

AN ABSTRACT OF THE DISSERTATION OF

Carol Seals Price for the degree of Doctor of Philosophy in Fisheries Science

presented on April 9, 2002. Title: Factors Affecting the Saltwater-entry Behavior and Saltwater Preference of Juvenile Chinook Salmon, *Oncorhynchus tshawytscha*.

Redacted for privacy

Abstract approved: \_\_\_\_\_

Carl B. Schreck

From 1998 - 2000, laboratory studies were conducted to examine factors that impact saltwater-entry behavior and saltwater preference (SWP) of juvenile chinook salmon, *Oncorhynchus tshawytscha*. These factors included bacterial kidney disease, stress and the presence of trout, *O. mykiss*. An additional study investigated the orientation of the startle response of chinook salmon within a salinity gradient. All experiments were conducted in 757-l tanks in which a stable, vertical salinity gradient was established. SWP was decreased in fish suffering from bacterial kidney disease ( $31 \pm 20.0\%$ ), compared with control fish ( $85 \pm 17.6\%$ ). A mild chasing stressor resulted in a 26% decrease in SWP relative to unstressed fish. After a severe handling stressor, only 20% of fish preferred salt water, compared with 100% of unstressed controls. After exposure to an overhead predator model, severely stressed fish descended into the saltwater

layer, but this response was transient. The presence of non-aggressive steelhead trout did not affect SWP of chinook salmon. Chinook salmon stocked with rainbow trout displayed decreased SWP. Aggression levels in tanks with rainbow trout were higher than in tanks with only chinook salmon. The orientation of the startle response was affected by the presence of salt water. Fish that preferred salt water within a gradient responded by moving horizontally within the saltwater layer. In contrast, control fish (held only in freshwater) moved vertically within the water column when startled. Prior preference for salt water superseded the inclination to move upward in the water column when startled.

Smoltification involves physiological, behavioral and morphological changes that prepare healthy chinook salmon for seawater residence. However, disease, stress and aggressive interactions can decrease the SWP of fish at this life history stage. Avoidance of salt water during estuarine outmigration is likely maladaptive, and may have ecological ramifications including increased risk of avian predation during outmigration and decreased fitness in the marine environment.

Doctor of Philosophy dissertation of Carol Seals Price presented on April 9, 2002

APPROVED:

**Redacted for privacy**

---

Major Professor, representing Fisheries Science

**Redacted for privacy**

---

Head of the Department of Fisheries and Wildlife

**Redacted for privacy**

---

Dean of Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

**Redacted for privacy**

---

Carol Seals Price, Author

## ACKNOWLEDGEMENTS

I thank all my committee members for their assistance, guidance and support throughout my time at Oregon State University. I am especially grateful to my advisor, Dr. Carl Schreck, for allowing me to do behavioral studies in his physiology lab, and for the opportunity to be a part of the Columbia River Salmon Project over the years. Dr. Sue Sogard's advice on conducting behavioral studies was invaluable, and she is a great role model. I thank the faculty, students and staff of the Oregon Cooperative Fish and Wildlife Unit who assisted in many facets of my research. I am especially grateful to Beth Siddons for her help in the lab and to Rob Chitwood for his excellent knowledge of fish husbandry. I am indebted to all the fish that took part in my experiments.

A very special thanks goes to my family for their continuous support throughout my academic endeavors. They have always been proud of my accomplishments, and they continue to encourage and inspire me to be my best. Apologies to my parents for choosing biology over medicine, thus effectively eliminating their chance for a long-ago-promised Mercedes!

Finally, a big hug to my husband, Blake, for his help throughout this project! He carried bags of salt to the lab, helped with plumbing projects, brought me lunch and hot tea on cold weekend workdays, and encouraged me always. Thanks for your patience, love and friendship.

# TABLE OF CONTENTS

	<u>Page</u>
INTRODUCTION.....	1
EFFECTS OF BACTERIAL KIDNEY DISEASE ON SALTWATER PREFERENCE OF JUVENILE SPRING CHINOOK SALMON, <i>ONCORHYNCHUS TSHAWYTSCHA</i> .....	13
Abstract.....	14
Introduction.....	15
Methods.....	17
Results.....	24
Discussion.....	27
Acknowledgements.....	35
Literature Cited.....	36

## TABLE OF CONTENTS (Continued)

	<u>Page</u>
STRESS AND SALTWATER-ENTRY BEHAVIOR OF JUVENILE CHINOOK SALMON ( <i>ONCORHYNCHUS TSHAWYTSCHA</i> ): CONFLICTS IN PHYSIOLOGICAL MOTIVATION.....	39
Abstract.....	40
Introduction.....	42
Methods.....	45
Results.....	52
Discussion.....	56
Acknowledgements.....	65
Literature Cited.....	66

## TABLE OF CONTENTS (Continued)

	<u>Page</u>
EFFECTS OF TROUT, <i>ONCORHYNCHUS MYKISS</i> , ON THE SALTWATER-ENTRY BEHAVIOR OF JUVENILE CHINOOK SALMON, <i>O. TSHAWYTSCHA</i> .....	72
Abstract.....	73
Introduction.....	74
Methods.....	78
Results.....	85
Discussion.....	97
Acknowledgements.....	104
Literature Cited.....	105

## TABLE OF CONTENTS (Continued)

	<u>Page</u>
PREFERENCE FOR SALT WATER ALTERS THE STARTLE RESPONSE OF JUVENILE CHINOOK SALMON ( <i>ONCORHYNCHUS TSHAWYTSCHA</i> ).....	110
Abstract.....	111
Introduction.....	112
Methods.....	114
Results.....	122
Discussion.....	128
Literature Cited.....	133
CONCLUSION.....	139
BIBLIOGRAPHY.....	152



## TABLE OF CONTENTS (Continued)

	<u>Page</u>
APPENDIX: TEMPORAL PATTERNS IN SALTWATER PREFERENCE OF HATCHERY-REARED JUVENILE CHINOOK SALMON (ONCORHYNCHUS TSHAWYTSCHA) SMOLTS.....	172
Introduction.....	173
Statistical Analysis.....	177
Results.....	180
Discussion.....	181
Acknowledgements.....	187
Literature Cited.....	188

## LIST OF FIGURES

<u>Figure</u>		<u>Page</u>
1.1	Diagram of saltwater preference tanks.....	21
1.2	Mean percentage of sick <b>(A)</b> or control <b>(B)</b> chinook salmon holding in fresh (FW) and salt (SW) water, or moving between the layers in BKD experiments.....	26
1.3	Mean ( $\pm$ SD) ELISA absorbance levels of the <i>R. salmoninarum</i> antigen from juvenile spring chinook salmon used in saltwater preference experiments.....	28
1.4	Linear regression of the mean ELISA absorbance levels of <i>R. salmoninarum</i> antigen versus the percent of juvenile spring chinook salmon holding in salt water for each tank in the preference experiments.....	29
2.1	<b>(A)</b> Mean percentage of control fish holding in fresh (FW) and salt water (SW), or moving between the layers in Experiment I. <b>(B)</b> Mean percentage of chased fish holding in fresh and salt water, or moving between the layers in Experiment I.....	55
2.2	<b>(A)</b> Mean percentage of control fish holding in fresh (FW) and salt water (SW), or moving between the layers in Experiment II. <b>(B)</b> Mean percentage of stressed fish holding in fresh and salt water, or moving between the layers in Experiment II.....	58

## LIST OF FIGURES (Continued)

<u>Figure</u>		<u>Page</u>
3.1	Mean percentage of chinook salmon held with or without trout holding in fresh and salt water, or moving between the layers in aggression experiment...	91
4.1	Diagram (top view) of the two tanks and startle apparatuses used in experiments. Each tank was isolated behind a black plastic curtain.....	119
4.2	Saltwater-entry behavior of juvenile chinook salmon during saltwater introduction and prior to presentation of the startle apparatus.....	124
4.3	The mean number of juvenile chinook salmon (n=6 replicate trials) present in the left, middle and right block of the saltwater layer in relation to the position of the startle apparatus.....	126
D.1	A conceptual model of the intrinsic and extrinsic factors that affect saltwater-entry behavior and saltwater preference of juvenile chinook salmon.....	146

## LIST OF TABLES

<u>Table</u>		<u>Page</u>
1.1	Saltwater preference of juvenile spring chinook salmon with or without external signs of BKD.....	25
2.1	Saltwater preference of chased and control juvenile chinook salmon in Experiment I.....	54
2.2	Saltwater preference of stressed and control juvenile chinook salmon in Experiment II.....	57
3.1	Saltwater preference data for juvenile spring chinook held with and without steelhead trout on four dates in 1999.....	87
3.2	Saltwater preference data for chinook salmon held with or without rainbow trout on three dates in 1999.....	90
3.3	The number of aggressive acts in tanks with chinook salmon and steelhead trout, or chinook only on four dates in 1999.....	94
3.4	The number of aggressive acts in tanks with chinook salmon and rainbow trout, or chinook salmon only on three dates in 1999.....	96
4.1	Behavior of juvenile chinook salmon analyzed from videotape recordings made during startle experiments...	123

## LIST OF APPENDIX FIGURES

<u>Figure</u>		<u>Page</u>
A.1	Saltwater preference of juvenile chinook salmon in laboratory tests conducted from 1997-2000.....	175
A.2	Saltwater preference of juvenile chinook salmon in laboratory tests conducted from 1997-2000.....	176
A.3	Percent of fish showing preference for salt water in experiments conducted from 1997-1999.....	178

**FACTORS AFFECTING THE SALTWATER-ENTRY BEHAVIOR  
AND SALTWATER PREFERENCE OF JUVENILE CHINOOK  
SALMON, *ONCORHYNCHUS TSHAWYTSCHA***

**INTRODUCTION**

Before entering the ocean, outmigrating juvenile chinook salmon (*Oncorhynchus tshawytscha*) must navigate a novel estuarine environment, and successful transition into salt water is crucial for these fish (Wedemeyer et al. 1980, Thorpe 1994). Spring chinook salmon typically spend the first year or more of their lives as parr in fresh water before migrating to the sea, and generally have a short estuarine residence time (Healy 1991). Radio telemetry of Columbia River juvenile spring chinook salmon (originating in the Snake River) indicates that these fish travel through the estuary passively with tidal flow (Carl Schreck, unpublished data). Fish only held in the estuary during slack tides, and were regularly observed moving upstream during incoming tides. However, chinook salmon did not remain in the estuary for more than a few tidal cycles before moving out with the ebb tide. Thus, for these fish, the transition from fresh to saline water occurs in a matter of hours, with no extended period in which to

slowly acclimate to salt water. This contrasts with chinook salmon juveniles with alternate life histories (i.e., fall chinook salmon) or other salmon species, which may reside in the estuary for up to several months before moving into the ocean (Groot and Margolis 1991).

Because outmigrating spring chinook salmon in the Columbia River estuary are likely moving passively with the tidal current, the extent to which fish may be able to regulate their vertical position is uncertain. Given that radio signals attenuate at salinities greater than 3-5 ppt, it is inferred that many fish tracked using radio telemetry were traveling in the surface freshwater, rather than in the deeper salt water. These observations agree with data collected by Birtwell et al. (1983) and Birtwell and Kruzynski (1989) at estuarine sites in British Columbia. There, juvenile chinook, coho (*O. kisutch*) and chum (*O. keta*) salmon were oriented toward the surface (0 - 2.5 m depth) and avoided deeper, saline water. A telemetry study of sea trout (*Salmo trutta*) in North Wales also found that outmigrating juveniles traveled near the surface and moved passively with the ebb tide (Moore et al. 1998). While these salmon seem to be surface oriented, it is uncertain whether this is a directed action, and the mechanisms governing the vertical distribution of outmigrating salmonids in estuaries are poorly understood.

One of the most predominant estuarine characteristics that the fish may be utilizing to orient themselves vertically within the water column is salinity

(McInerny 1964, Birtwell and Krusynski 1989). In many estuaries, a saltwater wedge is formed when less-dense fresh water floats above heavier salt water, and a sharp halocline is often observed. Juvenile salmon may be using this feature to orient within the water column and it is necessary to consider the potential motivating forces that influence how fish make the transition from fresh to salt water.

The first factor to consider is an endogenous process common to anadromous salmonids. Prior to or during outmigration, juvenile salmonids undergo smoltification - a morphological, physiological, and behavioral transformation that prepares them for ocean residence (Folmar and Dickhoff 1980, Hoar 1988, Iwata 1995). Morphological changes during the parr-smolt transformation include alterations in skin pigmentation and body form. In parr, dark, vertical bands provide cryptic coloration and pattern to help fish blend with the rocky, riverine substrate. During smolting, the purines guanine and hypoxanthine are accumulated in the skin, turning the fish silvery. Condition factor (a ratio of weight to length) is decreased in smolts, resulting in a more streamlined fish.

Several important physiological changes occur. Plasma cortisol, growth hormones, thyroid hormones (triiodothyronine and thyroxine) and gill  $\text{Na}^+/\text{K}^+$ -ATPase activity increase. Plasma levels of prolactin, a freshwater-adapting hormone, decrease. Cortisol functions in water and ion balance, is associated



with increases in chloride cell proliferation and gill  $\text{Na}^+/\text{K}^+$ -ATPase activity (Madsen 1990, Shrimpton and McCormick 1998), and it reduces the total lipid and triacylglycerol content of the liver and dark muscle (Sheridan 1986). Growth hormone stimulates gill  $\text{Na}^+/\text{K}^+$ -ATPase activity and has been associated with changes in thyroid hormone levels (Prunet et al. 1989) in Atlantic salmon (*S. salar*) and coho salmon (Richman and Zaugg 1987, Bjornsson et al. 1987). Thyroid hormones are involved in metabolic and pigmentation changes, increased seawater tolerance, and are implicated in many behavioral shifts (Hoar 1988, Iwata 1995).

Several dramatic behavioral changes occur during smoltification. Territorial aggression decreases during smoltification, and fish become gregarious and form schools (Iwata et al. 1990). As parr, salmon are generally bottom dwelling, but move up in water column as they move downstream (Edmundson et al. 1968, Everest and Chapman 1972, Moore et al. 1998). Smolts are more phototactic and rheotactic (Iwata 1995) than parr and, as migration tendency increases smolts begin to leave their natal streams. Preference for salt over fresh water also develops as fish become progressively more smolted (McInerny 1964, Iwata 1995). In summary, smoltification can be viewed as an integration of various endogenous processes acting in concert to promote the successful transition of salmon from fresh to saline water, and it may be

considered the primary positive motivation for fish to volitionally enter salt water.

The process of smoltification itself may be deleteriously affected by several factors. Physiological stress may suppress some aspects of the smoltification process (Schreck 1982, Patino et al. 1986, Forsman 1998). Mesa et al. (1999) found that juvenile chinook salmon infected with *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease (BKD), increased plasma cortisol and lactate, and decreased plasma glucose. However, gill  $\text{Na}^+/\text{K}^+$ -ATPase activity and condition factor were not different from uninfected controls. Outbreaks of BKD often occur concurrently with smoltification and may cause chronic mortality at this life history stage. Parr that have not attained a critical size may delay smoltification for an entire year (Weatherley and Gill 1995). Factors such as these would act in opposition to the normal smoltification process, and would retard or even prevent saltwater readiness. Relatively little work has been done to fully investigate many of these effects, and this field of study will likely prove to be promising for future research.

One of the predominant challenges facing managers and scientists is determining which of the above physiological or behavioral variables associated with smoltification are most predictive of the readiness of juvenile salmon to survive and thrive in salt water (Wedemeyer et al. 1980, Iwama 1992). In the past, hatcheries have used fish age or size, and seawater challenge tests to decide

when fish should be released for outmigration. Yet, these methods have not proven to be a very reliable indicator of how juveniles will fare, and even less of future adult returns. Attention has recently been given to establishing multiple criteria by which the quality of juvenile salmonids may be assessed (Iwama 1992). Fish performance and survival during outmigration and early ocean residence will likely be dependent upon a variety of endogenous and exogenous factors, and complex interactions of variables at multiple scales.

One of the most commonly used tests to assess the readiness of salmon is a saltwater challenge. Fish are transferred directly from fresh water into full strength (30+ ppt) salt water, and osmoregulatory capacity is measured after 24 hours. Fish that can down regulate sodium below 170 meq/L are considered capable of surviving in seawater (Clarke and Blackburn 1978). However, several factors are known to decrease the ability of seemingly smolted fish to effectively ionoregulate.

The physiological stress response in fishes is an adaptive mechanism by which a perturbed animal attempts to reestablish homeostasis and ensure its survival (Seyle 1950, Barton 1997, McEwen 1998, Schreck 2000). An abrupt transfer from fresh to salt water is generally considered a stressful experience, even for smolting fish, and is known to elicit a rise in plasma cortisol (Strange and Schreck 1980, McDonald et al. 1988), a hormone released as part of the general stress response (Donaldson 1981). Smolted salmonids can overcome this

stress and regain ionic balance, but physiologically unprepared fish often do not adjust, and die. For example, when freshwater adapted yearling coho salmon smolts were placed in seawater immediately after a confinement stress, plasma sodium concentrations and osmolarity were elevated for 24 hours and survivorship was only 25% (Redding and Schreck 1983). When additional stressors are presented concomitantly with a salinity challenge, even smolted fish may not be able to compensate for the additive effects. Seawater challenge tests were used by Matthews et al. (1986) to determine the most stressful facets of handling procedures and the bypass system at Lower Granite dam on the Snake River for juvenile chinook salmon. The highest percent mortality was observed for fish that were severely stressed, either by the dam passage system or by the presence of steelhead trout, *O. mykiss*, in holding tanks. Similarly, Järvi (1989a) demonstrated 90% mortality of Atlantic salmon smolts moved directly from fresh to salt water containing a piscine predator, and then subjected to a 24-hr seawater challenge. Fish transferred to saltwater tanks with no predators had 100% survival in challenge tests. He concluded that the stress induced by the presence of the predator, rather than osmotic stress, was responsible for the low rate of survival between the treatments. Mortality of juvenile coho salmon subjected to a temperature stressor (4° C increase) combined with a seawater challenge ranged from 30 to 100%, while fish exposed only to the seawater challenge had 100% survival (McGeer et al. 1991). In stressed fish, water and ion

flux across the gill epithelium increases, (McDonald and Milligan 1997), and the decreased ability to maintain electrolyte balance would be exacerbated for stressed, freshwater-adapted fish placed into high salinity water.

BKD has been shown in several studies to decrease the ability of salmon to acclimate to and survive in salt water. Groups of spring and fall chinook salmon from three Oregon hatcheries were assessed for BKD incidence (direct fluorescent antibody technique, or FAT) before transfer to full strength seawater (Banner et al. 1983). Incidence of the disease was initially very low, but outbreaks of the disease were quickly observed after salt water exposure. Horizontal transmission of the disease occurred, and mortality attributable to infection was as high as 81% for up to six months. Spring chinook were more susceptible than fall chinook. FAT is a relatively insensitive assay that can only be used to assess the incidence of the disease, and has been replaced by ELISA (enzyme-linked immunosorbent assay), which can quantify the level of infection *R. salmoninarum*. Using this method, Elliott et al. (1995) found that the progeny of adults with low levels of BKD also tended to have low infection, while the opposite was true for the progeny of highly infected adults. Furthermore, after transfer to seawater, no low-BKD juveniles died as a result of the disease, but 85% of mortality in the high-BKD group was attributable to BKD. Moles (1997) subjected infected and uninfected groups of coho salmon smolts to saltwater challenge tests. After 8 days, plasma sodium levels of infected fish were

significantly higher. Survival was 95 to 100% for uninfected fish, but ranged from 0 to 90% for fish with BKD.

Results of saltwater challenge studies lend insight into the physiological capacity of smolted salmon to adjust to increased salinity. However, very little is known about the behavioral scope of spring chinook salmon while outmigrating through estuaries. Several fundamental questions may be posed regarding this subject. First, are these fish capable of perceiving salinity differences and determining their vertical positions relative to a salinity gradient? If so, are smolted fish immediately attracted to salt water, or do they prefer to gradually acclimate to it? Will smolted fish always enter salt water or is this behavior flexible? Which factors might influence the willingness of fish to enter salt water? What are the possible ecological consequences of abnormal saltwater-entry behavior? The answers to these questions are largely unknown.

It is known that various fishes, especially intertidal species, can move along salinity gradients (Pitcher 1986) and studies by McNerney (1964), Iwata and Komatsu (1984), and Iwata et al. (1990) indicate that Pacific salmon species (chinook; chum; coho; pink, *O. gorbuscha*; and sockeye, *O. nerka*) are capable of responding to salinity gradients. Tolerance and preference for salt water tend to increase as fish become progressively smolted, and salinity preference is stimulated by several hormones known to be important during smoltification, including cortisol, growth hormone, and thyroid hormones (Iwata et al. 1990).

Given the life history of salmon, it follows that as they develop into a marine fish, they would be increasingly inclined toward salt water. However, the nuances of saltwater-entry behavior have yet to be explored.

If outmigrating spring chinook salmon are regulating their depth in response to salinity, then the answers to the above questions become quite salient to our understanding of the importance of estuarine passage to successful ocean entry. It has been suggested that the first few days of ocean residence are critical (Pearcy 1992, Ryding and Skalski 1999,) and chinook salmon that have difficulty making the transition may be unlikely to survive. The shift from fresh to salt water may be deleteriously impacted in several ways. Fish that are not sufficiently smolted and intolerant of high salinity may prefer to hold in the freshwater lens as long as possible, before the current forces them into the suboptimal seawater. If the saltwater preference of fully smolted fish can be reversed by other environmental factors, fish may also be driven into freshwater. Additionally, if chinook salmon perceive saltwater-entry as a stressful event, their ability to perform other vital functions such as feeding and predator avoidance may be inhibited. Finally, fish near the surface may be more susceptible to predation by birds that feed at or near the surface, due to both proximity and reduced avoidance capability.

The volitional entry of chinook salmon into salt water, may be an important step during this life history stage, but relatively little is known about

this specific behavior in the estuarine environment. The value of behavioral studies is becoming increasingly more apparent as we try to understand our environment at multiple scales. In studies of animal ecology, behavior can provide the explanatory link between large-scale, ecosystem patterns, population characteristics, and individual, endogenous processes (Wedemeyer and Mcleay 1981, Schreck 1990, Shuter 1990, Wedemeyer et al. 1990, Schreck et al. 1997, Shumway 1999). Behavioral assays also provide the ecological context within which physiological perturbations become significant. Furthermore, by knowing how a specific behavior may be impacted by a variety of factors, we can better interpret observations of the variability of this behavior in the wild.

The following research program was designed to gather baseline data about factors that might be affecting the saltwater preference and saltwater-entry behavior of juvenile chinook salmon. Experiments were conducted with fish that were in the process of smoltification, and treatments were chosen that were relevant to salmon populations in the Columbia River basin. The factors that were investigated include infection with *R. salmoninarum* (the causative agent of BKD) and stress. The stressors imposed included social stress (fish were stocked with rainbow or steelhead trout), a mild chasing stress, and a confinement stress. In conjunction with the confinement stress, chinook salmon were exposed to an avian predator model. Another experiment investigated the orientation of the startle response of fish that preferred salt water. Collectively, the results of these



experiments provide insight into the possible factors that may be influencing the saltwater-entry behavior of juvenile chinook salmon upon encountering saline water. Many of the observed behavioral changes are likely maladaptive to fish traveling through the estuarine environment, and may pose a serious threat to the survival of juvenile chinook salmon during this life history stage.

**EFFECTS OF BACTERIAL KIDNEY DISEASE ON  
SALTWATER PREFERENCE OF JUVENILE SPRING CHINOOK  
SALMON, *ONCORHYNCHUS TSHAWYTSCHA***

Carol Seals Price and Carl B. Schreck

Submitted to *Aquaculture*, 09 March 2001

**ABSTRACT**

In March 1998, we conducted a laboratory experiment to assess the effect of bacterial kidney disease (BKD) on the saltwater preference of juvenile spring chinook salmon, *Oncorhynchus tshawytscha*. Experiments were done in 757-l tanks in which a vertical salinity gradient was established. Fish with external symptoms of BKD were designated as sick and those without external symptoms of disease served as controls. Two hours after saltwater introduction,  $85 \pm 17.6\%$  (SD) of controls held in salt water, compared to  $31 \pm 20.0\%$  of sick fish. There was a significant negative relationship between mean infection level (quantified by ELISA) and saltwater preference. Such behavior may increase the risk of avian predation for chinook salmon outmigrating through the estuary.

## INTRODUCTION

Bacterial kidney disease (BKD), caused by *Renibacterium salmoninarum*, is responsible for significant mortality of salmonids of both wild and hatchery origin in the Pacific Northwest (Fryer & Sanders 1981, Fryer & Lannan 1993). Infection among Columbia River chinook salmon (*Oncorhynchus tshawytscha*) populations is virtually ubiquitous; from 1988 to 1991, 86 - 100% of outmigrating juvenile chinook salmon sampled at major dams tested positive for the *R. salmoninarum* antigen by ELISA (enzyme-linked immunosorbent assay; Elliott et al. 1997). BKD can cause chronic mortality during the freshwater portion of the salmonid life history and we have observed that severe outbreaks in hatcheries often occur in conjunction with smoltification. Smoltification occurs prior to or during outmigration. This is a critical life history stage for salmonids during which they undergo a number of morphological, physiological and behavioral changes in preparation for ocean residence (Hoar 1976, Folmar & Dickhoff 1980, Wedemeyer et al. 1990). Mesa et al. (1999) demonstrated that even as BKD severity increased over the course of smoltification in chinook salmon, several key indicators of smoltification were not hindered. Changes in condition factor and gill  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase were comparable to uninfected control fish, and infected fish ate and grew normally. Despite this evidence that BKD may not impede the smolting process, high mortality is often associated with the transfer of infected

fish to salt water (Banner et al. 1983, Elliott et al. 1995, Moles 1997). Oftentimes, fish that have survived even relatively high infection in fresh water die when transferred to salt water.

While the lethal consequences of BKD to salmon have been widely reported and investigated, very little is known about sublethal or behavioral effects. Mesa et al. (1998) recently found that more than twice as many chinook salmon experimentally challenged with *R. salmoninarum* were predated by either smallmouth bass, *Micropterus dolomieu*, or northern pikeminnow, *Ptchocheilus oregonensis*, compared to unexposed controls. The researchers did not observe any obvious differences in the behavior of challenged versus control fish, however their findings strongly indicate that fish with sublethal infections of *R. salmoninarum* are at high risk of death which is not directly pathogen related. More research of the secondary effects of the disease is required to fully understand the significance of BKD to salmonid populations, and analysis of the behavioral ramifications of the disease may be key in understanding the role of BKD within an ecological context.

In a previous study in our laboratory, we monitored the saltwater preference of chinook salmon during smoltification (C. Seals, C.B. Schreck & W. W. Dickhoff, National Marine Fisheries Service, 2725 Montlake Blvd. East, Seattle, WA, 98112; unpublished data). Preference for salt water is known to increase during the course of smoltification (McInerny 1964), and represents a

crucial adjustment for salmonids migrating from fresh to saline environments. Although several indices suggested that fish in that study were at advanced stages of smoltification, we observed a decrease in salinity preference following a severe outbreak of BKD (75% of dissected chinook salmon smolts had kidney lesions indicative of high infection levels). Our observations agree with those of Mesa et al. (1999) that BKD does not hinder the smolting process. However, the decrease in preference for salt water suggested a behavioral manifestation of the disease warranting further investigation. We conducted the following laboratory experiment to examine the effects of BKD on the saltwater preference of juvenile spring chinook salmon during smoltification.

## **METHODS**

### **Test Fish**

Yearling spring chinook salmon used in these experiments were reared at Oregon State University's Fish Performance and Genetics Laboratory, Corvallis, OR. Brood stock from which these fish were spawned was obtained from Oregon's South Santiam Fish Hatchery. Fish were held indoors with natural

lighting, in 2-m, circular tanks and fed to satiation twice per day (BiOregon® semi-moist pellets) until needed for experiments. During rearing, water temperature fluctuated between 12.8 and 14°C. Several months prior to experiments, fish in one holding tank experienced a severe outbreak of BKD (confirmed by state fish pathologists) with high mortality from the disease (25-30%). Once the outbreak had progressed, mortality declined, but many of the survivors continued to show external signs of the disease; these fish were used for this experiment. For experiments, fish were selected that showed at least one external symptom of infection, including exophthalmia, lesions, skin haemorrhaging, or abdominal swelling (Bruno 1986). However, fish that exhibited behavioral abnormalities such as listlessness, erratic swimming, and disorientation were not used. The intent was to choose fish that were highly infected, yet not moribund. Only one sick fish died during the course of the experiments, despite the stress associated with netting, transport and introduction to a novel tank, suggesting that this objective was achieved. Control fish came from tanks that had not experienced BKD outbreak and individuals used were carefully inspected for any external symptoms of the disease. Both control and sick fish had undergone morphological changes associated with smoltification including silvering, loss of parr marks and darkening of fin margins. For saltwater preference experiments, fish were transported for 15 minutes from the Fish Performance and Genetics Laboratory

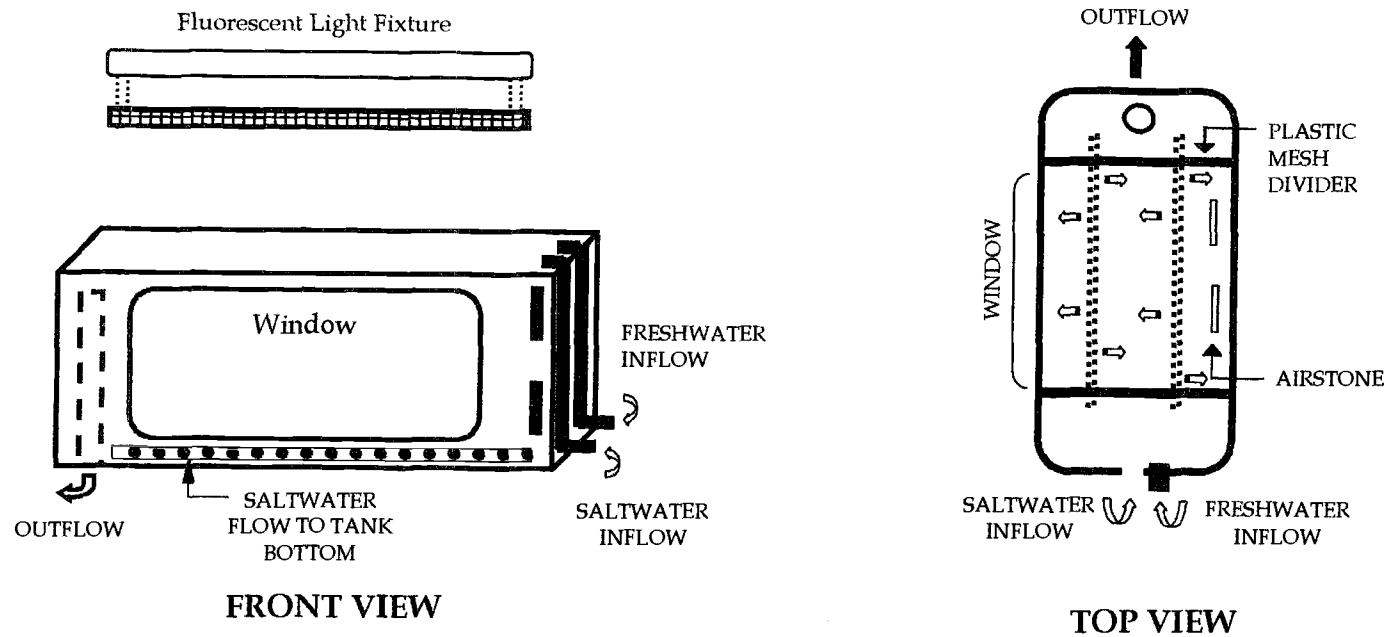
to the Oak Creek Laboratory in 130-L plastic cans supplied with oxygen. Water temperature at the Oak Creek Laboratory ranged from 9 - 10°C. Sick fish averaged  $18.9 \pm 2.86$  cm and  $80.8 \pm 32.1$  g (n=62) and control fish averaged  $17.3 \pm 1.64$  cm and  $55.2 \pm 17.17$  g (n=60). The slightly higher weight of sick fish is probably accounted for by abdominal fluid retention, which is a sign of BKD.

### **Saltwater Preference**

Saltwater preference experiments were carried out in four rectangular 757-l tanks (1.83 x 0.66 x 0.60 m) with Plexiglas front windows (Figure 1.1). The tanks were supplied with airstones, flow-through water ( $3 \text{ l min}^{-1}$ ) and mesh covers. Each was enclosed behind a black plastic curtain with holes cut out to allow for visual observation. Fluorescent lights were suspended above each tank. Light was diffused by fiberglass netting on frames suspended below the bulbs. During experiments, salt water was introduced into the bottom of each saltwater preference tank from one of two header tanks located outside of the plastic curtain. Header tanks were 1-m, circular tanks and were elevated approximately 1.5 m above the bottom of the experimental tanks. Each header was connected to one of the preference tanks by PVC pipes running along the bottom of the experimental tanks. These pipes had small holes drilled in two rows, allowing



salt water to be slowly introduced. Preliminary work found that after 48 hours fish began normal feeding, so we considered them to be acclimated after this time. Once acclimated, fish were not observed resting directly on the tank bottoms. Stationary fish tended to position themselves 5-30 cm off the bottom. Fish were dispersed throughout the tanks and actively explored all areas of the tank including the surface. The photoperiod was set to the ambient light cycle. Fish were not fed during acclimation to reduce disturbance to the tank. After acclimation, 379 l of 30 ppt salt water was mixed in each header tank using Instant Ocean® artificial salt, and was aerated. Ten minutes prior to saltwater introduction, freshwater supply and aeration were turned off to prevent mixing. The valves from the header tanks were then opened and salt water began to flow into the bottom of the preference tanks, pushing the lighter, fresh water out of the top-draining standpipe. This created a stable saltwater layer in the bottom of the water column, with a freshwater layer on top. The halocline was visible, so it was easy to distinguish whether fish were in salt or fresh water. Every 10 minutes the number of fish holding in fresh water or salt water was recorded as well as the total amount of salt water that had entered the tank. Some fish would not hold in one layer or the other, but instead, moved between the two layers. These were tallied separately from the other two groups. It took just under 1 hour for all the salt water to drain into the tanks. At this time, the bottom half of the water column (30 cm deep) was saline and the top half was fresh.



**Figure 1.1** Diagram of saltwater preference tanks. Tanks are fiberglass with Plexiglas front windows, and measure 1.83 x 0.66 x 0.60 m. A black plastic curtain surrounded each tank. Elevated header tanks outside of the curtained area supplied salt water via gravity flow. The pipe from header tanks split into two lines along the bottom of the preference tanks. Salt water was slowly introduced through holes drilled every 5 cm in two rows along the sides of the pipes, shown by the open arrows in the top view. Plastic mesh dividers were used to keep fish within the tank area in front of the window. Fluorescent lights were suspended above the tanks and light was diffused by netting suspended on frames below the light fixture.

Preliminary experiments with dyed salt water showed that the halocline was stable for several days when no fish were present and no mixing occurred. Early tests also found that with fish in the tanks (and some potential mixing from swimming), salinity in the top half remained below 3 ppt for at least 24 hours. Observations were continued for another hour after saltwater introduction.

### **Saltwater Preference Experiments**

On 20, 23 and 27 March 1998, 20 control and 20 sick fish were transported to Oak Creek Laboratory and immediately stocked into the four experimental tanks. Each tank received either 10 sick or 10 control fish. Fish were acclimated for 48 hours, and on each of the three experimental days, two consecutive sets of observations were done per day, each consisting of one control and one treatment tank. This gave a total of six replicate trials per treatment. Salt water was introduced from the header tanks and fish positions were noted every 10 minutes. Observations were continued for another hour after saltwater introduction after which fish were removed and killed by anesthesia overdose (tricaine methanesulfonate, 200mg l<sup>-1</sup>). Kidneys were removed from all fish and stored at -80°C until analysis for *R. salmoninarum*. Tissue samples were sent to the USGS-BRD Western Fisheries Research Center, Seattle, Washington to

quantify *R. salmoninarum* infection levels by the ELISA method as described by Pascho et al. (1991). The ELISA is a very sensitive method which allows for qualitative assessment of infection level based upon quantitative absorbance values: fish considered to be negative for the pathogen have absorbances  $\leq 0.073$ , absorbances between 0.074 - 0.199 are considered low, absorbances between 0.200 - 0.999 are considered medium, and absorbances greater than 1.000 are considered high infection levels.

### **Statistical Analysis**

Each tank was considered an independent replicate. For each tank, the percentages of fish holding in salt and fresh water or moving between the layers at various times were calculated. These data were arcsine transformed to make them continuous. Multifactor ANOVA ( $P < 0.05$ ) was used to determine any treatment, tank, or date effects. If data were skewed, the non-parametric Kruskal-Wallis comparison of medians was performed to determine treatment effect. For pairwise comparisons of ELISA values, *t* tests were used.

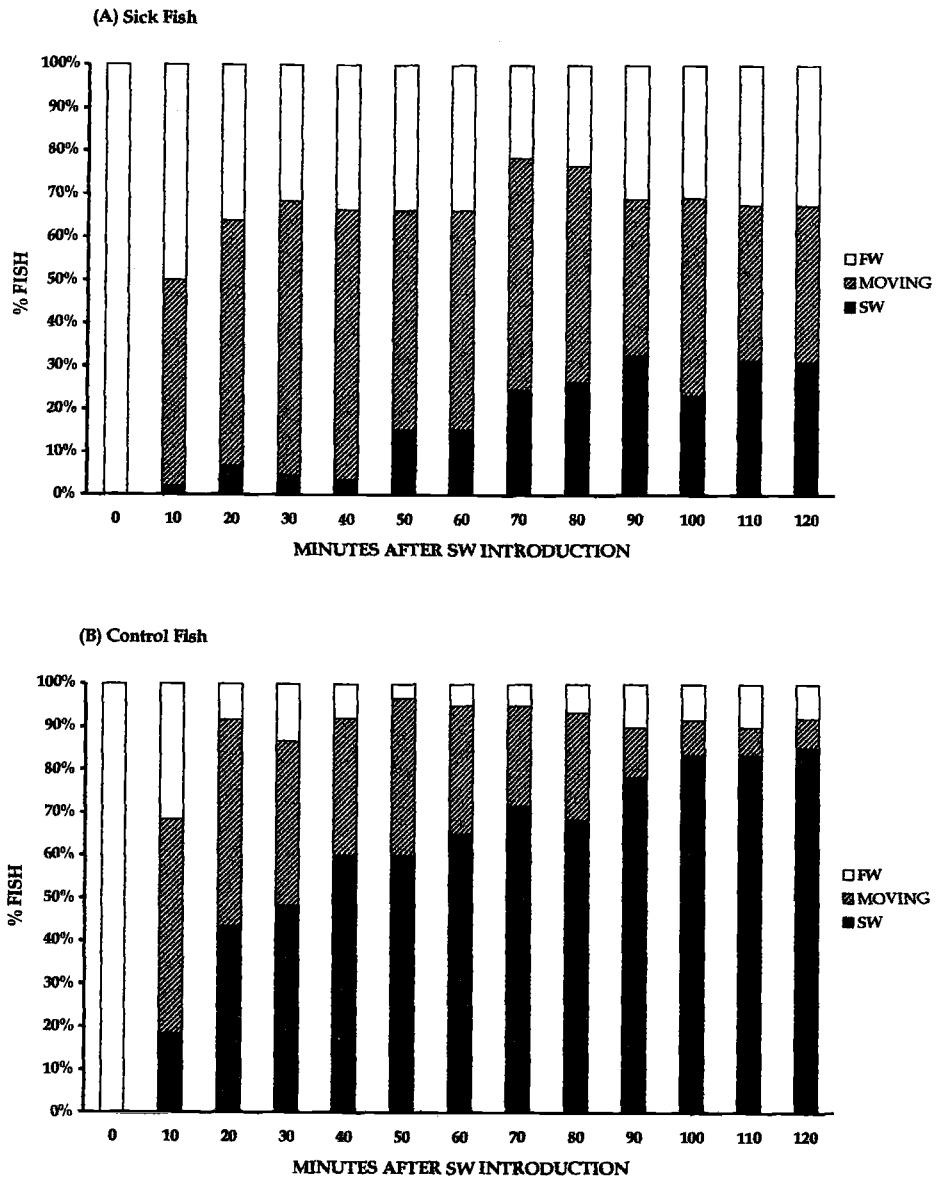
## RESULTS

### Saltwater Preference

Multifactor ANOVA indicated no date or tank effects ( $P = 0.9523$ ,  $P = 0.8591$ ), so data for the two replicates of each treatment were pooled for all three dates. Significantly fewer sick fish ( $15 \pm 10.8\%$ ) were holding in salt water after 1 hour compared to controls ( $65 \pm 40.4\%$ ; ANOVA,  $P = 0.0220$ ; Table 1.1; Figure 1.2). This trend continued, and after 2 hours,  $85 \pm 17.6\%$  of controls were holding in salt water compared to only  $31 \pm 20.0\%$  of sick fish (ANOVA,  $P = 0.0016$ ). Not only did fewer sick fish choose to remain in salt water, but it also took them longer to make that choice (Kruskal-Wallis,  $P = 0.0056$ ). In control tanks, half of the fish were holding in salt water within 49 minutes. In only two of the tanks with sick fish did  $>50\%$  of the fish hold in salt water and then only after 95 and 105 minutes.

**Table 1.1** Saltwater preference of juvenile spring chinook salmon with or without external signs of BKD. Also shown is the time at which at least 50% of fish were holding in salt water. ANOVA indicated that control fish showed higher preference for salt water at both 60 minutes ( $P = 0.0220$ ) and 120 minutes ( $P = 0.0016$ ). Control fish held in salt water more quickly than sick fish (Kruskal-Wallis;  $P = 0.0056$ ).

TREATMENT	DATE	TANK	% FISH HOLDING IN SW AFTER:		MINUTES UNTIL > 50% FISH WERE HOLDING IN SW
			60 MIN	120 MIN	
SICK	22 MAR	1	10	40	95
		2	0	0	> 120
	25 MAR	1	27	55	105
		2	27	27	>120
	29 MAR	1	9	18	> 120
		2	18	45	> 120
CONTROL	22 MAR	1	30	60	90
		2	50	100	60
	25 MAR	1	100	80	15
		2	100	100	10
	29 MAR	1	10	70	100
		2	100	100	20
MEAN±SD		SICK	15 ± 10.8	31 ± 20.0	> 120
		CONT.	65 ± 40.4	85 ± 17.6	49 ± 39.8



**Figure 1.2.** Mean percentage of sick (A) or control (B) chinook salmon holding in fresh (FW) and salt (SW) water, or moving between the layers in BKD experiments. For both charts, bars at each time represent pooled data from six tanks per treatment on three dates from 22-29 March 1998. Time = 0 represents the beginning of saltwater introduction. ANOVA found that saltwater preference was significantly different between sick and control fish at both 60 minutes ( $P = 0.0220$ ) and 120 minutes ( $P = 0.0016$ ).

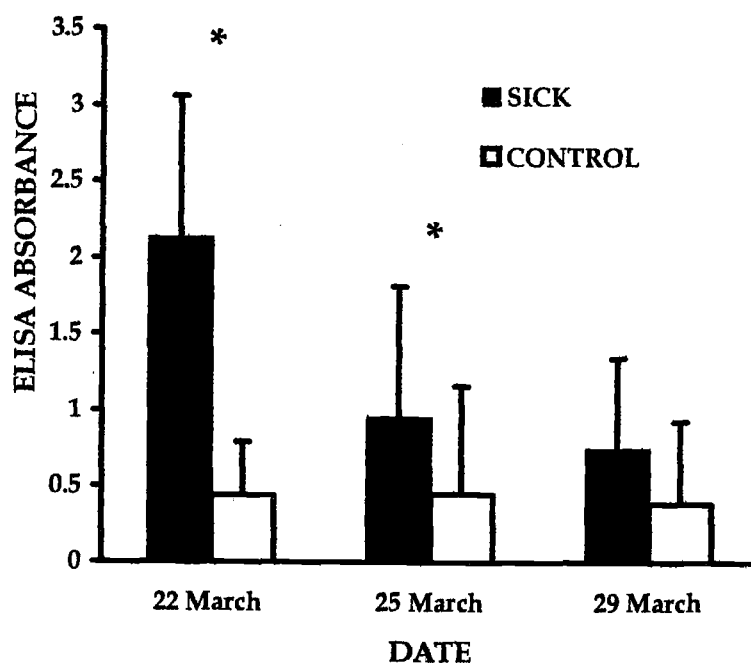
## ELISA Analysis

There were no tank effects (ANOVA,  $P = 0.1968$ ); therefore, ELISA absorbance data from replicates were pooled on each day for the two treatments for *t test* comparison (Figure 1.3). On 22 and 25 March, sick fish had significantly higher absorbances than did control fish ( $P < 0.0001$  and  $P = 0.03$ , respectively). On 29 March, there was no statistically significant difference between ELISA absorbances of control and sick fish ( $P = 0.08$ ). This was accounted for by one control fish that had a very high absorbance of 2.58. When this outlier was removed from the analysis, sick fish had higher absorbances than controls ( $P = 0.0107$ ). Linear regression indicated a significant negative relationship between the mean ELISA absorbance of fish from each tank and the percent of fish holding in salt water after 2 hours (ANOVA,  $P = 0.0074$ ;  $R^2 = 0.5284$ ; Figure 1.4).

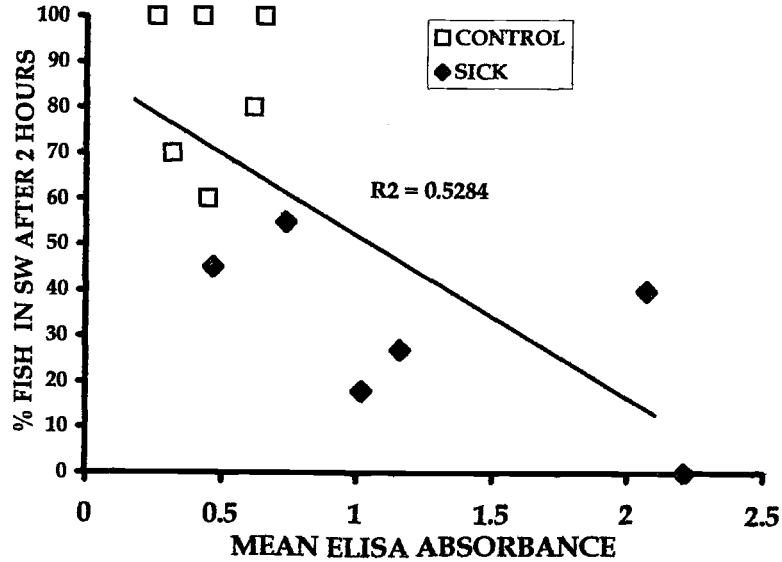
## DISCUSSION

The results of this experiment indicate that infection with *R. salmoninarum* can significantly decrease the saltwater preference of juvenile chinook salmon at a time in their life history when healthy fish largely prefer high salinity. Both sick and control fish used in this experiment displayed morphological





**Figure 1.3.** Mean ( $\pm$  SD) ELISA absorbance levels of the *R. salmoninarum* antigen from juvenile spring chinook salmon used in saltwater preference experiments. Asterisks denote mean values within a date that are significantly different (*t* test;  $P < 0.05$ ;  $n=20$  fish per treatment). On the last date, one control fish had a very high absorbance of 2.58. After removal of this outlier from the analysis, control fish on the last date had lower absorbances than sick fish ( $P = 0.0107$ ).



**Figure 1.4.** Linear regression of the mean ELISA absorbance levels of *R. salmoninarum* antigen versus the percent of juvenile spring chinook salmon holding in salt water for each tank in the preference experiments. ANOVA indicates a significant negative relationship between the variables ( $P = 0.0074$ ;  $R^2 = 0.5284$ ).

characteristics consistent with the latter stages of smoltification and, as expected, control fish predominantly chose to enter and remain in full-strength salt water. In contrast, nearly a third of sick fish completely avoided salt water and remained in the freshwater layer of the tank. Another third of the fish chose to hold in salt water. However, these fish took much longer to make a permanent move to the saltwater layer than did the control fish (Table 1, Figure 2). The other third of the sick fish moved between the fresh and saltwater layers. This final observation suggests that although some sick fish were unwilling to remain in salt water, they maintained an orientation toward high salinity.

Our observations are in agreement with the findings of Mesa et al. (1999) that BKD does not inhibit some key changes of smoltification, including a behavioral orientation toward salt water. This latter observation is intriguing, given the increased mortality of fish suffering from BKD following transfer to seawater (Banner et al. 1983, Sanders et al. 1992, Elliott et al. 1995). The inability of fish with BKD to thrive in salt water is likely the result of decreased ionoregulatory capacity. Moles (1997) reported that following a 24 h saltwater challenge, juvenile coho salmon (*O. kisutch*) with BKD had plasma sodium levels 15% above uninfected fish, and that full osmocompetence was not achieved even after 8 days in seawater. Further insight is provided by Mesa et al. (1999) who concluded that increased cortisol levels in fish suffering from BKD might account for decreased ionocompetence. Because they found no affect of BKD on gill Na<sup>+</sup>,

K<sup>+</sup>-ATPase activity, these researchers believed this protein's role in smolting remained relatively intact. Cortisol levels typically increase slightly during smoltification (from <10 ng ml<sup>-1</sup> up to 40 ng ml<sup>-1</sup>). However, cortisol levels reported for fish with BKD were much higher and indicative of chronically stressed fish (Mesa et al. 1998, 1999).

Cortisol has long been studied for its hormonal role in decreased hypoosmoregulatory capacity during the stress response in fishes (Mazeaud et al. 1977, Barton 1997) and chronically elevated levels of this hormone in smolting salmon with BKD may well provide a mechanistic explanation for their poor survival in salt water. If this is the case, then sick fish are faced with two conflicting physiological motivations when encountering salt water. While the process of smoltification would tend to promote their orientation toward and preference for salt water, decreased osmocompetence resulting from a physiological stress response associated with BKD would decrease their ability to survive in saline environments. Such a conflict is suggested by the saltwater-entry behavior observed and quantified in this study. Despite a general decrease in preference for full-strength salt water, varying degrees of orientation toward salinity was displayed by two thirds of the sick fish (Figure 1.2).

The negative correlation between infection level and salinity preference found in our study indicates that affinity for salt water is proportional to the severity of BKD. This relationship might have been even more distinct had our

control fish been completely disease free. Unfortunately, BKD is a pervasive disease and other studies (Mesa et al. 1998, 1999) have also found low to moderate antigen levels in control groups. Control fish in our experiments had medium levels of *R. salmoninarum*, yet did not exhibit any external signs of the disease when selected for use in the experiment. Similarly, Elliott et al (1997) reported that fish with low and medium infection levels may exhibit none of the external symptoms associated with BKD, although highly infected fish typically do. Despite being moderately infected, our control fish largely chose to move into salt water (Table 1.1, Figure 1.2), while only a third of sick fish (showing external symptoms of BKD) with high infection levels remained in salt water. In combination, this information would suggest that only fish with advanced BKD may show severely altered saltwater-entry behavior, and that the presence of external symptoms on chinook salmon smolts may be a good non-lethal indicator of fish performance capacity relative to ocean entry.

Decreased saltwater preference may have profound consequences as juvenile salmonids outmigrate through estuarine environments. Freshwater floats above more dense saline water, providing a potential refuge for fish unwilling to immediately enter full-strength salt water. Such behavior has been documented for chum salmon (*O. keta*) in Japan (Iwata & Komatsu 1984). Underwater observations revealed that outmigrating juveniles held in the upper, brackish (10-15 ppt and <1 m below the surface) layer for up to two days before

migrating seaward. This estuary was relatively small (<1 km wide at the mouth of the river), and flows were presumably low enough that fish could actively govern their position and migration rate. Subsequent laboratory experiments found that 12 hour acclimation to 12 ppt seawater improved osmoregulatory performance in full-strength seawater. Thus, holding in brackish water may be a behavioral response allowing salmon to slowly adjust to high salinity. This might be especially relevant for fish with decreased osmoregulatory capacity associated with BKD. While such behavior allows fish time to acclimate, it may also increase their risk of predation. If the freshwater lens is very shallow, salmon may be more prone to birds feeding at or near the surface. Mesa et al. (1998) showed that piscine predators ate nearly twice as many juvenile chinook salmon that suffered from BKD, compared to uninfected fish under laboratory conditions. Sick fish choosing to avoid salt water may find temporary osmoregulatory respite in the lens, but their chance of being eaten is also increased.

From 1988 - 1991, Elliott et al. (1997) monitored *R. salmoninarum* infection levels of outmigrating juvenile chinook salmon by ELISA at four dams in the Columbia and Snake Rivers. The percentage of fish with high infection levels ranged from 2-20% and averaged 7%. A similar monitoring study from 1988 - 1992 found that 65 to >90% of spring chinook salmon in Columbia and Snake River hatcheries were infected with *R. salmoninarum* (Maule et al. 1996). Thus, a

large number of fish may exhibit abnormal behavior upon reaching the estuary, thereby exposing themselves to increased predation risk. The ecological relevance of physiological perturbations, including disease, may best be reflected in behavioral analysis (Schreck et al. 1997). The findings of this study indicate that although fish may survive and undergo smoltification despite severe levels of BKD, secondary behavioral manifestations of the disease are potentially lethal for outmigrating salmonids.

## ACKNOWLEDGEMENTS

We thank the U.S. Army Corps of Engineers for providing the funding for this research. Rob Chitwood provided excellent fish husbandry. Ron Pascho, Dorothy Chase, and Diane Elliott of the USGS-BRD Western Fisheries Research Center in Seattle, Washington kindly conducted the ELISA assays free of charge.



## LITERATURE CITED

- Banner, C.R., Rohovec, J.S. & Fryer, J.L. 1983. *Renibacterium salmoninarum* as a cause of mortality among chinook salmon in salt water. *J. World Maricult. Soc.* 14: 236-239.
- Barton, B.A. 1997. Stress in finfish: past, present and future - a historical perspective. pp. 1-34. *In: Iwama, G.K., Pickering, A.D., Sumpter, J.P. & Schreck, C.B. (eds.) Fish stress and health in aquaculture, Society for Experimental Biology Seminar Series: 62, Cambridge University Press, Cambridge.*
- Bruno, D.W. 1986. Histopathology of bacterial kidney disease in laboratory infected rainbow trout, *Salmo gairdneri* Richardson, and Atlantic salmon, *Salmo salar* L., with reference to naturally infected fish. *J. Fish Dis.* 9: 523-537.
- Elliott, D.G., Pascho, R.J. & Palmisano, A.N. 1995. Brood stock segregation for the control of bacterial kidney disease can affect mortality of progeny chinook salmon (*Oncorhynchus tshawytscha*) in seawater. *Aquaculture* 132: 133-144.
- Elliott, D.G., Pascho, R.J., Jackson, L.M., Matthews, G.M. & Harmon, J.R. 1997. *Renibacterium salmoninarum* in spring-summer chinook salmon smolts at dams on the Columbia and Snake Rivers. *J. Aquat. Anim. Health* 9: 114-126.
- Folmar, L.C. & Dickhoff, W.W. 1980. The parr-smolt transformation (smoltification) and seawater adaptation in salmonids: a review of selected literature. *Aquaculture* 21: 1-37.

- Fryer, J.L. & Lannan, C.N. 1993. The history and current status of *Renibacterium salmoninarum*, the causative agent of bacterial kidney, disease in Pacific salmon. *Fish. Res.* 17: 15-33.
- Fryer, J.L. & Sanders, J.E. 1981. Bacterial kidney disease of salmonid fish. *Annu. Rev. Microbiol.* 35: 273-298.
- Hoar, W.S. 1976. Smolt transformation: evolution, behavior, and physiology. *J. Fish. Res. Bd. Can.* 33: 1234-1252.
- Iwata, M. & Komatsu, S. 1984. Importance of estuarine residence for adaptation of chum salmon (*Oncorhynchus keta*) fry to seawater. *Can. J. Fish. Aquat. Sci.* 41: 744-749.
- Maule, A.G., Rondorf, D.W., Beeman, J. & Haner, P. 1996. Incidence of *Renibacterium salmoninarum* infections in juvenile hatchery spring chinook salmon in the Columbia and Snake Rivers. *J. Aquat. Anim. Health* 8: 37-46.
- Mazeaud, M.M., Mazeaud, F. & Donaldson, E.M. 1977. Primary and secondary effects of stress in fish: some new data with a general review. *Trans. Am. Fish. Soc.* 106: 201-212.
- Mesa, M.G., Poe, T.P., Maule, A.G. & Schreck, C.B. 1998. Vulnerability to predation and physiological stress response in juvenile chinook salmon (*Oncorhynchus tshawytscha*) experimentally infected with *Renibacterium salmoninarum*. *Can. J. Fish. Aquat. Sci.* 55: 1599-1606.
- Mesa, M.G., Maule, A.G., Poe, T.P. & Schreck, C.B. 1999. Influence of bacterial kidney disease on smoltification in salmonids: is it a case of double jeopardy? *Aquaculture* 174: 25-41.

- McInerny, J.E. 1964. Salinity preference: an orientation mechanism in salmon migration. *J. Fish. Res. Bd. Can.* 21: 995-1018.
- Moles, A. 1997. Effect of bacterial kidney disease on saltwater adaptation of coho salmon smolts. *J. Aquat. Anim. Health* 9: 230-233.
- Pascho, R.J., Elliott, D.G. & Streufert, J.M. 1991. Brood stock segregation of spring chinook salmon by use of the enzyme-linked immunosorbent assay (ELISA) and the fluorescent antibody technique (FAT) affects the prevalence and levels of *Renibacterium salmoninarum* infection in progeny. *Dis. Aquat. Org.* 12: 25-40.
- Sanders, J.E., Long, J.J., Arakawa, C.K., Bartholomew, J.L. & Rohovec, J.S. 1992. Prevalence of *Renibacterium salmoninarum* among downstream-migrating salmonids in the Columbia River. *J. Aquat. Anim. Health* 4: 72-75.
- Schreck, C.B., Olla, B.L. & Davis, M.W. 1997. Behavioral responses to stress. pp. 145-170. *In: Iwama, G.K., Pickering, A.D., Sumpter, J.P. & Schreck, C.B. (eds.), Fish stress and health in aquaculture, Society for Experimental Biology Seminar Series, Cambridge University Press, New York.*
- Wedemeyer, G.A., Barton, B.A. & McLeay, D.J. 1990. Stress and acclimation. pp. 451-489. *In: Schreck, C.B. and Moyle, P.B. (eds.), Methods for fish biology, American Fisheries Society, Bethesda, Maryland.*

**STRESS AND SALTWATER-ENTRY BEHAVIOR OF  
JUVENILE CHINOOK SALMON (*ONCORHYNCHUS  
TSHAWYTSCHA*): CONFLICTS IN PHYSIOLOGICAL  
MOTIVATION**

Carol Seals Price and Carl B. Schreck

## ABSTRACT

Juvenile chinook salmon (*Oncorhynchus tshawytscha*) outmigrating through estuarine habitats may be exposed to a variety of stressors, yet little research has investigated the influence of stress on seawater-entry behavior. We conducted two laboratory experiments to determine the effects of a mild or a more severe stressor on the willingness of fish to enter and remain in salt water. To observe the response of the fish to an overhead threat, we also presented stressed fish with a model simulating an avian predator in the second experiment. Experiments were conducted in 757-l tanks containing a stable vertical salinity gradient. Fish stressed by being chased (mild stressor) prior to saltwater introduction showed a 26% decrease in saltwater preference compared with unstressed fish. After a more severe handling stressor, only 20% of fish entered and remained in salt water, compared with 100% of unstressed controls. After the presentation of the avian model, all stressed fish moved into the lower saltwater layer, but this behavioral response was transient. We conclude that stress significantly decreases the saltwater preference of juvenile chinook salmon that would otherwise select full-strength salt water, and may affect behavior in the estuary. While smoltification primes these fish for seawater residence, stress may induce a conflicting physiological motivation. The resultant behavior may

be maladaptive with ecological ramifications such as increased risk to avian predation.

## INTRODUCTION

Juvenile spring chinook salmon (*Oncorhynchus tshawytscha*) outmigrating through novel estuarine environments may be faced with an array of environmental stressors prior to entering seawater, but little is known about how stress might alter the behavior of these fish with respect to saltwater entry. The physiological characteristics of the stress response have been thoroughly studied in salmonids (Pickering 1981, Adams 1990, Iwama et al. 1997); however, behavioral assays of stress are less common. Many authors promote behavioral studies as a link between small-scale, individual physiological processes and large-scale, population processes (Wedemeyer and Mcleay 1981, Schreck 1990, Shuter 1990, Wedemeyer et al. 1990, Schreck et al. 1997). Thus, investigating behavioral aberrations may lend insight into the ecological significance of stress for fishes

Laboratory studies involving behavioral effects of stress have often focused on interactions of stressed salmonids with predators. Kruzynski and Birtwell (1994) found that juvenile chinook salmon exposed to the antisapstain fungicide, 2-(thiocyanomethylthio)benzothiazole (TCMTB), were five times more likely than unexposed fish to be eaten by yellowtail rockfish (*Sebastes flavidus*). Juvenile coho salmon (*O. kisutch*) subjected to a simple handling stressor and allowed a 1 minute recovery period were more susceptible to predation by

lingcod (*Ophiodon elongatus*) than unstressed controls (Olla et al. 1992). In that study, no difference in predation rate was evident if fish were allowed to recover for more than 90 minutes. Similarly, Mesa (1994) showed that stressed juvenile chinook salmon were preferentially preyed upon by northern pikeminnow (*Ptychocheilus oregonensis*), but after 1 hour stressed and unstressed fish were consumed equally. Studies by Järvi (1989a, b) support the notion that stress also inhibits normal predator-avoidance behavior in Atlantic salmon (*Salmo salar*).

Additional studies have investigated other behavioral deviations resulting from a variety of stressors. Cover-seeking time was increased for juvenile chinook salmon exposed to multiple acute handling stressors (Sigismondi and Weber 1988). Pulp mill effluent altered the schooling behavior of chum (*O. keta*) and coho salmon (Birtwell and Kruzynski 1989). Feeding activity of salmonids has been shown to decrease in response to several stressors including acidity (Jones et al. 1987), toxicants (Folmar et al. 1981, Jones and Hara 1988) and handling (Pickering et al. 1982, McCormick et al. 1998). Chinook salmon smolts suffering from advanced bacterial kidney disease showed a decreased preference for salt water (Chapter 1) and an increased risk of predation (Mesa et al. 1999).

The physiological stress response is generally considered an adaptive mechanism by which a perturbed organism attempts to regain homeostasis and increase survival (Seyle 1950, Barton 1997, McEwen 1998, Schreck 2000).

However, the above examples indicate that stress-induced behavioral changes



may place fish at a higher risk of predation. In nature, fish are often exposed concomitantly to several conflicting stimuli or stressors, and must make choices to maximize survival. Although the stress itself may not be lethal, 'ecological death' may ensue when the synergistic effects of multiple stressors result in maladaptive behavior inappropriate within the fish's ecological context (Schreck et al. 1997).

We conducted two laboratory experiments to determine the effects of relatively mild stressors on the saltwater-entry behavior of juvenile chinook salmon smolts. We wanted to simulate stress levels that might be experienced by fish naturally migrating through the estuary and encountering salt water for the first time. Additionally, we were interested in the combined effects of stress and an avian predator model on saltwater-entry behavior. Our results suggest that the conflicting physiological demands of stressed salmon encountering salt water can lead to suboptimal behavior, and we discuss possible ecological ramifications of this behavior.

## METHODS

### Test fish

Juvenile chinook salmon used in these experiments were obtained as fingerlings (5-10 cm) from Oregon's South Santiam (Experiment I) and Marion Forks (Experiment II) hatcheries and reared at Oregon State University's Fish Performance and Genetics Laboratory at Smith Farm, Corvallis, OR. Fish were held indoors with natural lighting in 2-m, circular tanks and fed to satiation twice per day for at least four months until needed for experiments. During rearing, water temperature fluctuated between 12.8 and 14° C. In Experiment I, yearling chinook measured  $16.9 \pm 2.9$  (SD) cm and weighed  $59.6 \pm 26.5$  g. In Experiment II, yearling chinook salmon measured  $16.8 \pm 2.2$  cm and weighed  $65.4 \pm 28.6$  g. The fish had undergone morphological changes associated with smoltification: partial or full loss of parr marks, silvering, and darkening of fin margins. Additionally, hatchery cohorts are historically released at the time of these experiments. Experiments were conducted at Oregon State University's Oak Creek Laboratory, Corvallis, OR. As needed, fish were transported for 15 minutes from the Fish Performance Laboratory in 130-l plastic cans supplied

with oxygen. Water temperature at the Oak Creek Laboratory ranged from 8 to 10° C.

### **Saltwater Preference Tanks**

Saltwater preference experiments were carried out in four, 757-1 rectangular tanks (1.83 x 0.66 x 0.60 m) with Plexiglas® front windows and flowing (3 L/min) freshwater. Each was enclosed by a black plastic curtain with holes cut out to allow for visual observation. Fluorescent lights were suspended above each tank. Light was diffused by fiberglass netting on frames suspended below the bulbs. During experiments, salt water (30 ppt) was introduced into the bottom of each tank from one of two header tanks. Each header was connected to one of the preference tanks by PVC pipes running along the bottom of the experimental tanks. These pipes had small holes drilled in two rows, allowing salt water to slowly diffuse to the tank bottom.

Fish transported to the Oak Creek Laboratory were randomly placed into preference tanks and held in aerated, flowing water for 48 hours. Preliminary work found that after 48 hours fish began normal feeding, so we considered them to be acclimated after this time. During acclimation, fish were not observed resting directly on the tank bottoms. Stationary fish tended to

position themselves 5-30 cm off the bottom. Fish were dispersed throughout the tanks and actively explored all areas of the tank, including the surface. The photoperiod was set to the ambient light cycle. Fish were not fed during acclimation to reduce disturbance to the tank. After acclimation, 379 L of salt water was mixed in each header tank using Instant Ocean<sup>®</sup> artificial salt, and was aerated. Ten minutes prior to saltwater introduction, water supply and aeration were turned off to prevent mixing. The valves from the header tanks were then opened and salt water began to flow into the bottom of the preference tanks, pushing the lighter freshwater out of the top-draining standpipe. This created a stable saltwater layer in the bottom of the water column, with a freshwater layer on top. The halocline was visible, so it was easy to distinguish whether fish were in salt or fresh water. Every 10 minutes the number of fish holding in fresh or salt water was observed and recorded, as well as the total amount of salt water that had entered the tank. Some fish would not hold in one layer or the other, but instead, moved between the two layers. These were tallied separately from the other two behavioral groups. A fish was considered to be moving between the layers if it left the layer it was seen in at the beginning of each counting observation one or more times. Behavioral counts took about 1 minute to tally. Although individual fish were not marked, there were few enough fish in the tank that their movement patterns (i.e., holding in a layer or moving between them) could be readily assessed. It took just under 1 hour for

all the salt water to flow into the tanks. At this time, the bottom half of the water column (30 cm deep) was saline and the top half was fresh. Observations were continued for another 60 minutes after saltwater introduction. Preliminary experiments with dyed salt water showed that the halocline was stable for several days when no fish were present, and no mixing occurred. Early tests also found that with fish in the tanks (and some potential mixing from swimming), salinity in the top half remained below 3 ppt for at least 24 hours.

#### **Experiment I: Effects of a mild chasing stress on saltwater preference of juvenile chinook salmon**

We conducted this experiment to determine the effects of a mild stressor, which very roughly simulated a fish being pursued by a predator, on the saltwater preference of chinook salmon. A preliminary experiment was conducted to establish that a physiological stress response would be elicited by chasing fish with a large net. On 27 January 1999, 15 fish were stocked into each of two preference tanks and allowed to acclimate for 48 hours. In one tank, fish were chased with a dip net (opening was 30 x 45 cm) for 2 minutes. Fish were not held in the net or removed from the water. Fish in the other tank served as controls and were not disturbed. After 45 minutes, fish from both tanks were quickly (approximately 1 minute) netted out and immediately killed by overdose

of tricaine methanesulfonate (200 mg/l). Blood was collected from all fish in heparinized capillary tubes after severing the caudal peduncle. The blood was centrifuged, plasma was collected, and samples were stored at -80°C until analysis. Plasma cortisol levels were assessed as an indicator of stress using a radioimmunoassay (Foster and Dunn, 1974 and modified by Redding et al., 1984).

On 21, 25 and 29 March and 02 April 1999, 52 novel fish were transported to the Oak Creek Laboratory, divided equally between the four preference tanks, and allowed to acclimate for 48 hours. On each experimental day, four tanks could be observed; however, only two could be viewed simultaneously. Therefore, two consecutive sets of experiments were done per day, each consisting of one control (not chased) and one treatment (chased). In total, eight tanks with chased fish and eight tanks with unchased fish were observed. Tanks were alternately assigned to serve for either control or treatment. After acclimation, fish in treatment tanks were chased with a dip net for 2 minutes as described above. Salt water was then introduced and fish positions were recorded as previously described.

## **Experiment II: Effects of a handling stress on saltwater preference of juvenile chinook salmon and response to avian model**

This experiment was conducted to investigate the effects of a more severe handling stressor on saltwater preference and to determine the response of stressed fish to an avian predator. On 02, 07 and 09 February 2000, 40 novel fish were transported to the Oak Creek Laboratory, equally divided between the four preference tanks, and allowed to acclimate for 48 hours. The design of the experiment was similar to that used in Experiment I. After acclimation, fish in treatment tanks were quickly removed by dip net and placed for 15 minutes in a 21-l bucket containing 8 cm of water and an airstone. They were then returned to the preference tank, salt water was introduced and fish positions were recorded. In the treatment tanks, once all salt water had entered the tank, an avian predator model was suddenly introduced through a hole in the plastic curtain. The model was constructed to mimic a tern (*Sterna* sp.), a surface-feeding bird common in estuaries and known to feed on salmonid smolts (Collis et al. 2001). The model was cut from 2.5 cm thick Styrofoam, covered with white colored tape and painted to resemble a bird viewed from below. The model was 36 cm long from beak to tail, its wingspan was 31 cm, and it was suspended by clear monofilament line from a thin, 1.3-m long pole. The model was suspended over the tank for 3 seconds, dropped momentarily on the mesh tank cover (8 cm above water surface), and then held above the tank for another second before

being removed from the tank area. Tank covers were left in place so that conditions in the tank after the stress experience were identical to pre-stress conditions and to control tanks. Prior observations found that fish were more oriented toward the tank bottom when covers were not used, and we wanted to avoid this as a potential confounding factor. After the model was presented, fish positions were recorded every 10 minutes for another hour. Observations of control fish (no stress and no model) were conducted for 1 hour during saltwater introduction and for 1 hour after introduction.

### **Statistical Analysis**

Each tank was considered an independent replicate. For each tank, the percentages of fish holding in salt and fresh water or moving between the layers at 60 and 120 minutes were calculated. These data were arcsine transformed to make them continuous. Multifactor ANOVA ( $P < 0.05$ ) was used to determine any treatment, tank, and date effects. In both experiments, the only significant factor was treatment; therefore, the eight replicates for each of the two treatments were pooled for one-way ANOVA. If data were skewed, the non-parametric Kruskal-Wallis comparison of medians was performed to determine treatment effect. For pairwise comparisons, post-hoc *t*-tests were used.



## RESULTS

### **Experiment I: Effects of a mild chasing stress on saltwater preference of juvenile chinook salmon**

The preliminary stress experiment found that chasing fish with a dip net for 2 minutes did elicit a stress response. After 45 minutes, the mean cortisol concentration of the chased fish was  $145 \pm 30.8$  (SD) ng/ml, compared with  $60 \pm 39.0$  ng/ml for controls (*t*-test;  $P < 0.0001$ ).

When salt water was first introduced and perceived, control fish initially avoided it and then gradually began moving into the saltwater layer. They usually spent 10 to 20 minutes moving between the two layers. Some fish immediately entered and remained in salt water, which often meant holding in a layer <5 cm from the bottom. Once a fish held in salt water, it usually did not return to fresh water for the duration of the experiment. As the saltwater layer deepened, fish holding there moved throughout the layer. Most fish that avoided salt water and chose to hold in freshwater, had explored the saltwater layer multiple times. Fish holding in both salt and fresh water were frequently observed swimming to the halocline, but not across it. Often, fish that were "undecided" would position themselves directly on the halocline as the saltwater layer moved up the water column.

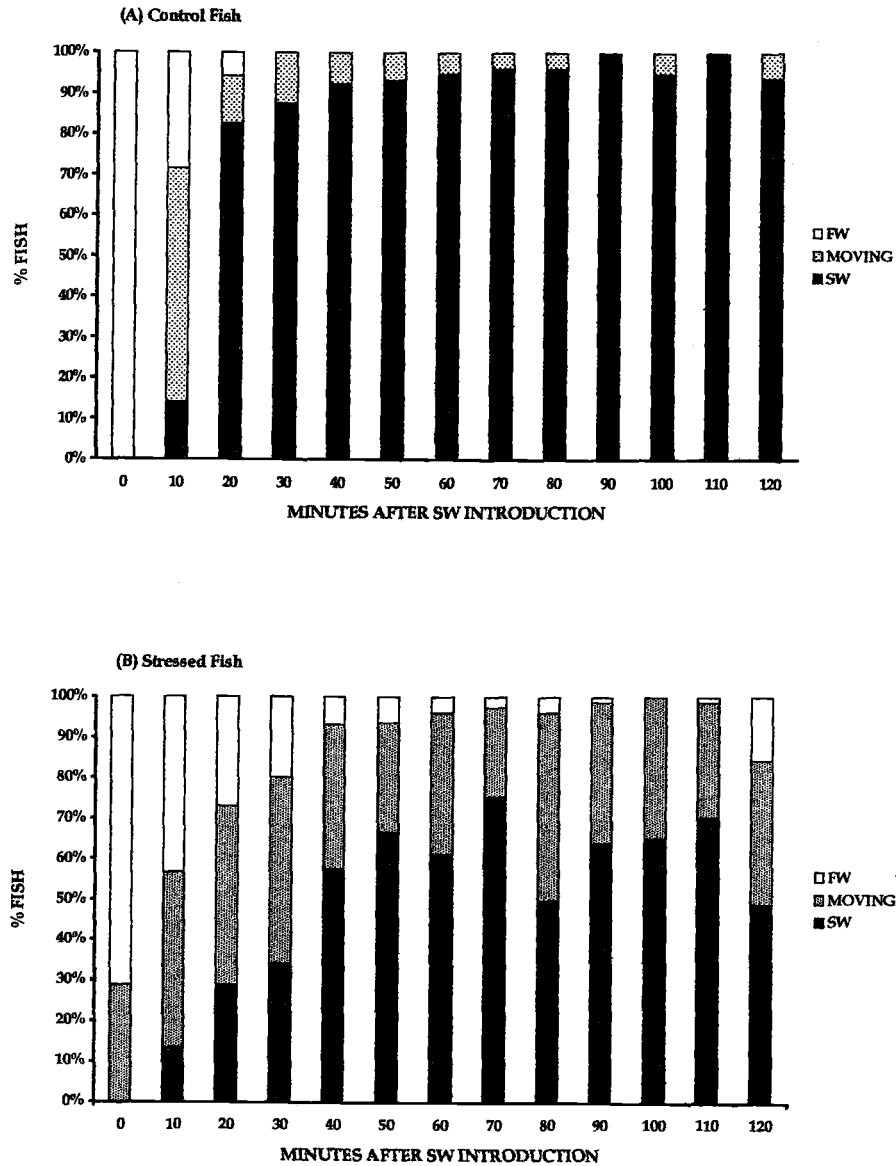
No date or tank effects were found (Multifactor ANOVA;  $P = 0.3454$  and  $0.2014$ , respectively), so the eight replicates of chased or unchased fish were pooled for the four dates. After 60 minutes,  $63 \pm 29\%$  of fish that had been chased were holding in salt water, compared to  $96 \pm 11\%$  of control fish (Table 2.1; Figure 2.1; Kruskal-Wallis;  $P = 0.0128$ ). This trend continued, and after 2 hours,  $69 \pm 15\%$  of chased fish and  $95 \pm 9\%$  of controls were holding in the saltwater layer (Kruskal-Wallis;  $P = 0.0035$ ). Not only did fewer chased fish choose to remain in salt water, but it also took them longer to make that choice; in control tanks, half of the fish had entered and remained in salt water within  $24 \pm 15$  minutes, compared to  $65 \pm 36$  minutes for chased fish (Kruskal-Wallis,  $P = 0.0056$ ).

### **Experiment II: Effects of a handling stress on saltwater preference and response to avian model of juvenile chinook salmon**

General behavior patterns were similar to fish in Experiment I. No date or tank effects were found (Multifactor ANOVA;  $P = 0.7917$  and  $0.9883$ , respectively), and replicates from the two treatments were pooled for the three dates. In all six control tanks, 100% of the fish were holding in salt water after 60 minutes (Table 2.2; Figure 2.2 A), and more than half of them held there within  $25 \pm 8$  minutes. After saltwater introduction, only  $20 \pm 13\%$  of stressed chinook

**Table 2.1.** Saltwater preference of chased and control juvenile chinook salmon in Experiment I. Also shown is the time at which at least 50% of fish were holding in salt water. Chased fish showed a decreased preference for salt water at both 60 and 120 minutes (Kruskal-Wallis;  $P = 0.0128$  and  $0.0035$ , respectively). Control fish were quicker to hold in salt water than chased fish (Kruskal-Wallis;  $P = 0.0056$ ).

TREATMENT	DATE	TANK	% FISH HOLDING IN SW AFTER:		MINUTES UNTIL >50% FISH HOLD IN SW
			60 MIN	120 MIN	
CONTROL	23 MAR	1	100	100	20
		2	100	100	10
	27 MAR	1	100	86	20
		2	100	100	20
	31 MAR	1	69	77	60
		2	100	100	20
	04 APR	1	100	100	20
		2	100	100	20
CHASED	23 MAR	1	31	54	110
		2	100	100	30
	27 MAR	1	46	54	80
		2	23	62	120
	31 MAR	1	77	69	50
		2	54	69	60
	04 APR	1	69	77	50
		2	100	69	20
MEAN $\pm$ SD		CONTROL	96 $\pm$ 63	95 $\pm$ 69	24 $\pm$ 65
		STRESS	63 $\pm$ 63	69 $\pm$ 69	65 $\pm$ 36



**Figure 2.1.** (A) Mean percentage of control fish holding in fresh (FW) and salt water (SW), or moving between the layers in Experiment I. Nearly 100% of the fish held in salt water, and did so quickly. Time = 0 represents the beginning of saltwater introduction. (B) Mean percentage of chased fish holding in fresh and salt water, or moving between the layers in Experiment I. About 65% of the fish held in salt water, and a very small percentage (5%) chose to hold in the freshwater layer. Throughout, 30-40% of the chased fish retained some orientation toward higher salinity and moved continuously between the two layers. Time = 0 represents the beginning of saltwater introduction.

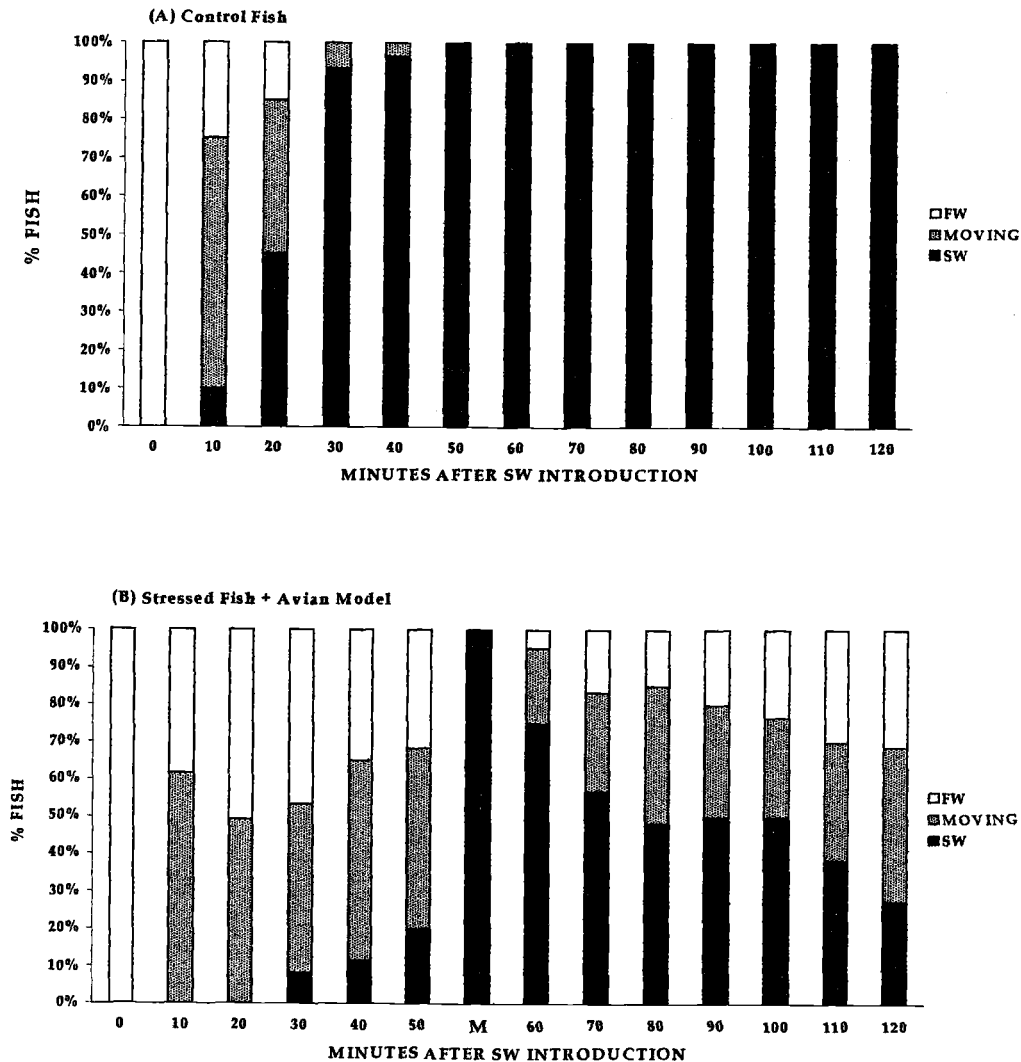
were holding in salt water (Table 2.2; Figure 2.2 B; Kruskal-Wallis;  $P = 0.0019$ ). In none of the tanks did more than half the stressed fish hold in salt water by the time it was fully introduced. Once the model was dropped, 100% of the stressed fish in all six replicates immediately moved into the saltwater layer (Figure 2 B). In five of the tanks, stressed chinook began returning to the freshwater layer within 10 minutes of exposure to the predator model. After 1 hour, only  $26 \pm 11\%$  of these fish remained in the salt water. In the remaining tank, 90% of the stressed fish remained in the saltwater layer for 1 hour after exposure to the model. By comparison, 100% of control fish were holding in salt water 1 hour after saltwater introduction (Kruskal-Wallis;  $P = 0.0020$ ).

## DISCUSSION

Our experiments found that both a mild chasing stressor and a more severe confinement stressor decreased the willingness of juvenile spring chinook salmon to remain in salt water at a developmental stage when unstressed fish prefer salt to fresh water. The mild chasing stressor evoked less of a response in terms of saltwater preference. In Experiment I, about half the chased fish did not hold in salt water (Figure 1B). While a small percentage of these fish completely avoided salt water and held in the freshwater layer (about 5%), most of them

**Table 2.2.** Saltwater preference of stressed and control juvenile chinook salmon in Experiment II. Also shown is the time at which at least 50% of fish were holding in salt water. An avian predator model was presented to stressed fish 60 minutes after the beginning of saltwater introduction...Stressed fish showed a decreased preference for salt water at both 60 and 120 minutes (Kruskal-Wallis;  $P = 0.0019$  and  $0.0020$ , respectively).

TREATMENT	DATE	TANK	% FISH HOLDING IN SW AFTER:		MINUTES UNTIL >50% FISH HOLD IN SW
			60 MIN	120 MIN	
CONTROL	04 FEB	1	100	100	30
		2	100	100	20
	09 FEB	1	100	100	30
		2	100	100	10
	11 FEB	1	100	100	30
		2	100	100	30
STRESS + AVIAN MODEL	04 FEB	1	10	10	-
		2	30	20	-
	09 FEB	1	40	90	After Model
		2	10	40	-
	11 FEB	1	10	30	-
		2	20	30	-
MEAN $\pm$ SD		CONTROL	100 $\pm$ 0	100 $\pm$ 0	25 $\pm$ 8
		STRESS	20 $\pm$ 13	37 $\pm$ 28	-



**Figure 2.2.** (A) Mean percentage of control fish holding in fresh (FW) and salt water (SW), or moving between the layers in Experiment II. After 50 minutes, 100% of the fish were holding in salt water and continued to do so for the observation period. Time = 0 represents the beginning of saltwater introduction. (B) Mean percentage of stressed fish holding in fresh and salt water, or moving between the layers in Experiment II. Once all salt water had been introduced, only 20% of fish were holding in it, a third of the fish were holding in freshwater, and about half were moving between the layers. At this time (represented by M on the graph) an avian predator model was dropped. 100% of the fish moved immediately into the saltwater layer. However, fish quickly returned to the freshwater or resumed moving between layers. Time = 0 represents the beginning of saltwater introduction.

retained some orientation toward high salinity. Throughout the observation period, 30 to 40% of the chased fish moved continuously between the two layers. It is important to note that while they did not hold in salt water, they did not necessarily avoid it. In contrast, 20 to 40% of stressed chinook salmon in Experiment II held in the freshwater layer and did not move into the saltwater layer even briefly (Figure 2B). The percentage of stressed fish holding in salt water was highest immediately after the avian predator model was presented, but half the fish quickly returned to the freshwater or resumed moving between the layers. Although the stressed fish retained the ability to perceive a threat and displayed appropriate avoidance behavior by moving into deeper water, their avoidance response was quite transient. In only one tank did most of the stressed fish remain in the saltwater layer for an extended period after the model was introduced (Table 2.2); these fish remained tightly grouped for the hour after the model was presented. Schooling behavior in prey fish often increases in the presence of predatory threat and is a group response to perceived danger (Pitcher 1986, Diana 1995). This behavior contrasted sharply with that of the other stressed fish whose behavior was more influenced by each individual's physiological state.

Stress is known to cause electrolyte imbalance in salmonids (Eddy 1981, McDonald and Milligan 1997) and we suggest that this is the most likely explanation for the avoidance of salt water by stressed fish in our experiments.



Stressed fish have higher water and ion flux across the gill epithelium (McDonald and Milligan 1997); freshwater fish tend to lose electrolytes and gain water while the opposite occurs in saltwater-adapted species (Mazeaud et al. 1977). Redding and Schreck (1983) subjected yearling coho salmon acclimated in freshwater to a confinement stress and immediately placed them in seawater. Plasma sodium concentration and osmolarity remained elevated for 24 hours, and there was a high mortality rate for these fish. While isotonic concentrations of salt have been found to be beneficial to fish recovering from stress (Wedemeyer 1972, Strange and Schreck 1980, Redding and Schreck 1983), high salinity may be a serious threat to recovery and survival.

Avoidance of salt water by stressed chinook salmon has been reported elsewhere (Kruzynski and Birtwell 1994). Fish exposed to the toxicant TCMTB were placed together with predators in a vertically stratified (fresh over salt water) 15,500-L tank. Preliminary experiments found that although fish survived toxicant exposure in freshwater, subsequent exposure to seawater resulted in 90% mortality. While non-exposed chinook held in the saltwater layer, exposed fish were typically observed holding in freshwater or just below the halocline. Exposed fish also showed reduced orientation to cover and swam erratically. As a result of these behavioral changes, predation by yellowtail rockfish was greater on exposed fish.

A previous study in our laboratory (C. B. Schreck, unpublished data) investigated the delayed behavioral effects of multiple stressors on smolted juvenile chinook salmon. In this study, fish were subjected, over 16 days, to a series of eight severe handling stressors to simulate passage through multiple hydroelectrical dams, and a stress response, indicated by elevated cortisol concentrations, was observed. Three hours after the last stressor, cortisol levels had returned to baseline values. The fish were allowed two weeks recovery and saltwater preference tests were performed on multiple-stressed fish and unstressed controls (following the same protocol as in this experiment). Over 90% of the control fish held in salt water compared to only 30% of stressed fish. These percentages are consistent with the results from the present study. While recovery of the primary physiological responses from the stress occurred within hours, delayed behavioral effects were still apparent more than two weeks later.

In the present experiments, the control fish quickly made the choice to hold in saline water, whereas most stressed fish likely perceived the salt water as an additional stressor. Thus, stressed fish were forced to make a choice between two conflicting physiological motivations. Chinook salmon in these experiments were in the latter stages of smoltification, which is defined as the sum of physiological, morphological and behavioral changes that anadromous salmonids undergo to prepare them for ocean residence (Hoar, 1988; Folmar and Dickhoff, 1980; Wedemeyer et al., 1980). While their ultimate physiological state

would tend to facilitate entry into salt water, the proximate physiological restrictions imposed by stress seemed more important in determining their preference behavior. Furthermore, the degree of behavioral shift reflected the severity of the stressor.

The stressed chinook salmon were again faced with conflicting stimuli when an additional stressor (i.e., the avian model) was introduced. By moving into the saltwater layer, fish seemingly made the appropriate choice with regards to immediate threat. However, they were not willing to remain in salt water once the model had been removed. In preliminary trials using a mild chasing stressor, fish that had initially avoided salt water also responded to overhead disturbance by moving into the lower, saltwater layer. These fish typically remained in salt water for up to 1 hour, but this was not investigated further in formal experiments. However, this difference in response behavior reinforces our observation that the severity of the initial stressor affects the willingness of fish to hold in salt water.

Our results suggest that if juvenile chinook salmon are stressed during the estuarine phase of outmigration, their orientation toward salt water may be compromised. Based on our laboratory findings, one might expect that mildly stressed fish would only briefly delay volitional entry into seawater. More severely stressed fish might avoid salt water altogether. In either case, this behavior is likely maladaptive and would expose fish to increased predation risk.

Many estuaries have a stratified water column with less-dense freshwater floating on the denser salt water. If this freshwater lens is very shallow, salmonid smolts using it as a refuge from osmoregulatory stress may be more susceptible to avian predators feeding at or near the surface. Stressed fish would be compromised in several ways. Both their position in the upper freshwater lens and a stress-induced decrease in predator avoidance response would put these fish at higher risk. Finally, an efficient shift from fresh to salt water may be a critical factor in determining early ocean survival for salmon. Speculatively, fish that experience difficulty while traveling through the estuarine environment may be more likely to succumb to adverse conditions after ocean entry.

While management practices can by no means eliminate all potential stressors for outmigrating juvenile chinook salmon, efforts to improve overall fish quality or condition may maximize the ability of these fish to appropriately respond to environmental stimuli (Wedemeyer et al. 1980, Iwama 1992). For example, our results suggest that ensuring the fish released from hatcheries have reached a sufficient stage of smoltification will improve their physiological motivation to enter seawater. Improved engineering of dam passage facilities to reduce stress to fish during the riverine phase of outmigration would be advantageous for decreasing delayed stress effects. Increased awareness of the interactions between physiology, stress and behavior, and experimental designs

incorporating the effects of stress at multiple scales will improve our knowledge of the ecological relevance of stress.

## ACKNOWLEDGEMENTS

We thank the U.S. Army Corps of Engineers for the funding to pursue this research, Rob Chitwood for assistance with fish husbandry, Dr. Cliff Pereira for statistical advice, and Beth Siddens for conducting the cortisol assays.

**LITERATURE CITED**

- Adams, S.M. (ed.). 1990. Biological indicators of stress in fish. American Fisheries Society Symposium 8.
- Barton, B.A. 1997. Stress in finfish: past, present and future - a historical perspective. pp. 1-34. *In: Iwama, G.K., Pickering, A.D., Sumpter, J.P. & Schreck, C.B. (eds.), Fish stress and health in aquaculture. Society for Experimental Biology Seminar Series: 62. Cambridge University Press, Cambridge.*
- Birtwell, I.K. & Kruzynski, G.M. 1989. *In situ* and laboratory studies on the behavior and survival of Pacific salmon (genus *Oncorhynchus*). *Hydrobiologia* 188/189: 543-560.
- Collis, K., Roby D.D., Craig, D.P., Ryan, B.A., & Ledgerwood, R.D., 2001. Colonial Waterbird Predation on Juvenile Salmonids Tagged with Passive Integrated Transponders in the Columbia River Estuary: Vulnerability of Different Salmonid Species, Stocks, and Rearing Types. *Trans. Amer. Fish. Soc.* 130: 385-396.
- Diana, J.S. 1995. *Biology and Ecology of Fishes*. Cooper Publishing Group LLC, Carmel, IN.
- Eddy, F.B. 1981. Effects of stress on osmotic and ionic regulation in fish. pp. 77-102. *In: Pickering, A.D. (ed.), Stress and Fish*. Academic Press Inc., New York.
- Folmar, L.C. & Dickhoff, W.W. 1980. The parr-smolt transformation (smoltification) and seawater adaptation in salmonids: a review of selected literature. *Aquaculture* 21: 1-37.

- Folmar, L.C., Craddock, D.R., Blackwell, J.W., Joyce, G. & Hodgins, H.O. 1981. Effects of petroleum exposure on predatory behavior of coho salmon (*Oncorhynchus kisutch*). Bull. Environ. Contam. And Toxic. 27: 458-462.
- Foster, L.C. & Dunn, R.T. 1974. Single-antibody technique for radioimmunoassay of cortisol in extracted serum or plasma. Clin. Chem. 20: 365-368.
- Hoar, W.S. 1988. The physiology of smolting salmonids. pp. 275-343 In: Hoar, W.S. & Randall, D.J., (eds.), Fish Physiology, Volume XIB. Academic Press, San Diego, California.
- Iwama, G.K. 1992. Smolt quality: a special section. World Aquacult. 23: 38-39.
- Iwama, G.K., Pickering, A.D., Sumpter, J.P. & Schreck, C.B. (eds.). 1997. Fish stress and health in aquaculture. Society for Experimental Biology Seminar Series: 62. Cambridge University Press, Cambridge.
- Järvi, T. 1989a. The effects of osmotic stress on the anti-predator behavior of Atlantic salmon smolts: a test of the 'maladaptive anti-predator behavior' hypothesis. Nord. J. Fresh. Res. 65: 71-79.
- Järvi, T. 1989b. Synergistic effect on mortality in Atlantic salmon, *Salmo salar*, smolt caused by osmotic stress and presence of predators. Env. Biol. Fish. 26: 149-152.
- Jones, K.A. & Hara, T.J. 1988. Behavioral alterations in Arctic charr (*Salvelinus alpinus*) briefly exposed to sublethal chlorine levels. Can. J. Fish. Aquat. Sci. 45: 749-753.



- Jones, K.A., Brown, S.B. & Hara, T.J. 1987. Behavioral and biochemical studies of onset and recovery from acid stress in Arctic char (*Salvelinus alpinus*). *Can. J. Fish. Aquat. Sci.* 44: 373-381.
- Kruzynski, G.M. & Birtwell, I.K. 1994. A predation assay to quantify the ecological significance of sublethal responses of juvenile chinook salmon (*Oncorhynchus tshawytscha*) to the antisapstain fungicide TCMTB. *Can. J. Fish. Aquat. Sci.* 51: 1780-1790.
- Mazeaud, M.M., Mazeaud, F. & Donaldson, E.M. 1977. Primary and secondary effects of stress in fish: some new data with a general review. *Trans. Am. Fish. Soc.* 106: 201-212.
- McCormick, S.D, Shrimpton, J.M., Carey, J.B., O'Dea, M.F., Sloan, K.E., Moriyama, S. & Björnsson, B.Th. 1998. Repeated acute stress reduces growth rate of Atlantic salmon parr and alter plasma levels of growth hormone, insulin-like growth factor I and cortisol. *Aquaculture* 168: 221-235.
- McDonald, G. & Milligan, L. 1997. Ionic, osmotic and acid-base regulation in stress. pp. 119-144. *In: Adams, S.M. (ed.), Biological Indicators of Stress in Fish. American Fisheries Society Symposium* 8.
- McEwen, B.S. 1998. Protective and damaging effects of stress mediators. *New Eng. J. Med.* 338: 171-179.
- Mesa, M.G. 1994. Effects of multiple acute stressors on the predator avoidance ability and physiology of juvenile chinook salmon. *Trans. Am. Fish. Soc.* 123: 786-793.
- Mesa, M.G., Maule, A.G., Poe, T.P. & Schreck, C.B. 1999. Influence of bacterial kidney disease on smoltification in salmonids: is it a case of double jeopardy? *Aquaculture* 174: 25-41.

- Olla, B.L., Davis, M.W. & Schreck, C.B. 1992. Comparison of predator avoidance salmon. *Trans. Am. Fish. Soc.* 121: 544-547.
- Pickering, A.D. (ed.). 1981. *Stress and Fish*. Academic Press Inc., New York.
- Pickering, A.D., Pottinger, T.G. & Christie, P. 1982. Recovery of the brown trout, *Salmo trutta* L., from acute handling stress: a time-course study. *J. Fish Biol.* 20: 229-244.
- Pitcher, T.J. 1986. Functions of shoaling behavior in teleosts. pp. 294-337. *In*: Pitcher, T.J. (ed.), *The Behavior of Teleost Fish*. The Johns Hopkins Univ. Press, Baltimore.
- Redding, J.M. & Schreck, C.B. 1983. Influence of ambient salinity on osmoregulation and cortisol concentration in yearling coho salmon during stress. *Trans. Am. Fish. Soc.* 112: 800-807.
- Redding, J.M., Schreck, C.B., Birks, E.B. & Ewing, R.D. 1984. Cortisol and its effects on plasma thyroid hormone and electrolyte concentrations in fresh water and during seawater acclimation in yearling coho salmon. *Oncorhynchus kisutch*. *Gen. and Comp Endoc.* 56: 146-155.
- Schreck, C.B. 1990. Physiological, behavioral, and performance indicators of stress. pp. 29-37. *In*: Adams, S.M. (ed.), *Biological Indicators of Stress in Fish*. American Fisheries Society Symposium 8.
- Schreck, C.B. 2000. Accumulation and long-term effects of stress in fish. pp. 147-158. *In*: Moberg, G.P. & Mench, J.A. (eds.), *The biology of animal stress*. CAB International, Wallingford.

- Schreck, C.B., Olla, B.L. & Davis, M.W. 1997. Behavioral responses to stress. pp. 145-170. *In*: Iwama, G.K., Pickering, A.D., Sumpter, J.P. & Schreck, C.B. (eds.), *Fish stress and health in aquaculture*. Society for Experimental Biology Seminar Series: 62. Cambridge University Press, Cambridge.
- Seyle, H. 1950. Stress and the general adaptation syndrome. *Brit. Med. Jour.* 1(4667): 1383-1392.
- Shuter, B.J. 1990. Population-level indicators of stress. pp. 145-166. *In*: Adams, S.M. (ed.), *Biological Indicators of Stress in Fish*. American Fisheries Society Symposium 8.
- Sigismondi, L.A. & Weber, L.J. 1988. Changes in avoidance response time of juvenile chinook salmon exposed to multiple acute handling stresses. *Trans. Am. Fish. Soc.* 117: 196-201.
- Strange, R.J. & Schreck, C.B. 1980. Seawater and confinement alters survival and cortisol concentration in juvenile chinook salmon. *Copeia* 1980: 351-353.
- Wedemeyer, G.A. 1972. Some physiological consequences of handling stress in the juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*). *J. Fish. Res. Bd. Can.* 33: 2699-2702.
- Wedemeyer, G.A., Barton, B.A. & McLeay, D.J. 1990. Stress and acclimation. pp. 451-489. *In*: Schreck, C.B. & Moyle, P.B. (eds.), *Methods for Fish Biology*. American Fisheries Society,.
- Wedemeyer, G.A., Saunders, R.L. & Clarke, W.C. 1980. Environmental factors affecting smoltification and early marine survival of anadromous salmonids. *Mar. Fish. Rev.* 42: 1-14.

Wedemeyer, G.A. & McLeay, D.J. 1981. Methods for determining the tolerance of fishes to environmental stressors. pp. 247-276. *In*: Pickering, A.D. (ed.), *Stress and Fish*. Academic Press Inc., New York.

**EFFECTS OF TROUT, *ONCORHYNCHUS MYKISS*, ON THE  
SALTWATER-ENTRY BEHAVIOR OF JUVENILE CHINOOK  
SALMON, *O. TSHAWYTSCHA***

Carol Seals Price and Carl B. Schreck

## ABSTRACT

Two experiments were conducted to examine the effects of steelhead or rainbow trout, *Oncorhynchus mykiss*, on the saltwater preference of juvenile chinook salmon, *O. tshawytscha*. Experiments were conducted in 757-l tanks in which a stable salinity gradient was established. In the first experiment, steelhead trout had no effect on the saltwater preference of chinook salmon smolts, and more than 75% of chinook salmon chose to remain in the saltwater layer. Steelhead trout were not observed to be highly aggressive, and we conclude that the mere presence of steelhead was not sufficient to decrease saltwater preference of chinook salmon. In the second experiment, chinook salmon stocked with rainbow trout displayed a significantly decreased preference for salt water. Aggression levels in tanks with trout were higher than in tanks with only chinook salmon. Our results suggest that the stress associated with trout aggression can have deleterious effects on the saltwater-entry behavior of juvenile chinook salmon.

## INTRODUCTION

Spring chinook salmon (*Oncorhynchus tshawytscha*) are sympatric with rainbow and steelhead trout (*O. mykiss*) throughout much of their distributional range. In the riverine environment, differences in microhabitat selection may serve to minimize aggressive interactions between juvenile trout and chinook salmon (Everest and Chapman 1972, Mc Michael and Pearsons 1998). However, in the Columbia River basin outmigration times of juvenile steelhead trout and spring chinook salmon overlap (Johnsen et al. 1986, Gessel et al. 1991, Giorgi et al. 1997), and these two species are routinely loaded together onto barges for transport through the river's hydropower system. Little research has investigated any potential effects that such stocking of interspecifics may have on successful ocean entry of chinook salmon. Steelhead trout are highly aggressive, whereas chinook salmon are considered a more passive species (Abbott and Dill 1985, Abbott et al. 1985, Hutchinson and Iwata 1997, Kelsey 1997). Interspecific aggression is known to cause both acute and chronic stress in subordinate salmonids (Ejike and Schreck 1980, Kelsey 1997). It also impacts behaviors such as habitat selection, territoriality and feeding under natural and laboratory conditions (Li and Brocksen 1977, Nakano 1995a,b, Huntingford and De Leaniz 1997, Tinus 2001).

Interactions with steelhead trout may be physiologically stressful to outmigrating chinook salmon. Maule et al. (1988) found that spring chinook salmon barged through the Columbia River hydroelectric dams are physiologically more sensitive to stress and take longer to recover from it than fall chinook salmon. They suggest that this difference is due to the presence of steelhead trout, which are barged with spring, but not fall, chinook salmon. This notion is supported by data collected by Congleton et al. (2000), which shows that cortisol levels of spring chinook salmon transported with steelhead trout are elevated compared to chinook salmon transported only with conspecifics. Additionally, Matthews (1986) reported that steelhead trout negatively influenced the ability of spring chinook salmon to osmoregulate when exposed to saltwater challenge. Finally, Kelsey (1997) observed that steelhead trout had deleterious physiological and behavioral effects on spring chinook salmon in laboratory experiments.

The delayed behavioral ramifications that aggression-related stress might impose are not well understood. The purpose of this study was to determine the effects that aggression-related stress experienced during barging with steelhead trout may have on the performance of chinook salmon during outmigration. Additional aggression may also be occurring as the two species migrate downriver after being released from the barges. Although no definitive studies of overlap in riverine habitat distribution during outmigration have been



conducted, several studies indicate a spatial overlap in the outmigration of steelhead trout and spring chinook salmon. Purse seine catches in the upper Columbia River estuary indicated a midriver orientation with peak catches (and presumably migration) during midday for both species (Ledgerwood et al. 1990). Studies of salmonid movements at dams on the Columbia River also indicate that steelhead trout and chinook salmon are traveling in the same parts of the water column at the same times (Long 1968, Smith 1974). A study conducted in the Columbia River estuary (Bottom and Jones 1990) found an overlap in vertical distribution and in the prey taxa consumed by both chinook salmon and steelhead trout, suggesting the potential for aggressive interactions during competitive feeding events. We were interested in the delayed behavioral ramifications that aggressive interactions might have on chinook salmon when reaching estuarine environments. Specifically, we investigated the effects of *O. mykiss* on the willingness of chinook salmon to enter full-strength salt water under laboratory conditions. We hypothesized that the presence of *O. mykiss* might influence the chinook salmon in two possible ways. First, the presence of the trout might drive chinook salmon out of their preferred environment, in this case, their preferred salinity. Secondly, aggression might induce a physiological stress response in the chinook salmon.

We conducted two experiments to investigate these possibilities. The first was designed to simulate the conditions of fully smolted chinook salmon that

might delay or avoid saltwater entry into as a result of aggression by steelhead trout. We hypothesized that if steelhead trout smolts moved into the saltwater layer of a stratified (fresh over salt water) tank, the chinook salmon would avoid the saltwater layer. The second experiment was designed to simulate the conditions of unsmolted chinook salmon that might avoid salt water for osmoregulatory reasons, yet enter salt water to avoid aggression by a freshwater aggressor. Gill  $\text{Na}^+\text{-K}^+$  ATPase levels (a physiological indicator of smoltification) assessed prior to and during outmigration have revealed that many outmigrating salmon may not be fully smolted (Muir et al. 1994a,b). We used both smolted and unsmolted chinook salmon in our experiments so that the effects of aggression on fish of both physiological states could be assessed. Our intentions for these experiments were to simulate aggression levels that chinook salmon might experience during barging or possibly during outmigration. Finally, we needed both freshwater and saltwater-preferring aggressors. Because steelhead trout are barged with chinook salmon they were the logical choice for the first experiment. We chose rainbow trout for the second experiment because 1) they would be unlikely to enter salt water, and 2) their phylogenetic similarity to steelhead trout would provide some morphological and behavioral uniformity between the two aggressors. Although it is uncertain that rainbow trout actually pose a threat to chinook salmon in the estuary, we chose them to serve as a model for aggressive species that outmigrating salmon might encounter.

## METHODS

### Experimental Fish

Spring chinook salmon used in these experiments were reared at Oregon State University's Fish Performance and Genetics Laboratory (FPGL) at Smith Farm, Corvallis, OR. Brood stock from which these fish originated was obtained from Oregon's South Santiam Fish Hatchery. Several months prior to experiments, fish were held indoors in 2-m, circular tanks and fed to satiation twice per day until needed for experiments. In the steelhead trout experiments, yearling chinook salmon measured  $18.0 \pm 1.8$  cm and weighed  $67.7 \pm 20.1$  g. In the rainbow trout experiments, subyearling chinook salmon measured  $11.2 \pm 12.6$  cm and weighed  $16.0 \pm 5.2$  g. Steelhead trout chosen for these experiments were similar in size to those that would be found outmigrating with chinook salmon. Steelhead trout ( $23.4 \pm 1.8$  cm and  $122.0 \pm 20.0$  g) were obtained from Oregon's Fall Creek hatchery one month prior to experimental use and were held at the FPGL. Rainbow trout ( $14.7 \pm 1.1$  cm and  $36.9 \pm 9.9$  g) were obtained from the Salmon Disease Laboratory, Corvallis, OR two weeks prior to experimental use, and held at Oregon State University's Oak Creek Laboratory (OCL), Corvallis,

OR, where the experiments took place. Fish were transported for 15 minutes from the FPGL to the OCL in 130-l plastic trashcans supplied with oxygen.

### **Saltwater Preference Tanks**

Saltwater preference experiments were carried out in four rectangular tanks (1.83 x 0.66 x 0.60 m) with Plexiglas® front windows and water volume of 757 l. The tanks were supplied with airstones, flow-through water supply (3 l/min) and mesh covers. Each was enclosed behind a black plastic curtain with holes cut out to allow for visual observation and videotaping. Fluorescent lights were suspended above each tank, and light was diffused by fiberglass netting on frames suspended below the bulbs. During experiments saltwater was introduced into the bottom of each saltwater preference tank from one of two header tanks located outside of the plastic curtain. Header tanks were 1-m, circular tanks and were elevated approximately 1 m above the bottom of the experimental tanks. Each header was connected independently to two of the preference tanks by PVC pipes running along the bottom of the experimental tanks. These pipes had small holes drilled every 5 cm in two lines, which allowed salt water to be slowly introduced.

In all experiments, fish were randomly placed into each preference tank and held in aerated, flowing water for 48 hours. Preliminary work found that after 48 hours fish began normal feeding, so we considered them to be acclimated after this time. The photoperiod was set to the ambient light cycle. Fish were not fed during acclimation or experiments to reduce disturbance to the tank. After this acclimation period, 378.5 l of 30 ppt salt water was mixed in each header tank using Instant Ocean® artificial salt, and was aerated. Videotaping began 20 minutes before saltwater introduction. Water supply and aeration were turned off to prevent mixing 10 minutes prior to saltwater introduction. The valves from the header tanks were then opened and salt water began to flow into the bottom of the preference tanks, pushing the lighter freshwater out of the top-draining standpipe. A stable saltwater layer was created in the bottom of the water column, with a freshwater layer on top. The halocline was visible, so it was easy to distinguish whether fish were in salt or fresh water. Every 10 minutes the number of fish in freshwater or salt water was recorded. Some fish would not hold in one layer or the other, but instead, moved between the two layers. These were tallied separately from the other two groups. It took about one hour for all the saltwater to drain into the tanks. At this time, the bottom half (30 cm deep) of the water column was saline and the top half was fresh. Observations were continued for one hour after saltwater introduction. Previous

trials with dyed salt water showed that the halocline was stable for at least 24 hours.

On each experimental day, fish in four tanks could be observed. However, only two could be run simultaneously. Therefore, two consecutive sets of experiments were done per day, each consisting of one control (tank without steelhead or rainbow trout) and one treatment (tank with steelhead or rainbow trout). On each day, only the first set of tanks was videotaped.

### **Steelhead Trout Experiment**

This experiment was conducted to determine whether aggression by steelhead trout had any affect upon the saltwater-entry behavior of juvenile chinook salmon. Chinook salmon used in this experiment had undergone morphological changes associated with smoltification: complete loss of parr marks, silvering and darkening of fin margins. Hatchery cohorts are historically released from hatcheries at this time of year. We hypothesized that chinook salmon would avoid the more aggressive steelhead trout, and that if the trout held in the saltwater layer, the salmon would remain in freshwater.

Experiments were conducted on 30 April and 03, 09 and 17 May 1999 at the OCL. To preacclimate the steelhead trout to salt water, six trout were

transported to the laboratory four days prior to the experiment, placed in a static, 80-l, aerated tank, and the salinity in the tank was adjusted to 10 ppt by the addition of Instant Ocean® artificial salt. The following day, the salinity was raised to 20 ppt. The next day, chinook salmon were transported to the lab and immediately placed in the four preference tanks and three preacclimated steelhead were placed into each of two tanks. The other two preference tanks served as "no steelhead" controls. In order to observe any effects of increased density, the following four stocking densities and ratios were used on each experimental day: 13 chinook + 3 steelhead, 13 chinook + no steelhead, 10 chinook + 3 steelhead, and 16 chinook + no steelhead. A blocked design was used to assign density to the tanks. After a 48 hour acclimation, saltwater was introduced and fish behavior was recorded as described.

### **Rainbow Trout Experiment**

This experiment was conducted to determine whether aggression by rainbow trout had any effect upon the saltwater-entry behavior of juvenile chinook. Chinook salmon used in this experiment were believed to be unsmolted parr (based upon morphological features and age), and therefore unlikely to enter or hold in salt water. Rainbow trout and chinook salmon used

in this experiment were held at the Oak Creek Laboratory and moved from holding to experimental tanks as needed. 50 rainbow trout were held in an 80-l circular tank with flow-through water and airstones. 250 chinook salmon were held in a rectangular (1.2 x 1.2 x 0.6 m) tank with flow-through water and airstones. Fish were fed daily to satiation. Experiments were conducted on 24, 27 and 30 July 1999 at the Oak Creek Laboratory. Stocking densities and ratios were the same as those used in the steelhead experiment. Fish were acclimated for 48 hours after being stocked into the saltwater preference tanks. Salt water was introduced and fish behavior was observed.

### **Videotape Recording Of Aggression In Saltwater Preference Experiments**

To assess the levels of aggression that fish experienced, the first 10 minutes of each videotape were watched. During this period, conditions in the tank were as they had been during the 48-hour acclimation. An aggressive act was defined as any act by an individual directed at another specific individual that elicited fleeing, and included charges, nips and chases. The total number of aggressive acts was tallied and the species of the attacker and the attacked was noted. For the steelhead experiment four control and four steelhead trout tanks



were videotaped. For the trout experiment three control and three rainbow trout tanks were taped.

### **Effects of Rainbow Trout on Stress Physiology of Juvenile Chinook Salmon**

This experiment was conducted to determine if the presence of rainbow trout caused a stress response in the chinook salmon that would be physiologically evident after 48 hours (to simulate the stress level of fish used in experiments). On 10 and 16 August, two of the preference tanks were each stocked with 10 chinook, and each day one tank also received three trout. After a 48-hour acclimation, the fish were quickly (30 - 45 sec) netted out of the tanks and killed by overdose of tricaine methanesulfonate (200 mg/l). Additionally, 10 fish were sampled from the holding tank on each date. Blood was collected in heparinized capillary tubes after severing the caudal peduncle, centrifuged and the plasma was collected. Plasma cortisol levels were analyzed as an indicator of stress using a radioimmunoassay (Foster and Dunn, 1974 as modified by Redding et al., 1984).

## Statistical Analysis

Each tank was considered an independent replicate. For each tank, the percentages of fish holding in salt and fresh water or moving between the layers at 60 and 120 minutes were calculated. These data were arcsine transformed to make them continuous. Multifactor ANOVA ( $P < 0.05$ ) was used to determine any treatment, tank, density or date effects. In both experiments, the only significant factor was treatment; therefore, the six replicates for each of the two treatments were pooled for one-way ANOVA. If data were skewed, the non-parametric Kruskal-Wallis comparison of medians was performed to determine treatment effect. For pairwise comparison of cortisol data, a *t*-test was used.

## RESULTS

### Steelhead Trout Experiment

During acclimation, chinook salmon and steelhead trout swam throughout the water column and did not form tight groups. Repeated observations during this acclimation period were done to monitor whether the

two species were segregated, but this was not the case. Steelhead and chinook mingled both horizontally and vertically, and no observable territories (discrete areas of the tank defended by individuals against other fish) were established by either species. Fish were not observed resting directly on the tank bottom and stationary fish tended to position themselves 5 - 20 cm off the bottom. Fish were dispersed throughout the tank and actively explored all areas of the tank, including the surface. When salt water was first introduced and perceived, both species usually spent 10 to 20 minutes moving between it and the freshwater. In many of the tanks, fish immediately entered and remained in salt water. This often meant holding in a layer only 5-7 cm from the bottom. Once fish held in salt water, they typically did not return to fresh water for the duration of the experiment. As the saltwater layer deepened, fish holding there moved throughout the layer. Most fish that avoided salt water and held in fresh water, had explored the saltwater layer multiple times. Fish holding in both salt and fresh water were frequently observed swimming up or down to the halocline, but not across it. Often, fish that were undecided, would position themselves directly on the halocline as the saltwater layer moved up the water column. In all experiments, the steelhead trout held in the saltwater layer.

Overall, steelhead trout had no effect on the saltwater preference of chinook salmon (Table 3.1). Control chinook salmon and those in tanks with

**Table 3.1.** Saltwater preference data for juvenile spring chinook held with and without steelhead trout on four dates in 1999. There was no significant difference in the percentage of chinook holding in salt water between the two treatments at 60 (Kruskal-Wallis,  $P = 0.5378$ ) or 120 (ANOVA,  $P = 0.5433$ ) minutes after saltwater introduction. There was no effect of density on saltwater preference at 60 or 120 minutes (ANOVA,  $P = 0.7960$  and  $0.8119$ , respectively).

TREATMENT	DATE	DENSITY Chinook + Steelhead	% FISH HOLDING IN SW AFTER:		MINUTES UNTIL > 50% FISH HOLD IN SW	
			60 MIN	120 MIN		
STEELHEAD	30 APR	13 +3	100	100	10	
		10+3	100	100	10	
	03 MAY	10+3	10	50	70	
		13+3	100	100	10	
	09 MAY	13 +3	38	38	>120	
		10+3	100	10	>120	
	17 MAY	10+3	100	100	10	
		13+3	100	100	10	
	CONTROL	30 APR	13+0	100	100	10
			16+0	100	100	10
03 MAY		16+0	25	56	110	
		13+0	100	100	10	
09 MAY		13+0	100	31	10	
		16+0	100	100	10	
17 MAY		16+0	100	100	10	
		13+0	100	100	10	
MEAN ± SD		SH	81 ± 36	75 ± 37	45 ± 52	
		CONTROL	91 ± 27	86 ± 25	23 ± 33	

steelhead trout showed a high degree of saltwater preference. In 6 of the 8 steelhead tanks and in 7 of the 8 control tanks, 100% of the chinook were holding in the saltwater layer after 1 hour (Kruskal-Wallis,  $P = 0.5378$ ). Two hours after saltwater introduction,  $75 \pm 37\%$  of chinook salmon with steelhead trout and  $86 \pm 25\%$  of control chinook salmon were holding in salt water (ANOVA,  $P = 0.5433$ ). When the percentages of fish holding in salt water or moving between the two layers were combined,  $97 \pm 8\%$  of control chinook salmon and  $94 \pm 11\%$  of chinook salmon with steelhead trout were holding in salt water after 2 hours (Kruskal-Wallis,  $P = 0.3002$ ). Additionally, the time that it took for more than half of the fish to make the decision to stay in salt water was similar in both treatments (Kruskal-Wallis,  $P = 0.2403$ ; Table 3.1). ANOVA indicated that there was no statistically significant effect of density on saltwater preference at 60 or 120 minutes ( $P = 0.7960$  and  $P = 0.8119$ ). in the saltwater layer after 1 hour (Kruskal-Wallis,  $P = 0.5378$ ). Two hours after saltwater introduction,  $75 \pm 37\%$  of chinook salmon with steelhead trout and  $86 \pm 25\%$  of control chinook salmon were holding in salt water (ANOVA,  $P = 0.5433$ ). When the percentages of fish holding in salt water or moving between the two layers were combined,  $97 \pm 8\%$  of control chinook salmon and  $94 \pm 11\%$  of chinook salmon with steelhead trout were holding in salt water after 2 hours (Kruskal-Wallis,  $P = 0.3002$ ). Additionally, the time that it took for more than half of the fish to make the decision to stay in salt water was similar in both treatments (Kruskal-Wallis,  $P =$

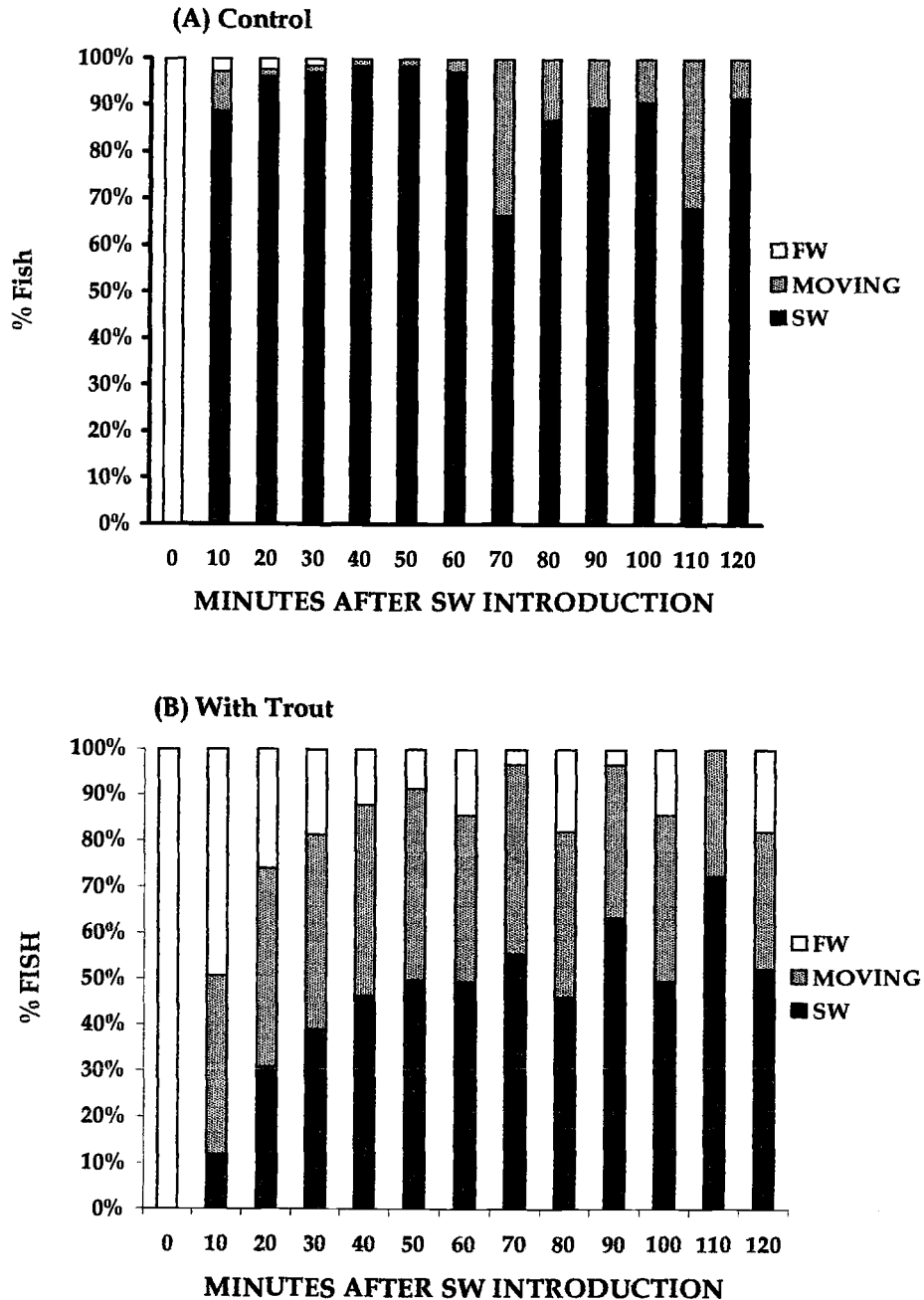
0.2403; Table 3.1). ANOVA indicated that there was no statistically significant effect of density on saltwater preference at 60 or 120 minutes ( $P = 0.7960$  and  $P = 0.8119$ ).

### **Rainbow Trout Experiment**

During acclimation, fish behavior was similar to that in the steelhead trout experiment. Fish were dispersed throughout the tank, and trout and salmon mingled. However, once salt water was introduced, chinook salmon in tanks with trout behaved differently than controls. In the control tanks,  $99 \pm 3\%$  of salmon were holding in salt water after 60 minutes (Table 3.2, Figure 3.1). In contrast, only  $47 \pm 21\%$  of the salmon with trout held in salt water after 60 minutes (Kruskal-Wallis,  $P = 0.0027$ ). Without trout, more than half of chinook held in salt water within 10 minutes. In four of the tanks with trout, it took  $55 \pm 44$  minutes for half the chinook salmon to hold in salt water, and in two of the tanks with trout 50% saltwater preference was not exhibited by chinook even after 2 hours (Kruskal-Wallis,  $P = 0.0073$ ). The trend in decreased saltwater preference by chinook salmon with trout was still evident after two hours, but we found no statistical difference. In control tanks,  $80 \pm 30\%$  of chinook salmon

**Table 3.2.** Saltwater preference data for chinook salmon held with or without rainbow trout on three dates in 1999. The percentage of salmon holding in salt water was significantly lower in tanks with trout 60 minutes after saltwater introduction (Kruskal-Wallis,  $P = 0.0027$ ). While this trend persisted to 120 minutes, the difference was not significant (ANOVA,  $P = 0.1971$ ). Similarly, the combined percentages of chinook holding in or exploring salt water were higher and more consistent for fish held with only conspecifics at 60 and 120 minutes (Kruskal-Wallis,  $P = 0.5853$  and  $0.5853$ , respectively).

TREATMENT	DATE	DENSITY Chinook+ Trout	% FISH HOLDING IN SW AFTER		% FISH HOLDING OR EXPLORING SW AFTER		MINUTES UNTIL >50% FISH HOLD IN SW
			60 MIN	120 MIN	60 MIN	120 MIN	
TROUT	24 JULY	13+3	77	100	100	100	30
		10+3	30	30	60	60	>120
	27 JULY	10+3	30	60	90	90	110
		13+3	69	77	100	100	10
	30 JULY	13+3	33	50	100	100	70
		10+3	40	40	80	70	>120
CONTROL	24 JULY	13+0	100	23	100	100	10
		16+0	100	75	100	100	10
	27 JULY	16+0	100	100	100	100	10
		13+0	92	100	100	100	10
	30 JULY	13+0	100	82	100	100	10
		16+0	100	100	100	100	10
	MEAN	TROUT	47±21	60±26	88±16	87±18	10±0
	±SD	CONTROL	99±3	80±30	100±0	100±0	>55±44



**Figure 3.1** Mean percentage of chinook salmon held with or without trout holding in fresh and salt water, or moving between the layers in aggression experiment. For both charts, bars at each time represent pooled data from six tanks per treatment from 24 - 30 July 1999. Time = 0 represents the beginning of saltwater introduction. Chinook in tanks with trout had a decreased preference for salt water (Kruskal-Wallis,  $P = 0.0027$ ).



were holding in salt water, compared to only  $60 \pm 26\%$  in tanks with trout (ANOVA,  $P = 0.1971$ ). The statistical insignificance of this difference is explained by the low percentage (23%) of fish holding in salt water in only one control tank. Interestingly, all the fish in this tank had immediately entered salt water and remained there for the first hour as salt water was introduced. Thereafter, the fish in that tank unaccountably started moving back and forth between the two layers. This behavioral change was also observed in other control tanks, but saltwater preference tended to remain high for the duration of the experiment in all but one tank. If this tank is removed from the analysis, chinook salmon saltwater preference was higher ( $91 \pm 12\%$ ) in control tanks (ANOVA,  $P = 0.0483$ ).

Although chinook salmon with trout were less likely to hold in salt water, they did not completely avoid it. Rather, 25 - 40% of the fish were constantly moving between the fresh and saltwater layers. This was the most prominent behavioral difference between the two treatments. In control tanks, fish moved back and forth only briefly, before generally settling into the salt water (Figure 3.1). A few times trout were observed briefly moving into salt water, but they only remained there for several seconds before moving back into freshwater. Most trout avoided the saltwater layer completely.

## **Aggression Data From Videotapes**

Statistical analysis was not performed on data collected from videotapes due to the small number of replicates for each treatment (four taped steelhead experiments and three taped trout experiments).

### **Steelhead Experiment**

There was a relatively low level of aggression observed in this experiment. In tanks with steelhead, 0 - 6 aggressive acts were recorded per tank, while in the control tanks 0 - 5 acts were recorded (Table 3.3). A total of 26 aggressive acts was noted from all 8 tapes. Interestingly, only one was initiated by a steelhead and it was toward a chinook salmon. All other 25 acts of aggression were perpetrated by salmon, 24 of which were directed toward another salmon.

**Table 3.3.** The number of aggressive acts in tanks with chinook salmon and steelhead trout, or chinook only on four dates in 1999. Ch = chinook salmon; SH = steelhead trout.

TREATMENT	DATE	TOTAL # AGGRESSIVE ACTS	ATTACKER : ATTACKED		
			Ch:Ch	Ch:SH	SH:Ch
STEELHEAD	28 APR	5	4	0	1
	02 MAY	6	5	1	0
	09 MAY	0	0	0	0
	17 MAY	6	6	0	0
CONTROL	28 APR	1	1	-	-
	02 MAY	0	0	-	-
	09 MAY	5	5	-	-
	17 MAY	3	3	-	-
	Total	26	24	1	1

### Trout Experiment

Tanks with rainbow trout had higher levels of aggression (Table 3.4) than tanks with only chinook salmon. There were 5 - 10 aggressive acts in each of the control tanks, while trout tanks showed 14 -18 acts each. In the 3 control tanks there was a total of 21 aggressive acts. Tanks with trout had more than twice the number of aggressive acts with a combined total of 46. In two of the trout tanks, the aggression was primarily from trout (11 of 14, and 12 of 14 acts). In both cases, one seemingly dominant trout was responsible for most of the aggression which was largely directed toward chinook salmon. In the remaining trout tank, salmon were responsible for most of the aggression (13 of 18 acts). This was due mostly to one salmon and was primarily directed toward other salmon. Overall, chinook salmon were the recipients of 57 of the 67 total aggressive acts in the 6 videos analyzed.

### **Stress Physiology**

Because there was no effect of date (Multifactor ANOVA;  $P = 0.9645$ ), the cortisol values for each treatment were pooled. Chinook salmon cortisol levels in the control tanks averaged  $20.9 \pm 40.29$  ng/ml, and were similar to those of fish

**Table 3.4.** The number of aggressive acts in tanks with chinook salmon and rainbow trout, or chinook salmon only on three dates in 1999. Ch = chinook salmon; T = rainbow trout; U = unknown.

TREATMENT	DATE	TOTAL # AGGRESSIVE ACTS	ATTACKER : ATTACKED					
			Ch:Ch	T:Ch	U:Ch	T:T	Ch:T	Ch:U
TROUT	24 JUL	14	1	10	1	1	0	1
	27 JUL	18	9	4	0	1	4	0
	30 JUL	14	2	10	0	2	0	0
CONTROL	24 JUL	10	10	-	-	-	-	-
	27 JUL	6	6	-	-	-	-	-
	30 JUL	5	5	-	-	-	-	-
Total		67	33	24	1	4	4	1

## DISCUSSION

The results of these experiments provide insight into the role that aggression toward chinook salmon might play in determining their salinity preference. While the presence of rainbow trout decreased the willingness of chinook salmon holding to hold in salt water, the presence of steelhead trout did not. We believe that this difference is accounted for by the higher levels of aggression observed in tanks with rainbow trout. Contrary to our expectations, the steelhead trout showed very little aggressive behavior. This may be explained by several factors. First, the preacclimation of the steelhead trout and subsequent return to freshwater for 48 hours might have been a stressful experience, thus decreasing aggressive behavior. This explanation seems unlikely because their behavior during visual observations was consistent with that of acclimated fish. Distressed fish (for example, when fish were first stocked into the tanks after netting and transport) tended to huddle in corners and at the bottom of the tank, they swam very little or not at all, and their ventilation rates were increased. Steelhead trout used in this experiment displayed none of these behaviors after the 48-hour acclimation.

Instead, the physiological state of the steelhead trout should be considered. These fish showed morphological signs of smoltification and their hatchery cohorts were released at the time these experiments took place. It is

therefore likely that the steelhead trout were at an advanced level of smoltification. One of the most prominent physiological characteristics of smolts is an increase in thyroxine levels (Hoar, 1988; Boeuf, 1994; Iwata, 1995). In addition to metabolic functions, this hormone has been also been implicated in behavioral changes associated with smolting (Folmar and Dickhoff, 1980). Relevant to this study, elevated thyroxine levels are associated with a decrease in aggressive behavior and an increase in shoaling or schooling behavior in steelhead and other anadromous salmonids (Hutchinson and Iwata, 1998). This is thought to be an adaptive mechanism as fish go from the territorial, riverine stage of their life history to the more gregarious, oceanic stage. It is most likely that the steelhead trout used in this experiment showed decreased aggression because of their natural physiological state. If this is the case, then our results imply that the mere presence of steelhead trout is not sufficient to cause a behavioral change in chinook salmon encountering salt water.

Contrary to our original hypothesis, the control chinook salmon in the rainbow trout experiment did not avoid salt water. Although chinook salmon used in this experiment appeared physically to be unsmolted, both control chinook salmon and those stocked with rainbow trout consistently exhibited considerable preference for, or orientation toward, high salinity. Though this preference for salinity was unexpected, similar observations have been documented by other researchers. In yearround saltwater challenge tests

conducted on hatchery (Cole Rivers Hatchery on the Rogue River, OR) chinook salmon, Ewing and Birks (1982) found that all fish greater than 9 cm survived for up to 72 hours, regardless of time of year. They concluded that for chinook salmon, saltwater tolerance was uncoupled from the cyclic parr-smolt transformation and was size dependent. Birtwell and Kruzynski (1989) observed that subyearling, freshwater-adapted chinook salmon (Fraser River, British Columbia) readily moved between waters ranging from 0.3 to 26 ppt in a vertically stratified laboratory tank from April through June. In their experiment, as hypoxic conditions were slowly established in the upper freshwater layer, the chinook salmon distribution shifted downward into the salty layer in response. As in our experiment, the authors of both studies noted that the high tolerance of subyearling chinook salmon for salt water was unanticipated, but highly reproducible.

Behavioral changes were quite evident for chinook salmon held with rainbow trout, and aggression levels were highest in tanks with trout. Although chinook salmon held only with conspecifics were also aggressive, the nature of the interactions was qualitatively different. Aggression by rainbow trout included obvious charges, nips and extended chases. Salmon aggression was often very subtle and rarely involved chasing. Salmon attacked by trout usually moved several body lengths away, but chinook attacked by conspecifics normally moved less than a body length. These qualitative differences in



aggressive behavior are in agreement with those observed by Kelsey (1997). The aggression by steelhead trout in her experiment was also more intense than aggression by chinook salmon. She observed that aggression between salmon seemed to be associated primarily with inter-fish distance, whereas trout aggression was motivated by territoriality and dominance displays. This pattern also characterized the chinook salmon and trout interactions in our study.

The physiological mechanism responsible for the decrease in saltwater preference by chinook salmon held with rainbow trout is uncertain. We hypothesized that trout aggression would be a stressor, and that plasma cortisol levels would be elevated as a result. In our experiment, the chinook salmon stocked with either conspecifics or trout had cortisol values that were consistent with smoltification (30 - 50 ng/ml), but not with stress (> 100 ng/ml). Subordinate salmon have been shown to have higher cortisol levels than dominant fish (Ejike and Schreck, 1980). When Kelsey (1997) monitored plasma cortisol levels of chinook salmon in response to the addition of steelhead trout to tanks, values peaked around two hours and remained elevated for up to 34 hours. The lower number of trout used in our experiments compared to Kelsey's (she used 10 of each species), may account for the differences in physiological response. Some of Kelsey's salmon returned to normal cortisol levels after 8 hours, and she noted that aggression in these tanks was less than in those with longer recovery times. It is probable that although our fish recovered

physiologically from any stress imposed by trout within 48 hours, the decrease in salinity preference reflected a delayed stress effect.

The most apparent behavioral difference between the two treatments was that chinook salmon held with rainbow trout spent much more time moving between the fresh and saltwater layers. It is important to make the distinction that fish not holding in salt water did not necessarily avoid it. This behavior indicates that the orientation toward salt water, which is characteristic of smoltification, is retained. In another experiment, chinook salmon that had been mildly stressed exhibited a similar behavior pattern (Chapter 2). A mild chasing stressor decreased the percentage of fish holding in salt water, but many stressed fish moved continuously between fresh and salt water. This response is less drastic than that of severely stressed or diseased fish that avoid salt water altogether (Chapters 1 and 2).

Studies of aggression in salmonids have primarily focused on short and long-term consequences in riverine ecosystems (Li and Brocksen 1977; Nakano 1995a,b; Huntingford and De Leaniz 1997; Yamomato et al. 1998), but the immediate or delayed affects of aggression for outmigrating salmonids are largely unknown. Researchers have investigated physiological impacts that steelhead might impose upon chinook, especially with regards to barging practices on the Columbia River. At Lower Granite Dam on the Snake River, Matthews et al. (1986) found that mortality during a 48-hour saltwater challenge

was doubled for juvenile chinook held in raceways with steelhead at the collection facility (16.3%), compared to chinook held only with conspecifics (8.2%). He noted that steelhead trout were often observed initiating aggressive behavior toward chinook in the holding raceways at the dam. They concluded that the stress of steelhead trout aggression added to the osmoregulatory challenge of adjustment to full strength seawater was responsible for increased mortality. Congleton et al. (2000) monitored physiological stress indices of juvenile chinook salmon that had been barged from Lower Granite Dam to their release site downstream of Bonneville Dam (the last dam on the Columbia River). Plasma cortisol and glucose levels were positively correlated with steelhead trout loading density throughout the trip and indicated that chinook salmon were more physiologically stressed when transported with steelhead trout. Similarly, Kelsey (1997) reported increased cortisol levels for up to 34 hours in juvenile chinook salmon held in tanks to which steelhead trout were added at densities mimicking those found in transport barges.

The steelhead in our experiments were not very aggressive and did not affect saltwater preference of juvenile chinook salmon. We were surprised by the unaggressive nature of the steelhead trout in our experiments considering the previously cited papers. This behavioral difference may be explained by high loading density at holding facilities and on barges, which possibly increases aggressive tendencies in steelhead trout. Additionally, fish collected at dams in

the upper Columbia River may not yet be fully smolted, and thus the thyroxine-mediated decrease in aggression may not be fully achieved.

However, the results of experiments with rainbow trout provide a good model for the aggression that outmigrating chinook salmon might experience at holding facilities, in barges, or downriver. Successful adjustment to the marine environment is essential to the early survival of salmon migrating through the estuarine environment (Wedemeyer et al., 1980). Chinook salmon that are unable or unwilling to enter salt water may travel in the shallow, upper freshwater lens, which is characteristic of the Columbia River estuary (Jay and Smith, 1990; Carl Schreck, unpublished data). Such behavior may make them more susceptible to avian predators that feed at or near the surface. The negative behavioral consequences of interspecific aggression for chinook salmon may be an important consideration in future management strategies for juvenile chinook salmon, especially in the Columbia River.

## ACKNOWLEDGEMENTS

We would like to thank Rob Chitwood for his outstanding husbandry and assistance at the Smith Farm Research Hatchery, and Don Stevens and Harriet Lorz for providing the trout used in these experiments. The statistical advice of Drs. Cliff Pereira and Fred Ramsey was greatly appreciated. Special thanks goes to Miranda Dodd for conducting the cortisol assays. This research was funded by the U.S. Army Corps of Engineers.

## LITERATURE CITED

- Abbott, J.C. & Dill, L.M. 1985. Patterns of aggressive behavior in juvenile steelhead trout (*Salmo gairdneri*). *Can. J. Fish. Aquat. Sci.* 42: 1702-1706.
- Abbott, J.C., Dunbrack, R.L. & Orr, C.D. 1985. The interaction of size and experience in dominance relationships of juvenile steelhead trout (*Salmo gairdneri*). *Behaviour* 92: 241-253.
- Birtwell, I.K. & Kruzynski, G.M. 1989. *In situ* and laboratory studies on the behavior and survival of Pacific salmon (genus *Oncorhynchus*). *Hydrobiologia* 188/189: 543-560.
- Boeuf, G. 1994. Salmonid smolting: a preadaptation to the oceanic environment. pp. 105-136. *In*: G.C. & Jenson, G.B. (eds.), *Fish Ecophysiology*. Chapman and Hall Publishers.
- Bottom, D.L. & Jones, K.K. 1990. Species composition, distribution, and invertebrate prey of fish assemblages in the Columbia River estuary. *Prog. Ocean.* 25: 243-270.
- Congleton, J., Schreck, C.B. & Davis, L. 2000. Stress indices in migrating juvenile chinook salmon and steelhead of wild and hatchery origin before and after barge transportation. *Trans. Amer. Fish. Soc.* 129: 946-961.
- Ejike, C. & Schreck, C.B. 1980. Stress and social hierarchy rank in coho salmon. *Trans. Amer. Fish. Soc.* 109: 423-426.
- Everest, F.H. & Chapman, D.W. 1972. Habitat selection and spatial interaction by juvenile chinook salmon and steelhead trout in two Idaho streams. *J. Fish. Res. Bd. Can.* 29: 91-100.

- Ewing, R.D. & Birks, E.K. 1982. Criteria for parr-smolt transformation in juvenile chinook salmon (*Oncorhynchus tshawytscha*). *Aquaculture* 28: 185-194.
- Folmar, L.C. & Dickhoff, W.W. 1980. The parr-smolt transformation (smoltification) and seawater adaptation in salmonids: a review of selected literature. *Aquaculture* 21: 1-37.
- Foster, L.C. & Dunn, R.T. 1974. Single-antibody technique for radioimmunoassay of cortisol in extracted serum or plasma. *Clin. Chem.* 20: 365-368.
- Gessel, M.H., Williams, J.G., Brege, D.A., Krcma, R. F. & Chambers, D.R. 1991. Juvenile salmonid guidance at the Bonneville Dam second powerhouse, Columbia River, 1983-1989. *N. Amer. J. Fish. Man.* 11: 400-412.
- Giorgi, A.E., Hillman, T.W., Stevenson, J.R., Hays, S.G. & Peven, C.M. 1997. Factors that influence the downstream migration rates of juvenile salmon and steelhead through the hydroelectric system in the mid-Columbia River basin. *N. Amer. J. Fish. Man.* 17: 268-282.
- Hoar, W.S. 1988. The physiology of smolting salmonids. pp. 275-343. *In*: Hoar, W.S. & Randall, D.J. (eds.), *Fish Physiology, Volume XIB*. Academic Press, San Diego, California.
- Huntingford, F.A. & De Leaniz, C.G. 1997. Social dominance, prior residence and the acquisition of profitable feeding sites in juvenile Atlantic salmon. *J. Fish Biol.* 51: 1009-1014.
- Hutchinson, M.J. & Iwata, M. 1997. A comparative analysis of aggression in migratory and non-migratory salmonids. *Env. Biol. Fish.* 50: 209-215.

- Hutchinson, M.J. & Iwata, M. 1998. Effect of thyroxine on the decrease of aggressive behavior of four salmonids during the parr-smolt transformation. *Aquaculture* 168: 169-175.
- Iwata, M. 1995. Downstream migratory behavior of salmonids and its relationship with cortisol and thyroid hormones: a review. *Aquaculture* 135: 131-139.
- Jay, D.A. & Smith, J.D. 1990. Circulation, density distribution and neap-spring transitions in the Columbia River estuary. *Prog. Ocean.* 25: 81-112.
- Johnsen, R.C., Wood, L.A. & Smith, W.W. 1986. Monitoring of downstream salmon and steelhead at federal hydroelectric facilities - 1986. Annual report. Bonneville Power Administration, Portland, OR.
- Kelsey, D.A. 1997. Effects of steelhead trout (*Oncorhynchus mykiss*) on chinook salmon (*O. tshawytscha*) behavior and physiology. Master's thesis. Oregon State University, Corvallis, OR.
- Ledgerwood, R.D., Thrower, F.P. & Dawley, E.M. 1990. Diel sampling of migratory juvenile salmonids in the Columbia River estuary. *Fish. Bull.* 89:69-78.
- Li, H.W. & Brocksen, R.W. 1997. Approaches to the analysis of energetic costs of intraspecific competition for space by rainbow trout (*Salmo gairdneri*). *J. Fish Biol.* 11: 329- 341.
- Long, C. 1968. Diel movements and vertical distribution of juvenile anadromous fishes in turbine intakes. *Fish. Bull.* 66: 599-609.
- Matthews, G.M., Park, D.L., Achord, S. & Ruehle, T.E. 1986. Static seawater challenge test to measure relative stress levels in spring chinook salmon smolts. *Trans. Amer. Fish. Soc.* 115: 236-244.



- Maule, A.G., Schreck, C.B., Bradford, C.S. & Barton, B.A. 1988. Physiological effects of collecting and transporting juvenile chinook salmon past dams on the Columbia River. *Trans. Amer. Fish. Soc.* 117: 245-261.
- McMichael, G.A. & Pearsons, T.N. 1998. Effects of juvenile spring chinook salmon on growth and abundance of wild rainbow trout. *Trans. Amer. Fish. Soc.* 127: 261-274.
- Muir, W.D., Giorgi, A.E. & Coley, T.C. 1994a. Behavioral and physiological changes in yearling chinook salmon during hatchery residence and downstream migration. *Aquaculture* 127: 69-82.
- Muir, W.D., Zaugg, W.S., Giorgi, A.E. & McCutcheon, S. 1994b. Accelerating smolt development and downstream movement in yearling chinook salmon with advanced photoperiod and increased temperature. *Aquaculture* 123: 387-399.
- Nakano, S. 1995a. Competitive interactions for foraging microhabitats in a size-structured interspecific dominance hierarchy of two sympatric stream salmonids in a natural habitat. *Can. J. Zool.* 73: 1845-1854.
- Nakano, S. 1995b. Individual differences in resource use, growth and migration under the influence of a dominance hierarchy in fluvial red-spotted masu salmon in a natural habitat. *J. Anim. Ecol.* 64: 75-84.
- Redding, J.M., Schreck, C.B., Birks, E.B. & Ewing, R.D. 1984. Cortisol and its effects on plasma thyroid hormone and electrolyte concentrations in fresh water and during seawater acclimation in yearling coho salmon. *Oncorhynchus kisutch*. *Gen. Comp. Endocrin.* 56: 146-155.
- Smith, J.R. 1974. Distribution of seaward migrating chinook salmon and steelhead trout in the Snake River above Lower Monumental dam. *Mar. Fish. Rev.* 36: 42-45.

- Tinus, C.A & Reeves, G.H. 2001. Redside shiner (*Richardsonius balteatus*) shoals provide a behavioral competitive refuge for subordinate juvenile steelhead trout (*Oncorhynchus mykiss*). *Can. J. Fish. Aquat. Sci.* 58: 319-324.
- Wedemeyer, G.A., Saunders, R.L. & Clarke, W.C. 1980. Environmental factors affecting smoltification and early marine survival of anadromous salmonids. *Mar. Fish. Rev.* 42: 1-14.
- Yamamoto, T., Ueda, H. & Higashi, S. 1998. Correlation among dominance status, metabolic rate and otolith size in masu salmon. *J. Fish Biol.* 52: 281-290.

**PREFERENCE FOR SALT WATER ALTERS THE  
STARTLE RESPONSE OF JUVENILE CHINOOK SALMON  
(*ONCORHYNCHUS TSHAWYTSCHA*)**

Carol Seals Price and Carl B. Schreck

**ABSTRACT**

A variety of factors may interact to determine the behavior of fish within environmental gradients. Often, tradeoffs are made to maximize homeostasis, reproduction or survival. The startle response of fish is an important predator evasion behavior, and the degree to which it is exhibited may be influenced by the animal's intrinsic and extrinsic conditions. We conducted an experiment to determine the orientation of the startle response of juvenile chinook salmon within a vertical salinity gradient. Specifically, we wished to determine whether fish that had chosen to hold in salt water would move into freshwater when startled within the preferred water layer. For comparison, the behavior of fish held only in freshwater was observed. We found that the presence of salt water significantly altered the startle response of chinook salmon. Fish held only in freshwater readily moved vertically within the water column when startled, whereas fish that preferred salt water responded by moving horizontally within the saltwater layer. In this experiment, prior preference for salt water superseded the fish's inclination to move upward in the water column when startled. These observations highlight the interactions between environmental stimuli, physiology and behavior.

## INTRODUCTION

The behavioral response of an organism to environmental stimuli may vary in concert with intrinsic and extrinsic conditions (Tinbergen 1963).

The motivation underlying specific behaviors of an animal should be considered within the context of its endogenous, physiological state and the abiotic or biotic factors it responds to in its environment. Because fish are restricted to and in intimate contact with the aquatic environment, they serve as an excellent model for studying complex interactions between behavior and environmental variables (Barlow 1993). For example, fish are adept at positioning themselves preferentially with respect to environmental gradients such as temperature (Sogard and Olla 1993, Bevelhimer 1996, Bohle 1998, Krause et al. 1998), light (Jain and Sahai 1994, Zigler and Dewey 1995, Champalbert and Diresch-Boursier 1998, McNicol et al. 1999), current velocity (Beecher et al. 1995, Knights et al. 1995, Sekine et al. 1997, Gerstner 1998), and salinity (McInerny 1964, Iwata et al. 1990, Maes et al. 1998, Wagner and Austin 1999).

In nature, however, multiple and often conflicting variables are likely influencing the distribution and behavior of fishes, and little is known about the relative importance of the many stimuli that they encounter. This experiment was one of several studies investigating the influence of various factors on the saltwater-entry behavior of juvenile chinook salmon (*Oncorhynchus tshawytscha*)

during smoltification - a process prior to or during outmigration in which anadromous salmonids undergo physiological, morphological and behavioral changes that prepare them for ocean residence (Hoar, 1988; Folmar and Dickhoff, 1980; Wedemeyer et al., 1980). One of the primary changes is an increased tolerance and preference for salt water, and we are interested in possible factors that affect or interact with this behavioral shift. Specifically, we wanted to determine if juvenile chinook salmon that preferred salt water in a vertically stratified tank would seek refuge in freshwater if startled in the saline layer.

The startle response in fishes is an important behavior, especially for eluding predators (Pitcher 1986). The degree to which this avoidance behavior is exhibited may be influenced by the fish's neurology (Roberts 1992), developmental stage (Williams et al. 1996), physiological stress (Sigismondi and Weber 1985) and health (Poulin 1993). External factors such as such as temperature (Webb and Zhang 1994, Temple and Johnston 1997), turbidity (Gregory 1993), habitat complexity (Domenici et al. 1994, Ekloev and Persson 1994) or the presence of toxicants (Zhou and Weis 1998) may also impact this behavior. Our purpose was to determine how prior preference for salt water might influence the startle response of chinook salmon.

The following laboratory experiment was conducted to observe the behavioral response of juvenile chinook salmon to two conflicting environmental stimuli. First, the fish were allowed to volitionally enter and remain in salt

water in a vertically stratified tank. Next, an apparatus was presented in the saltwater layer to evoke a startle response. Our predominant interest was the orientation of the fishes' response to the startle apparatus. Would the fish move vertically and leave salt water to avoid the apparatus, or would they avoid it only horizontally to maintain their position within the salt water? For comparison, we also presented the startle apparatus to fish held only in freshwater.

## **METHODS**

### **Experimental fish**

Juvenile spring chinook salmon used in these experiments were obtained as fingerlings from Oregon's Marion Forks hatchery and reared at Oregon State University's Fish Performance and Genetics Laboratory at Smith Farm, Corvallis, OR. Fish were held indoors with natural lighting in 2-m, circular tanks and fed to satiation twice per day until needed for experiments. During rearing, water temperature fluctuated between 12.8 and 14° C. Subyearling chinook salmon used in this experiment averaged  $12.8 \pm 8.8$  cm and  $24.0 \pm 5.1$  g, and were

undergoing morphological changes associated with smoltification. Parr marks on all fish were very faint or absent, fish were becoming silvery, and body shape was more fusiform. Experiments were conducted at Oregon State University's Oak Creek Laboratory, Corvallis, OR. As needed, fish were transported for 15 minutes from the Fish Performance Laboratory. Water temperature at Oak Creek Laboratory ranged from 10.5 to 12° C.

### **Experimental Tanks**

Experiments were carried out in two rectangular tanks (1.83 x 0.66 x 0.60 m) with Plexiglas® front windows and water volume of 757 l. The observation tanks were supplied with airstones, flow-through water (3 l/min.) and mesh covers. Each was enclosed behind a black plastic curtain with holes cut out to allow for visual observation and videotaping. Fluorescent lights were suspended above each tank. Light was diffused by fiberglass netting on frames suspended below the bulbs. During experiments, salt water was introduced into the bottom of each observation tank from one of two header tanks located outside of the plastic curtain. Each header was connected to one of the observation tanks by PVC pipes running along the bottom of the tanks. These



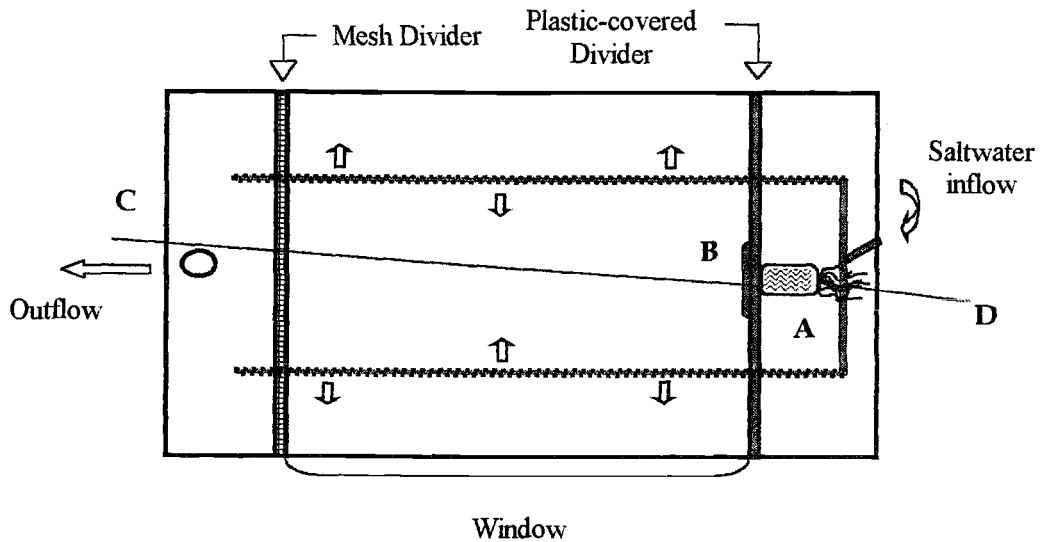
pipes had small holes drilled in two rows, allowing salt water to slowly diffuse to the tank bottom.

Fish transported to the Oak Creek Laboratory were randomly placed into observation tanks and held in aerated, flowing water for 48 hours. Preliminary work found that after 48 hours fish began normal feeding, so we considered them to be acclimated after this time. During acclimation, fish were never observed resting directly on the tank bottoms. Stationary fish tended to position themselves 5-30 cm off the bottom. Fish were dispersed throughout the tanks, and actively explored all areas of the tank, including the surface. The photoperiod was set to the ambient light cycle. Fish were not fed during acclimation to reduce disturbance to the tank. After acclimation, 379 l of 30-ppt salt water was mixed in each header tank using Instant Ocean<sup>®</sup> artificial salt, and was aerated. Twenty minutes prior to saltwater introduction, the videotape recorder was turned on. Ten minutes later, freshwater supply and aeration were turned off to prevent mixing. After another 10 minutes, the valves from the header tanks were then opened and saltwater began to flow into the bottom of the observation tanks, pushing the lighter freshwater out of the top-draining standpipe. This created a stable saltwater layer in the bottom of the water column, with a freshwater layer on top. The halocline was visible, so it was easy to distinguish whether fish were in salt or freshwater. Every 10 minutes the number of fish holding in freshwater or salt water was recorded as well as the

total amount of salt water that had entered the tank. Some fish would not hold in one layer or the other, but instead, moved between the two layers. These were tallied separately from the other two groups. It took just under one hour for all the salt water to drain into the tanks. At this time, the bottom half of the water column (30 cm deep) was saline and the top half was fresh. Preliminary experiments with dyed salt water showed that the halocline was stable for several days when no fish were present and no mixing occurred. Earlier tests also found that with fish in the tanks (and some potential mixing from swimming), salinity in the top half remained below 3 ppt for at least 24 hours.

Plastic mesh dividers were placed in the tank to keep fish in the tank area in front of the window. One of the dividers was covered with heavy black plastic and a trapdoor was cut into the bottom of this divider (Figure 4.1). The door could be opened from outside the curtained area by pulling on a monofilament line. When the line was pulled, the door flipped up; weights were attached to the bottom of the door, so that it would fall shut when the line was released. The area behind the divider was completely inaccessible, both physically and visibly, to the fish during acclimation, and the startle apparatus was located in this area. The startle apparatus consisted of a flat, silvery metal bar (17.5 cm x 7 cm x 3 mm) with opalescent reflective tape on the upper surface. Attached to one end was a 'tail' fashioned from 50 white, iridescent plastic strips (3 mm wide and 12 - 17 cm long). The apparatus was placed into the tank prior

to stocking fish. It could be pulled along the middle of the tank bottom by monofilament lines, attached at front and back, which ran along the tank bottom and outside the curtained area. This system enabled us to maneuver the object from outside the curtained tank area.



**Figure 4.1.** Diagram (top view) of the two tanks and startle apparatuses used in experiments. Each tank was isolated behind a black plastic curtain. The startle apparatus (A, see text for detailed description) was visually and physically isolated from the fish during acclimation behind a mesh divider completely covered with heavy black plastic. After saltwater introduction, a door (B) in the divider was opened from above by pulling on a monofilament line that ran outside of the curtained area. The apparatus was pulled along the bottom of the tank by monofilament line (C) that also ran outside the curtained area. Once the model reached the other mesh divider, it was pulled back across the tank bottom by monofilament line (D) and back through the door. Open arrows indicate the flow of salt water from the tank bottom.

## Startle Experiment

On 10, 14, 17, and 27 October 1999, 26 fish were transported to the Oak Creek Laboratory and equally divided between two observation tanks, giving a total of eight replicate trials for this treatment. Fish were allowed to acclimate for 48 hours. Salt water was introduced to both tanks as described above. Once all the salt water had been introduced (just under one hour), the trapdoor was opened and the startle apparatus was slowly pulled along the middle of the tank bottom until it reached the other divider. Then it was pulled back across the tank bottom and through the door where it was again out of the fishes' sight. It took about 30 seconds to pull the apparatus each way across the tank, so the fish were exposed to the object for one minute total. All experiments were videotaped to allow for detailed analysis of fish behavior in response to the startle apparatus. Due to malfunctioning of the startle apparatus (10 Oct) and poor videotape quality (14 Oct), behavioral observations could not be done on two replicate tanks. Hence, the number of replicates for behavioral analysis was reduced to six trials.

For comparison, we also observed the response of fish not exposed to salt water to the startle apparatus. On 01 and 06 November 1999, 26 juvenile chinook salmon were transported to the Oak Creek Laboratory and equally divided between two observation tanks. Fish were allowed to acclimate for 48 hours.

Tank conditions were identical to those described above, but no salt water was introduced. The startle apparatus was pulled through the tank exactly as in the previous experiments, and the four replicate trials for this treatment were videotaped for behavioral analysis.

### **Videotape analysis**

Several behaviors were analyzed by watching each videotape recording. The first 10 minutes of each tape showed fish during acclimation conditions and prior to any experimental stimuli. We counted the number of fish movements into the upper half of the tank during the first 5 minutes of this portion, to assess the general vertical movement patterns of fish acclimated to freshwater in the observation tanks. Next, we counted the number of fish moving into the upper half of the tank as the startle apparatus was pulled through. Finally, we further analyzed the avoidance behavior of fish in tanks to which salt water was added. For each of these videotapes, the bottom half of the tank (the saltwater layer) was conceptually divided into three blocks – left, middle and right. As the startle apparatus was being pulled from left to right (from the far side of the tank back into the hidden area), the numbers of fish in all blocks were counted at the time that the apparatus was directly in the center of each block.

## Statistical analysis

Each tank was considered an independent replicate. Multifactor ANOVA ( $P < 0.05$ ) was used to determine any treatment (salt water or freshwater), tank, or date effects. Because the only significant factor was treatment, the replicate trials from tanks with or without salt water added were pooled. For pairwise comparison of vertical avoidance behavior, a *t*-test was used.

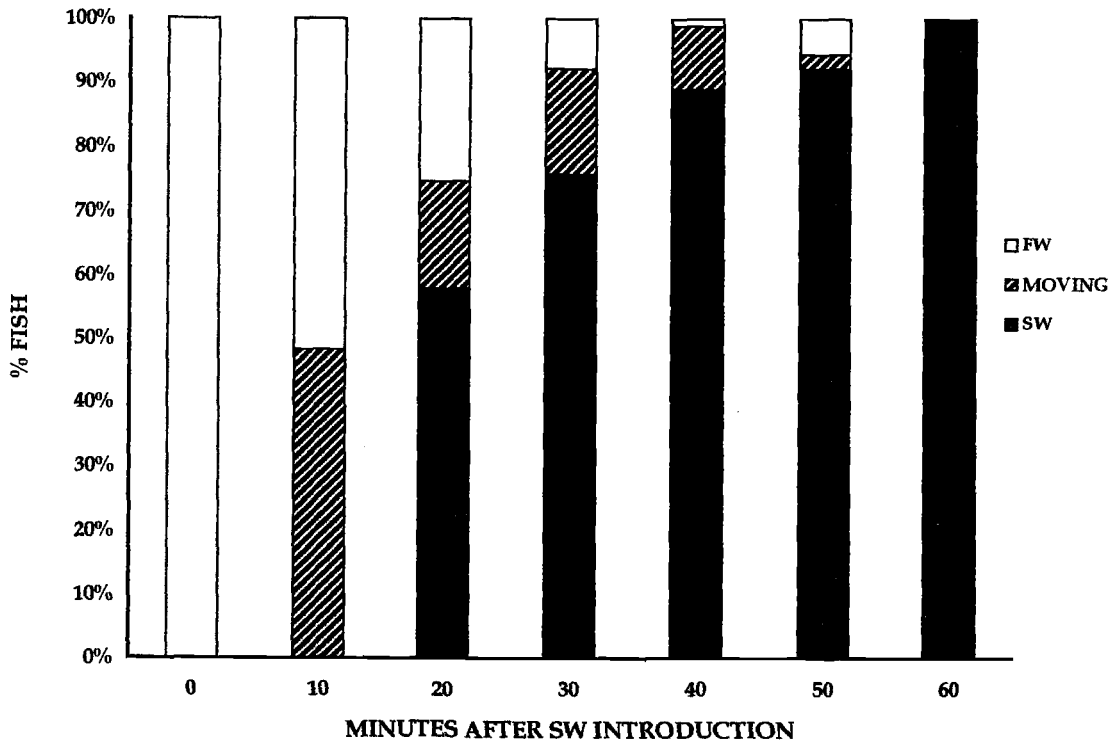
## RESULTS

During acclimation conditions (the first 5 minutes of each videotape), vertical movement patterns were similar between the treatments. (Table 4.1; *t*-test,  $P = 0.4232$ ;  $n = 8$  tanks with salt water,  $n = 4$  tanks with only freshwater). In the eight tanks to which salt water was added, all the chinook salmon exhibited a strong orientation to saline water (Figure 4.2). By the time all the salt water had flowed into the observation tanks, 100% of the fish were holding in salt water. Chinook salmon holding in salt water moved throughout that layer, and did not return to the freshwater. These fish were observed swimming up to the halocline, but not across it.

**Table 4.1.** Behavior of juvenile chinook salmon analyzed from videotape recordings made during startle experiments. Tanks either received salt water or did not. The number of fish movements into the upper half of the water column was counted during the first 5 minutes of video recording (salt water not yet added). There were no differences in the numbers of fish moving into the upper tank half during the first 5 minutes of videotaping between the treatments (*t*-test;  $P = 0.4232$ ). In tanks to which salt water was added, fewer fish moved into the upper tank half to avoid the apparatus, compared to fish in tanks that had not received salt water (*t*-test;  $P < 0.0001$ ).

TREATMENT	DATE	NO. OF MOVEMENTS INTO UPPER HALF IN FIRST 5 MIN.	% FISH HOLDING IN SW AFTER ONE HR.	NO. OF MOVEMENTS INTO UPPER HALF TO AVOID APPARATUS
EXPOSED TO SALT WATER	10 OCT	26	100	0
		6	100	
	14 OCT	19	100	1
		15	100	
	17 OCT	16	100	0
		10	100	2
	27 OCT	4	100	0
		3	100	0
NO SALT WATER EXPOSURE	03 NOV	2	-	9
		19	-	15
	08 NOV	2	-	10
		10	-	13
MEAN ± SD		12.4 ± 8.1		0.5 ± 0.8
		8.25 ± 8.1		11.8 ± 2.8

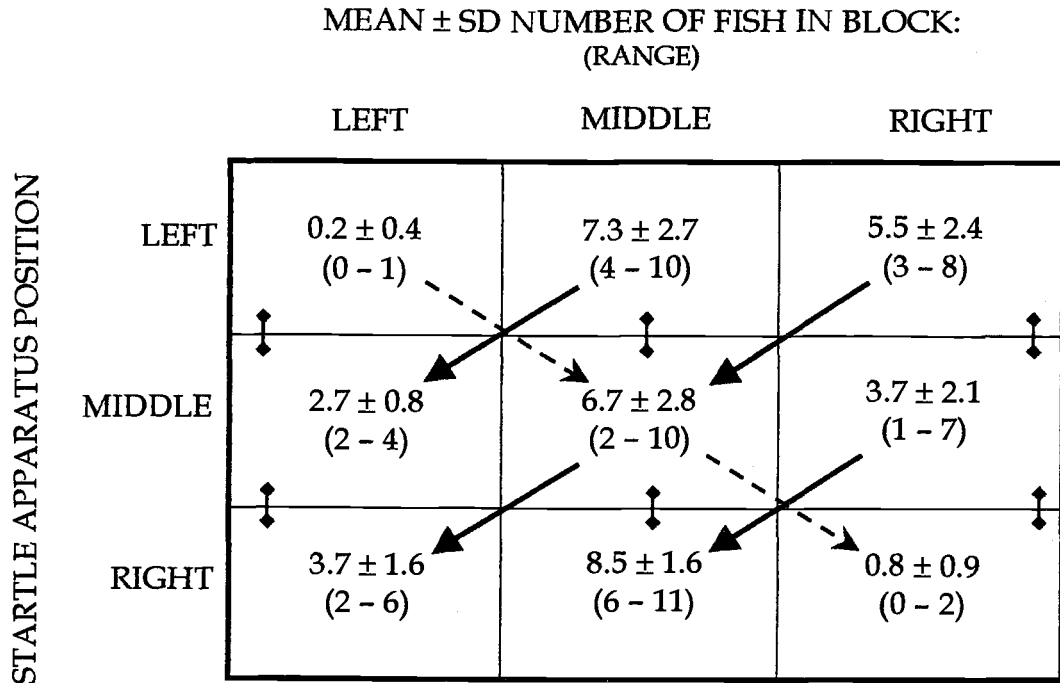




**Figure 4.2.** Saltwater-entry behavior of juvenile chinook salmon during saltwater introduction and prior to presentation of the startle apparatus. At each time, the mean percentage ( $n=8$  replicate trials) of fish holding in salt or freshwater, or moving between the layers is shown. Time = 0 represents the beginning of saltwater introduction.

When the apparatus was presented in salt water, a startle response was elicited and all the fish darted away from the object. Detailed analysis of the tapes found that fish avoidance behavior predominantly took place within the saltwater layer (Figure 4.3). Videotape observations were conducted once the apparatus had been pulled across the tank for the first time, and the object was on the tank side opposite the trap door. On average, only  $0.2 \pm 0.4$  (Figure 4.3, LEFT-LEFT block) fish remained in the left block when the object was centered there on its return to its original position. The remaining fish were in the middle ( $7.3 \pm 2.7$ ) and right ( $5.5 \pm 2.4$ ) blocks. As the object was pulled toward the center of the tank, most of the fish in the middle block darted toward the left side of the tank. Several fish ( $6.7 \pm 2.8$ ) were seen in the center block. Fish in the right block ( $3.67 \pm 2.1$ ) either moved toward the left side of the tank or held in the right block. As the object was moved into the right block, the majority of fish in the center and right blocks darted toward the left side of the tank.

Two distinct behavioral responses were observed. All fish darted away from the object and toward the opposite side of the tank when the object was first pulled through the trap door. The fish then tended to position themselves along the mesh divider such that they could see the object approaching. As the apparatus was pulled further across the tank bottom and toward the fish, the fish quickly darted around the object to the opposite tank side, often along the walls of the tank. By the time the apparatus had reached the far side of the tank,



**Figure 4.3.** The mean number of juvenile chinook salmon ( $n=6$  replicate trials) present in the left, middle and right block of the saltwater layer in relation to the position of the startle apparatus. Dashed arrows show the directional progression of the apparatus through the tank. Solid arrows indicate the direction of fish movement in the tank. As the object moved from left to right, the fish movement was in the opposite direction. The short, diamond-capped lines indicate that a small number of fish held their position within the block as the apparatus approached. Most of these fish eventually darted to evade the object, but 3 fish adopted 'freezing' behavior and huddled into corners.

nearly all the fish were in either the middle or right blocks (Figure 4.3). However, one fish (LEFT-LEFT block) responded by freezing, dropping to the bottom of the tank, and positioning itself in the corner made by the divider and the tank wall. As the object was being pulled back toward the door, most fish positioned there again quickly darted around the object, toward the opposite tank side. The freezing-type behavior was again exhibited by two fish (RIGHT/RIGHT block) that remained near the door side, but positioned themselves in bottom corners. Fish darting across the tank within the saltwater layer did swim upward, but only within that layer. An especially interesting behavior was observed in an average of  $3.2 \pm 2.2$  (range: 0 - 6) fish in the six tanks. These fish skimmed the halocline with their backs as they moved to avoid the object, but did not actually move across it into freshwater. In the six trials that were viewed, only 3 of the 78 total fish swam above the halocline (into freshwater) to avoid the apparatus (Table 4.1).

During trials conducted in freshwater, fish avoidance behavior toward the startle apparatus was markedly different from behavior observed in fish in salt water. Many fish that were held only in freshwater readily moved into the upper half of the tank (Table 4.1), as well as darting laterally, to avoid the object. Fish avoidance behavior occurred in both the vertical and horizontal dimensions. Several fish that moved into the upper half of the water column remained near the surface until the object had been pulled back through the door and out of

sight. In the four trials, an average of  $11.8 \pm 2.8$  fish movements into the upper half of the tank were counted in response to the object (Table 4.1), and this is significantly higher than for saltwater trials ( $0.5 \pm 0.8$ ; *t*-test;  $P < 0.0001$ ).

## DISCUSSION

The startle response and avoidance behavior of juvenile chinook salmon in these experiments was significantly influenced by the presence of salt water. Virtually no fish that had chosen to hold in the lower, saltwater layer moved into the upper, freshwater layer in response to the startle apparatus. Fish movements were predominantly lateral, and the avoidance behavior pattern of these fish can be conceptually thought of as occurring in the horizontal dimension within the saltwater layer. Our general impression of their behavior is that the fish were keenly aware of the boundary between fresh and salt water, as reflected in the halocline skimming behavior that we observed. Although these fish moved upward in the water column to avoid the startle apparatus, they were unwilling to actually cross the halocline. In contrast, chinook salmon exposed to the startle apparatus in freshwater reacted in both the horizontal and vertical dimensions. As videotape analysis found that the general vertical movements patterns of fish during acclimation were similar in all tanks, we conclude that the suppression of

vertical avoidance behavior by fish holding in salt water was directly related to salinity differences between the treatments.

The juvenile chinook salmon in this experiment had volitionally entered and remained in salt water – a behavior consistent with the life history of this species. During smoltification, anadromous salmon species display an increased preference for saline over freshwater (McInerny 1964, Iwata et al. 1990). This physiological and behavioral transformation serves to prepare fish that rear in freshwater habitats for oceanic residence as adults. Our principle interest in conducting this experiment was to discern whether fish that had chosen to enter full-strength salt water would leave it to avoid an unfamiliar stimulus. The startle apparatus was constructed to pose a relatively mild threat to the fish - the object was roughly only twice the size of the fish and was not maneuvered directly toward the fish in an overtly aggressive manner. Thus, we conclude from our results that the physiological predisposition to salt water took precedence over any threat the fish perceived from the startle apparatus.

Prior studies conducted in the same observation tanks used in this experiment, found that the saltwater preference of juvenile chinook salmon can be deleteriously affected by several factors such as disease and stress (Chapters 1 and 2). Fish suffering from bacterial kidney disease showed a 54% decrease in saltwater preference, compared to healthy controls. A mild chasing stressor administered just prior to the introduction of salt water to the tanks decreased

saltwater preference by 26%, compared to unchased control fish. Only 20% of fish exposed to a more severe handling and confinement stress (fish netted out of the tank and held in a small bucket for 15 minutes) preferred salt water, compared with 100% of unstressed controls. In the latter experiment, when an avian predator model was dropped on the top of the tank, all fish that had previously avoided salt water immediately descended into the lower, saline half of the tank. However, once the model was removed from the tank area, the fish resumed their positions in freshwater. In these three experiments, the physiological state of the fish had been compromised to some degree prior to saltwater exposure, and this impairment was reflected in their behavior. We hypothesize that salmon outmigrating through estuaries that are unwilling or unable to make the transition to salt water may be traveling in the upper, freshwater lens. This behavior may be maladaptive, as it could increase their risk of predation by birds feeding at or near the surface. Birds are considered a significant cause of mortality to outmigrating salmonids worldwide (Larsson 1985, Kennedy and Greer 1988, Krohn and Blackwell 1996, Russell et al. 1996,). In the Columbia River estuary, Collis et al. (2001) estimated that 16.7 million salmonid smolts were consumed by colonial nesting birds during the 1998 outmigration.

In the present experiment, the chinook salmon were not physiologically compromised, entered salt water, and adjusted their behavior (relative to fish in

freshwater) such that avoidance maneuvers took place within the layer of preferred salinity. Such context-dependent behavioral shifts in response to a variety of concomitant stimuli have been reported in other fish species. For example, the avoidance of chemical stressors may be suppressed when exposure is concurrent with positive stimuli such as preferred cover (McNicol et al. 1999), temperature (Giattina et al. 1981), food (Meldrim and Fava 1977) and spawning habitat (Meldrim and Fava 1977, Korver and Sprague 1989). Negative stimuli such as predators (Sogard and Olla 1993, Olla et al. 1996) or cold water (Pavlov et al. 1997) may shift the distribution of fish out of their preferred environments. Such behavioral shifts in response to multiple environmental factors may have significant ecological ramifications. For instance, fish exposed to hypoxic conditions may remain in their preferred habitat, but suffer reduced growth or death (Kramer 1987). On the other hand, fish may increase surface-breathing behavior, but thereby increase risk of avian predation. Fish leaving the hypoxic habitat may be faced with increased resource competition.

Chinook salmon in our experiments chose to remain in their preferred habitat when presented with a mild threat. In the estuary, this behavior may be advantageous. Considering the serious threat of avian predation, any behavioral adjustments that would keep the fish out of the upper, freshwater layer and thus further away from surface-feeding birds, would likely be an adaptive response. Our results suggest that healthy smolts perceiving unfamiliar stimuli in the



saline environment will continue to travel in the saltwater layer, unless such threats become physiologically stressful. However, the freshwater lens might also serve as a potential refuge from saltwater predators that are unwilling to cross the halocline in the pursuit of prey. If this is the case, then the suppression of vertical escape behavior might not always be beneficial.

Fish must respond to multiple intrinsic and extrinsic factors in a way that optimizes physiological homeostasis, reproduction and survival. For outmigrating juvenile salmonids, successful ocean entry is a critical step in their life cycle, and the process of smoltification prepares them, physiologically and behaviorally, for this event. In our experiment, the motivation to enter and remain in salt water superseded the compulsion to move upward in the water column to avoid the startle apparatus. This rather subtle behavioral shift accentuates the importance and complexity of the interplay between environmental stimuli (salt water), physiology (smoltification) and behavior (startle response).

## LITERATURE CITED

- Barlow, G.W. 1993. Fish behavioral ecology: pros, cons and opportunities. pp. 7-28. *In*: Huntingford, F.A. & Torricelli, P. (eds.), Behavioral ecology of fishes, Harwood Academic Publishers GmbH, Chur, Switzerland.
- Beecher, H.A., Carleton, J.P. & Johnson, T.H. 1995. Utility of depth and velocity preferences for predicting steelhead parr distribution at different flows. *Trans. Amer. Fish. Soc.* 124: 935-938.
- Bevelhimer, M.S. 1996. Relative importance of temperature, food and physical structure to habitat choice by smallmouth bass in laboratory experiments. *Trans. Amer. Fish. Soc.* 125: 274-283.
- Bohle, Bjorn. 1998. Temperature preferences of saltwater fish within horizontal gradients - a preliminary report. *Can. Transl. Fish. Aquat. Sci.* No. 5665.
- Champalbert, G. & Diresch-Boursier, L.L. 1998. Influence of light and feeding conditions on swimming activity rhythms of larval and juvenile turbot *Scophthalmus maximus* L.: an experimental study. *J. Sea Res.* 40: 333-345.
- Collis, K., Roby D.D., Craig, D.P., Ryan, B.A., & Ledgerwood, R.D., 2001. Colonial Waterbird Predation on Juvenile Salmonids Tagged with Passive Integrated Transponders in the Columbia River Estuary: Vulnerability of Different Salmonid Species, Stocks, and Rearing Types. *Trans. Amer. Fish. Soc.* 130: 385-396.
- Domenici, P., Harper, D.G. & Blake, R.W. 1994. Habitat type and the escape performance of fish. *Journal of Fish Biology* 45, Symposium on Predator-prey relationships in fishes, Glasgow, Scotland, 11-15 Jul 1994.

- Ekloev, P. & Persson, L. 1994. Predator-prey interactions: effects of prey refuges and species-specific capacities in juvenile fish. *Journal of Fish Biology* **45**, Symposium on Predator-prey relationships in fishes, Glasgow, Scotland, 11-15 Jul 1994.
- Folmar, L.C. & Dickhoff, W.W. 1980. The parr-smolt transformation (smoltification) and seawater adaptation in salmonids: a review of selected literature. *Aquaculture* **21**: 1-37.
- Gerstner, C.L. 1998. Use of substratum ripples for flow refuging by Atlantic cod, *Gadus morhua*. *Env. Biol. Fish.* **51**: 455-460.
- Giattina, D.S., Cairns Jr., J. & Stevens, D.G. 1981. Comparison of laboratory and field avoidance behavior of fish in heated chlorinated water. *Trans. Amer. Fish. Soc.* **110**: 526-535.
- Gregory, R.S. 1993. Effect of turbidity on the predator avoidance behavior of juvenile chinook salmon (*Oncorhynchus tshawytscha*). *Can. J. Fish. Aquat. Sci.* **5**: 241-246.
- Hoar, W.S. 1988. The physiology of smolting salmonids. pp. 275-343. *In*: Hoar, W.S. & Randall, D.J. (eds.), *Fish Physiology, Volume XIB*. Academic Press, San Diego, California.
- Iwata, M., Yamauchi, K., Nishioka, R.L. & Bern, H.A. 1990. Effects of thyroxine, growth hormone and cortisol on salinity preference of juvenile coho salmon (*Oncorhynchus kisutch*). *Mar. Behav. and Physiol.* **17**: 191-201.
- Jain, V.K. & Sahai, S. 1994. Phototactic response of some fishes. *Env. Ecol.* **12**: 123-127.

Kennedy, G.J.A. & Greer, J.E. 1988. Predation by cormorants, *Phalacrocorax carbo* (L.), on the salmonid populations of an Irish river. *Aquac. Fish. Man.* 19: 159-170.

Knights, B.C., Johnson, B.L. & Sandheinrich, M.B. 1995. Response of bluegills and black crappies to dissolved oxygen, temperature, and current in backwater lakes of the upper Mississippi River during winter. *N. Amer. J. Fish. Man.* 15: 390-399.

Korver, R.M. & Sprague, J.B. 1989. Zinc avoidance by fathead minnow (*Pimephalas promelas*): computerized tracking and greater ecological relevance. *Can. J. Fish. Aquat. Sci.* 46: 494-502.

Kramer, D. L. 1987. Dissolved oxygen and fish behavior. *Env. Biol. Fish.* 18: 81-92.

Krause, J., Staaks, G. & Mehner, T. 1998. Habitat choice in shoals of roach as a function of water temperature and feeding rate. *J. Fish Biol.* 53: 377-386.

Krohn, W.B. & Blackwell, B.F. 1996. The double-crested cormorant in Maine. *Maine Fish and Wild.* 3: 8-12.

Larsson, P.O. 1985. Predation on migrating smolt as a regulating factor in Baltic salmon, *Salmo salar* L., populations. *J. Fish Biol.* 26: 391-397.

Maes, J., Van Damme, P.A., Taillieu, A. & Ollevier, F. 1998. Fish communities along an oxygen-poor salinity gradient. *J. Fish Biol.* 52: 534-546.

McInerny, J.E. 1964. Salinity preference: an orientation mechanism in salmon migration. *J. Fish. Res. Brd. Can.* 21: 995-1018.

- McNicol, R.E., Bégout-Anras, M.-L., Scherer, E. 1999. Influence of light preference on the avoidance response of lake whitefish, *Coregonus clupeaformis*, to cadmium. *Env. Biol. Fish.* 55: 295-306.
- Meldrim, J.W. & Fava Jr., J.A. 1977. Behavioral avoidance responses of estuarine fishes to chlorine. *Ches. Sci.* 18: 154-157.
- Mesa, M.G. 1994. Effects of multiple acute stressors on the predator avoidance ability and physiology of juvenile chinook salmon. *Trans. Amer. Fish. Soc.* 123:786-793.
- Olla, B.L., Davis, M.W., Ryer, C.H. & Sogard, S.M. 1996. Behavioral determinants of distribution and survival in early stages of walleye pollock, *Theragra chalcogramma*: a synthesis of experimental studies. *Fish. Ocean.* 5(Suppl. 1): 167-178.
- Pavlov, D.S., Sadkovskii, R.V., Kostin, V.V. & Lupandin, A.I. 1997. Influence of photo, thermo and barogradients on cyprinid fry fish behavior and vertical distribution. *J. Ichthyol.* 37: 66-71.
- Pitcher, T. J. 1986. Functions of shoaling behavior in teleosts. pp. 294-337. *In*: Pitcher, T. J. (ed.), *The Behavior of teleost fish*, The Johns Hopkins Univ. Press, Baltimore, Maryland.
- Poulin, R. 1993. Age-dependent effects of parasites on anti-predator responses in two New Zealand freshwater fish. *Oecologia* 96: 431-438.
- Roberts, B.L. 1992. Neural mechanisms underlying escape behavior in fishes. *Rev. Fish Biol. Fish.* 2: 243-266.

- Russell, I.C., Dare, P.J., Eaton, D.R. & Armstrong, J.D. 1996. Assessment of the problem of fish-eating birds in inland fisheries in England and Wales. Report to the Ministry of Agriculture, Fisheries and Food (MAFF Project VC0104).
- Sekine, M., Imai, T. & Ukita, M. 1997. A model of fish distribution in rivers according to their preference for environmental factors. *Ecol. Model.* 104: 215-230.
- Sigismondi, L.A. & Weber, L.J. 1988. Changes in avoidance response time of juvenile chinook salmon exposed to multiple acute handling stresses. *Trans. Amer. Fish. Soc.* 117: 196-201.
- Sogard, S.M. & Olla, B.L. 1993. Effects of light, thermoclines and predator presence on vertical distribution and behavioral interactions of juvenile walleye pollock, *Theragra chalcogramma* Pallas. *J. Exp. Mar. Biol. Ecol.* 167: 179-195.
- Temple, G.K. & Johnston, I.A. 1997. The thermal dependence of fast-start performance in fish. *J. Therm. Biol.* 22: 391-401.
- Tinbergen, N. 1963. On aims and methods of ethology. *Zeitschrift für Tierpsychologie* 20. pp. 410-433. *In:* Houck, L.D. & Drickamer, L.C. (eds.), 1996. *Foundations of animal behavior: classic papers with commentaries*, University of Chicago Press, Chicago.
- Wagner, C.M. & Austin, H.M. 1999. Correspondence between environmental gradients and summer littoral fish assemblages in low salinity reaches of the Chesapeake Bay, USA. *Mar. Ecol. Prog. Ser.* 177: 197-212.
- Webb, P.W. & Zhang, H. 1994. The relationship between responsiveness and elusiveness of heat-shocked goldfish (*Carassius auratus*) to attacks by rainbow trout (*Oncorhynchus mykiss*). *Can. J. Zool.* 72: 423-426.

- Wedemeyer, G.A., Saunders, R.L. & Clarke, W.C. 1980. Environmental factors affecting smoltification and early marine survival of anadromous salmonids. *Mar. Fish. Rev.* 42: 1-14.
- Williams, P.J., Brown, J.A., Gotceitas, V. & Pepin, P. 1996. Developmental changes in escape response performance of five species of marine larval fish. *Can. J. Fish. Aquat. Sci.* 53: 1246-1253.
- Zhou, T. & Weis, J.S. 1998. Swimming behavior and predator avoidance in three populations of *Fundulus heteroclitus* larvae after embryonic and/or larval exposure to methylmercury. *Aquat. Toxicol.* 43: 131-148.
- Zigler, S.J. & Dewey, M.R. 1995. Phototaxis of larval and juvenile northern pike. *N. Amer. J. Fish. Man.* 15: 651-653.

## CONCLUSION

These studies provide insights into intrinsic and extrinsic factors that may contribute to the vertical distribution of outmigrating juvenile spring chinook salmon traveling through the estuarine environment. These laboratory experiments clearly demonstrate that chinook salmon are capable of responding differentially to a saltwater gradient, and that their motivation to enter salt water may be significantly and negatively impacted by processes acting in opposition to smoltification.

Severe infection with *R. salmoninarum* decreased preference for salt water by more than 50%, and greatly increased the time that it took for fish to make that choice. One of the more intriguing observations in this experiment was that our control fish had medium infection levels and showed no external signs of BKD, yet the majority (85%) chose to hold in salt water. This was a somewhat surprising result considering that infected fish tend to fare very poorly in seawater (Banner et al. 1983, Sanders et al. 1992, Elliott et al. 1995, Moles 1997). Even sick fish that survive an initial transfer to seawater often do not thrive or grow, and delayed mortality is common. It is uncertain whether infected fish that volitionally enter salt water would survive or thrive in salt water. It is possible that for such fish the drive to enter salt water at this life history stage supercedes their disease-related aversion to it. Because of the widespread



incidence of BKD among Pacific Salmon, efforts to understand the behavioral effects of infection are important to understanding the role that this disease may play.

Three stressors were tested for their effects on saltwater preference of chinook salmon, and all were found to reduce affinity for salt water. The most severe stressor in terms of decreased salinity preference was the 15-minute confinement. After 60 minutes, only 20% of stressed chinook held in salt water, compared with 100% of the unstressed controls. Avoidance behavior continued even after the avian predator model was presented. Severe stress apparently represents a significant threat to normal saltwater-entry behavior. The milder chasing stressor also decreased preference for salt water, but to a lesser extent. Nearly 70% of the chased fish held in salt water, compared with 95% of unstressed controls. The large difference in avoidance response between the mildly and severely stressed fish suggests that the severity of the stressor is important in determining the extent to which saltwater preference will be inhibited.

The mildest of the three stressors was social aggression imposed by rainbow trout. After 60 minutes, significantly fewer chinook salmon stocked with trout entered salt water relative to chinook salmon held only with conspecifics. While this trend was still evident after 120 minutes, the differences were no longer significant. When the percentage of fish holding in and exploring

(i.e., moving between fresh and salt water) salt water were combined, 100% of salmon held only with conspecifics were holding in or exploring the saltwater layer. By contrast, after 120 minutes 13% of salmon held with trout were avoiding salt water and holding in the freshwater layer. Chinook salmon held with steelhead trout showed no difference in saltwater preference compared to controls held only with conspecifics, and there was less aggression among fish in that experiment. Collectively, the results of the steelhead and rainbow trout experiments suggest that the mere presence of an aggressive species is not sufficient to alter chinook salmon behavior, and that the changes in preference behavior are a result of social aggression. This impression is further supported by the fact that qualitatively milder aggression among chinook salmon was observed in both experiments, but salinity preference was high in control tanks.

Finally, preference for salt water was found to significantly alter the startle response of chinook salmon. Fish held only in fresh water readily moved both vertically and horizontally within the water column to avoid a simulated threat presented along the tank bottom. In contrast, saltwater-preferring fish in a vertical gradient did not move upwards into the freshwater layer in response to the threat. Rather, they moved laterally within the saltwater layer, and a few fish adopted a 'freezing' behavior. In this experiment, a trade-off between physiological preference and the orientation of the startle response was observed.

Other studies have also reported changes in saltwater preference in juvenile chinook salmon as a result of stress or toxicant exposure. Schreck et al. (in prep) found that chinook salmon exposed to eight consecutive stressors (simulating passage through dams of the Columbia River hydropower system) showed reduced preference for salt water more than two weeks following the final stressor. Stressed fish had exhibited physiological recovery, as evidenced by their return to normal cortisol levels, within several hours of the final stressor, yet behavioral ramifications of the stress were evident for an extended time afterwards. Juvenile chinook salmon exposed to the antisapstain fungicide 2-(thiocyanomethylthio) benzothiazole (TCMTB) and subsequently placed in a vertically stratified tank held in freshwater or just below the halocline while unexposed salmon held in the saltwater layer.

The volitional entry of juvenile chinook salmon into salt water may best be conceptualized as the resultant behavior of various, and often conflicting, motivating factors. The process of smoltification is the dominant driving force that primes fish morphologically, physiologically and behaviorally for the transition to salt water. However, even well smolted fish may be hesitant or unwilling to enter salt water due to other intrinsic and extrinsic influences such as disease, immediate or delayed effects of physiological stress, or threat. The cumulative results of these experiments suggest that healthy, unstressed smolts will readily and quickly enter salt water, but both the timing and extent of this

behavior may be variably affected by a wide range of factors. Severe perturbations such as confinement stress and advanced BKD greatly reduced the willingness of chinook salmon to hold in salt water, but mild stressors (chasing and social stress) did so to a much lesser degree. A high percentage (85%) of fish with moderate levels of BKD retained preference for salt water. Modest threat (startle apparatus) had no affect on preference for salt water, and instead, fish altered their avoidance behavior to maintain their position within the preferred salinity. It is also important to note that in several of these experiments, fish that were unwilling to hold in salt water did not necessarily completely avoid it. Instead, fish in treatment tanks tended to move between the fresh and saltwater layers to a greater extent than did fish in control tanks. Although these fish were unwilling to hold in salt water, their orientation to high salinity was maintained.

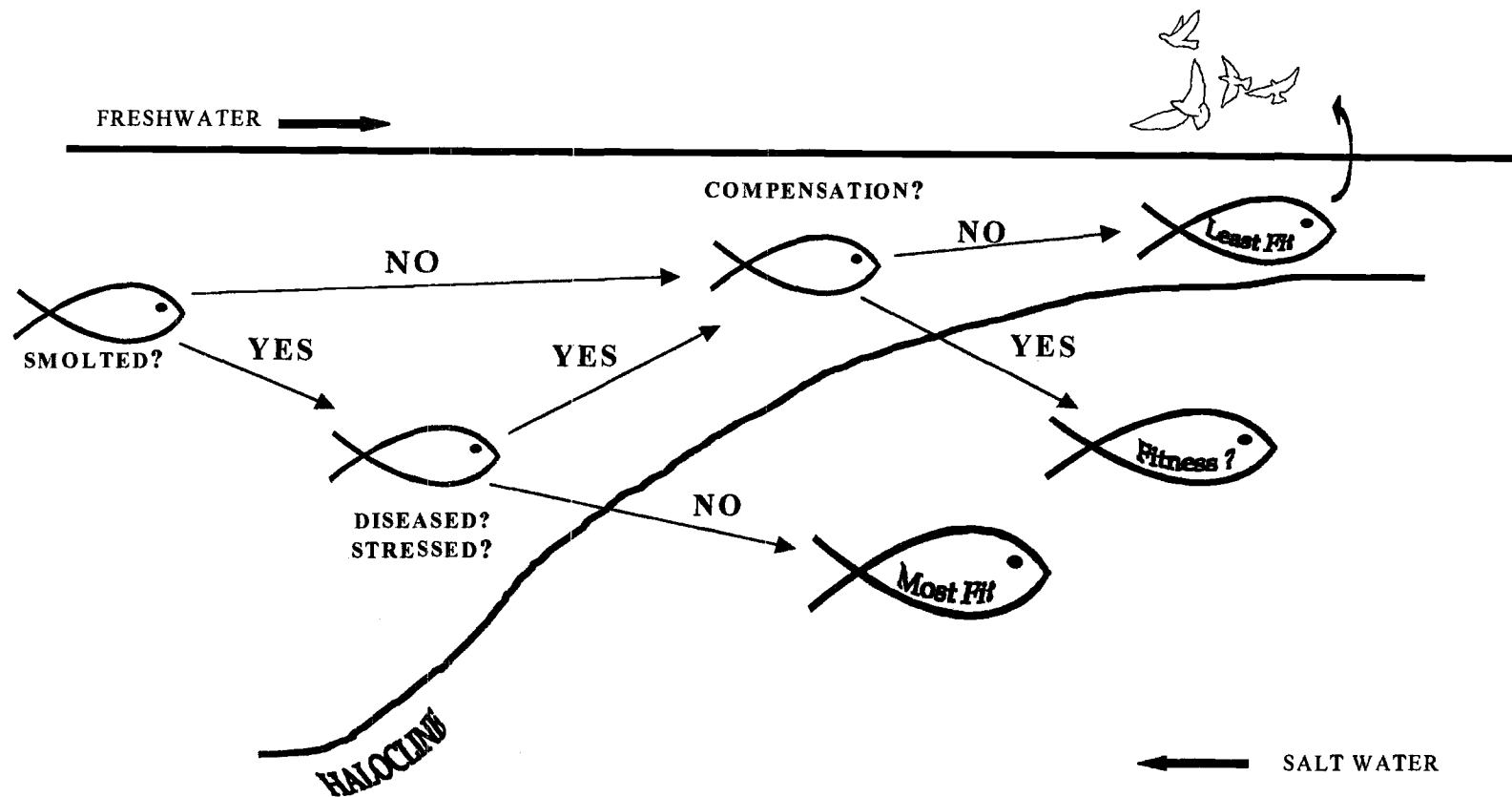
This is a particularly interesting insight because it allows for an additional, though not contradictory, interpretation of these experimental results. In the experiments with BKD or stress as treatments, preference for salt water was greatly reduced in impacted fish, compared to controls. However, in each experiment some fish did hold in the saltwater layer. After 2 hours, an average of 69% of chased fish, 60% of chinook salmon held with rainbow trout, and 31% of fish suffering from BKD were holding in salt water. Even a severe confinement stress did not completely reverse their preference relative to controls (20% of stressed fish held in salt water). When one combines the

percentages of fish holding in and exploring (moving between the layers) the saltwater layer, the percentages of fish showing some orientation toward salt water are very high - 70% of confined fish, 97% of chased fish, 87% of fish with trout, and 71% of fish with severe BKD infection. Roughly, only a quarter of stressed or diseased chinook salmon completely avoided salt water and chose to remain in fresh water. This is rather interesting because it reveals the strength of the drive to enter salt water, even in perturbed smolts.

This series of studies investigated several factors that affect the saltwater-entry behavior and saltwater preference of juvenile chinook salmon. The results suggest that an individual's behavior with respect to salt water is dependent upon various intrinsic and extrinsic motivations (Figure D.1). Extrinsic factors may include salinity, the presence of aggressors or predators, and other biotic and abiotic components of the environment. Intrinsic factors such as infection with disease, physiological stress, level of smoltification and genetics are also important. Upon encountering salt water, a fish's behavior reflects the integration of all these factors. Smolted, healthy and unstressed fish will respond optimally by readily entering and adjusting to salt water. However, fish must often make trade-offs between conflicting motivations and factors. Fish that are not sufficiently smolted, sick or stressed may differ in their behavioral response to salt water because they must compensate for one or more factors that conflict with the appropriate behavior for this life history stage. Severely compromised

fish that are unable or unwilling to enter saline water may be forced into freshwater. Fish that are able to physiologically or behaviorally adjust to negative factors, may delay entry into salt water. The salient question then becomes: How do impaired fish that move into salt water thrive and survive in comparison to healthy, unstressed fish?

The answer to this question is largely unknown. While downstream migration and survival of smolts has long been monitored (Stasko 1975; Fried et al. 1978; McCleave 1978; Tytler et al. 1978; Moser et al. 1991; Moore 1998), the fate of fish once they have entered the ocean is logistically much more difficult to assess (Pearcy 1992). Several years of studies radiotracking outmigrating juvenile chinook salmon through the Columbia River estuary found that survival from Bonneville Dam (the most downstream dam on the Columbia River) to the upper estuary was nearly 100% (Schreck et al., in prep). However, avian predation in the estuary accounted for 5-30% mortality. In a separate study, Caspian terns were estimated to eat between 7.4 and 15.2 million juvenile salmonids per year, accounting for approximately 11% of the run (Collis, et al. 2001). High predation rates of avian predators on outmigrating juvenile salmonids have been reported worldwide (Larsson 1985; Wood 1987a,b; Kennedy and Greer 1988; Greenstreet et al. 1993; Dieperink 1995; Suter 1995; Russell et al. 1996). Although predation obviously poses an extensive threat to



**Figure D.1.** A conceptual model of the intrinsic and extrinsic factors that affect saltwater-entry behavior and saltwater preference of juvenile chinook salmon. The fit fish are smolted, healthy and unstressed. These are most likely to outmigrate successfully and have high early-ocean survival. The least fit fish are unsmolted, diseased, stressed, or a combination of these. Fish that avoid salt water are very susceptible to avian predators that feed at or near the surface. Fish that can compensate for negative factors may delay entry into salt water. Their early-ocean survival may be low, reflecting a delayed effect of difficulty adjusting to salt water.

these fish, it is important to consider the role that smolt quality may play in this ecological phenomenon.

Salmon that are in good condition and sufficiently smolted will likely make a smooth and efficient transition into saline, ocean waters. On the contrary, stressed, diseased, or otherwise impaired fish may be avoiding salt water and remaining in fresh water as long as possible due to osmoregulatory limitations. These fish may be more susceptible to avian predation for two reasons. First, in the upper water layer they are proximal to the surface, where many avian predators feed. Second, stressed and diseased salmonid smolts are known to be less adept at evading predators than healthy fish. For example, Mesa et al. (1998) reported that juvenile chinook salmon experimentally challenged with *R. salmoninarum* were nearly twice as likely to be preyed upon by either northern pikeminnow or smallmouth bass. Additionally, stressed coho smolts were found to be more susceptible to predation by lingcod than unstressed controls (Olla et al. 1992). Finally, stressed smolts, which avoided salt water, would only temporarily dive downward into saline water when threatened with an avian predator model (Chapter 2). These studies strongly suggest that decreased ability to avoid predators may be a significant issue for outmigrating salmon traveling through estuaries. Salmon that are unwilling or unable to make the transition into salt water, even temporarily, may face greater danger of predation.



Few field studies have investigated the behavior of outmigrating salmon smolts just prior to ocean entry. During underwater observations, Iwata and Komatsu (1984) found that juvenile chum salmon held in the upper, brackish layer (10-15 ppt and < 1m below the surface) of a small estuary for up to two days before entering the ocean. Further laboratory experiments confirmed that after brief acclimation to 12 ppt seawater, osmoregulatory capacity was increased for these fish. The authors also note that this behavior constituted a tradeoff between physiological capacity and threat of avian predation.

The first few days or weeks in the ocean, may constitute a critical period for outmigrating juvenile salmon. Mortality rates during this time are high, and environmental factors such as ocean temperature, salinity and food availability have been correlated with early survival of Pacific salmonids (Pearcy 1992). Salmon that are in prime condition upon reaching the sea, will be most likely to survive initial hardship, while diseased or stressed fish that experienced difficulty (in terms of decreased osmoregulatory capacity or predator threat) during the estuarine phase of outmigration may be less fit in the marine environment. Thus, even if impaired fish survive the initial transition into salt water, their early-ocean survival may still be jeopardized.

Such early-ocean mortality would represent a delayed effect of stress, which is experienced temporally and spatially during the freshwater portion of their life cycle, but manifested in the ocean environment. While the stress

response is typically considered an adaptive process whereby fish regain homeostasis, the long-term effects are often difficult to assess. This is especially true of juvenile salmon whose life history incorporates habitats separated by hundreds or even thousands of miles. Many studies of the stress response in fishes focus upon its physiological aspects, predominantly the increase in plasma cortisol. However, recovery to baseline levels in cortisol is often observed within several hours of the application of the stressor (Schreck et al. 1997). Several authors promote the use of behavioral assays to gain better understanding of the behavioral alterations that may persist after physiological recovery from stress (Wedemeyer and McLeay 1981, Schreck 1990, Shuter 1990, Wedemeyer et al. 1990, Schreck et al. 1997). Behavioral studies are especially useful in that they provide an explanatory link between small-scale physiological processes and large-scale ecological trends. The results herein make it possible to present one hypothesis to explain the extensive predation on smolts by birds in the estuary: upon reaching the estuary stressed or diseased smolts differ in physiological readiness from fish in good condition, their saltwater-entry preference and behavior are altered, and these behavioral changes likely make them easier targets for avian predators. The behavioral observations made in these experiments provide a framework for undertaking further investigations into the physiological, behavioral and ecological factors that are interacting to determine the fate of outmigrating juvenile salmon.

Efforts are underway to develop improved methods for assessing the overall quality of smolts and their readiness for ocean residence (Iwama 1992, Clarke 1992, Olla et al. 1994, Beckman et al. 1999). Many variables are involved in such assessments, and discerning the predominate factors governing successful fish performance is a difficult task. Physiological and morphological parameters of smoltification are often unreliable indicators of smolt survival and subsequent adult returns (Wedemeyer et al. 1980, Beckman et al. 1999). Even saltwater challenge tests may not reflect the true ability of smolts to adjust to the saline environment (MacDonald et al. 1988). Though the physiological capacity of fish may be evaluated by biochemical markers and challenge tests, the behavioral capacity of fish may not be reflected in the results of such assays. Ultimately, the behavior and performance of fish during outmigration may be the most important factors dictating their immediate survival.

While management practices cannot eliminate all the stressors and dangers that outmigrating juvenile salmonids might face, several steps can be taken to optimize smolt performance. First, hatchery fish should be at advanced stages of smoltification and healthy upon release to maximize their orientation toward salt water. Second, dam passage facilities should be engineered to minimize delayed effects of stress. Finally, barging protocols can be developed to decrease any effects of social stress associated with high densities or interspecific aggression.

In the Columbia River, predator management programs include the removal of predators that feed on smolts, such as northern pikeminnows (National Research Council 1996) and Caspian terns (Collis et al. 2001). Poor quality fish are probably behaving in ways that make them easy prey and eliminating select predator species from the ecosystem will only shift predatory pressure to other piscine, avian or mammalian species. The long-term solution will likely result from management practices that result in heartier, healthier salmonid smolts that are better able to survive the gauntlet of threats that they encounter during outmigration.

## BIBLIOGRAPHY

- Abbott, J.C. & Dill, L.M. 1985. Patterns of aggressive behavior in juvenile steelhead trout (*Salmo gairdneri*). *Can. J. Fish. Aquat. Sci.* 42: 1702-1706.
- Abbott, J.C., Dunbrack, R.L. & Orr, C.D. 1985. The interaction of size and experience in dominance relationships of juvenile steelhead trout (*Salmo gairdneri*). *Behaviour* 92: 241-253.
- Adams, S.M. (ed.). 1990. Biological indicators of stress in fish. American Fisheries Society Symposium 8.
- Banner, C.R., Rohovec, J.S. & Fryer, J.L. 1983. *Renibacterium salmoninarum* as a cause of mortality among chinook salmon in salt water. *J. World Maricult. Soc.* 14: 236-239.
- Barlow, G.W. 1993. Fish behavioral ecology: pros, cons and opportunities. pp. 7-28. *In*: Huntingford, F.A. & Torricelli, P. (eds.), *Behavioral ecology of fishes*, Harwood Academic Publishers GmbH, Chur, Switzerland.
- Barton, B.A. 1997. Stress in finfish: past, present and future - a historical perspective. pp. 1-34. *In*: Iwama, G.K., Pickering, A.D., Sumpter, J.P. & Schreck, C.B. (eds.), *Fish stress and health in aquaculture*, Society for Experimental Biology Seminar Series: 62, Cambridge University Press, Cambridge.
- Beecher, H.A., Carleton, J.P. & Johnson, T.H. 1995. Utility of depth and velocity preferences for predicting steelhead parr distribution at different flows. *Trans. Amer. Fish. Soc.* 124: 935-938.

- Bevelhimer, M.S. 1996. Relative importance of temperature, food and physical structure to habitat choice by smallmouth bass in laboratory experiments. *Trans. Amer. Fish. Soc.* 125: 274-283.
- Birtwell, I.K., Nelles, S. & Harbo, R.M. 1983. A brief investigation of fish in the surface waters of the Somass estuary, Port Alberni, British Columbia. *Can. MS Rep. Fish. Aquat. Sci.* 1744: 31 p.
- Birtwell, I.K. & Kruzynski, G.M. 1989. *In situ* and laboratory studies on the behavior and survival of Pacific salmon (genus *Oncorhynchus*). *Hydrobiologia* 188/189: 543-560.
- Boeuf, G. 1993. Salmonid smolting: a preadaptation to the oceanic environment. pp. 105-136. *In: Rankin, G.C. & Jenson, G.B. (eds.), Fish ecophysiology.* Chapman and Hall Publishers.
- Bohle, Bjorn. 1998. Temperature preferences of saltwater fish within horizontal gradients - a preliminary report. *Can. Transl. Fish. Aquat. Sci.*, No. 5665.
- Bottom, D.L. & Jones, K.K. 1990. Species composition, distribution, and invertebrate prey of fish assemblages in the Columbia River estuary. *Prog. Ocean.* 25: 243-270.
- Bruno, D.W. 1986. Histopathology of bacterial kidney disease in laboratory infected rainbow trout, *Salmo gairdneri* Richardson, and Atlantic salmon, *Salmo salar* L., with reference to naturally infected fish. *J. Fish Dis.* 9: 523-537.
- Champalbert, G. & Diresch-Boursier, L.L. 1998. Influence of light and feeding conditions on swimming activity rhythms of larval and juvenile turbot *Scophthalmus maximus* L.: an experimental study. *J. Sea Res.* 40: 333-345.

- Clarke, W.C. & Blackburn, J. 1978. Seawater challenge test performed on hatchery stocks of chinook and sockeye salmon in 1977. Tech. Rep. - Fish. Mar. Serv (Can). 761: 1-19.
- Clarke, W.C., Withler, R.E. & Shelbourn, J.E. 1992. Genetic control of life history pattern in chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. 49: 2300-2306.
- Clarke, W.C., Withler, R.E. & Shelbourn, J.E. 1994. Inheritance of smolting phenotypes in backcrosses of hybrid stream-type X ocean-type chinook salmon (*Oncorhynchus tshawytscha*). Estuaries 17: 13-25.
- Collis, K., Roby D.D., Craig, D.P., Ryan, B.A., & Ledgerwood, R.D., 2001. Colonial Waterbird Predation on Juvenile Salmonids Tagged with Passive Integrated Transponders in the Columbia River Estuary: Vulnerability of Different Salmonid Species, Stocks, and Rearing Types. Trans. Amer. Fish. Soc. 130: 385-396.
- Congleton, J., Schreck, C.B. & Davis, L. 2000. Stress indices in migrating juvenile chinook salmon and steelhead of wild and hatchery origin before and after barge transportation. Trans. Amer. Fish. Soc. 129: 946-961.
- Conte, F.P. & Wagner, H.H. 1965. Development of osmotic and ionic regulation in juvenile steelhead trout *Salmo gairdneri*. Comp. Biochem. Physiol. 14: 603-620.
- Conte, F.P., Wagner, H.H., Fessler, J. & Gnose, C. 1966. Development of osmotic and ionic regulation in juvenile coho salmon, *Oncorhynchus kisutch*. Comp. Biochem. Physiol. 18: 1-15.
- Diana, J.S. 1995. Biology and ecology of fishes. Cooper Publishing Group LLC, Carmel, IN.

- Diepernick, C. 1995. Depredation of commercial and recreational fisheries in a Danish fjord by cormorants, *Phalacrocorax carbo sinensis*, Shaw. *Fish. Man. Ecol.* 2: 197-207.
- Domenici, P., Harper, D.G. & Blake, R.W. 1994. Habitat type and the escape performance of fish. *Journal of Fish Biology* 45, Symposium on Predator-prey relationships in fishes, Glasgow, Scotland, 11-15 Jul 1994.
- Donaldson, E.M. 1981. The pituitary-interrenal axis as an indicator of stress in fish. pp. 11-48. *In: Pickering, A.D. (ed.), Stress and fish*, Academic Press, London.
- Eddy, F.B. 1981. Effects of stress on osmotic and ionic regulation in fish. pp. 77-102. *In: Pickering, A.D. (ed.), Stress and Fish*. Academic Press Inc., New York.
- Edmundson, E., Everest, F.E. & Chapman, D.W. 1968. Permanence of station in juvenile chinook salmon and steelhead trout. *J. Fish. Res. Brd. Can.* 25: 1453-1464.
- Ejike, C. & Schreck, C.B. 1980. Stress and social hierarchy rank in coho salmon. *Trans. Amer. Fish. Soc.* 109: 423-426.
- Ekloev, P. & Persson, L. 1994. Predator-prey interactions: effects of prey refuges and species-specific capacities in juvenile fish. *Journal of Fish Biology* 45, Symposium on predator-prey relationships in fishes, Glasgow, Scotland, 11-15 Jul 1994.
- Elliott, D.G., Pascho, R.J. & Palmisano, A.N. 1995. Brood stock segregation for the control of bacterial kidney disease can affect mortality of progeny chinook salmon (*Oncorhynchus tshawytscha*) in seawater. *Aquaculture* 132: 133-144.



- Elliott, D.G., Pascho, R.J., Jackson, L.M., Matthews, G.M. & Harmon, J.R. 1997. *Renibacterium salmoninarum* in spring-summer chinook salmon smolts at dams on the Columbia and Snake Rivers. *J. Aquat. Anim. Health* 9: 114-126.
- Everest, F.H. & Chapman, D.W. 1972. Habitat selection and spatial interaction by juvenile chinook salmon and steelhead trout in two Idaho streams. *J. Fish. Res. Bd. Can.* 29: 91-100.
- Ewing, R.D. & Birks, E.K. 1982. Criteria for parr-smolt transformation in juvenile chinook salmon (*Oncorhynchus tshawytscha*). *Aquaculture* 28: 185-194.
- Folmar, L.C. & Dickhoff, W.W. 1980. The parr-smolt transformation (smoltification) and seawater adaptation in salmonids: a review of selected literature. *Aquaculture* 21: 1-37.
- Folmar, L.C., Craddock, D.R., Blackwell, J.W., Joyce, G. & Hodgins, H.O. 1981. Effects of petroleum exposure on predatory behavior of coho salmon (*Oncorhynchus kisutch*). *Bull. Environ. Contam. And Toxic.* 27: 458-462.
- Forsman, L., Pirhonen, J. & Soivio, A. 1998. Effect of long-term stress on the smolting of two forms of brown trout (*Salmo trutta* L.). *Aquaculture* 168: 49-55.
- Foster, L.C. & Dunn, R.T. 1974. Single-antibody technique for radioimmunoassay of cortisol in extracted serum or plasma. *Clin. Chem.* 20: 365-368.
- Fried, S.M., McCleave, J.D. & LeBar, G.W. 1978. Seaward migration of hatchery-reared Atlantic salmon, *Salmo salar*, smolts in the Penobscot River estuary, Maine: riverine movements. *J. Fish. Res. Bd. Can.* 35: 76-87.

- Fryer, J.L. & Sanders, J.E. 1981. Bacterial kidney disease of salmonid fish. *Annu. Rev. Microbiol.* 35: 273-298.
- Fryer, J.L. & Lannan, C.N. 1993. The history and current status of *Renibacterium salmoninarum*, the causative agent of bacterial kidney, disease in Pacific salmon. *Fish. Res.* 17: 15-33.
- Gerstner, C.L. 1998. Use of substratum ripples for flow refuging by Atlantic cod, *Gadus morhua*. *Env. Biol. Fish.* 51: 455-460.
- Gessel, M.H., Williams, J.G., Brege, D.A., Krcma, R. F. & Chambers, D.R. 1991. Juvenile salmonid guidance at the Bonneville Dam second powerhouse, Columbia River, 1983-1989. *N. Amer. J. Fish. Man.* 11: 400-412.
- Giattina, D.S., Cairns Jr., J. & Stevens, D.G. 1981. Comparison of laboratory and field avoidance behavior of fish in heated chlorinated water. *Trans. Amer. Fish. Soc.* 110: 526-535.
- Giorgi, A.E., Hillman, T.W., Stevenson, J.R., Hays, S.G. & Peven, C.M. 1997. Factors that influence the downstream migration rates of juvenile salmon and steelhead through the hydroelectric system in the mid-Columbia River basin. *N. Amer. J. Fish. Man.* 17: 268-282.
- Greenstreet, S.P.R., Morgan, R.I.G., Barnett, S. & Redhead, P. 1993. Variation in the numbers of shags *Phalacrocorax aristotelis* and common seals *Phoca vitulina* near the mouth of an Atlantic salmon *Salmo salar* river at the time of the smolt run. *J. Anim. Ecol.* 1993. 62: 565-576.
- Gregory, R.S. 1993. Effect of turbidity on the predator avoidance behavior of juvenile chinook salmon (*Oncorhynchus tshawytscha*). *Can. J. Fish. Aquat. Sci.* 5: 241-246.

- Groot, C., Margolis, L. & Clarke, W.C. (eds.). 1995. Physiological ecology of Pacific salmon. UBC Press, Vancouver, Canada.
- Healy, M.C. 1991. Life history of chinook salmon (*Oncorhynchus tshawytscha*). In: Groot, C. & Margolis, L. (eds.), Pacific salmon life histories. UBC Press, Vancouver, Canada, pp. 311-394.
- Hoar, W.S. 1976. Smolt transformation: evolution, behavior, and physiology. J. Fish. Res. Bd. Can. 33: 1234-1252.
- Hoar, W.S. 1988. The physiology of smolting salmonids. pp. 275-343 In: Hoar, W.S. & Randall, D.J., (eds.), Fish physiology, Volume XIB. Academic Press, San Diego, California.
- Huntingford, F.A. & De Leaniz, C.G. 1997. Social dominance, prior residence and the acquisition of profitable feeding sites in juvenile Atlantic salmon. J. Fish Biol. 51: 1009-1014.
- Hutchinson, M.J. & Iwata, M. 1997. A comparative analysis of aggression in migratory and non-migratory salmonids. Env. Biol. Fish. 50: 209-215.
- Hutchinson, M.J. & Iwata, M. 1998. Effect of thyroxine on the decrease of aggressive behavior of four salmonids during the parr-smolt transformation. Aquaculture 168: 169-175.
- Iwama, G.K. 1992. Smolt quality: a special section. World Aquacult. 23: 38-39.
- Iwama, G.K., Pickering, A.D., Sumpter, J.P. & Schreck, C.B. (eds.). 1997. Fish stress and health in aquaculture. Society for Experimental Biology Seminar Series: 62. Cambridge University Press, Cambridge.

- Iwata, M. 1995. Downstream migratory behavior of salmonids and its relationship with cortisol and thyroid hormones: a review. *Aquaculture* 135: 131-139.
- Iwata, M. & Komatsu, S. 1984. Importance of estuarine residence for adaptation of chum salmon (*Oncorhynchus keta*) fry to seawater. *Can. J. Fish. Aquat. Sci.* 41: 744-749.
- Iwata, M., Yamauchi, K., Nishioka, R.L. & Bern, H.A. 1990. Effects of thyroxine, growth hormone and cortisol on salinity preference of juvenile coho salmon (*Oncorhynchus kisutch*). *Mar. Behav. and Physiol.* 17: 191-201.
- Jain, V.K. & Sahai, S. 1994. Phototactic response of some fishes. *Env. Ecol.* 12: 123-127.
- Järvi, T. 1989a. The effects of osmotic stress on the anti-predator behavior of Atlantic salmon smolts: a test of the 'maladaptive anti-predator behavior' hypothesis. *Nord. J. Fresh. Res.* 65: 71-79.
- Järvi, T. 1989b. Synergistic effect on mortality in Atlantic salmon, *Salmo salar*, smolt caused by osmotic stress and presence of predators. *Env. Biol. Fish.* 26: 149-152.
- Jay, D.A. & Smith, J.D. 1990. Circulation, density distribution and neap-spring transitions in the Columbia River estuary. *Prog. Ocean.* 25: 81-112.
- Johnsen, R.C., Wood, L.A. & Smith, W.W. 1986. Monitoring of downstream salmon and steelhead at federal hydroelectric facilities - 1986. Annual report. Bonneville Power Administration, Portland, OR.
- Jones, K.A., Brown, S.B. & Hara, T.J. 1987. Behavioral and biochemical studies of onset and recovery from acid stress in Arctic char (*Salvelinus alpinus*). *Can. J. Fish. Aquat. Sci.* 44: 373-381.

- Jones, K.A. & Hara, T.J. 1988. Behavioral alterations in Arctic charr (*Salvelinus alpinus*) briefly exposed to sublethal chlorine levels. *Can. J. Fish. Aquat. Sci.* 45: 749-753.
- Kelsey, D.A. 1997. Effects of steelhead trout (*Oncorhynchus mykiss*) on chinook salmon (*O. tshawytscha*) behavior and physiology. Master's thesis. Oregon State University, Corvallis, OR.
- Kennedy, G.J.A. & Greer, J.E. 1988. Predation by cormorants, *Phalacrocorax carbo* (L.), on the salmonid populations of an Irish river. *Aquac. Fish. Man.* 19: 159-170.
- Knights, B.C., Johnson, B.L. & Sandheinrich, M.B. 1995. Response of bluegills and black crappies to dissolved oxygen, temperature, and current in backwater lakes of the upper Mississippi River during winter. *N. Amer. J. Fish. Man.* 15: 390-399.
- Korver, R.M. & Sprague, J.B. 1989. Zinc avoidance by fathead minnow (*Pimephalas promelas*): computerized tracking and greater ecological relevance. *Can. J. Fish. Aquat. Sci.* 46: 494-502.
- Kramer, D. L. 1987. Dissolved oxygen and fish behavior. *Env. Biol. Fish.* 18: 81-92.
- Krause, J., Staaks, G. & Mehner, T. 1998. Habitat choice in shoals of roach as a function of water temperature and feeding rate. *J. Fish Biol.* 53: 377-386.
- Krohn, W.B. & Blackwell, B.F. 1996. The double-crested cormorant in Maine. *Maine Fish and Wild.* 3: 8-12.

- Kruzynski, G.M. & Birtwell, I.K. 1994. A predation assay to quantify the ecological significance of sublethal responses of juvenile chinook salmon (*Oncorhynchus tshawytscha*) to the antisapstain fungicide TCMTB. *Can. J. Fish. Aquat. Sci.* 51: 1780-1790.
- Larsson, P.-O. 1985. Predation on migrating smolt as a regulating factor in Baltic salmon, *Salmo salar* L., populations. *J. Fish Biol.* 26: 391-397.
- Ledgerwood, R.D., Thrower, F.P. & Dawley, E.M. 1990. Diel sampling of migratory juvenile salmonids in the Columbia River estuary. *Fish. Bull.* 89:69-78.
- Li, H.W. & Brocksen, R.W. 1977. Approaches to the analysis of energetic costs of intraspecific competition for space by rainbow trout (*Salmo gairdneri*). *J. Fish Biol.* 11: 329- 341.
- Long, C. 1968. Diel movements and vertical distribution of juvenile anadromous fishes in turbine intakes. *Fish. Bull.* 66: 599-609.
- MacDonald, J.S., Levings, C.D., McAllister, C.D., Fagerlund, U.H.M. & McBride, J.R. 1988. A field experiment to test the importance of estuaries for chinook salmon (*Oncorhynchus tshawytscha*) survival: short-term results. *Can. J. Fish. Aquat. Sci.* 45: 1366-1377.
- Madsen, S.S. 1990. Effect of repetitive cortisol and thyroxine injections on chloride cell number and Na<sup>+</sup>/K<sup>+</sup>-ATPase activity in gills of freshwater acclimated rainbow trout, *Salmo gairdneri*. *Comp. Biochem. and Phys., A.* 95A: 171-175.
- Maes, J., Van Damme, P.A., Taillieu, A. & Ollevier, F. 1998. Fish communities along an oxygen-poor salinity gradient. *J. Fish Biol.* 52: 534-546.

- Matthews, G.M., Park, D.L., Achord, S. & Ruehle, T.E. 1986. Static seawater challenge test to measure relative stress levels in spring chinook salmon smolts. *Trans. Amer. Fish. Soc.* 115: 236-244.
- Maule, A.G., Schreck, C.B., Bradford, C.S. & Barton, B.A. 1988. Physiological effects of collecting and transporting juvenile chinook salmon past dams on the Columbia River. *Trans. Amer. Fish. Soc.* 117: 245-261.
- Maule, A.G., Rondorf, D.W., Beeman, J. & Haner, P. 1996. Incidence of *Renibacterium salmoninarum* infections in juvenile hatchery spring chinook salmon in the Columbia and Snake Rivers. *J. Aquat. Anim. Health* 8: 37-46.
- Mazeaud, M.M., Mazeaud, F. & Donaldson, E.M. 1977. Primary and secondary effects of stress in fish: some new data with a general review. *Trans. Am. Fish. Soc.* 106: 201-212.
- McCleave, J.D. 1978. Rhythmic aspects of estuarine migration of hatchery-reared Atlantic salmon (*Salmo salar*) smolts. *J. Fish. Biol.* 12: 559-570.
- McCormick, S.D., Shrimpton, J.M., Carey, J.B., O'Dea, M.F., Sloan, K.E., Moriyama, S. & Björnsson, B.Th. 1998. Repeated acute stress reduces growth rate of Atlantic salmon parr and alter plasma levels of growth hormone, insulin-like growth factor I and cortisol. *Aquaculture* 168: 221-235.
- McDonald, G. & Milligan, L. 1997. Ionic, osmotic and acid-base regulation in stress. pp. 119-144. *In*: Adams, S.M. (ed.), *Biological indicators of stress in fish*. American Fisheries Society Symposium 8.
- McEwen, B.S. 1998. Protective and damaging effects of stress mediators. *New Eng. J. Med.* 338: 171-179.

- McGreer, J.C., Baranyi, L. & Iwama, G.K. 1991. Physiological response to challenge tests in six stocks of coho salmon (*Oncorhynchus kisutch*). *Can. J. Fish. Aquat. Sci.* 48: 1761-1771.
- McInerney, J.E. 1964. Salinity preference: an orientation mechanism in salmon migration. *J. Fish. Res. Bd. Can.* 21: 995-1018.
- McMichael, G.A. & Pearsons, T.N. 1998. Effects of juvenile spring chinook salmon on growth and abundance of wild rainbow trout. *Trans. Amer. Fish. Soc.* 127: 261-274.
- McNicol, R.E., Bégout-Anras, M.-L. & Scherer, E. 1999. Influence of light preference on the avoidance response of lake whitefish, *Coregonus clupeaformis*, to cadmium. *Env. Biol. Fish.* 55: 295-306.
- Meldrim, J.W. & Fava Jr., J.A. 1977. Behavioral avoidance responses of estuarine fishes to chlorine. *Ches. Sci.* 18: 154-157.
- Mesa, M.G. 1994. Effects of multiple acute stressors on the predator avoidance ability and physiology of juvenile chinook salmon. *Trans. Amer. Fish. Soc.* 123:786-793.
- Mesa, M.G., Poe, T.P., Maule, A.G. & Schreck, C.B. 1998. Vulnerability to predation and physiological stress response in juvenile chinook salmon (*Oncorhynchus tshawytscha*) experimentally infected with *Renibacterium salmoninarum*. *Can. J. Fish. Aquat. Sci.* 55: 1599-1606.
- Mesa, M.G., Maule, A.G., Poe, T.P. & Schreck, C.B. 1999. Influence of bacterial kidney disease on smoltification in salmonids: is it a case of double jeopardy? *Aquaculture* 174: 25-41.
- Moles, A. 1997. Effect of bacterial kidney disease on saltwater adaptation of coho salmon smolts. *J. Aquat. Anim. Health* 9: 230-233.



- Moore, A., Ives, M., Scott, M. & Bamber, S. 1998. The migratory behavior of sea trout (*Salmo trutta* L.) in the estuary of the River Conway, North Wales. *Aquaculture* 168: 57-68.
- Moser, M.L., Olson, A.F. & Quinn, T.P. 1991. Riverine and estuarine migratory behavior of coho salmon (*Oncorhynchus kisutch*) smolts. *Can. J. Fish. Aquat. Sci.* 48: 1670-1678.
- Muir, W.D., Giorgi, A.E. & Coley, T.C. 1994a. Behavioral and physiological changes in yearling chinook salmon during hatchery residence and downstream migration. *Aquaculture* 127: 69-82.
- Muir, W.D., Zaugg, W.S., Giorgi, A.E. & McCutcheon, S. 1994b. Accelerating smolt development and downstream movement in yearling chinook salmon with advanced photoperiod and increased temperature. *Aquaculture* 123: 387-399.
- Myers, J.M., Kope, R.G., Bryant, G.J., Teel, D., Lierheimer, L.J., Wainwright, T.C., Grand, W.S., Waknitz, F.W., Neely, K., Lindley, S.T. & Waples, R.S. 1998. Status review of chinook salmon from Washington, Idaho, Oregon and California. U.S. Dept. Commerce, NOAA Tech. Memo. NMFS-NWFSC-35, 443 pp.
- Nakano, S. 1995a. Competitive interactions for foraging microhabitats in a size-structured interspecific dominance hierarchy of two sympatric stream salmonids in a natural habitat. *Can. J. Zool.* 73: 1845-1854.
- Nakano, S. 1995b. Individual differences in resource use, growth and migration under the influence of a dominance hierarchy in fluvial red-spotted masu salmon in a natural habitat. *J. Anim. Ecol.* 64: 75-84.
- National Research Council. 1996. Upstream: salmon and society in the Pacific Northwest. National Academy Press, Washington, D.C.

- Olla, B.L., Davis, M.W. & Schreck, C.B. 1992. Comparison of predator avoidance in salmon. *Trans. Am. Fish. Soc.* 1221: 544-547.
- Olla, B.L., Davis, M.W., Ryer, C.H. & Sogard, S.M. 1996. Behavioral determinants of distribution and survival in early stages of walleye pollock, *Theragra chalcogramma*: a synthesis of experimental studies. *Fish. Ocean.* 5(Suppl. 1): 167-178.
- Pascho, R.J., Elliott, D.G. & Streufert, J.M. 1991. Brood stock segregation of spring chinook salmon by use of the enzyme-linked immunosorbent assay (ELISA) and the fluorescent antibody technique (FAT) affects the prevalence and levels of *Renibacterium salmoninarum* infection in progeny. *Dis. Aquat. Org.* 12: 25-40.
- Patino, R., Schreck, C.B., Banks, J.L. & Zaugg, W.S. 1986. Effects of rearing conditions on the developmental physiology of smolting coho salmon. *Trans. Amer. Fish. Soc.* 115: 828-837.
- Pavlov, D.S., Sadkovskii, R.V., Kostin, V.V. & Lupandin, A.I. 1997. Influence of photo, thermo and barogradients on cyprinid fry fish behavior and vertical distribution. *J. Ichthyol.* 37: 66-71.
- Pearcy, W.C. 1992. *Ocean ecology of North Pacific salmon*, University of Washington Press.
- Pickering, A.D. (ed.). 1981. *Stress and Fish*. Academic Press Inc., New York.
- Pickering, A.D., Pottinger, T.G. & Christie, P. 1982. Recovery of the brown trout, *Salmo trutta* L., from acute handling stress: a time-course study. *J. Fish Biol.* 20: 229-244.

- Pitcher, T.J. 1986. Functions of shoaling behavior in teleosts. pp. 294-337. *In*: Pitcher, T.J. (ed.), The behavior of teleost fish. The Johns Hopkins Univ. Press, Baltimore.
- Poulin, R. 1993. Age-dependent effects of parasites on anti-predator responses in two New Zealand freshwater fish. *Oecologia* 96: 431-438.
- Prunet, P., Boeuf, G., Bolton, J.P. & Young, G. 1989. Smoltification and seawater adaptation in Atlantic salmon (*Salmo salar*): prolactin, growth hormone, and thyroid hormones. *Gen Comp. Endocrinol.* 74:355-364.
- Redding, J.M. & Schreck, C.B. 1983. Influence of ambient salinity on osmoregulation and cortisol concentration in yearling coho salmon during stress. *Trans. Am. Fish. Soc.* 112: 800-807.
- Redding, J.M., Schreck, C.B., Birks, E.B. & Ewing, R.D. 1984. Cortisol and its effects on plasma thyroid hormone and electrolyte concentrations in fresh water and during seawater acclimation in yearling coho salmon, *Oncorhynchus kisutch*. *Gen. and Comp Endoc.* 56: 146-155.
- Richamn, N.H., III & Zaugg, W.S. 1987. Effects of cortisol and growth hormone on osmoregulation in pre- and desmoltified coho salmon (*Oncorhynchus kisutch*). *Gen. Comp. Endocrinol.* 65: 189-198.
- Roberts, B.L. 1992. Neural mechanisms underlying escape behavior in fishes. *Rev. Fish Biol. Fish.* 2: 243-266.
- Russell, I.C., Dare, P.J., Eaton, D.R. & Armstrong, J.D. 1996. Assessment of the problem of fish-eating birds in inland fisheries in England and Wales. Report to the Ministry of Agriculture, Fisheries and Food (MAFF Project VC0104).

- Ryding, K.E. & Skalski, J.R. 1999. Multivariate regression relationships between ocean conditions and early marine survival of coho salmon (*Oncorhynchus kisutch*). *Can J. Fish. Aquat. Sci.* 56: 2374-2384.
- Sanders, J.E., Long, J.J., Arakawa, C.K., Bartholomew, J.L. & Rohovec, J.S. 1992. Prevalence of *Renibacterium salmoninarum* among downstream-migrating salmonids in the Columbia River. *J. Aquat. Anim. Health* 4: 72-75.
- Schreck, C.B. 1982. Stress and rearing of salmonids. *Aquaculture* 28: 241-249.
- Schreck, C.B. 1990. Physiological, behavioral, and performance indicators of stress. pp. 29-37. *In: Adams, S.M. (ed.), Biological indicators of stress in fish. American Fisheries Society Symposium* 8.
- Schreck, C.B. 2000. Accumulation and long-term effects of stress in fish. pp. 147-158. *In: Moberg, G.P. & Mench, J.A. (eds.), The biology of animal stress. CAB International, Wallingford.*
- Schreck, C.B., Olla, B.L. & Davis, M.W. 1997. Behavioral responses to stress. pp. 145-170. *In: Iwama, G.K., Pickering, A.D., Sumpter, J.P. & Schreck, C.B. (eds.), Fish stress and health in aquaculture, Society for Experimental Biology Seminar Series, Cambridge University Press, New York.*
- Sekine, M., Imai, T. & Ukita, M. 1997. A model of fish distribution in rivers according to their preference for environmental factors. *Ecol. Model.* 104: 215-230.
- Seyle, H. 1950. Stress and the general adaptation syndrome. *Brit. Med. Jour.* 1: 1383-1392.

- Sheridan, M.A. 1986. Effects of thyroxin, cortisol, growth hormone, and prolactin on lipid metabolism of coho salmon, *Oncorhynchus kisutch*, during smoltification. *Gen. Comp. Endocrinol.* 64: 220-238.
- Shrimpton, J.M. & McCormick, S.D. 1998. Seasonal differences in cortisol and gill corticosteroid receptors in upper and lower mode juvenile Atlantic salmon. *Aquaculture* 168: 205-219.
- Shumway, C. 1999. A neglected science: applying behavior to aquatic conservation. *Env. Bio. Fish.* 55: 183-201.
- Shuter, B.J. 1990. Population-level indicators of stress. pp. 145-166. *In: Adams, S.M. (ed.), Biological indicators of stress in fish. American Fisheries Society Symposium* 8.
- Sigismondi, L.A. & Weber, L.J. 1988. Changes in avoidance response time of juvenile chinook salmon exposed to multiple acute handling stresses. *Trans. Am. Fish. Soc.* 117: 196-201.
- Smith, J.R. 1974. Distribution of seaward migrating chinook salmon and steelhead trout in the Snake River above Lower Monumental Dam. *Mar. Fish. Rev.* 36: 42-45.
- Sogard, S.M. & Olla, B.L. 1993. Effects of light, thermoclines and predator presence on vertical distribution and behavioral interactions of juvenile walleye pollock, *Theragra chalcogramma* Pallas. *J. Exp. Mar. Biol. Ecol.* 167: 179-195.
- Stasko, A.B. 1975. Progress of migrating salmon (*Salmo salar*) along an estuary, observed by ultrasonic tracking. *J. Fish. Biol.* 7: 329-338.

- Strange, R.J. & Schreck, C.B. 1980. Seawater and confinement alters survival and cortisol concentration in juvenile chinook salmon. *Copeia* 1980: 351-353.
- Suter, W. 1995. The effect of predation by wintering cormorants *Phalacrocorax carbo* on grayling *Thymallus thymallus* and trout (Salmonidae) populations: two case studies from Swiss rivers. *J. Appl. Ecol.* 32: 29-46.
- Taylor, E.B. 1990a. Phenotypic correlates of life-history variation in juvenile chinook salmon, *Oncorhynchus tshawytscha*. *Journal of Animal Ecology* 59: 455-468.
- Taylor, E.B. 1990b. Variability in agonistic behavior and salinity tolerance between and within two populations of juvenile chinook salmon, *Oncorhynchus tshawytscha*, with contrasting life histories. *Can. J. Fish. Aquat. Sci.* 47: 2172-2180.
- Temple, G.K. & Johnston, I.A. 1997. The thermal dependence of fast-start performance in fish. *J. Therm. Biol.* 22: 391-401.
- Thorpe, J.E. 1994. Salmonid fishes and the estuarine environment. *Estuaries* 17: 76-93.
- Tinbergen, N. 1963. On aims and methods of ethology. *Zeitschrift für Tierpsychologie* 20. pp. 410-433. *In*: Houck, L.D. & Drickamer, L.C. (eds.), 1996. *Foundations of animal behavior: classic papers with commentaries*, University of Chicago Press, Chicago.
- Tinus, C.A & Reeves, G.H. 2001. Redside shiner (*Richardsonius balteatus*) shoals provide a behavioral competitive refuge for subordinate juvenile steelhead trout (*Oncorhynchus mykiss*). *Can. J. Fish. Aquat. Sci.* 58: 319-324.

- Tytler, P., Thorpe, J.E. & Shearer, W.M. 1978. Ultrasonic tracking of the movements of Atlantic salmon smolts (*Salmo salar* L.) in the estuaries of two Scottish rivers. *J. Fish. Biol.* 12: 575-586.
- Wagner, C.M. & Austin, H.M. 1999. Correspondence between environmental gradients and summer littoral fish assemblages in low salinity reaches of the Chesapeake Bay, USA. *Mar. Ecol. Prog. Ser.* 177: 197-212.
- Wagner, H.H., F.P. Conte & J.L. Fessler. 1969. Development of osmotic and ionic regulation in two races of chinook salmon *Oncorhynchus tshawytscha*. *Comp. Biochem. Physiol.* 29: 325 - 341.
- Webb, P.W. & Zhang, H. 1994. The relationship between responsiveness and elusiveness of heat-shocked goldfish (*Carassius auratus*) to attacks by rainbow trout (*Oncorhynchus mykiss*). *Can. J. Zool.* 72: 423-426.
- Wedemeyer, G.A. 1972. Some physiological consequences of handling stress in the juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*). *J. Fish. Res. Bd. Can.* 33: 2699-2702.
- Wedemeyer, G.A., Saunders, R.L. & Clarke, W.C. 1980. Environmental factors affecting smoltification and early marine survival of anadromous salmonids. *Mar. Fish. Rev.* 42: 1-14.
- Wedemeyer, G.A. & McLeay, D.J. 1981. Methods for determining the tolerance of fishes to environmental stressors. pp. 247-276. *In: Pickering, A.D. (ed.), Stress and fish.* Academic Press Inc., New York.
- Wedemeyer, G.A., Barton, B.A. & McLeay, D.J. 1990. Stress and acclimation. pp. 451-489. *In: Schreck, C.B. and Moyle, P.B. (eds.), Methods for fish biology,* American Fisheries Society, Bethesda, Maryland.

- Williams, P.J., Brown, J.A., Gotceitas, V. & Pepin, P. 1996. Developmental changes in escape response performance of five species of marine larval fish. *Can. J. Fish. Aquat. Sci.* 53: 1246-1253.
- Wood, C.C. 1987a. Predation of juvenile Pacific salmon by the common merganser (*Mergus merganser*) on eastern Vancouver Island. I: Predation during the seaward migration. *Can. J. Fish. Aquat. Sci.* 44:941-949.
- Wood, C.C. 1987b. Predation of juvenile Pacific salmon by the common merganser (*Mergus merganser*) on eastern Vancouver Island. II: Predation of stream-resident juvenile salmon by merganser broods. *Can. J. Fish. Aquat. Sci.* 44: 950-959.
- Yamamoto, T., Ueda, H. & Higashi, S. 1998. Correlation among dominance status, metabolic rate and otolith size in masu salmon. *J. Fish Biol.* 52: 281-290.
- Young, G., Björnsson, B.Th., Prunet, P., Lin, R.J., & Bern, H. 1989. Smoltification and seawater adaptation in coho salmon (*Oncorhynchus kisutch*): plasma prolactin, growth hormone, thyroid hormones and cortisol. *Gen. Comp. Endocrinol.* 74: 335-345.
- Zhou, T. & Weis, J.S. 1998. Swimming behavior and predator avoidance in three populations of *Fundulus heteroclitus* larvae after embryonic and/or larval exposure to methylmercury. *Aquat. Toxicol.* 43: 131-148.
- Zigler, S.J. & Dewey, M.R. 1995. Phototaxis of larval and juvenile northern pike. *N. Amer. J. Fish. Man.* 15: 651-653.



**APPENDIX**

## Temporal Patterns In Saltwater Preference Of Hatchery-Reared Juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) Smolts

### INTRODUCTION

Between February 1997 and February 2000, the experiments described in this dissertation were conducted to examine various factors that affected the willingness of juvenile chinook salmon to enter and remain in salt water. These laboratory experiments were conducted in large fiberglass tanks (1.83 x 0.66 x 0.60 m, 757 L) in which a stable, vertical salinity gradient could be established. In order to examine the temporal patterns in saltwater preference (SWP) of juvenile chinook salmon, the data from all the SWP tests are compiled here. Included are results from: control tanks used in experiments, preliminary studies, experiments not reported here, and routine monitoring of SWP. Results from tanks that received treatments (such as disease or stress) that were shown to decrease preference for salinity are not included. The fish whose SWP is presented here were believed to be healthy and unstressed. Due to the temporal cycling of the smoltification process, it is likely that levels of smoltification varied considerably. SWP data were collected for three years. In 1997, SWP tests were conducted from 14 Feb - 21 Mar, and from 21 Sep - 01 Oct. In 1998, tests were conducted from 08 Jan - 29 Mar, and from 04 Nov - 19 Dec. In 1999, tests were conducted

nearly yearlong excluding Jun, Aug and Nov. In 2000, tests were conducted in Jan and Feb.

Figure A.1 presents SWP results from 111 individual trials. Each point represents the percent of fish/tank holding in salt water 60 minutes after the beginning of saltwater introduction. At this time, all the salt water had flowed into the experimental tank, and a distinct halocline was established in which the bottom half of the water column was saline (30 ppt). In Figure A.2, the same data are compiled across all years to illustrate annual patterns.

Tests were conducted with either subyearling (age 0) or yearling (1+) chinook salmon. With the exception of six tanks (two each on 14 Feb, 03 Mar and 17 Mar 1997; from Gnat Creek Hatchery), all fish originated from the South Santiam or Marion Forks Hatcheries. The fish from the South Santiam and Marion Forks Hatchery were either spawned at the Oregon State University (OSU) Fish Performance and Genetics Laboratory, or were brought in as fingerlings (5 - 10 cm) and reared in that facility. These two groups of fish were treated as separate stocks in the analysis, to determine if differences in rearing conditions affected SWP. All fish were reared under natural photoperiod conditions, and artificial lighting during experiments was regulated to reflect the natural photoperiod.

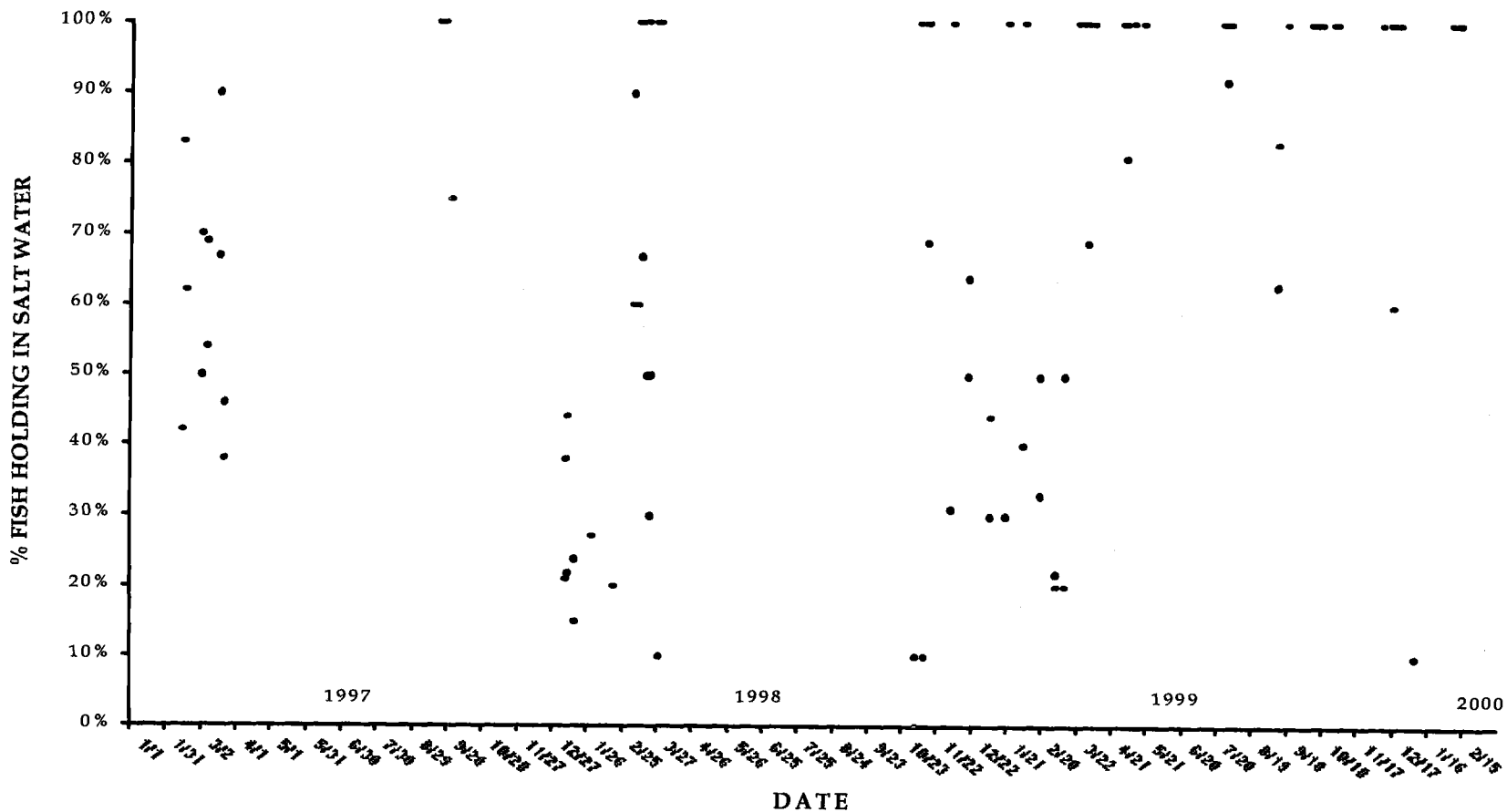
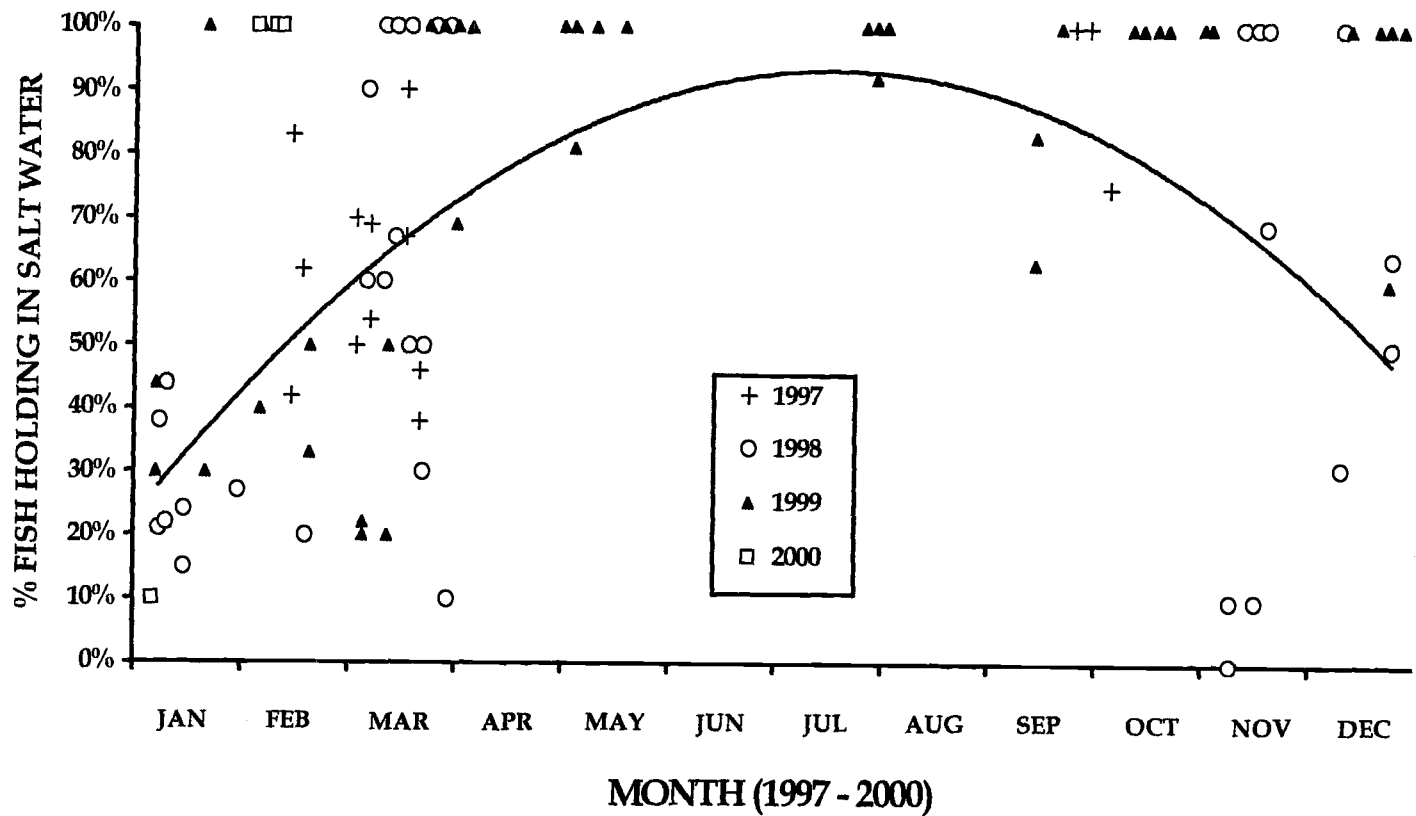


Figure A.1. Saltwater preference of juvenile chinook salmon in laboratory tests conducted from 1997-2000. Each point represents the percentage of fish/tank (n = 111 tanks with 9-13 fish) that were holding in salt water 60 minutes after the initiation of saltwater introduction. At this time, the top half of the water column was fresh and the bottom half was saline.



**Figure A.2.** Saltwater preference of juvenile chinook salmon in laboratory tests conducted from 1997-2000. Results are compiled by month to illustrate temporal patterns in salinity preference. Each point represents the percentage of fish/tank ( $n = 111$  tanks) that were holding in salt water 60 minutes after the initiation of saltwater introduction. At this time, the top half of the water column was fresh and the bottom half was saline. A second-order polynomial curve was fitted to the data ( $R^2 = 0.1798$ ).

## STATISTICAL ANALYSIS

The primary purpose of this analysis was to determine the temporal variation and patterns in SWP. It was also important to account for the factors that varied between the tanks - age of the fish, the stock of fish used, and the year the trial was conducted. Prior to statistical analysis, cross tabulations revealed that sampling was not evenly distributed among the four variables. For example, fish from Gnat Creek hatchery were only used in February of 2000. In 1997, 1998 and 2000, no sampling was conducted from April - September. Fish of age 0 were never used from January to August. To decrease these confounding interactions, results from the seven tanks tested in 2000 were removed from the data set, as were the results from the six tanks that used fish from Gnat's Creek Hatchery. In addition, the variable "month" was found to be seriously confounded with the other independent variables, stock, age and year. For this reason the months were categorized into seasons. Three season categories were created based upon Fisher's LSD *post hoc* test after one-way ANOVA between month and the arcsine of the percent SWP ( $P = 0.0001$ ; see Table A.1 for results of the *post hoc* test; Figure A.3). January comprised the first season (Winter;  $SWP = 38 \pm 38\%$ ); the second season included February and

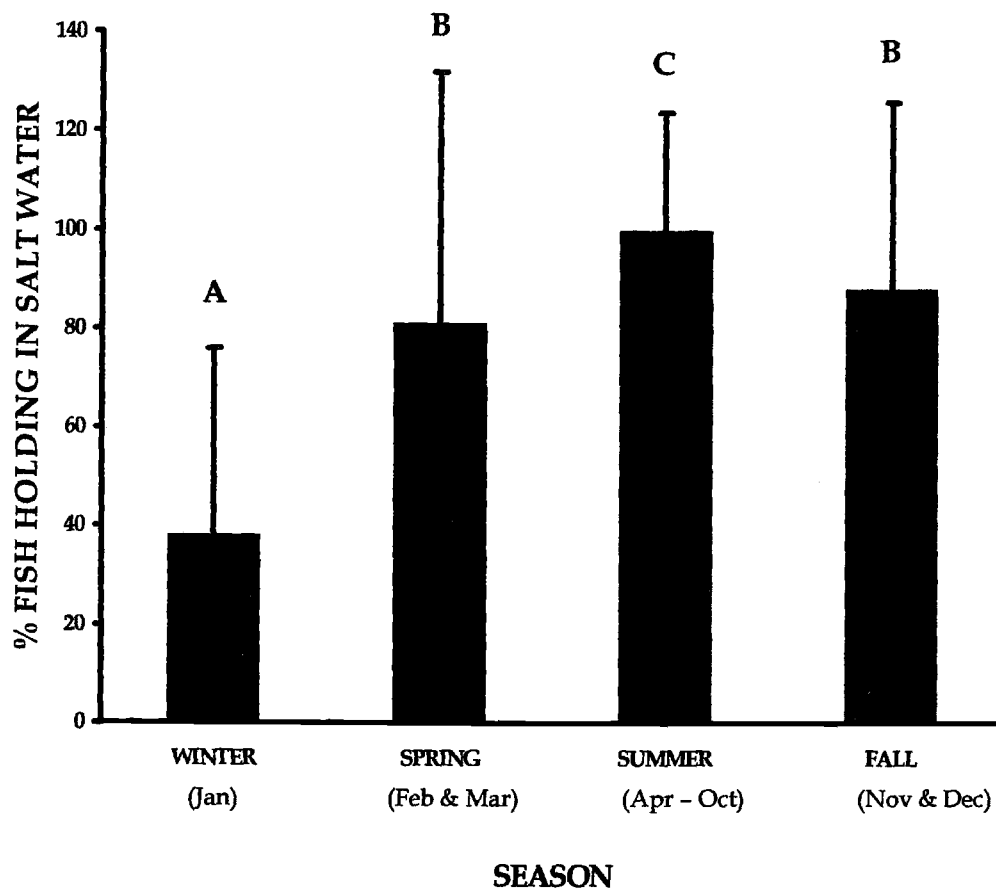


Figure A.3. Percent of fish showing preference for salt water in experiments conducted from 1997 - 1999. Four seasons categories were designated based upon *post hoc* testing after one-way ANOVA between month and saltwater preference. Seasons with shared letters are statistically similar.

**Table A.1.** Results of Fisher's LSD *post hoc* test to determine homogeneity in saltwater preference of juvenile chinook salmon among months. The test was conducted after one-way ANOVA indicated a significant effect of month on preference for salt water ( $P = 0.0001$ ). X is used to designate months that are grouped into the same season.

Month	Number Of Experimental Tanks	Mean % Fish Preferring Salt Water	Homogenous Groups		
Jan	12	38.0	X		
Nov	8	78.7	X		
Mar	32	79.0	X		
Feb	14	86.2	X	X	
Dec	11	93.1	X	X	X
Sep	6	97.0	X	X	X
May	6	99.5		X	X
Jul	6	99.8			X
Oct	12	99.8			X
Apr	4	100.0		X	X

Total = 111



March (Spring; SWP =  $83 \pm 51\%$ ); the third season contained April - October (Summer; SWP =  $99 \pm 24\%$ ); the fourth season was November and December (Fall; SWP = 84%). Spring and Fall were statistically similar. A General Linear Model Univariate ANOVA was then applied to determine the effects of season, age, stock and year on the SWP of juvenile chinook salmon. Homogenous groups were then identified using Fisher's LSD test.

## RESULTS

Stock ( $P = 0.2390$ ) and age ( $P = 0.3790$ ) were not significant variables in the model, while year ( $P = 0.0010$ ) and season ( $P = 0.0170$ ) were. The Fisher's LSD test found that 1999 had an overall higher SWP value compared to 1997 and 1998. This effect is explained by bias in sampling, as 1999 was the only year in which tests were conducted in the summer months when SWP was at its peak. Season (as described in the previous section) was then left as the only explanatory variable for the variability in SWP found in these studies.

## DISCUSSION

Preference for salt water varied throughout the year, and was highest between April and October. The analysis indicated that the fish from the South Santiam (both hatchery reared and spawned at the OSU Laboratory) and Marion Forks Hatcheries displayed similar annual patterns in SWP. This is not surprising as both are Willamette River chinook salmon stocks, and therefore it is probable that inter-stock variation in SWP is minimal.

The general pattern of the preference data is consistent with current understanding of spring chinook salmon life history. After hatching, spring chinook salmon reside in stream habitats for 14 - 16 months. Smoltification occurs several months prior to and during outmigration. In the Columbia River Basin, outmigration occurs primarily from April to June with a peak in May (Healy 1991). Juveniles that smolt, but do not outmigrate, will undergo parr reversion. This entails the loss of the physiological, behavioral and morphological characteristics that prepare them for saltwater residence, and the fish may revert to the freshwater form. Thus, in late fall and winter, experimental fish were expected to avoid salt water. Instead, high affinity for salt water was displayed throughout the year. Even in January, 38% (n = 12 tanks) of the fish entered and remained in salt water, and in eight of those tanks, 37% of the fish were still holding in saltwater another hour after saltwater

introduction was complete. This was an unexpectedly high percentage for this time of year when fish are expected to be freshwater adapted. Relatively high salinity preference was also displayed by fish in November and December.

This discrepancy between expected and observed behavior, may be explained by physiological mechanisms that have evolved in chinook salmon. Chinook salmon are divided into two races with very different life history strategies. Spring chinook salmon, also known as the stream-type race, spend one or more years in the freshwater environment before outmigration. Fall, or ocean-type, chinook salmon spend only a few days or months in freshwater before moving seaward and may reside in the estuarine environment for several months before moving into ocean (Healy 1991). The two races differ considerably in the size and age at which they enter salt water. Phenotypic variation within each of these races is also common and, in a stochastic environment, this likely serves to distribute the risk of high mortality across years (Healy 1991).

Interestingly, the ontogeny of hypoosmoregulation is similar in both races (Wagner et al., 1969). Individuals from three cohorts of both races were exposed, either immediately or gradually, to full-strength seawater over a 16-month period beginning immediately after hatching. Both races showed similar tolerance for salt water throughout the experiments. Neither spring nor fall chinook fry were able to tolerate direct transfer to seawater (30 ppt), and nearly

all fish less than 100 days old died after exposure. Fall chinook alevins were able to survive salinities of 15 - 20 ppt immediately after hatching. Tolerance to full strength salt water was achieved at 100 days post-hatching. Spring chinook alevins were not salinity tolerant, but by 40 days post-hatching about half were able to survive immediate transfer to 20 ppt seawater. Full tolerance to salt water was attained in fish over 140 days old. Both races showed high survival (>80%) in full strength seawater within 100 days of hatching if the transfer was gradual. Saltwater exposure trials were conducted for 540 days post-fertilization. Spring and fall chinook had nearly identical percent survival throughout the trials, and 230 days after hatching, survival was always greater than 50%. The highest percent survival (nearly 100%) was observed after 400 days. In comparable experiments, coho salmon (the closest phylogenetic relative of chinook salmon, Domanico et al. 1997) showed a chronologically similar development of salinity tolerance that was also permanent (Conte and Wagner 1965). Steelhead trout, in contrast, lost their acquired tolerance for salinity at 350 days, which corresponded to the end of their outmigration period (Conte et al. 1966). The overall conclusion of the authors was that both spring and fall chinook salmon juveniles can adapt to sea water very early in life, especially if acclimation to a hyperosmotic medium is gradual, and that this tolerance is maintained.

Most juvenile spring chinook salmon in the Wagner et al. (1969) study were not highly salt water tolerant, and mortality associated with immediate transfer to 30 ppt salt water was usually greater than 50% in young fish. However, the early onset and retention of salinity tolerance in a portion of these young chinook salmon, correlates to the salinity preference observed in the saltwater preference behavior trials. The ability of chinook salmon to tolerate salt water, even at times when they are "unsmolted," has been researched in other salmon species such as chum and coho salmon (Hoar 1988).

The tolerance and preference for salt water exhibited by salmon is a complex combination of environmental, developmental and physiological processes and it is likely that behavioral variability in saltwater preference is regulated by multiple genetic mechanisms. Spring and fall chinook salmon represent divergent phenotypes within the chinook salmon species. The genetic mechanisms underlying the behavioral and physiological differences expressed as different life history strategies between these two races are under investigation. Genetic differences in rheotaxis, growth rate, aggression (Taylor 1990a,b) and response to photoperiod (Clarke et al. 1992, Clarke et al. 1994) have been found between life history types of chinook salmon. Interestingly, intra-population variation made it difficult to assess inter-population differences in early saltwater tolerance between eight families (full sib progeny from single

male X female crosses) of chinook salmon juveniles displaying two life history strategies (Taylor 1990b).

In the Columbia River Basin, there is considerable phenotypic overlap between genetically distinct subpopulations of chinook salmon (Myers et al. 1998). For example, the timing of adult chinook salmon returns to natal rivers was not necessarily consistent with genetic subdivisions between races and straying between spring and fall types has been recorded. Additionally, the hatchery breeding practices may serve to mingle genes from the two life history types. Thus, it is likely that a considerable level of genetic heterogeneity exists for many phenotypes, including the response to salt water, of both races within the larger metapopulation.

The behavioral results of this study suggest that plasticity in tolerance and preference for salt water appears to have been conserved within the chinook salmon species as a whole. Within the spring chinook salmon studied, a small percentage of fish were willing to enter salt water at times when most of the population preferred freshwater. Perhaps, this reflects an adaptive mechanism to ensure that some of a cohort is able to survive premature outmigration. This may occur if flood events wash fish downstream too early, if drought conditions decrease available freshwater feeding territories, or if aggression (intra- and inter-specific) is unusually high in the stream environment (Healy 1991). Further research to investigate the relationships between the genetic and environmental

factors is needed to fully understand the temporal patterns that were observed in the saltwater preference of juvenile chinook salmon.

## ACKNOWLEDGEMENTS

I thank Dr. Don Holbert of East Carolina University, Greenville, NC, for his *gratis* statistical advice.



## LITERATURE CITED

- Clarke, W.C., Withler, R.E. & Shelbourn, J.E. 1992. Genetic control of life history pattern in chinook salmon (*Oncorhynchus tshawytscha*). *Can. J. Fish. Aquat. Sci.* 49: 2300-2306.
- Clarke, W.C., Withler, R.E. & Shelbourn, J.E. 1994. Inheritance of smolting phenotypes in backcrosses of hybrid stream-type X ocean-type chinook salmon (*Oncorhynchus tshawytscha*). *Estuaries* 17: 13-25.
- Conte, F.P. & Wagner, H.H. 1965. Development of osmotic and ionic regulation in juvenile steelhead trout *Salmo gairdneri*. *Comp. Biochem. Physiol.* 14: 603-620.
- Conte, F.P., Wagner, H.H., Fessler, J. & Gnose, C. 1966. Development of osmotic and ionic regulation in juvenile coho salmon *Oncorhynchus kisutch*. *Comp. Biochem. Physiol.* 18: 1-15.
- Domanico, M.J., Philips, R.B. & Oakley, T.H. 1997. Phylogenetic analysis of Pacific salmon (genus *Oncorhynchus*) using nuclear and mitochondrial DNA sequences. *Can. J. Fish. Aquat. Sci.* 54: 1865-1872.
- Healy, M.C. 1991. Life history of chinook salmon (*Oncorhynchus tshawytscha*). pp. 311-394. *In: Groot, C. and Margolis, L. (eds.), Pacific salmon life histories.* UBC Press, Vancouver, Canada.
- Hoar, W.S. 1976. Smolt transformation: evolution, behavior and physiology. *J. Fish. Res. Board Can.* 33: 1234-1252.

- Myers, J.M., Kope, R.G., Bryant, G.J., Teel, D., Lierheimer, L.J., Wainwright, T.C., Grand, W.S., Waknitz, F.W., Neely, K., Lindley, S.T. & Waples, R.S. 1998. Status review of chinook salmon from Washington, Idaho, Oregon and California. U.S. Dept. Commerce, NOAA Tech. Memo. NMFS-NWFSC-35, 443 pp.
- Taylor, E.B. 1990a. Phenotypic correlates of life-history variation in juvenile chinook salmon, *Oncorhynchus tshawytscha*. *Journal of Animal Ecology* 59: 455-468.
- Taylor, E.B. 1990b. Variability in agonistic behavior and salinity tolerance between and within two populations of juvenile chinook salmon, *Oncorhynchus tshawytscha*, with contrasting life histories. *Can. J. Fish. Aquat. Sci.* 47: 2172-2180.
- Wagner, H.H., Conte, F.P. & Fessler, J.L. 1969. Development of osmotic and ionic regulation in two races of chinook salmon, *Oncorhynchus tshawytscha*. *Comp. Biochem. Physiol.* 29: 325 - 341.