1985; Schreck et al. 1985), and may contribute to observed differences in wild versus hatchery fish.

The one exception in the hatchery-wild comparison was sockeye salmon. Hatchery fish sampled at Rock Island Dam had higher mean ATPase levels than wild fish. The rearing method

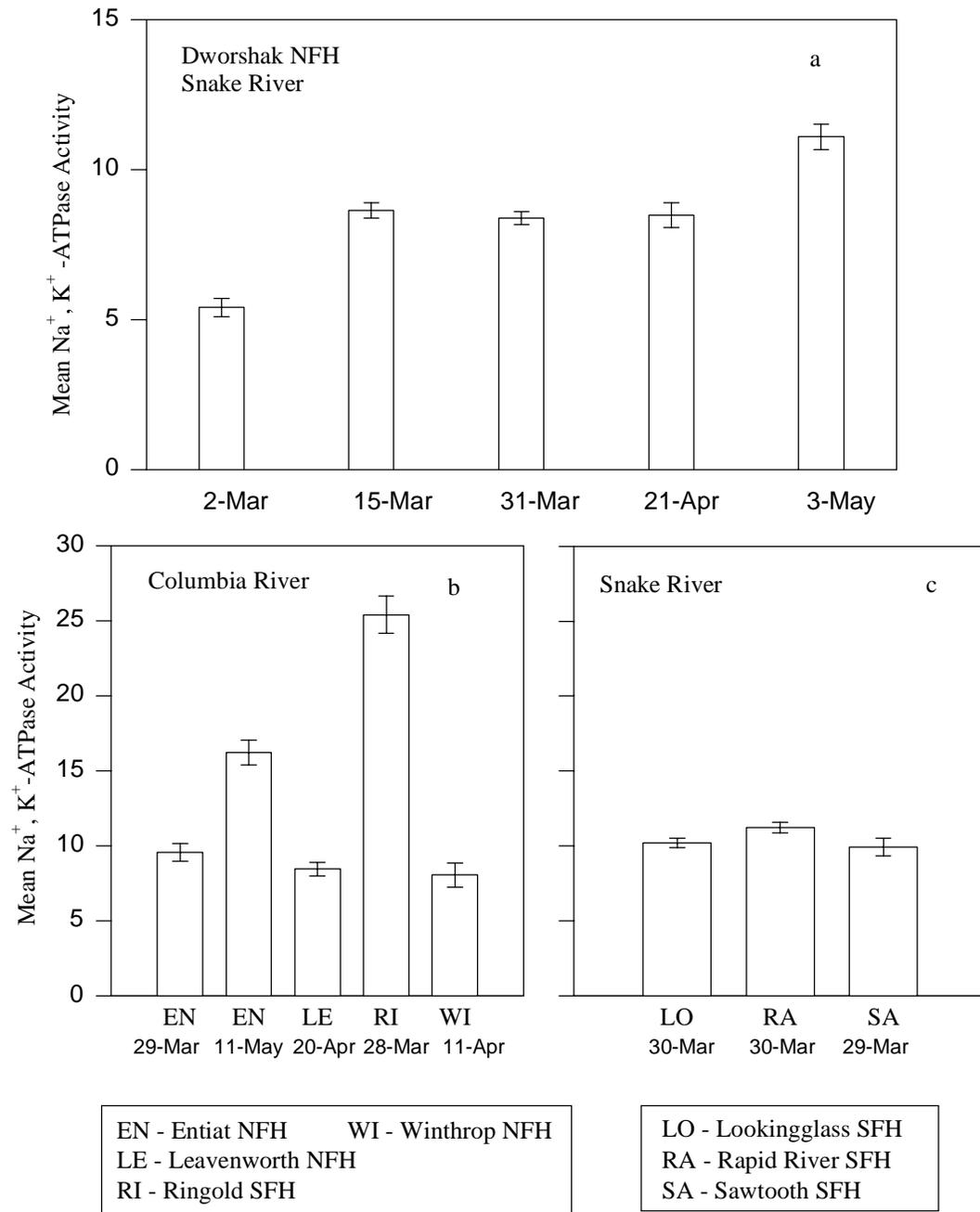


Figure 2. Mean (\pm SE) Na^+, K^+ -ATPase levels ($\mu\text{mol P}_i \cdot \text{mg protein}^{-1} \cdot \text{h}^{-1}$) for spring chinook salmon sampled at Columbia and Snake river basin hatcheries in 1994 (NFH = National Fish Hatchery; SFH = State Fish Hatchery).

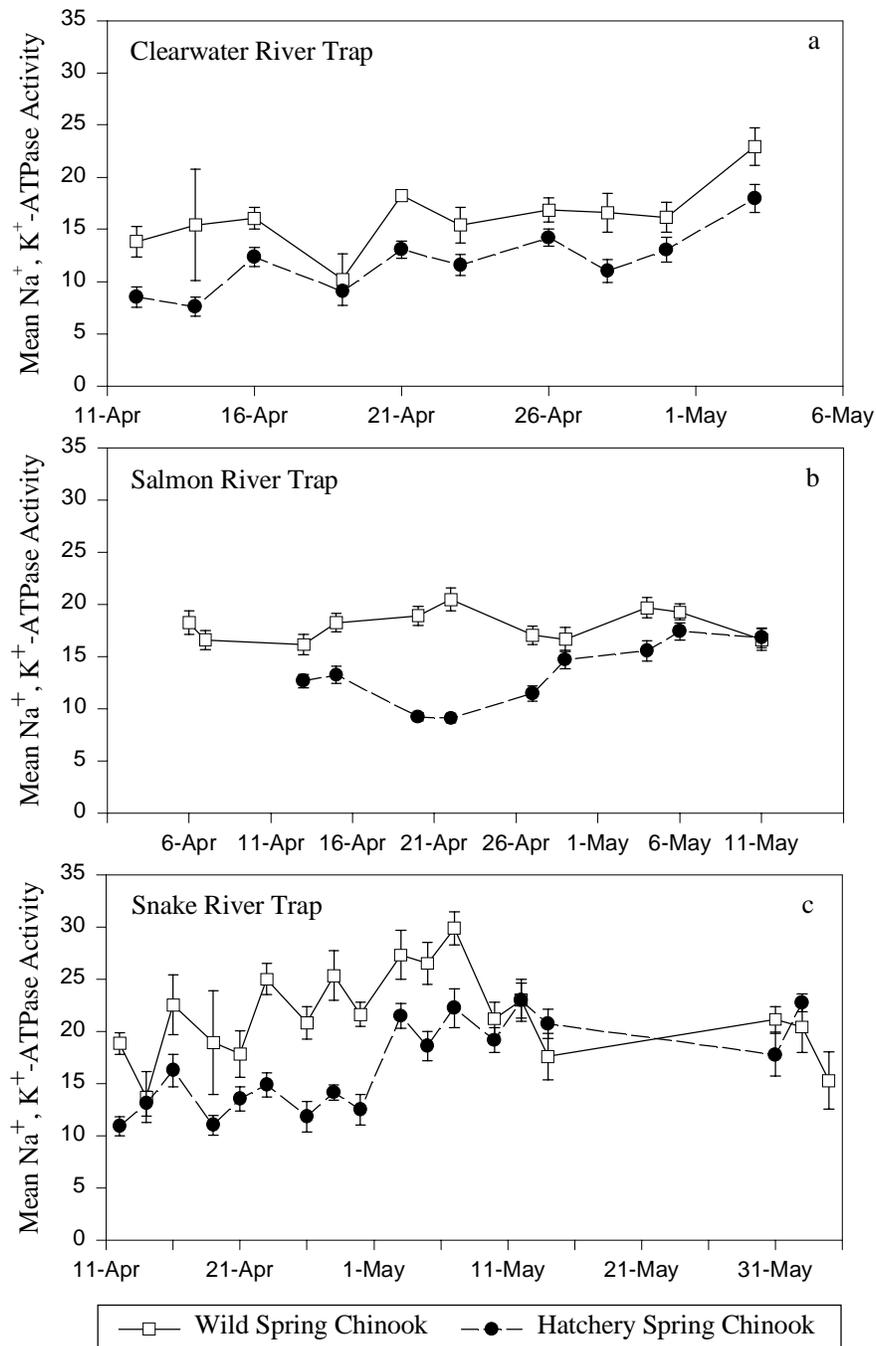


Figure 3. Mean (\pm SE) Na^+ , K^+ -ATPase ($\mu\text{mol P}_i \cdot \text{mg protein}^{-1} \cdot \text{h}^{-1}$) levels of wild and hatchery spring chinook salmon captured at traps in the Snake River basin in 1993.

for the hatchery fish is one possible explanation for this exception. All hatchery sockeye salmon sampled at Rock Island Dam in 1993 and 1994 came from a net pen operation at Lake Wenatchee, which also has a wild population of sockeye salmon. Sockeye were transferred to the net pens as fry in April, and released into the lake in late October to overwinter. These fish then migrate volitionally the following spring with the wild fish. Studies by Shrimpton et al. (1994) found hatchery coho salmon, released early to overwinter, were physiologically equivalent to wild fish with respect to ATPase level and seawater tolerance at the time of migration.

Differences between the mean ATPase levels of wild and hatchery spring chinook salmon on specific sample dates may have been the result of differences in the time of peak migration between hatchery and wild fish. Wild spring chinook salmon tend to begin migrating through the upriver sampling sites earlier than hatchery fish. For example, in 1994, wild fish were first collected at the Salmon River Trap eight days before the arrival of the first hatchery fish (Fish Passage Center 1993, 1994). Wild spring chinook salmon had higher gill ATPase levels than the hatchery fish at the same site on the same date. Differences in mean ATPase levels between the wild and hatchery fish were most pronounced early in the season and at the upper river sampling sites. As the season continued, mean ATPase levels of wild and hatchery fish were similar.

The differences between wild and hatchery fish were not as pronounced in 1994 as in 1993. This may be related to the difference in river flows between the two years; 1993 was considered a high flow year and 1994 a low flow year (U.S. Army Corps of Engineers 1993, 1994). Previous reports (Beeman and Rondorf 1992) found juvenile salmonid travel time decreased as flow increased. Ewing (1980), in a study of chinook salmon, speculated that high flows could move fish downstream without a corresponding increase in ATPase level. Wild and hatchery fish sampled at dams and traps could come from many sources and could reside in the river for varying lengths of time. The natural variation among individuals within a population, and the necessity to determine the origin and rearing environment of wild fish, makes it difficult to compare the physiological status of wild and hatchery migrants. However, data collected and analyzed for this report provides useful information about the physiological smoltification level of juvenile salmonids at hatcheries, and while migrating, during high and low flow years. This information, when combined with information on water availability, can be useful to fisheries management agencies in making decisions regarding minimization of travel times of outmigrating juvenile salmonids.

Lower Granite Dam Recapture Study 1996

In 1996, we conducted a study on juvenile yearling (spring and summer) chinook salmon and steelhead of wild and hatchery origin traveling between the Salmon River Trap or the Snake River Trap and Lower Granite Dam. We compared changes in the physiological indices of smoltification and differences between wild and hatchery fish, with respect to river flows, travel time, and sample date, between upstream and downstream sites. In a prior study, we determined

that mean gill ATPase activity remained low in steelhead and chinook salmon the last few weeks before fish were released from hatcheries but that ATPase increased rapidly after the fish began their seaward migration (Beeman et al. 1991). We also found that wild salmonids usually had higher mean ATPase activity than their hatchery counterparts during the same period. The difference was less pronounced in fish sampled further downstream. It had also been shown that the level of smoltification, as determined by gill ATPase or date of sampling, influenced the rate at which fish migrated downstream (Berggren and Filardo 1993; Beeman et al. 1994). Data used in the studies by Berggren and Filardo (1993) and Beeman et al. (1994) were mean values from all fish sampled on a given day and, therefore, did not consider individual variation or changes in smoltification during migration. The development of non-lethal sampling techniques (Schrock et al. 1994) allowed fish to be sampled for gill ATPase, implanted with PIT tags (Prentice et al. 1990a), released and re-sampled at downstream sites.

Methods

Spring chinook salmon of wild and hatchery origin and steelhead of wild and hatchery origin were sampled at the Salmon River Trap and the Snake River Trap. Fish were injected with PIT tags at the traps and released daily as part of the SMP. Implantation of PIT tags at the Salmon and the Snake River traps was performed by the Idaho Department of Fish and Game. The PIT tag codes of the sampled fish were entered into the PIT Tag Information System (PTAGIS). Fish were interrogated at Lower Granite Dam with PIT tag detectors described by Prentice et al. (1990b). When a fish interrogated at the Lower Granite Dam bypass facility was identified by PIT tag code as a fish sampled for gill ATPase at one of the traps, the fish was channeled to a holding tank. Personnel from the CRRL measured fork length and weight of each fish, examined fish for visual signs of bacterial kidney disease (BKD), and took a gill clip for ATPase measurement. Fish traveled 164 river kilometers (rkm) through mostly free flowing river (85 rkm in the Salmon River, 79 rkm in the Snake River) and 51 rkm of reservoir from the Salmon River Trap to Lower Granite Dam. Fish traveled 51 rkm of reservoir from the Snake River Trap to Lower Granite Dam.

Results and Discussion

Our results suggested a significant difference in ATPase levels between wild and hatchery spring chinook salmon at the Salmon River Trap, but not at the Snake River Trap or at Lower Granite Dam. Hatchery spring chinook salmon sampled at the Salmon River Trap had lower ATPase levels and slower travel times to Lower Granite Dam than the wild fish. At Lower Granite Dam, ATPase levels did not differ significantly between wild and hatchery fish. This suggests that the longer in-river migration to Lower Granite Dam of hatchery fish was related to degree of smoltification, and that the additional time in-river allowed the hatchery fish to become as physiologically developed as wild fish. There was a significant negative correlation between the ATPase level of wild and hatchery spring chinook salmon at the Salmon River Trap and the percent change in ATPase at Lower Granite Dam (Figure 4). The lower the ATPase level of a

fish at the Salmon River Trap, the greater the percent increase in ATPase level that occurred between the trap and arrival at Lower Granite Dam. This suggests that while most fish are actively smolting while migrating, fish with lower ATPase levels may compensate by developing at a higher rate. There was also a significant negative correlation between ATPase level at the Snake River Trap and percent change in ATPase for hatchery chinook salmon. The fish with the lowest ATPase levels still experienced the greatest increase. However, considering the short travel distance between the Snake River Trap and Lower Granite Dam, some of the fish had relatively long travel times; three hatchery fish took 18 to 22 days to travel the reach.

For hatchery spring chinook salmon, travel time was strongly correlated with sample date at the Salmon River Trap (Figure 5). The travel times of the wild fish at the Salmon River Trap were correlated with sample date, but the relationship was not as strong as for hatchery fish. There was no correlation of date of collection at the Snake River Trap with travel time to Lower Granite Dam for wild and hatchery fish. Wild spring chinook salmon had higher ATPase levels at the Salmon River Trap and faster travel times to Lower Granite Dam compared to hatchery fish. The data suggests that wild fish smolt earlier in the year, before hatchery fish are released, and therefore are already actively migrating when they pass the Salmon River Trap. Smoltification and the corresponding propensity to migrate are stimulated by lengthening photoperiod and are also influenced by increasing water temperature and flows (Jonsson 1991). There was no correlation between travel time and flows at the Salmon River Trap for the wild fish. Jonsson (1991) considered increased flows a direct modifier of fish migration, with photoperiod acting as the primary cue; there is possibly a combined effect of the two variables.

Data collected for this report illustrates the differences in travel time between steelhead and spring chinook salmon. Steelhead sampled at the Salmon River Trap had significantly faster mean travel times than either hatchery or wild spring chinook salmon. There was no correlation between travel time and sample date or flows at the Salmon River Trap for wild or hatchery steelhead. The steelhead also had significantly lower mean ATPase levels than wild or hatchery spring chinook salmon, but the change in ATPase for steelhead, by the time of their arrival at Lower Granite Dam, was as high as 77.4%. The steelhead began migration at low ATPase levels that quickly advanced once the fish moved downriver. Migrating steelhead are considerably larger than spring chinook migrants, and therefore may travel faster. ATPase level may differ in magnitude, activity pattern, or physiological effect between the two species. A travel time assessment study conducted in 1991 found ATPase was a good predictor of travel time for spring chinook salmon, but not for steelhead (Maule et al. 1994).

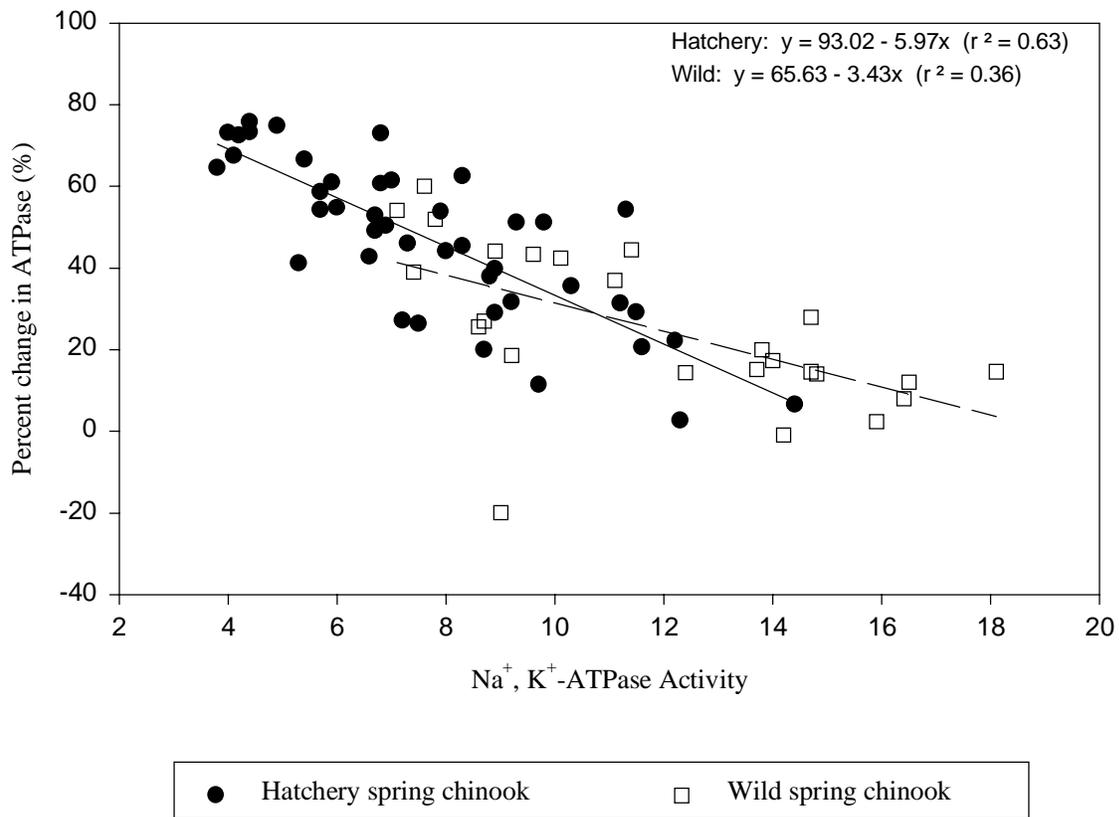


Figure 4. Correlations of gill Na⁺, K⁺-ATPase activity ($\mu\text{mol P}_i \cdot \text{mg protein}^{-1} \cdot \text{hour}^{-1}$) of individual wild and hatchery spring chinook salmon captured at the Salmon River Trap, to percent change in gill ATPase activity from initial sampling to recapture at Lower Granite Dam, in 1996.

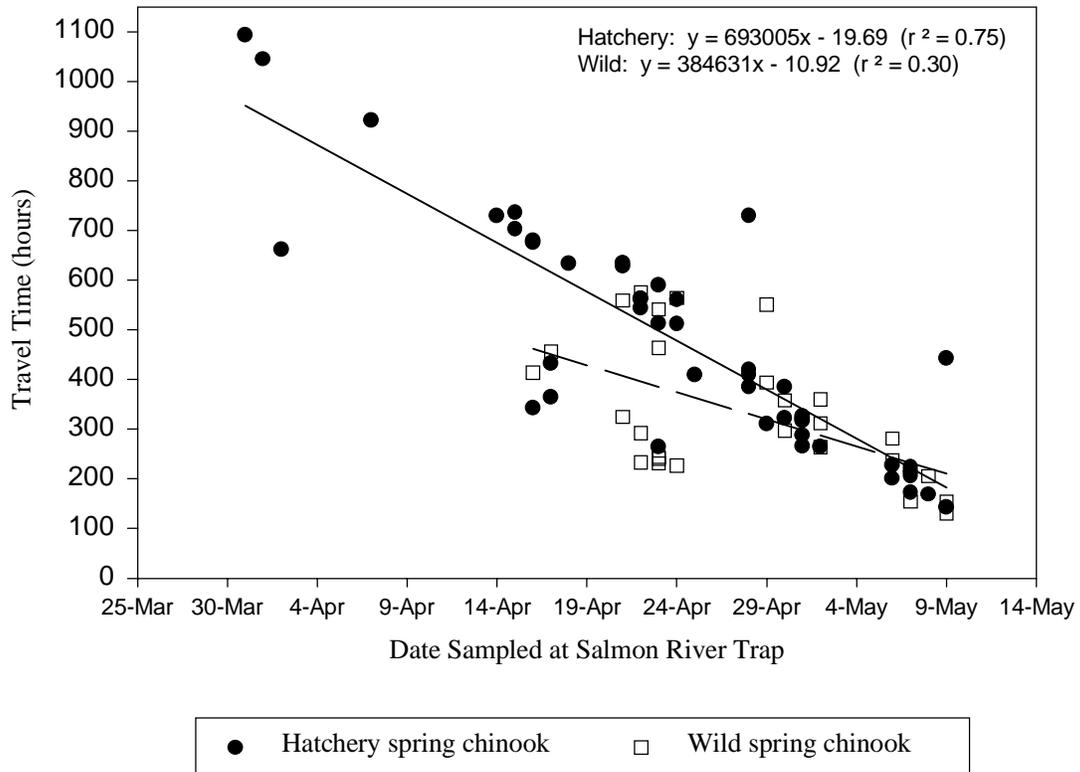


Figure 5. Correlations of travel time with date of initial sampling of wild and hatchery spring chinook salmon sampled at the Salmon River Trap and recaptured at Lower Granite Dam, in 1996. Travel time represents the number of hours between PIT tag release time at the Salmon River Trap and PIT tag interrogation time at Lower Granite Dam.

The Lower Granite Dam Recapture Study in 1996 represented the first time multiple ATPase samples had been taken from individual juvenile salmonids during migration. The results of this study illustrated the differences in the physiological development between wild and hatchery spring chinook salmon, and between spring chinook salmon and steelhead. Data collected for this study supported our previous research which concluded that hatchery spring chinook salmon were being released for migration at a different stage of physiological development compared to their wild counterparts, and that there were inter-species differences between migration patterns in spring chinook salmon and steelhead. However, small sample size, along with natural variations in ATPase and travel time of migrating juvenile salmonids, make inferential conclusions from this data difficult and results should be interpreted cautiously. Further study of individual fish migrating between the upriver reaches and lower river dams could provide more information on the physiological and behavioral changes experienced by these fish, and on the effect of river flows and other environmental factors on smolt development, as measured by gill ATPase and travel time.

Comparisons of Wild and Hatchery Spring Chinook Salmon and Steelhead, 1990 to 1996

Federal and state agencies have attempted to supplement natural runs of salmon and steelhead with large numbers of hatchery fish. In 1994, 24.55 and 22.37 million hatchery salmonids were released into the Columbia and Snake River basins, respectively (Fish Passage Center 1994). However, the number of adult fish returning to these basins continues to decline. An objective of the ASCTTA project was to compare levels of gill ATPase activity in wild and hatchery juvenile spring chinook salmon and steelhead during their seaward migration. Studies have shown that when techniques such as photoperiod manipulation were used to advance smoltification, hatchery fish migrated faster (Muir et al. 1994), and that fish with higher ATPase levels had a better survival rate when passing dams (Giorgi et al. 1988). High ATPase levels at release could be important in low flow years. Studies by Ewing et al. (1980) found fish could be moved downriver by high flows without increasing ATPase activity. Shrimpton et al. (1994) compared wild and hatchery coho smolts captured during seaward migration and found wild fish had a significantly increased tolerance to salt water. Based on Na^+ , K^+ -ATPase activity and chloride (Cl) cell concentration, wild and colonized (hatchery fish released as fry) fish were more physiologically advanced as smolts than fish released directly from the hatchery.

Methods

From 1990 to 1996, we collected gill ATPase data from wild and hatchery juvenile salmonid migrants at smolt monitoring sites in the Columbia and Snake river basins. Evaluation of this data provided many examples where, at the same site on the same date, chinook salmon and steelhead of wild origin had ATPase levels higher than their hatchery counterparts. Statistical comparisons of wild and hatchery steelhead were presented in previous reports (1989, 1990).

After 1993, all hatchery chinook salmon in the Snake River were marked, allowing comparison of wild and hatchery chinook salmon.

Results and Discussion

We are in the process of analyzing data to compare levels of gill ATPase activity in wild and hatchery juvenile spring chinook salmon and steelhead during their seaward migration. In this report we present some preliminary observations. We compared gill ATPase data for steelhead at Rock Island Dam, McNary Dam, Lower Granite Dam, and the Snake River Trap from 1990 to 1996, Salmon River Trap from 1993 to 1996, and the Grande Ronde and Imnaha River traps in 1994 and 1995. For spring chinook salmon, we analyzed data from the Salmon River, Snake River and Clearwater River traps and Lower Granite Dam from 1993 to 1996, and the Grande Ronde and Imnaha River traps in 1994 and 1995. Data from Little Goose Dam for steelhead and spring chinook was available only for 1993.

Wild spring chinook salmon and steelhead sampled at Columbia and Snake river locations generally had higher mean ATPase values than hatchery fish for the majority of the sample dates for all years studied. Differences between wild and hatchery fish were not consistent among all sample sites in all years. On many occasions, the difference between mean ATPase values from wild and hatchery fish was statistically significant. The temporal patterns of increasing and decreasing mean ATPase values for wild and hatchery fish ran parallel to each other at most sample sites. Wild fish generally had higher ATPase levels than hatchery fish, until the later part of the migration period, when levels were similar. However, throughout 1993, mean ATPase activity of wild spring chinook was significantly higher than that of hatchery spring chinook. The same was true of spring chinook and steelhead at the Snake River Trap. In 1993, a high flow year, ATPase activities of wild and hatchery spring chinook at Lower Granite Dam were significantly different for the first five sampling dates between April 27 and May 12. There was a significant difference between wild and hatchery steelhead in 1993 at Lower Granite Dam for the entire season, and for six of nine sample dates in May at Little Goose Dam (Figure 6). Data from this study suggested that wild fish begin migrating with higher ATPase levels than hatchery fish and experience a lower percent increase in ATPase activity than hatchery fish during migration. This observation was supported by data from the Lower Granite Dam Recapture Study in 1996.

There were a number of instances at traps and dams when wild and hatchery fish mean ATPase values differed significantly only in the early part of the sampling season. This was true for both spring chinook salmon and steelhead, at upper basin traps and dams, for all years. Generally, the earlier sampling began (late March versus early May), the more pronounced were the differences in mean ATPase values between wild and hatchery fish. This early season difference was seen in steelhead at Rock Island Dam from 1990 through 1993, at McNary Dam in 1993 and 1994, and at the Snake River Trap in 1990, 1993, and 1995. For spring chinook

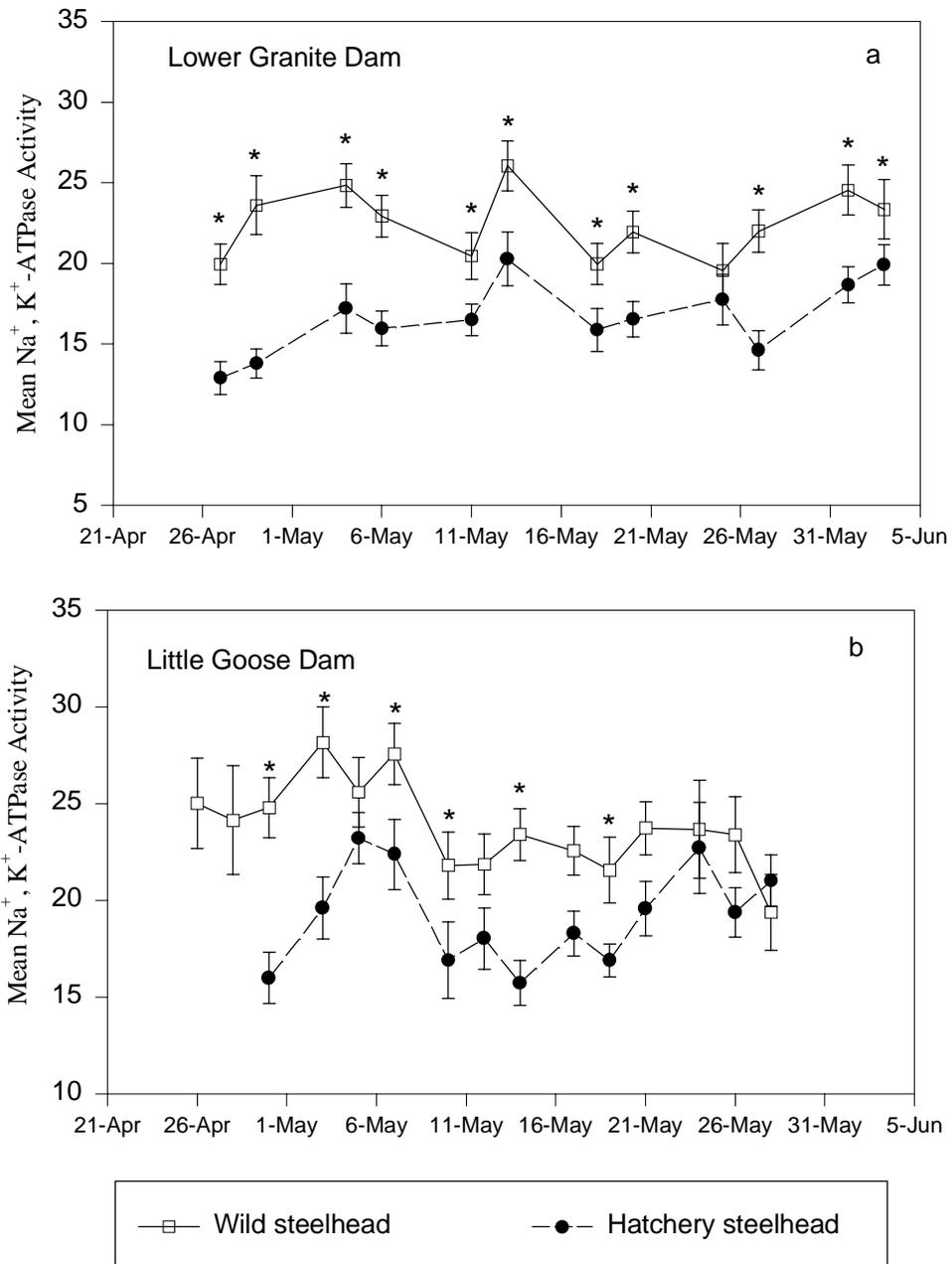


Figure 6. Mean Na^+ , K^+ -ATPase levels ($\mu\text{mol P}_i \cdot \text{mg protein}^{-1} \cdot \text{hour}^{-1}$) of steelhead sampled from run-at-large populations collected at a) Lower Granite Dam and b) Little Goose Dam in 1993. Asterisks mark dates when ATPase levels differed significantly.

salmon, early season differences were seen at the Salmon River Trap from 1993 through 1995, at the Snake River Trap in 1993, and at Lower Granite Dam from 1993 through 1995. However, in 1995 at the Salmon River Trap, significant differences between mean ATPase levels continued for most of the season. In comparison, there were only a few instances of significant difference between wild and hatchery spring chinook salmon sampled at the Clearwater River Trap.

Summary and Conclusions

Data collected by the ASCTTA project provides information about the smoltification of wild and hatchery spring chinook salmon and steelhead. This information, provided on a weekly basis, has been used by fish managers to set Water Budget requests for the Columbia and Snake river basins. Wild and hatchery fish at dams and traps can come from many sources and be in-river for varying lengths of time. The diversity of sources and length of in-river residence, as well as the natural variation within a population, make it difficult to draw conclusions about the smoltification of a population of fish and its effect on the readiness of juvenile salmon to migrate without physiological monitoring. However, data from this project provides useful information about the physiological condition of juvenile salmonids at hatcheries and at different times during their migration at sites in the Snake and Columbia river basins. Because the data spans a ten-year period, comparisons can be made between years with differing environmental conditions, such as high and low river flow. This information can be used with data on water availability to make management decisions regarding outmigrating juvenile salmonids.

Data from this project provides additional evidence in support of previous studies, which conclude hatchery fish are often released at a different level of smoltification as compared to their wild counterparts, a difference that appears to lessen as the fish migrate. Differences in the smoltification level of wild and hatchery fish are important because studies have shown spring chinook salmon with higher ATPase levels have faster travel time, and it is assumed that faster travel time translates into higher survival to the ocean. Wild coho salmon smolts captured during seaward migration had significantly greater tolerance to saltwater than hatchery fish (Shrimpton et al. 1994). Although there was no consistent pattern of differences between wild and hatchery fish for all sample sites in all years, there are recognizable trends in the data that support these conclusions.

The 1996 Lower Granite Dam Recapture Study represents the first time multiple ATPase samples have been taken collected from individual juvenile salmonids during their migration. The results of the study illustrate the differences in physiological development and migration patterns between wild and hatchery spring chinook salmon, and between spring chinook salmon and steelhead. Inter-species differences exist between patterns of smolt development and migration between spring chinook salmon and steelhead.

SECTION ONE PART FOUR

Prevalence of Bacterial Kidney Disease in Juvenile Chinook Salmon in the Columbia and Snake Rivers A Review 1987 to 1996

Scott P. VanderKooi

Bacterial kidney disease (BKD) is one of the most serious health problems affecting wild (Evelyn et al. 1973) and hatchery-reared salmonids (Fryer and Sanders 1981; Fryer and Lannan 1993). *Renibacterium salmoninarum* (RS), the pathogenic bacterium that causes BKD, can be transmitted both horizontally (Bell et al. 1984; Murray et al. 1992) and vertically (Bullock et al. 1978; Evelyn et al. 1986). The diverse modes of transmission allow the disease to be easily spread and make control in hatchery environments difficult. The lack of effective vaccines and the inability of antibiotics to eliminate RS infections (Fryer and Lannan 1993) have also hindered efforts to control this disease. The disease is not just a problem in hatchery environments. Large numbers of juvenile chinook salmon *Oncorhynchus tshawytscha* migrating to the ocean from the Snake and Columbia rivers were infected with RS (Elliot et al. 1997), which is of concern because infected fish may die after entering salt water (Sanders et al. 1992).

Over a ten-year period, the Assessment of Smolt Condition for Travel Time Analysis (ASCTTA) project monitored RS infections both in hatcheries and during migration as part of an effort to evaluate the health and condition of juvenile salmonids in the Columbia River basin. Our initial objective was to determine whether high mortality in migrating chinook salmon smolts used to estimate travel time and survival was due to RS infection. Over time, we expanded our study to incorporate several new objectives. These objectives were to determine whether: 1) the incidence of RS varied from year to year in fish in hatcheries, 2) the incidence of RS infections would change during the migration of fish from the Columbia and Snake rivers to the ocean, 3) the incidence of RS infections differed between Columbia River fish and Snake River fish during migration, 4) the incidence of RS changed with the timing of migration, and 5) changes in the incidence of RS infections were associated with altered hatchery practices or differences in river environments. For the last four years of the study, 1993 through 1996, we wanted to determine whether the incidence of RS infections at six of eight hatcheries would remain low or continue to decline, as had been observed from 1988 through 1992 (Maule et al. 1996). We also wanted to determine if the incidence of RS would decline at two hatcheries concurrent with the adoption of certain hatchery practices thought to be associated with lowered prevalence at other hatcheries.

We began monitoring the prevalence of RS infections in juvenile chinook salmon in 1987 to help determine if certain assumptions were being violated when calculating survival and travel time estimates for test groups (released directly from hatcheries) and control groups (truck-transported to downstream release sites) of salmonids migrating from the Columbia River basin to

the ocean (Rondorf et al. 1988). The assumptions in question were that, in groups of fish used to estimate survival and travel time, test and control fish were: 1) identical at time of release and 2) identical at the recovery site and therefore equally susceptible to collection. Kidney samples in 1987 were analyzed by both a fluorescent antibody technique (FAT) and an enzyme linked immunosorbent assay (ELISA). The greater sensitivity of the ELISA in detecting fish positive for RS led us to use the ELISA results for comparison of groups in 1987, and to use this assay exclusively for the detection of RS in all years of this study.

Results from 1987 indicated that the prevalence of RS infections was significantly higher in test fish than in controls at release from Winthrop National Fish Hatchery (NFH). We also found that the prevalence of RS was higher in test groups at release than among fish recaptured at McNary Dam. Another observation was that RS prevalence increased as the migration progressed. The prevalence of the disease was higher in fish collected in May compared to those sampled in April (Rondorf et al. 1988). Our results indicated the assumptions employed to estimate survival and travel time were being violated. We found that test and control groups differed in RS prevalence at release, and that prevalence had changed between release and recapture.

Monitoring of RS continued from 1988 through 1996, as part of our effort to monitor the condition of smolts used to estimate survival and travel times of migrating salmonids. In 1988, we expanded monitoring sites to include two Columbia River and two Snake River hatcheries as well as three hydroelectric dams, one on the Snake and two on the Columbia. By 1990, the number of monitored hatcheries had increased to four Snake River and four Columbia River sites. In-river monitoring of RS continued through 1992, and most hatcheries were sampled through 1996.

The most obvious trend observed from 1988 to 1992 was a decline in the prevalence of RS-positive fish at six of eight monitored hatcheries (Figures 1a and 1b). Prevalence declined from levels near 100% at most sites to as low as 3% at Entiat National Fish Hatchery. Concomitant with decreases in RS prevalence, the majority of these hatcheries were changing or adopting practices thought to stop the spread of RS or limit the severity of infection. Changes implemented, although not universally, included antibiotic injection of adults, destruction of eggs from severely infected females, juvenile segregation based on parental infection levels, antibiotic laced feed, and reduced rearing densities in raceways and ponds. It was suggested that the decline in prevalence was the result of changes in hatchery practices which acted to limit the vertical and horizontal transmission of RS (Maule et al. 1996). Over the same time period, we also found that the mean prevalence of RS was significantly higher in fish at Lower Granite and McNary dams than in fish just prior to release from monitored Snake River hatcheries. In contrast, prevalence in fish prior to release from Columbia River hatcheries was not significantly

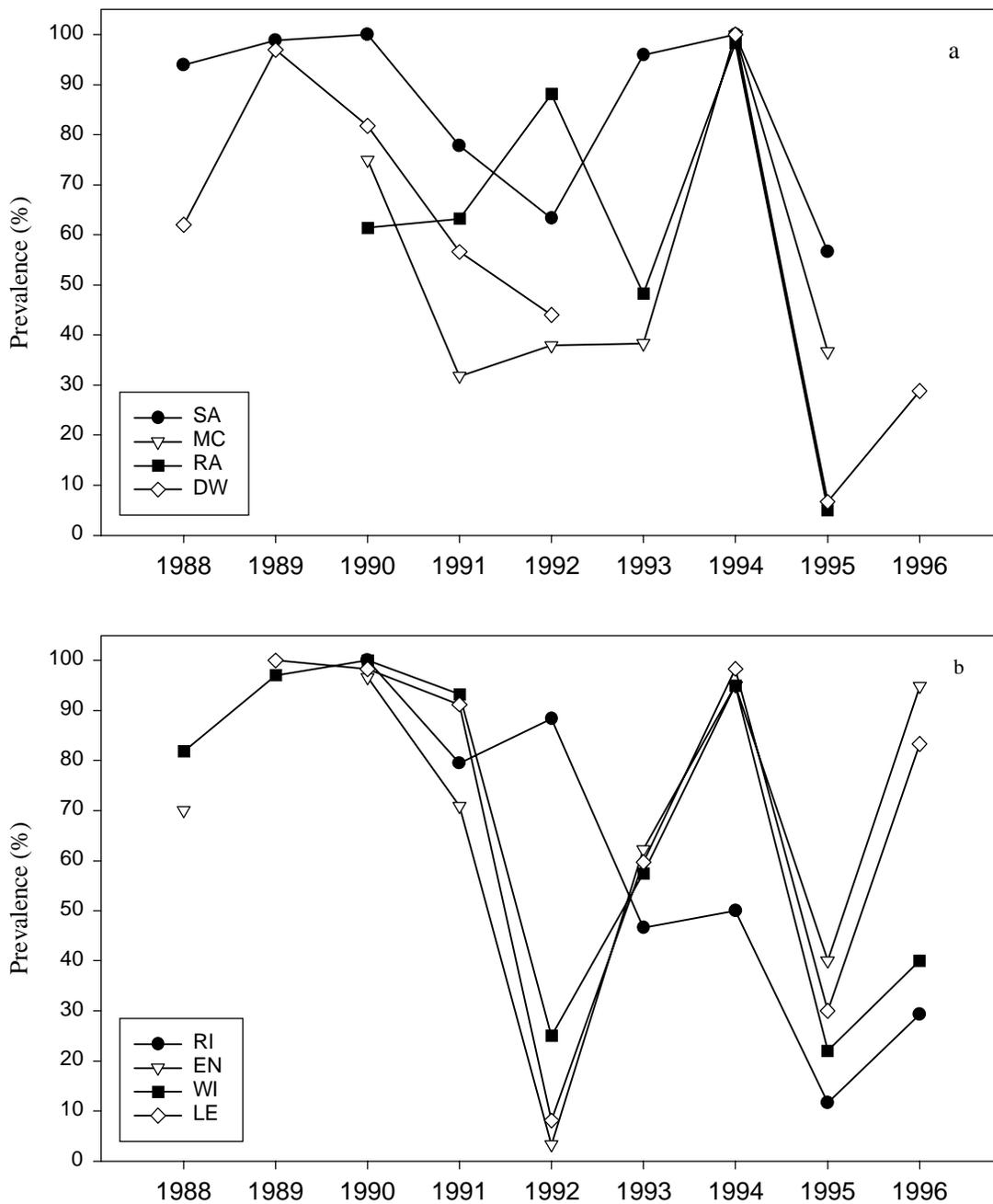


Figure 1. Prevalence of *Renibacterium salmoninarum*-positive spring chinook salmon sampled at a) Snake River hatcheries: Sawtooth SFH (SA), McCall SFH (MC), Rapid River SFH (RA), and Dworshak NFH (DW); and b) Columbia River hatcheries: Ringold SFH (RI), Entiat NFH (EN), Winthrop NFH (WI), and Leavenworth NFH (LE) from 1988 to 1996.

different from groups sampled at Rock Island and McNary dams. Similarly, we found that mean severity of infection was higher for Snake River fish in six of 16 monitored groups collected at Lower Granite and McNary dams compared with levels prior to release, but no differences were detected in fish released from Columbia River sites and collected at Rock Island and McNary. We concluded that RS prevalence and severity of infection could increase during migration. The variability in results between the Snake and Columbia rivers may be explained by environmental differences. Snake River fish were exposed to higher water temperatures and had longer distances to travel from release locations to collection sites at dams.

We continued to monitor hatcheries from 1993 through 1996 to determine if RS prevalence would remain low or continue to decline at locations where decreases had been observed from 1988 through 1992. We also wanted to determine if prevalence would decline at hatcheries following changes in practices after 1992. Our results showed that prevalence declined at the two hatcheries where practices were changed after 1992. The prevalence of RS infection at Rapid River State Fish Hatchery (SFH) dropped from near 90% in 1992 to below 50% in 1993 (Figure 1a), and prevalence at Ringold SFH went from just below 90% to less than 20% in 1995 (Figure 1b). These results further supported our hypothesis that changes in certain hatchery practices may reduce the prevalence of RS infections. Prevalence increased at four of seven sites in 1993 and seven of eight sites in 1994 (Figures 1a and 1b), indicating that declines in the prevalence of RS infections at these locations may have been temporary.

The increases in RS prevalence observed in 1993 and 1994, followed by declines at all hatcheries in 1995 and increases at all locations in 1996, suggest that the prevalence of RS infections is not solely controlled by hatchery practices. Our results showed that by 1993, the severity of RS infections as measured by the mean ELISA optical density (OD), was low at all monitored hatcheries and remained low at most locations through 1996 (Figures 2a and 2b). These results suggest that certain hatchery practices may limit the severity of RS infections. However, elevations in severity at Winthrop and Entiat NFH in 1994 and 1995 indicate these reductions may be temporary. It was suggested that the prevalence and severity of RS infections are controlled by many factors including, but not limited to, hatchery practices (VanderKooi and Maule 1998).

Although our results indicate that hatchery practices are not the only factor involved in controlling RS infections, we believe they do play an important role in limiting the spread of this and other diseases. Two practices implemented at several monitored hatcheries, juvenile segregation based on parental RS infection level (Pascho et al. 1991; Elliot et al. 1995) and antibiotic injection of adults (Lee and Evelyn 1994), have been shown to limit RS infections. Other factors that may influence RS infections include stress, antibiotic resistance, genetic factors, and environmental conditions. While certain hatchery practices can limit RS infections, it is clear more research is necessary to develop a full understanding of the factors involved and an ability to control this complex disease.

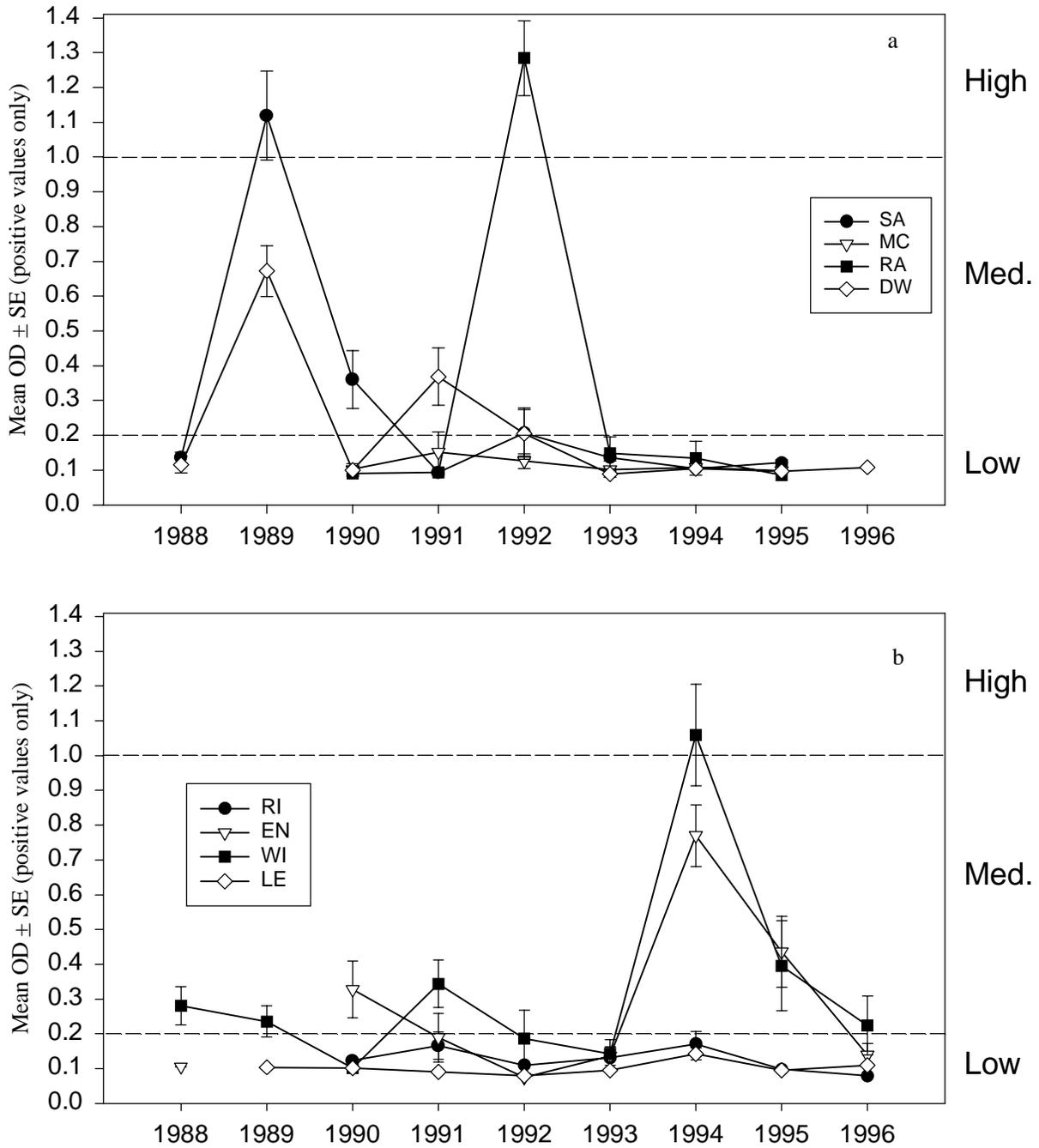


Figure 2. Mean (\pm SE) ELISA optical density (OD) of *Renibacterium salmoninarum*-positive spring chinook salmon sampled at a) Snake River Hatcheries: Sawtooth SFH (SA), McCall SFH (MC), Rapid River SFH (RA), and Dworshak NFH (DW), and b) Columbia River hatcheries: Ringold SFH (RI), Entiat NFH (EN), Winthrop NFH (WI), and Leavenworth NFH (LE), from 1988 to 1996. The high, medium (MED), and low levels of infection are based on values established by Pascho et al. (1991).

SECTION TWO PART ONE

Technical Assistance to Fishery Management Agencies

Robin M. Schrock

Introduction

Numerous federal, state, tribal and public utility agencies are conducting studies evaluating the effects of their management strategies on smolt physiology to optimize juvenile migration and adult returns. These studies (listed below) include measurements of physiological variables to predict the success of various treatments. The USGS, Biological Resources Division, Columbia River Research Laboratory (CRRL) receives requests to provide these agencies with assistance in the form of experimental design review and analytical laboratory support. Since 1987, results of project monitoring and evaluation activities have been provided directly to state and federal reference hatcheries, and to agencies conducting in-river marking and evaluation projects. Fourteen agency projects, many receiving direct Bonneville Power Administration (BPA) funding, were given analytical support in 1997, with ten projects scheduled for assistance in 1998. In 1997, the frequency of requests increased to the extent that a specific program designed to meet this need was essential. Foremost to the technical assistance program, a database needs to be established that will provide a unified means for information transfer to all fisheries managers in the basin. Results, considered in the framework of past studies, can be incorporated into future studies to avoid duplication of effort. Researchers are often not aware of the existence of projects similar to their own. The lack of information transfer results in projects that do not take advantage of available technology, and information collected may not be evaluated relative to the full array of data on Columbia River basin fish quality, wild fish surveys, water quality, and fishery management activities.

The CRRL has established the necessary database documenting management practices throughout the basin. The database will be available to regional managers to provide a reference for future studies by cooperating agencies, provide analysis capabilities which consider system-wide variables, and provide a framework for future technical assistance programs. The program will be composed of database support, research design evaluation, and specialized laboratory support services.

Basis of 1998 Priority

The priority for 1998 is based on renewed requests for assistance from ten projects that received assistance from our laboratory in 1997. The latest project objective involved creating a database from all projects in the region that document rearing practices designed to increase juvenile outmigration. We will include data on rearing variables (e.g., nutrition, prophylaxis, and water quality, among others), Columbia and Snake River water quality (e.g., temperature, flow, spill, etc.), and other system-wide variables that might explain differences in migration and survival between treatments, years, and stocks. The program will expand the value of existing projects by providing database and analytical laboratory support needed by fishery production and field managers to determine the basis of changes in migration and survival of salmon related to the physiology of referenced stocks. The database project will provide assessment capabilities to all fishery production and management practices in the basin, as they relate to juvenile emigration and adult returns. We will ensure that agencies that contact us use compatible techniques, research design, and data entry.

1997 Technical Assistance Projects

Each of the projects listed below has requested assistance in the form of recommendations for project design, and measurement of physiological parameters related to smoltification. We have also provided assistance to the USFWS California-Nevada Fish Health Center, the Minnesota Department of Natural Resources, and researchers at Purdue University in setting up similar research projects by providing consultations, and sampling and assay protocols.

Title: Post-release Behavior and Survival of Hatchery Fall Chinook Released into the Snake River
Agency: USGS-BRD, Columbia River Research Laboratory
Funding: BPA
Contact: Dennis Rondorf, Project Leader
Phone: (509)538-2299 ext. 228
FAX: (509)538-2843

Title: Movement and Behavior of Juvenile Salmonids at Three Lower Columbia River Dams
Agency: USGS-BRD, Columbia River Research Laboratory
Funding: US Army Corps of Engineers (COE)
Contact: Thomas Poe, Project Leader
Phone: (509)538-2299 ext. 237
FAX: (509)538-2843

Title: Gas Bubble Disease Monitoring and Research of Juvenile Salmonids
Agency: USGS-BRD, Columbia River Research Laboratory
Funding: BPA
Contact: John Beeman, Principal Investigator
Phone: (509)538-2299 ext. 257
FAX: (509)538-2843

Title: Post-release Attributes and Survival of Hatchery and Natural Fall Chinook Salmon in the Snake River
Agency: USFWS, Idaho Fishery Resource Office, P.O. Box 18, Ahsahka ID 83520-0018
Funding: COE-funded cooperative project under Lower Snake River Compensation Plan, Nez Perce Tribe, WDFW (BPA-funded for 1998)
Contact: Billy Connor, Co-Project Leader
Phone: (208)476-7242
FAX: (208)476-3252

Title: Study on the Influence of Water Temperature on Precocity and Residualism in Wenatchee River Steelhead Smolts
Agency: WDFW, Rock Island Evaluation, Mid-Columbia Field Office, 610 N. Mission Suite B8, Wenatchee WA 98801
Funding: WDFW
Contact: Kris Petersen, Project leader
Phone: (509)664-3149
FAX: (509)664-3148

Title: Survival Differences in Coho Salmon Reared Under Semi-natural Conditions and Standard Conditions in a Hatchery
Agency: WDFW, Hatcheries Program, 600 Capitol Way, Olympia WA 98501-1091
Funding: WDFW
Contact: Howard Fuss, Project Leader
Phone: (360)902-2664
FAX: (360)902-2943
Field Contact (Cathlamet, Elocham Hatchery): Jim Byrne, 28501 NW 7th Ave, Ridgefield WA 98642, Phone (360)887-1536

Title: Cowlitz Falls Anadromous Fish Reintroduction Project
Agency: WDFW, Fish Management, 600 Capitol Way, Olympia WA 98501-1091
Funding: WDFW
Contact: Charles Morrill, Project Leader
Phone: (360)753-3009
FAX: (360)586-2481
E-mail: cfmorrill@aol.com
Field Contacts (Cowlitz Falls Fish Facility): John Serl, Diane McKissick, Phone (360)497-5026
or (360)498-3003; FAX (360)493-3002

Title: Stress in Acclimated versus Direct Stream Released Fish: Tucannon River Spring Chinook
and Lyons Ferry Fall Chinook
Agency: WDFW, Hatcheries Program, Snake River Laboratory, 401 S. Cottonwood St.,
Dayton WA 99328
Funding: WDFW
Contact: Glen Mendel, Project leader
Phone: (509)382-4755
FAX: (509)382-2427
Field Contacts: Debbie Milks, Joe Baumgartner, Phone (509)382-4755

Title: Effectiveness of β -Glucans as Feed Additives For Increasing Disease Resistance:
Measured by Non-specific Immune Response and Survival after Disease Challenge
Agency: USFWS, Abernathy Salmon Culture Technology Center, 1440 Abernathy Road,
Longview WA 98632
Funding: USFWS
Contact: Ann Gannam, Project Leader
Phone: (360)425-6072
FAX: (360)636-1855

Title: Determination of the Seasonality of Mucus Lysozyme Associated with Smoltification and
Novel Water Rearing
Agency: USFWS, Abernathy Salmon Culture Technology Center, 1440 Abernathy Road,
Longview WA 98632
Funding: USFWS
Contact: Ann Gannam, Project Leader
Phone: (360)425-6072
FAX: (360)636-1855

Title: Evaluation of Fish Cultural Techniques at Dworshak National Fish Hatchery
Agency: USFWS, Idaho Fishery Resource Office, P.O. Box 18, Ahsahka ID 83520-0018
Funding: USFWS
Contact: Ray Jones, Project leader
Phone: (208)476-7242
FAX: (208)476-3252

Title: Cohabitation Experiment: Effects of Stress on Chinook Salmon Exposed to Chinook Salmon Infected with *Renibacterium salmoninarum*
Agency: USGS, BRD, Western Fisheries Research Center 6505 NE 65th St., Seattle WA 98115
Funding: USGS, BRD
Contact: Ron Pascho, Project Leader
Lab contact: Connie McKibben
Phone: (206)526-6282
FAX: (206)526-6654
Contact at Marrowstone Marine Station: Nancy Elder, Phone (360)385-1007, FAX (360)385-7207.

Title: Behavior and Fate of Juvenile Salmonids Entering Tailwaters of The Dalles Dam via Spill
Agency: USGS Oregon Cooperative Fishery Research Unit, Department of Fish and Wildlife, Room 104 Nash Hall, Oregon State University, Corvallis OR 97331
Funding: COE
Contact: John Snelling, Project leader
Phone: (541)737-2592

Title: Evaluation of Facilities for Collection, Bypass, and Transportation of Outmigrating Chinook Salmon
Agency: USGS Oregon Cooperative Fishery Research Unit, Department of Fish and Wildlife, Room 104 Nash Hall, Oregon State University, Corvallis OR 97331
Funding: COE
Contact: Larry Davis, Project leader
Phone: (541)737-2592

Summary

Technical assistance provided by the ASCTTA project includes sampling and analysis assistance to other BPA-funded projects at the CRRL, and to cooperating agencies in the region. The greatest amount of assistance has been provided to BPA-funded projects that monitor fall chinook salmon in the Columbia River basin. The most frequently requested activity is the determination of gill Na^+ , K^+ -ATPase in juvenile salmon at hatcheries and during migration.

Demand for physiological testing for other blood or tissue components associated with smoltification has increased. The ASCTTA project has provided all Na^+ , K^+ -ATPase analysis for BPA Project No. 91-029 that is also funded by the USFWS Lower Snake River Compensation Plan. Participation in NMFS survival studies has also included Na^+ , K^+ -ATPase analysis to assess smoltification and its relationship to travel times and survival. Further analyses that have been requested by other projects are blood electrolytes including sodium, potassium, and chloride, blood differentials, cortisol, lysozyme, and total body fat analysis. Because the listed assays require trained personnel, it is not cost-effective to hire skilled technicians for the short duration of most projects, or to train staff for activities that usually occur seasonally. While we have trained personnel from other projects and agencies to perform the assays themselves, the equipment necessary for the techniques is usually prohibitively expensive. The ability of the ASCTTA project to provide this service to other fishery agencies involved in monitoring the same stocks is advantageous because it is cost-effective and allows for a consistency of results for basin stocks. Results may be compared and evaluated without the concern of differences in handling of samples between laboratories.

SECTION TWO PART TWO

Non-Lethal Indices of Smolt Condition and Health

Robin M. Schrock

The Assessment of Smolt Condition for Travel Time Analysis Project (ASCTTA) developed within the framework of the Fish and Wildlife Program (FWP) to provide the Fish Passage Center (FPC) with information about physiological characteristics of juvenile salmonids in the Columbia River basin for in-season management of flows. The relation between the physiology, condition, and health of juvenile migrants to migration rates and survival was investigated. This information was applied to management of the Water Budget, the volume of water set aside to provide flows to facilitate juvenile migration. The level of smoltification, or physiological preparedness to osmoregulate in seawater, was tested at specific times during development.

Smoltification is a complex process involving progressive endocrine, morphological, immunological, and behavioral changes (Wedemeyer et al. 1980; Folmar and Dickhoff 1981; Hoar 1988; Barron 1986). Tissues differentiate to adapt the fish for life in seawater, and behavioral changes occur, prompting the fish to migrate. Use of a single measurement as an index of smoltification is questionable based on the prolonged and complex nature of the process. Elements of each adaptive change have been examined and described in great detail, but a complete understanding of the interactions and exact sequence of events remains obscure. Recognizable and repeated patterns of these physiological changes occur in all anadromous salmonid species, yet the magnitude and timing of these events differ among species, and between wild and hatchery fish. Environmental cues control the onset, progression, and magnitude of all the physiological and behavioral changes associated with smoltification. Characterization of the annual physiological development patterns of individual fish stocks allows for adaptive management of captive rearing protocols and hydropower operations during smolt and adult migrations. A major goal of the ASCTTA project has been to develop non-lethal techniques to measure physiological change in juvenile salmonids for field applications and stock management. As more stocks in the Columbia River basin reach critically low numbers, and are listed as threatened or endangered under the Endangered Species Act (ESA), the need for such techniques increases.

The measurement of gill sodium, potassium-activated adenosine triphosphatase (Na^+ , K^+ -ATPase), an enzyme involved with ionic regulation in the gills and, therefore, osmoregulatory ability, in combination with condition factor, has been the standard used to assess the stage of

physiological development of juvenile salmonids related to migration. The enzyme exhibits a characteristic and repeated pattern of seasonal change in activity seen in all juvenile anadromous salmonids. Gill Na^+ , K^+ -ATPase provides a quantitative measurement of smoltification, but levels at different stages of development vary among species and stocks. The characteristic profile of low values in the hatchery that increase slowly until fish are released, and increase substantially during the migration, is of a different magnitude in wild and hatchery fish. In keeping with our objective to find non-lethal methods of determining the level of smolt development, we developed a microassay for the gill Na^+ , K^+ -ATPase to allow us to continue smolt evaluations without sacrificing fish (Schrock et al. 1994).

Methods that measure other changes occurring during smoltification have been developed and correlated to gill Na^+ , K^+ -ATPase. A noticeable change in body shape occurs as juvenile salmon undergo smoltification, including lengthening of the caudal peduncle, enlargement of the head, and narrowing of the body. The changes in body shape can be used to measure smoltification level, by using distances between anatomical landmarks in photographs of fish to calculate morphometric indices. Several principal components were used to calculate a canonical variate that correlates significantly with gill ATPase activity in juvenile spring chinook salmon and steelhead (Beeman et al. 1994, 1995). The method allows for the non-lethal, non-invasive physiological evaluation of large numbers of fish. The fish can be photographed in water under light anesthesia, then released. The earliest use of the method was in studies of spring and fall chinook salmon juveniles by WDFW. Returns of 5-year old adults in 1998 will allow for publication of the results. The USGS Western Fisheries Research Laboratory, Seattle, WA, is using the method to evaluate smoltification in wild, hatchery, and wild \times hatchery crosses of Warm Springs National Fish Hatchery (NFH) spring chinook salmon. The Oregon Department of Fish and Wildlife (ODFW) is currently applying the method to compare and distinguish between wild chinook salmon parr and smolts in eastern Oregon. The method is suited to field applications, and will allow continued monitoring of threatened and endangered stocks where invasive techniques might critically affect fish health and survival.

Juvenile anadromous salmonids change color as guanine and other purines are deposited under the skin as the fish smolt. The degree of silvering, which indicates level of smoltification, has been measured by a subjective visualization method later found to be unreliable. Therefore, a new method of measuring reflectance from the guanine found in the skin was developed by the project (Haner et al. 1995). Uniform light, reflected from fish held in an opaque box to eliminate ambient light, is measured in reference to a photographic gray scale. Digital images are processed by an imaging system that calculates skin reflectance with the average shade of gray on a standardized area of the fish. Skin reflectance was correlated with measurements of both gill Na^+ , K^+ -ATPase and skin guanine for juvenile steelhead and spring and fall chinook salmon. Reflectance is being used in conjunction with the morphometric method in the Warm Springs NFH evaluation, and we have provided the procedure to ODFW for incorporation in their investigations. The USFWS Dworshak National Fish Hatchery is currently using the method to distinguish among parr and smolts of different length frequencies of production steelhead. A

further application of the method will be in the routine health monitoring of hatchery releases by the Dworshak Fish Health Center. We have also provided information about the techniques to a USFWS office in California, and to Yakama Tribe fishery biologists.

The purpose of developing non-lethal techniques is to provide methods that can be applied to production, field, and management evaluations without endangering fish health. The value of these methods to the region is increasing, especially under the increased constraints of additional ESA listings on monitoring efforts. No other regional program now exists to monitor the changes in juvenile salmonid health and condition that occur as a result of continuing changes in habitat, production, and fish passage management in the region. Current and proposed passage facility modification, changes in flow and spill management, proposed drawdown and dam removals, and the continuing decline of Columbia basin salmonid numbers creates not only a need for a coordinated monitoring effort to test the effects of these changes on regional stocks, but also a need for methods to accomplish monitoring without further harm to declining stocks.

SECTION TWO PART THREE

Smolt Physiology Comprehensive Database

Philip V. Haner

The Assessment of Smolt Condition for Travel Time Analysis (ASCTTA) project is in the process of compiling a database containing (1) physiological data collected from 1987 to 1996 on juvenile salmonids, including chinook salmon, steelhead, and sockeye salmon, (2) hatchery practices during that same time, (3) environmental variables, such as water flow and temperature, and (4) smolt-to-adult survival data for Columbia and Snake river basin hatcheries. We believe that thorough multivariate analyses of these variables will help to elucidate those factors most important to predicting survival.

Physiological measurements included in the database were related to a) smoltification, such as gill Na^+ , K^+ -ATPase (ATPase), guanine, morphology, reflectance, and plasma thyroxine; b) assessment of health, including bacterial kidney disease (BKD), lysozyme, gas bubble trauma (GBT), and the Goede health assessment index; and c) stress, for example plasma cortisol, glucose, and chloride (Table 1). Fish from hatcheries throughout the Columbia River basin were sampled, however, sampling effort was concentrated on four hatcheries from the mid-Columbia region (Entiat, Leavenworth, Winthrop, and Priest Rapids) and four from the Snake River basin (Dworshak, McCall, Sawtooth, and Rapid River) (Table 2). Samples were also taken from fish collected at dams and traps on the Columbia and Snake rivers, focused on a core set of sites (Rock Island, Lower Granite, and McNary dams, and Snake River (Lewiston) and Clearwater traps) (Table 3).

All data we collected were stored in a relational database (RBase, Microrim, Inc. 1996) for each year. Data were analyzed primarily with SAS (SAS Institute Inc., 1996), Lotus 1-2-3 (Lotus Development Corporation, 1994), and Excel (Microsoft Corporation, 1994). SigmaPlot (SPSS Inc., 1997), Freelance Graphics (Lotus Development Corporation, 1994), and Statgraphics (Statistical Graphics Corporation, 1992) were used for creating figures and presentations for annual reports and publications.

The database will incorporate results from cooperative research and technical assistance activities to broaden our ASCTTA database and the hatchery rearing conditions survey results (see Section Two, Part Four). Future analysis will characterize individual stocks in the context of rearing conditions, release strategies, migration patterns, hydrosystem operations and annual climatic changes. We believe that comparison of wild and hatchery stocks using the historical records will allow fishery managers to determine what practices will ensure survival of each unique hatchery stock under changing environmental conditions.

Table 1. Summary of assays conducted on samples collected from chinook salmon, sockeye salmon, and steelhead from 1987 through 1996 by the Assessment of Smolt Condition for Travel Time Analysis Project. Assays include those testing levels of smoltification (ATPase, morphology, reflectance, and thyroxine), general health (bacterial kidney disease, lysozyme, and gas bubble trauma), and stress (plasma cortisol, glucose and chloride).

Assay	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996
Gill Na ⁺ , K ⁺ -ATPase	X	X	X	X	X	X	X	X	X	X
Bacterial Kidney Disease (BKD) visual	X	X	X	X	X	X	X	X	X	X
BKD ELISA	X	X	X	X	X	X	X	X	X	X
BKD FAT		X	X							
Plasma Chloride	X	X	X	X						
Plasma Cortisol	X	X	X							
Electromagnetic Scan (total body lipids)					X					
Gas Bubble Trauma									X	
Plasma Glucose	X	X	X							
Goede Health Assessment		X	X							
Guanine		X	X	X	X	X				
Lysozyme								X		
Morphology	X	X	X	X						
Potassium					X					
Reflectance					X	X				
Potassium after Seawater Challenge	X									
Sodium after Seawater Challenge	X									
Chloride after Seawater Challenge	X									
Osmolality	X									
Thyroxine	X	X	X	X						

Table 2. Summary of hatcheries sampled from 1987 through 1996 by the Assessment of Smolt Condition for Travel Time Analysis project (NFH = National Fish Hatchery; SFH= State Fish Hatchery; ID = Idaho; OR = Oregon; WA = Washington).

Hatcheries Sampled	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996
Bonneville SFH (OR)						X				
Carson NFH (WA)				X						
Dworshak NFH (ID)		X	X	X	X	X	X	X	X	X
Entiat NFH (WA)		X	X	X	X	X	X	X	X	X
Hagerman NFH (ID)		X								
Irrigon SFH (OR)		X	X	X	X	X	X			
Kooskia NFH (ID)										X
Leavenworth NFH (WA)		X	X	X	X	X	X	X	X	X
Lookingglass SFH (OR)								X	X	X
Lyons Ferry Trout SFH (WA)	X	X	X	X	X	X				
Lyons Ferry Salmon SFH (WA)		X	X	X						X
Magic Valley SFH (ID)			X							
McCall SFH (ID)		X	X	X	X	X	X	X	X	X
Niagara Springs SFH (ID)		X	X							
Priest Rapids SFH (WA)		X	X	X	X	X	X	X	X	X
Rapid River SFH (ID)		X	X	X	X	X	X	X	X	X
Ringold SFH (WA)		X		X	X	X	X	X	X	X
Sawtooth SFH (ID)		X	X	X	X	X	X	X	X	X
Spring Creek NFH (WA)			X	X	X					
Wells Salmon SFH (WA)								X	X	X
Wells Trout SFH (WA)	X	X	X	X	X					
Winthrop NFH (WA)	X	X	X	X	X	X	X	X	X	X

Table 3. Summary of dams and traps on the Columbia and Snake rivers, sampled from 1987 through 1996 by the Assessment of Smolt Condition for Travel Time Analysis project.

Dams and Traps Sampled	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996
Bonneville Dam		X		X	X	X	X			
Clearwater Trap				X	X	X	X	X	X	
Grande Ronde River Trap								X	X	
Imnaha River Trap								X	X	
John Day Dam				X	X	X	X	X	X	
Snake River Trap			X	X	X	X	X	X	X	X
Little Goose Dam						X	X	X		
Lower Granite Dam			X	X	X	X	X	X	X	X
Lower Monumental Dam		X		X				X	X	
Lower Granite Reservoir				X	X		X	X		
McNary Dam		X	X	X	X	X	X	X	X	
Rock Island Dam		X	X	X	X	X	X	X	X	X
Salmon River Trap							X	X	X	X

SECTION TWO PART FOUR

A Survey to Evaluate the Effects of Hatchery Rearing Conditions on Smoltification

Jack D. Hotchkiss

The Assessment of Smolt Condition for Travel Time Analysis (ASCTTA) project developed a survey to gather information from reference hatcheries in the Columbia River basin (Appendix 1). Data from the survey will be used to determine how rearing conditions might affect measurements of smolt physiology and development. The survey form was sent to state and federal hatcheries where we had previously taken physiological measurements of fish prior to release. Fish from these hatcheries were also monitored during emigration. Hatchery personnel were asked to fill out the survey, or to provide data that were pertinent to the survey questions. Physiological data from the ASCTTA project (described previously in Section Two, Part Three) will be compared with data from the hatchery survey to evaluate how hatchery variables influence specific measures of juvenile salmon physiology. The relation of hatchery conditions and smolt physiology during emigration to smolt-to-adult survival will also be examined.

Hatchery survey data is stored in a relational database (Rbase, Microrim, Inc. 1996). The data will be combined with the ASCTTA smolt physiology database, and analysis will be conducted to determine which hatchery rearing variables contribute to promoting smolt-to-adult returns.

The survey documents hatchery information for the ASCTTA project from 1987 to 1996. Each hatchery is a complex rearing system that requires a vast amount of diverse data for thorough study; therefore the survey had to be comprehensive. The survey requested genetic information, such as source(s) of hatchery stocks, stock transfers, and spawning ratios. Facility design was addressed, including raceway and pond design, and lighting. Rearing variables such as water source, water temperature and flow, stocking densities, and feed types and schedules were requested. The occurrence of disease outbreaks and timing of disease treatments before release were also considered. Release strategies, important to acclimation and imprinting, were of special concern. Records of adult returns will also be an important part of the analysis and will be added as the data becomes available.

Gathering all the information we needed for our analysis was problematic. Documentation was not uniform among hatcheries due to differences in data availability and data storage capabilities. Hatchery data received from the survey will be supplemented with data from other sources when possible, but we anticipate our analyses will need to be tailored to individual

hatcheries to accommodate differences in available data. A reference hatchery with the most complete record will be analyzed first, to provide a model for analysis of other hatcheries.

Our survey offers the opportunity to increase knowledge of the interaction of hatchery fish with changing environmental conditions during emigration, by combining the survey with the ASCTTA Columbia River salmon physiology database. We recommend further combining the ASCTTA and hatchery data into a consolidated Columbia River Basin hatchery database, which will improve access to historical data, and help standardize future data collection at hatcheries. A carefully designed database that addresses the specific information needs of all hatcheries will provide regional access to innovations in production, and will help establish basin-wide protocols for data collection and analysis. A centralized database and standardized documentation of hatchery conditions will allow ready access to information regarding variables that are important to promoting fish health, condition, migration, and smolt-to-adult survival.

SECTION TWO PART FIVE

Thyroxine Activity and the Role of Water Temperature in Smolt Physiology of Chinook Salmon (*Oncorhynchus tshawytscha*)

Sally T. Sauter
Alec G. Maule

Introduction

Smoltification in Pacific salmon (*Oncorhynchus* spp.) is a developmental process which prepares freshwater juveniles for emigration and seawater entry (Hoar 1976). Initiation of smoltification is a complex phenomenon which involves developmental readiness, environmental stimulation, and physiological coordination. Considerable differences occur in these variables between different stocks and species of Pacific salmon.

Thyroid hormone involvement was implicated very early in the study of smolt transformation (Hoar 1976). However, the permissive and synergistic physiological effects of thyroid hormones, the developmental plasticity of salmonids, and the multiple levels of organismal reorganization involved in smoltification prevent a clear and concise summation of the thyroidology of smoltification (Eales 1988; Specker 1988). Several studies have followed tissue or plasma levels of thyroxine (T_4) through smoltification. Dickhoff et al. (1978) investigated plasma T_4 levels of coho salmon (*O. kisutch*) and found that increased levels of circulating T_4 were associated with critical phases of development, a result previously documented for anuran amphibians undergoing metamorphosis. Yearling coho salmon display a prolonged increase in plasma T_4 levels in the spring. Ura et al. (1994) reported a similar pattern of T_4 activity for masou salmon (*O. masou*), which, like coho salmon, typically smolt as yearlings in the spring. Chum salmon (*O. keta*), which migrate to the ocean immediately following emergence, concomitantly show an increase in tissue levels of T_4 (Tagawa and Hirano 1990).

Seasonal increases in plasma T_4 levels of smolting salmonids appear to follow an endogenous activity pattern independent of changes in photoperiod and environmental temperature (Grau 1988). However, nutritional status and water temperature influence thyroid hormone metabolism in fish (Eales 1985), thus the physiological and developmental expression of endogenous thyroid activity.

Variation in the early life history characteristics of Pacific salmon encouraged further description of endogenous T_4 activity, and its role in smolt development and energy utilization. Inland stocks of chinook salmon (*O. tshawytscha*) in the Columbia River drainage show two

distinct freshwater residence patterns: spring chinook salmon rear for a full year in freshwater, whereas upriver bright fall chinook salmon rear for 3 to 6 months in the near-shore riverine environment before migrating to the ocean as subyearlings (Carl and Healey 1984). In the present study, we held spring and upriver bright fall chinook salmon under two temperature regimens, a constant low water temperature (8°C), and an increasing water temperature schedule which generally followed seasonal water temperatures in the Columbia River. Our objectives were to characterize seasonal T₄ activity patterns under controlled laboratory conditions during smoltification of spring and fall chinook salmon. We also investigated the relationship between plasma T₄ activity and gill Na⁺, K⁺-ATPase activity in subyearling fall chinook salmon during the period when they normally rear and emigrate. Mean plasma T₄ levels, mean gill Na⁺, K⁺-ATPase activity, and mean condition factor are presented for fish from each water temperature treatment and each race at approximately weekly intervals during the period when these fish undergo smoltification. Changes in the thermal preference of smolts, which suggest shifts in metabolism and energy utilization, were also noted.

Methods

Juvenile spring and upriver bright fall chinook salmon were obtained from the Little White Salmon Fish Hatchery, Cook, Washington, prior to smolt development. Fish were transported to the Columbia River Research Laboratory, Cook, Washington, and each race was stocked into one 1500-L outdoor tank, and two 285-L indoor tanks. Fish held indoors were exposed to a simulated natural photoperiod. Water to the outdoor tanks was unheated, and remained near 8°C throughout the study. Water temperature of the indoor tanks was held at 8°C during March; thereafter, water temperature was increased by 2°C at the beginning of each month until mid-June (14°C) for spring chinook and mid-August (18°C) for fall chinook salmon. The experimental design consisted of four groups of fish: spring chinook held at increasing water temperatures and at 8°C, and fall chinook held at increasing water temperatures and at 8°C. Fish were fed to satiation twice daily, in the morning and late afternoon.

Sampled fish were placed in a lethal dose (200 mg · liter⁻¹) of tricaine methane sulfonate (MS-222), measured for fork length (mm), and weighed (g). The caudal peduncle was blotted dry and severed with a razor blade. Blood was collected in a heparinized collection tube, centrifuged, and the plasma was removed from the pellet. Surgical scissors were used to remove a small amount of gill tissue from each fish, and the tissue was placed in a 0.5 ml aliquot of sucrose - disodium ethylenediamine tetraacetate - imidazole solution (SEI). Plasma and gill samples were immediately frozen at -80°C. Plasma T₄ levels were determined using the radioimmunoassay method of Dickhoff et al. (1978). To obtain an adequate blood sample for T₄ assay from fall chinook salmon, it was necessary to pool weekly plasma samples from three to five fish through most of the experimental period. We collected 14 pools of plasma from fall chinook salmon held at 8°C, and nine pools of plasma from fish held at increasing water temperatures. Gill Na⁺, K⁺-ATPase activity was measured using the method of Schrock et al. (1994), and is

expressed in units of micromoles of inorganic phosphate per milligram protein per hour ($\mu\text{mol P}_i \cdot \text{mg protein}^{-1} \cdot \text{h}^{-1}$).

Condition factor was calculated using the following formula:

$$K = 10^5 \cdot W \cdot L^{-3}$$

where K = condition factor, W = mean weight of sample group, and L = mean length of sample group.

Results

The temporal pattern of mean gill Na^+ , K^+ -ATPase activity and mean plasma T_4 were used to evaluate the degree of smolt development in spring and fall chinook salmon. Spring and fall chinook salmon displayed different patterns in timing of physiological events during smoltification in this study; additionally, these patterns were modified by the water temperature at which fish were held.

Spring chinook salmon held at increasing water temperatures showed a significant increase in mean gill Na^+ , K^+ -ATPase activity between March 4 ($5.6 \mu\text{mol P}_i \cdot \text{mg protein}^{-1} \cdot \text{h}^{-1}$) and April 12 ($12.4 \mu\text{mol P}_i \cdot \text{mg protein}^{-1} \cdot \text{h}^{-1}$) ($P = 0.1$, Tukey) (Figure 1). Plasma T_4 correlated positively with sample date from March 4 through April 19, and declined thereafter. Low levels of mean plasma T_4 ($5.6 \text{ ng} \cdot \text{ml}^{-1}$) and mean gill Na^+ , K^+ -ATPase activity ($5.0 \mu\text{mol P}_i \cdot \text{mg protein}^{-1} \cdot \text{h}^{-1}$), as well as the highest mean condition factor (1.33) occurred on June 14, indicating these fish had completed smoltification, and were reverting to parr. These fish showed no change in thermal preference during the experimental period (Sauter 1996).

Fall chinook salmon held at increasing water temperatures demonstrated recurrent peaks in mean gill Na^+ , K^+ -ATPase activity on June 3, July 8, and July 20, of 18.6, 17.7, and 19.8 $\mu\text{mol P}_i \cdot \text{mg protein}^{-1} \cdot \text{h}^{-1}$ respectively; all significantly different from April values ($P = 0.1$, Tukey) (Figure 2). Mean plasma T_4 levels peaked on May 5, at $11.7 \text{ ng} \cdot \text{ml}^{-1}$, preceding an increase in mean gill Na^+ , K^+ -ATPase activity. The need to pool plasma samples precluded statistical analysis of these data. Mean plasma T_4 levels decreased after May 5, and leveled off after May 20, while mean gill Na^+ , K^+ -ATPase activity continued to increase through August 7 ($17.8 \mu\text{mol P}_i \cdot \text{mg protein}^{-1} \cdot \text{h}^{-1}$). Mean condition factor increased to 1.23 by April 28, leveled off in May, and declined sharply to 1.02 by June 22, prior to an increase in mean gill Na^+ , K^+ -ATPase activity, and increased again between June 22 (1.02) and August 7 (1.21).

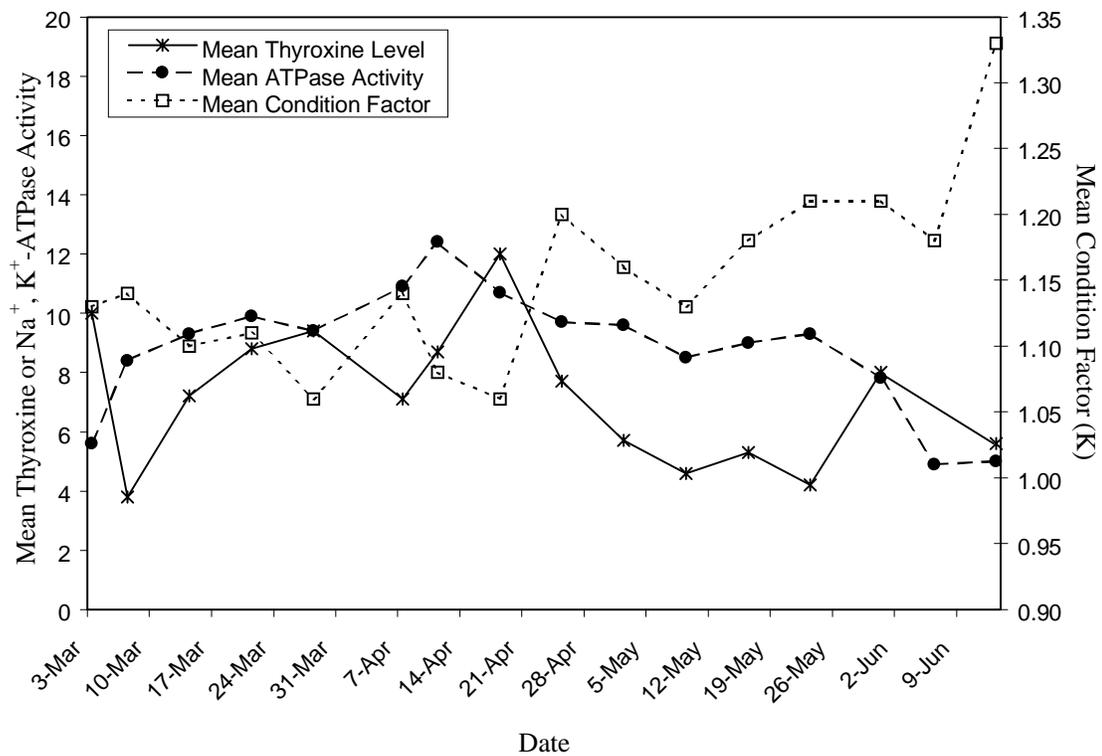


Figure 1. Mean thyroxine level ($\text{ng} \cdot \text{ml}^{-1}$), mean gill sodium, potassium-activated ATPase activity ($\mu\text{mol P}_i \cdot \text{mg protein}^{-1} \cdot \text{hr}^{-1}$), and mean condition factor (K) of spring chinook salmon held at increasing water temperatures.

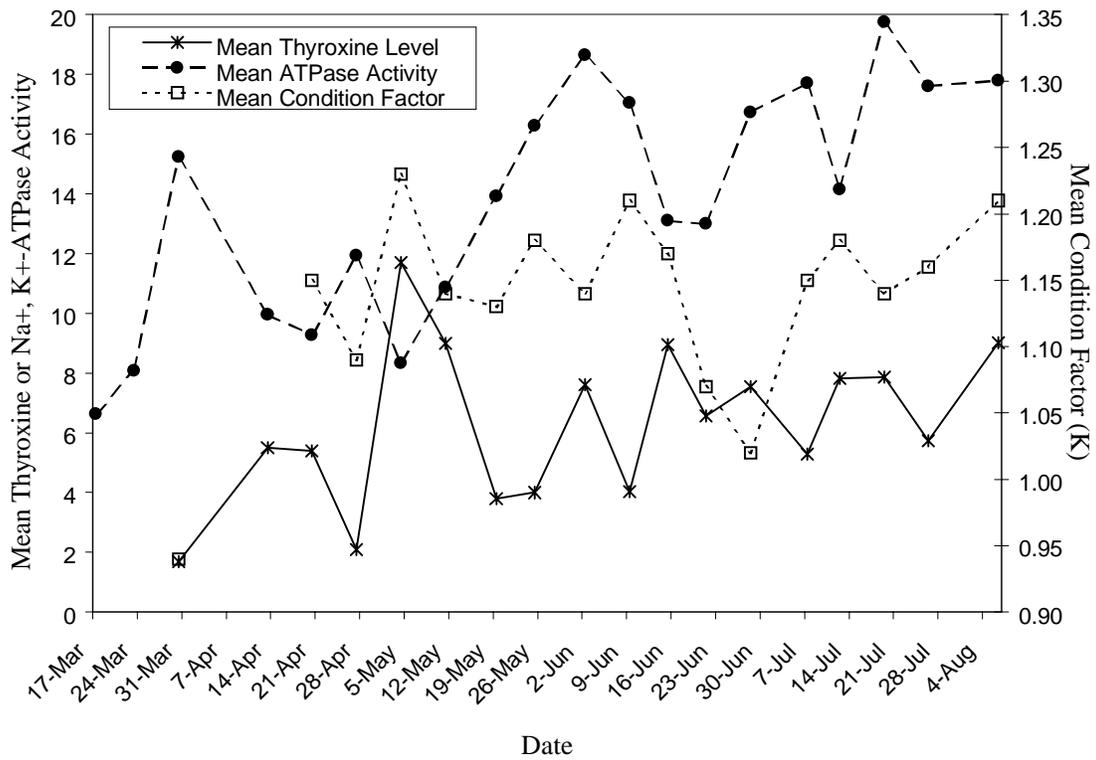


Figure 2. Mean thyroxine level (ng· ml⁻¹), mean gill sodium, potassium-activated ATPase activity (μmol P_i· mg protein⁻¹· hr⁻¹), and mean condition factor (K) of fall chinook salmon held at increasing water temperatures.

Spring chinook salmon held at 8°C showed a significant increase in mean gill Na⁺, K⁺-ATPase activity between March 9 (7.6 μmol P_i · mg protein⁻¹ · h⁻¹) and March 30 (13.5 μmol P_i · mg protein⁻¹ · h⁻¹) (P = 0.1, Tukey), followed by a significant decline in activity on April 13 (6.5 μmol P_i · mg protein⁻¹ · h⁻¹) (P = 0.1) (Figure 3). Mean gill Na⁺, K⁺-ATPase activity increased significantly again between May 19 (8.2 μmol P_i · mg protein⁻¹ · h⁻¹) and June 8 (19.4 μmol P_i · mg protein⁻¹ · h⁻¹) (P = 0.1). Concurrent with the initial rise in mean gill Na⁺, K⁺-ATPase activity, mean condition factor dropped to 0.99 on March 30, while mean plasma T₄ levels increased, but not significantly so, between March 23 (6.9 ng · ml⁻¹) and April 9 (16.2 ng · ml⁻¹). Mean condition factor increased between April 20 (1.11), and April 27, 1994 (1.22) prior to an increase in mean plasma T₄ level on May 11 (14.5 ng · ml⁻¹), and a significant peak in mean gill Na⁺, K⁺-ATPase activity on June 8 (19.3 μmol P_i · mg protein⁻¹ · h⁻¹).

Fall chinook salmon held at 8°C displayed recurrent increases in mean plasma T₄ levels on April 29 (18.4 ng · ml⁻¹), June 17 (5.7 ng · ml⁻¹), July 15 (7.3 ng · ml⁻¹), and August 10 (11.3 ng · ml⁻¹) (Figure 4). Plasma samples were pooled due to small fish size, so increases in mean plasma T₄ level could not be statistically validated. Increases in plasma T₄ levels preceded increases in mean gill Na⁺, K⁺-ATPase activity through the summer months, indicating these fish were actively smolting (Figure 4). A significant increase in mean gill Na⁺, K⁺-ATPase activity occurred between May 13 (7.1 μmol P_i · mg protein⁻¹ · h⁻¹), and August 30 (14.4 μmol P_i · mg protein⁻¹ · h⁻¹). Mean condition factor reached its highest point on May 27 (1.14) as mean gill Na⁺, K⁺-ATPase activity increased to 11.9 μmol P_i · mg protein⁻¹ · h⁻¹ on June 11. Concurrent increases in mean condition factor, and mean plasma T₄ on June 17 were followed by an increase in mean gill Na⁺, K⁺-ATPase activity on July 1 (13.0 μmol P_i · mg protein⁻¹ · h⁻¹).

Discussion

In this study, differences in the temporal sequence of gill Na⁺, K⁺-ATPase and T₄ activity were observed between spring and fall chinook salmon, and between fish held at 8°C and those held at increasing water temperatures. All groups of laboratory-held chinook salmon showed seasonal increases in mean gill Na⁺, K⁺-ATPase activity indicative of smolt development.

Spring chinook salmon held at 8°C demonstrated two significant peaks in gill Na⁺, K⁺-ATPase activity during the spring, while spring chinook salmon held at increasing water temperatures showed only one. The temperature differential between the two groups of spring chinook salmon was 4°C when fish at increasing water temperatures began to show a decline in gill Na⁺, K⁺-ATPase and plasma T₄ activity. Fall chinook salmon held at 8°C and at increasing water temperatures displayed several similarly-timed increases in gill Na⁺, K⁺-ATPase activity during the spring and summer months.

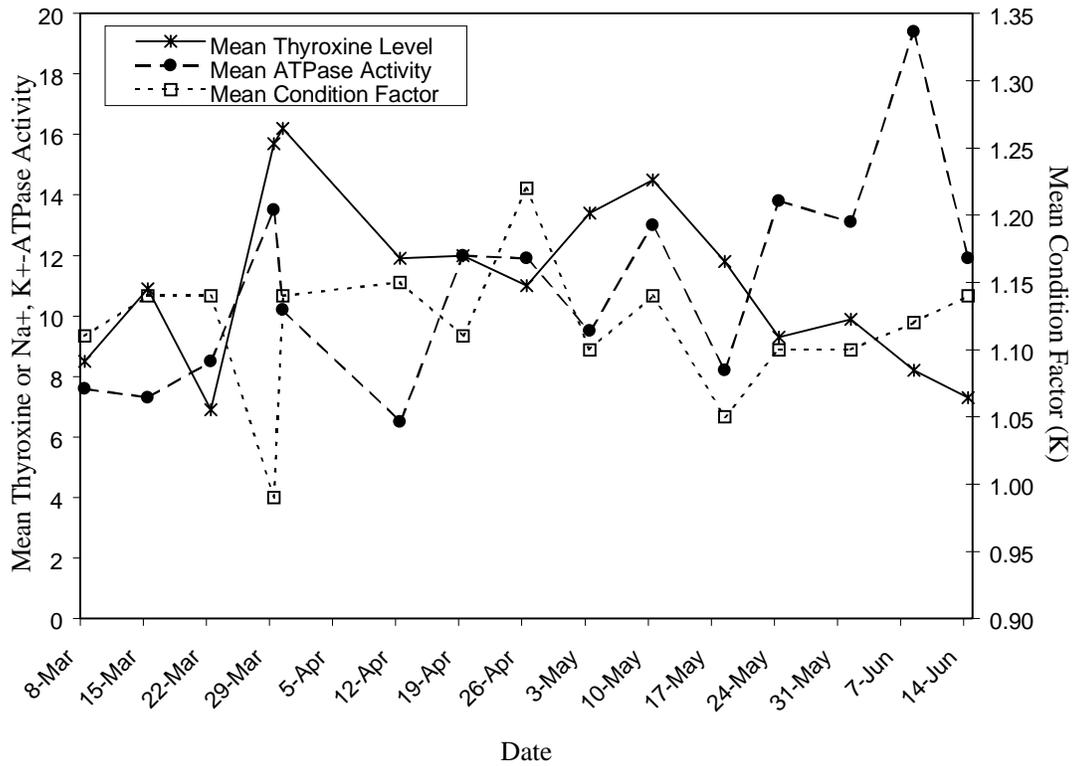


Figure 3. Mean thyroxine level (ng· ml⁻¹), mean gill sodium, potassium-activated ATPase activity (μmol P_i· mg protein⁻¹· hr⁻¹), and mean condition factor (K) of spring chinook salmon held at 8°C.

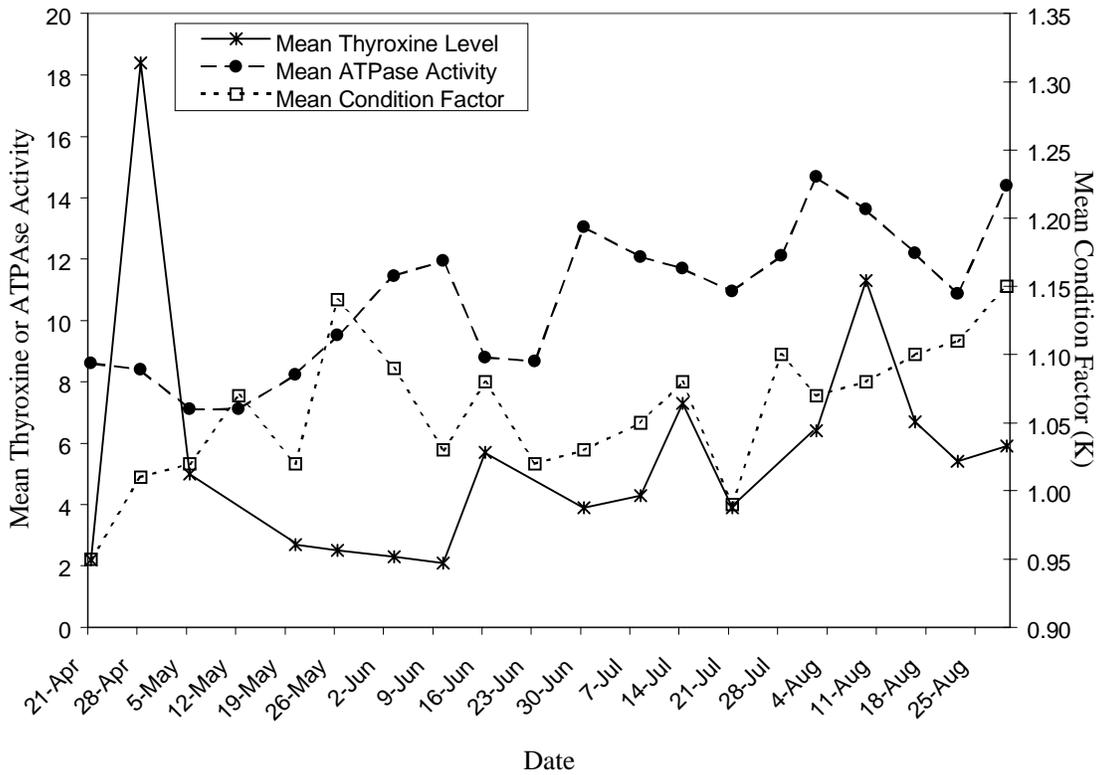


Figure 4. Mean thyroxine level (ng· ml⁻¹), mean gill sodium, potassium-activated ATPase activity (μmol P_i· mg protein⁻¹· hr⁻¹), and mean condition factor (K) of fall chinook salmon held at 8°C.

Levels of mean gill Na⁺, K⁺-ATPase activity were generally higher in spring chinook salmon held at 8°C compared to spring chinook salmon held at increasing water temperatures. Interestingly, we found the inverse situation in fall chinook salmon. Water temperatures of 16°C for 30 days in July did not appear to diminish gill Na⁺, K⁺-ATPase activity in fall chinook salmon. Inhibition of gill Na⁺, K⁺-ATPase activity has been reported in laboratory-held steelhead (*O. mykiss*) at 13°C for 20 days (Zaugg 1981b), and in coho salmon held at 15° and 20°C compared to fish held at 6° and 10°C (Zaugg and McLain 1976).

A rise in mean plasma T₄ level coincided with the initial peak in mean gill Na⁺, K⁺-ATPase activity of spring chinook salmon, a pattern previously reported for smolting coho salmon (Folmar and Dickhoff 1980). The initial T₄ peak in fall chinook salmon, and the second peak in spring chinook salmon held at 8°C, immediately preceded prolonged increases in gill Na⁺, K⁺-ATPase activity; this pattern is similar to that reported for Atlantic salmon (*Salmo salar*) smolts (Pruent et al. 1989).

Increases in mean plasma T₄ levels were not dependent on rising water temperatures; in fact, spring and fall chinook salmon held at 8°C showed larger initial peaks in mean plasma T₄ level than cohorts held at higher water temperatures. Slower mobilization and cellular uptake and/or intracellular conversion of T₄ to T₃ due to the inhibitory effect of 8°C water on metabolism could account for the higher initial peaks in plasma T₄ in fish held at 8°C (Eales 1985; Grau 1988).

Low water temperature magnified a cyclical relationship between seasonal increases in gill Na⁺, K⁺-ATPase and T₄ rhythms in fall chinook salmon. Near monthly increases in plasma T₄ levels which preceded increased gill Na⁺, K⁺-ATPase activity were evident for fall chinook salmon held at 8°C. The relationship between gill Na⁺, K⁺-ATPase and T₄ activity was not apparent in fall chinook salmon held at increasing water temperatures, presumably because T₄ was rapidly picked up by target cells and converted to T₃, the biologically active form of the hormone. Both groups of fall chinook salmon showed a pattern of increasing gill Na⁺, K⁺-ATPase activity over the summer, although fish held at increasing water temperatures had higher enzyme activity levels. In contrast, spring chinook salmon held at 8°C did not show cyclic regularity in T₄ and gill Na⁺, K⁺-ATPase activity. Differences in T₄ and gill Na⁺, K⁺-ATPase activity patterns between spring and fall chinook salmon may be due to the photoperiod sensitivity of spring chinook salmon (Clarke et al. 1989, 1992). Spring chinook salmon fry require exposure to a short-day photoperiod prior to long-day photoperiod to grow normally and develop seawater tolerance; fall chinook salmon fry grow well and adapt to seawater without photoperiod cues (Clarke et al. 1989). The presence of a photoperiod-dependent smoltification switch apparently results in a limited window for smolt development in yearling spring chinook salmon. The length of this developmental period is determined by water temperature (Wedemeyer et al. 1980).

Smolt development is an energetically demanding process, associated with increased oxygen consumption and energy catabolism (Sheridan 1986, 1988; Hoar 1988). Nutritional state influences growth rate, gill Na^+ , K^+ -ATPase activity and plasma T_4 levels during smoltification (Wedemeyer et al. 1980; Eales 1988; Grau 1988; CRRL, unpublished data). In addition, ample research confirms that an increase in plasma T_4 level reflects mobilization of stored body lipids in smolting salmonids (Barrington et al. 1961; Narayansingh and Eales 1975; Sheridan 1986; Cowley et al. 1994). Shifts in the thermoregulatory behavior of smolting chinook salmon provides additional indirect evidence of the relationship between smoltification and energy availability and utilization. After water temperature reached 16°C , thermal preference decreased by almost 3°C in fall chinook salmon held at increasing water temperatures (Sauter 1996). The author suggested that a drop in thermal preference late in smolt development reflects changes in metabolism adaptive to seawater entry and optimal energy utilization. Spring and fall chinook salmon held at 8°C increased their thermal preference by about 1°C during the period when these fish normally smolt, and may have selected the higher water temperature to accelerate smolt differentiation once sufficient size was attained and adequate energy resources were available for smoltification at the low water temperature (Sauter 1996). Many authors report evidence of minimum size thresholds for smoltification in juvenile salmonids (Conte et al. 1966; Clarke et al. 1978; Ewing et al. 1979; Wedemeyer et al. 1980; Brannon et al. 1982; Weatherley and Gill 1995), and Wedemeyer et al. (1980) report that the hypoosmoregulatory ability of juvenile salmonids increases with growth during the parr-to-smolt transformation. It seems likely that lower body size limits for smolt differentiation reflect the energetic advantage of increasing allometry between body size and metabolic rate as fish grow, which creates an expanding energy surplus (Post 1990; Post and Lee 1996; Hewett and Johnson 1996). Successful smolt differentiation will occur in juvenile anadromous salmonids under appropriate photoperiod cues, and when a combination of water temperature, nutritional status, and body size result in an adequate available energy surplus for the process.

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APPENDIX 1
Hatchery Survey Questions

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Hatchery Survey Questions

Smolt Monitoring Project
Columbia River Research Laboratory
Fish Hatchery Survey 1997

Stock Information

- 1) Are there historical records of your hatchery's founding stocks?
- 2) Was your hatchery started with stocks from the basin where your hatchery is located?
- 3) Is your stock now considered unique to your hatchery?
- 4) Number of adult males trapped in the following years: 1987-1996 (answers given by year)
- 5) Number of adult females trapped in the following years: 1987-1996
- 6) Number of jacks trapped in the following years: 1987-1996
- 7) Number of egg takes done in the following years: 1987-1996
- 8) Number of males spawned in the following years: 1987-1996
- 9) Number of females spawned in the following years: 1987-1996
- 10) What are your established spawning ratios (i.e., 2 males to 2 females)? If these ratios changed in the following years, please indicate below: 1987-1996.
- 11) Number of eggs you received from outside sources in the following years: 1987-1996 (list number received and source of eggs by year).
- 12) Number of fish you received from outside sources in the following years: 1987-1996 (list number received and source of fish by year).
- 13) Number of eggs you shipped to outside sources in the following years: 1987-1996 (list number of eggs shipped and destination by year).
- 14) Number of fish you shipped to outside sources in the following years: 1987-1996 (list number of fish shipped and destination by year).

- 15) Adult return timing in the following years: 1987-1996 (list start, peak, and end dates by year).
- 16) Age structure of adult returns, reported in actual numbers or percentages, of 3, 4, 5, and 6 year old fish in runs from the following years: 1987-1996. Please include how this data was obtained (i.e., scale analysis, length\age models).

Facility Design

- 17) What is your egg tray design?
- 18) Describe your nursery system, or any intermediate holding system between the trays and raceway (pond) rearing.
- 19) Describe your raceways (numbers, types, sizes).
- 20) Describe your ponds (numbers, shapes, sizes, bottom type lining or substrate).
- 21) Lighting: For each area, please describe the primary lighting system, and indicate if the area is under the influence of a night light, yard light, ambient light from windows, or any other lighting that may affect only part of the production group. Please indicate if these have changed in the last ten years, and if so, when.
 - a) Describe the lighting in the hatching trays.
 - b) Describe the lighting in the nursery raceways.
 - c) Describe the lighting in the outdoor raceways or ponds
 - d) Describe the cover of any outside ponds or raceways, and indicate if equal shade is provided to perimeter and interior tanks.

Water Sources and Temperature

- 22) Please describe your primary source of water for your hatchery (spring, well, river, etc.).
- 23) If you use another source of water for your trays please describe it.
- 24) If you use another source of water for your nursery please describe it.
- 25) If you use another source of water for your ponds or raceways please describe it.
- 26) Does your hatchery use acclimation ponds, if so, please describe them.

- 27) We would like to request a copy of your monthly temperature records for your trays, nursery, and raceways. We will gladly accept any spreadsheet or database format, photocopies, or handwritten summaries, if available, or use the table below. If you do not have monthly records, please give annual temperatures. Please indicate if and when any extreme changes in temperature occurred, and the duration of the episode. (list tray, nursery, and pond or raceway temperatures for each month for the years 1987-1996).
- 28) Number of days until fish eyed in the following years: 1987-1996.
- 29) Number of days fish spent in tray after swim-up in the following years: 1987-1996.
- 30) Number of days after swim-up until first feeding in the following years: 1987-1996.
- 31) Number of days fish spent in indoor nursery after swim-up before being transferred to ponds or raceways in the following years: 1987-1996.

Feeding Schedule

- 32) Please give the type of feed, size of feed, and factor used at swim-up in the following years: 1987-1996.
- 33) Please give the type of feed, size of feed, and factor used in your indoor nursery in the following years: 1987-1996.
- 34) Please give the type of feed, size of feed, and factor used for your production fish in the following years: 1987-1996.
- 35) Pounds of feed given to swim-up fish in the following years: 1987-1996 (listed by month and total annual, for each year).
- 36) Pounds of feed given to nursery fish in the following years: 1987-1996 (listed by month and total annual, for each year).
- 37) Pounds of feed given to production fish in the following years: 1987-1996 (listed by month and total annual, for each year).

Disease Management in Production Fish

- 38) By year (1987-1996), what treatments, if any, were given to eggs and fry to prevent disease during egg tray rearing (i.e., chemical baths, antibiotic treatments, etc.)? Please include frequency of treatments.

- 39) By year (1987-1996), what treatments, if any, were given to juveniles to prevent disease during nursery rearing (i.e., chemical baths, antibiotic feed, etc.)? Please include frequency of treatments.
- 40) By year (1987-1996), what treatments, if any, were given to juveniles to prevent disease during raceway rearing (i.e., chemical baths, antibiotic feed, etc.)? Please include frequency of treatments.
- 41) By year (1987-1996), what was the approximate percent survival of production groups through the main stages of development: eggs to eyed, eyed to swim-up, swim-up to fry, fry to parr, parr to smolt (and total eggs to smolt)?

Disease Outbreaks in Production Fish

- 42) Were there any disease epizootics in production fish? If yes, please report by year: (1) the disease, the groups of fish affected (i.e., raceways, ponds, etc.), (2) what treatment(s), if any, were applied in response to the outbreak (i.e., formalin treatment, etc.), (3) if specific pathogens were identified for each outbreak, and (4) the method(s) used to identify the disease organism (i.e., external signs, gross pathology, diagnostic test, Goede's index, or an organosomatic index).

Disease Management in Broodstock

- 43) Were adults screened for Bacterial Kidney Disease (BKD)? If yes, please indicate the method of detection and actions taken based on this information (i.e., eggs destroyed, not spawning adult with high BKD titers, juvenile segregation): 1987-1996.
- 44) By year (1987-1996), what treatments, if any, were given to returning adults to control or prevent disease during holding (i.e., chemical baths, antibiotic injections, etc.)?

Hatchery Predation

- 45) Please list the predators that are problems at your hatchery.
- 46) Please list the predator control methods used at your hatchery to protect fish (i.e., netting).
- 47) Do you use any noise making devices to deter predators (i.e., air cannons)?

Hatchery Stocking Densities and Water Flow Rates

- 48) What were the stocking densities in your ponds and raceways in the following years (1987-1996). Please indicate density, density unit of measure, and the number of ponds or raceways that had this density.

- 49) Please comment on stocking density changes that have occurred over the following years: 1987-1996.
- 50) What were the typical flow rates into the ponds and raceways in the following years. Please comment on any significant changes you may have made to flows over those years.
- 51) Please comment on changes in flows over the following years (i.e., storm events causing high flows): 1987-1996.

Fish Releases

- 52) Over the following years, describe how your fish have been released (i.e., forced, volitional, transported): 1987-1996.
- 53) If you have used volitional releases, indicate how long fish were allowed to leave and estimate the number or percentage of fish that had to be forced out in the following years: 1987-1996.
- 54) If fish have been transported to release sites please indicate 1) the length of transport, 2) the mode of transportation, 3) if air was supplied to fish, 4) what was the water temperature, and 5) concentration of salt, if used in the water (list by year 1986-1997).
- 55) It is our belief that hatchery personnel have an incredible amount of firsthand knowledge that never gets recorded. Please make comments on anything that you think is important or that we should know.