Freshwater growth and survival in AYK Chinook salmon, maternal health, and the ultimate effects on stock productivity

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Abstract: This study focuses on the freshwater factors affecting Western Alaska Chinook salmon productivity through three complementary studies: a retrospective study of salmon scales examining the relationship between freshwater growth and survival to adulthood, a study of the persistent effects of infection of adults with the parasite *Ichthyophonus* on their offspring, and laboratory rearing experiments supporting the first two studies by validating scale and otolith-based indicators of growth and comparing growth and survival between offspring from *Ichthyophonus*-infected and *Ichthyophonus*-free parents. All project components are progressing, although there have been some delays due to weak Chinook returns in 2011 and a rearing-facility malfunction. In the retrospective study, scale growth measurements from stocks on both the Yukon and Kuskokwim Rivers have been taken, and these data are being analyzed to determine patterns in growth over time and the relationship of growth to survival. *Ichthyophonus*-infected and *Ichthyophonus*-free fish have been collected, samples analyzed and compared, and some preliminary results are included here. However, sample sizes are small because a weak Chinook return in 2011 precluded some of our anticipated sampling. Laboratory rearing experiments for validation of the relationship between scales, otoliths, ration, and freshwater growth were successfully concluded. Scales and otoliths will be read in the near future, and the experimental data statistically analyzed to quantify the growth relationships. Laboratory comparisons of growth and survival of offspring of infected and healthy parents were not possible because of an equipment failure in the first year and the unavailability of eggs in the second year. We anticipate requesting an extension of support to finish the *Ichthyophonus*-focused component of our project, which has been hampered by these unforeseen events.

Component Title: Effect of *Ichthyophonus* infection on Yukon River Chinook salmon hatching success, growth, and survival

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Abstract

In recent years, Chinook salmon (*Oncorhynchus tshawytscha)* returns to the Yukon River have been inadequate. As a result, fisheries managers had to enforce unprecedented restrictions on Chinook salmon harvest for subsistence users and commercial fishermen, including a no-buy policy for Chinook salmon during chum salmon (*Oncorhynchus keta*) commercial openings, as well as reductions in subsistence fishing time. Reasons for these run failures are poorly understood, but *Ichthyophonus hoferi*, a protozoan parasite, is known to adversely affect stamina of adults during their spawning migration. In summer 2010, 51 Chinook salmon (27 females and 24 males) were collected, and in 2011, 32 Chinook salmon (15 females and 17 males) were sampled from the Salcha River, a tributary in the Yukon River drainage, to determine prevalence and effects of *Ichthyophonus* on hatching success and offspring quality. Eggs and milt were collected, and gametes of "healthy" and *Ichthyophonus*-infected parents were cross-fertilized and transported to the University of Alaska Fairbanks. Infection with *Ichthyophonus* was first determined by visual inspection of fish in the field and was then confirmed by culture of the parasite in cardiac muscle. Prevalence of *Ichthyophonus* continued to be low and was 7.8% (3 females, 1 male) in 2010 and 6.3% (2 males) in 2011. Fertilized ova were raised to the yolk-sac fry stage and dead eggs were enumerated and removed weekly. The average percent survival of eggs to hatching for *Ichthyophonus*-negative offspring was 41.02%, while the average percent survival of eggs to hatching for *Ichthyophonus*-positive offspring was lower at 24.44%, although not significant ($p>0.05$). Proximate composition (%water, %lipid, and %crude protein) and morphometric parameters (length, weight, and height of yolk-sac) of yolk-sac fry were measured to determine if "healthy" and infected crosses have the same energetic composition and grow at similar rates ($p>0.05$ for all parameters). Once hatched, yolk-sac fry have the same composition and chance of survival as offspring from "healthy" parents. Blood parameters were different between *Ichthyophonus*-infected and "healthy" adult fish, and organ damage indicators, e.g., creatine kinase (CK) and aspartate aminotransferase (AST) were unexpectedly higher in "healthy" salmon. Understanding the effect of pathogens, such as *Ichthyophonus*, on survival and growth of juvenile fish hatching from gametes of infected parents is critical to foster pro-active management of all salmon life stages.

Introduction/Background

Ichthyophonus hoferi (*Ichthyophonus* here after) is a marine-derived protozoan parasite infecting a variety of marine and anadromous fish species, including salmonids (McVicar, 1999; Kocan et al., 2004; Tierney and Farrell, 2004; Gavryuseava, 2007). In the 1990s, mass mortalities of Atlantic herring (*Clupea harengus*) were attributed to *Ichthyophonus* epizootics (Mellergaard and Spanggaard, 1997). Once infected with the pathogen, fish are not thought to recover and the majority will eventually die from the disease (Sindermann, 1965; Marty et al., 1998; Kocan et al., 1999; Hershberger et al., 2002). Recent poor production and low abundance of Arctic-Yukon-Kuskokwim (AYK) Chinook salmon stocks has been a concern to commercial and subsistence fishers and fisheries managers. This species is a staple of Alaska's fishery, and Yukon River Chinook salmon is particularly prized due to its high oil content. Over 60 Yukon River communities rely on salmon as part of their subsistence lifestyle, and the Chinook salmon commercial fishery often provides the only economic influx into lower Yukon River villages. Chinook salmon returns in 2007 through 2011 were lower than expected based on reported spawning escapements in the brood years (JTC, 2011). In response, a Chinook salmon directed commercial fishery on the Yukon River mainstem did not occur in 2008 and 2009 for the first time in almost a decade (JTC, 2011) and the subsistence fishery was curtailed by up to 50%. The management plan in 2009 included the protection of the first pulse of Chinook salmon entering the Yukon River and closure of all fisheries; early fish are thought to contain mainly salmon bound for Canadian tributaries. While severely impacting the U.S. subsistence fishery along the Yukon River, these actions proved successful in meeting the interim management escapement goal into Canada in 2009. In 2010, subsistence fishermen were again asked to voluntarily reduce their catch to meet treaty obligations (similar to 2008). Low Chinook salmon returns in 2011 again made management actions necessary, and two subsistence periods were cancelled. In addition, the reduced gillnet mesh size of 7.5-inch (adopted by the Alaska Board of Fisheries in 2010) was implemented for the first time. In the lower Yukon River, mesh size was further restricted to 6-inch at the end of June to protect a late pulse of Chinook salmon entering the river. These marked changes in salmon abundance in recent years raise questions about the possible involvement of disease in these declines, either due to pathogen-induced mortality, reduced fecundity, or the inability of Chinook salmon to successfully migrate and spawn in tributaries.

In the mid 1980's, *Ichthyophonus* was identified by the Alaska Department of Fish and Game (ADF&G) and the U.S. Fish and Wildlife Service (USFWS) after fishermen reports of white pustules on the heart, liver, and muscle began to increase. In addition, fishermen noticed an unpleasant fruity smell when affected fish were processed and noted failure of salmon strips to dry properly in smokehouses. *Ichthyophonus* was proposed as a newly emerging parasite in Alaskan salmonids and current evidence suggests that disease can have significant effects on pre-spawning mortality of Chinook salmon (Kocan et al. 2004, 2006, 2009). The potential for *Ichthyophonus* to cause significant pre-spawning mortality in Chinook salmon remains heavily debated. Direct studies utilizing radio-telemetry to track diseased fish to the spawning grounds are difficult as generally only robust fish in good condition are selected for transmitter implantation, inherently biasing outcomes about consequences of disease. However, the more important effects of *Ichthyophonus* on salmon spawning success, egg quality and juvenile survival remain poorly understood. Fish egg and embryo vitality is correlated to body condition of spawning females, and it has been shown that vitellogenesis is adversely influenced by high levels of corticosteroids (Pankhurst and van der Kraak, 2000; King et al., 2003). Even healthy Yukon River Chinook salmon make almost complete use of their considerable energy reserves to

undertake one of the longest salmon migrations in the world. In diseased fish, energy demands are increased due to high physiological stress and costs associated with the immune response. In herring, ichthyophoniasis is associated with reduced body reserves and emaciation (Rahimian, 1998). Lipids may therefore be re-routed from gonads of *Ichthyophonus*-positive fish to complete the spawning migration and then they either produce less or lower quality eggs. It is therefore critical to determine energy and lipid content of ova to establish if *Ichthyophonus*-positive fish (compared to healthy salmon) allocate the same energy stores to eggs, or use energy stored in gonads in addition to body-fat reserves to reach the spawning grounds. Further, studies investigating vitality, survival, and growth of juveniles hatching from gametes of infected parents are critically needed to foster pro-active management of all salmon life-stages. Currently, management decisions on the Yukon River are based on numbers of fish escaping onto the spawning grounds, and historical Biological Escapement Goals (BEGs), run outlooks, and predictions are based on Ricker and sibling models. However, BEGs do not take into account that stressed or diseased fish may not have the same fecundity as healthy fish or that gametes (eggs and milt) may not be of the same quality. In addition, survival of eggs deposited and fertilized by diseased or parasitized fish may be compromised, leading to low or no survival and decreased hatching success. If disease has a measurable deleterious effect on reproduction of Chinook salmon, management actions will need to take this potentially diminished reproductive capacity into account.

Objectives

This study aims to i) determine egg quality (via analysis of dry matter, lipid, and protein content) in female Chinook salmon to evaluate if infected salmon allocate the same energy stores to ova as healthy fish; ii) establish sperm viability of infected and healthy male Chinook salmon; iii) investigate hatching success of eggs deposited and fertilized by *Ichthyophonus*-positive parents compared to negative controls; iv) quantify morphometrics, growth, and condition of emerging yolk-sac and swim-up fry; v) validate and quantify cortisol (as an indicator of stress) in adult and juvenile Chinook salmon; and vi) examine biomarkers of disease in blood/plasma (e.g., enzymes indicative of tissue necrosis) to aid in the development of non-lethal *Ichthyophonus* testing.

Study Area/Field Sampling

The Tanana River and its clear water tributaries, the Chena and Salcha rivers, are some of the largest tributaries and producers of Chinook salmon on the Yukon River in Interior Alaska. Escapement of salmon into these systems is monitored annually by counting towers. The biological escapement goal (BEG) for the Chena River is between 2,800 and 5,700 fish and between 3,300 and 6,500 fish for the Salcha River. A sample of 51 Chinook salmon was collected from the Salcha River from July 29 to August 1, 2010 (Permit #SF2010-129), and a 32 fish were collected on July 19, 2011 (Permit #SF2011-068). The work was approved under IACUC protocol # 170553-5 and 170553-6.

The BEG on the Chena River was not met in either 2010 or 2011, as a result of poor Chinook salmon returns on the Yukon River. The BEG on the Salcha River was not met in 2011 and counting was hampered by flooding and high waters. A stipulation for the lethal take of Chinook salmon under our Fish Resource Permit is that sampling will only be allowed if the BEGs into

the tributaries are met. As a result of the poor Chinook salmon run in 2011, we did not receive permission to sacrifice fish for our study. We were however fortunate enough to be invited by ADF&G to participate in the egg takes for the State hatchery. While we were not able to obtain and fertilize gametes for our study, we were able to sample the adult salmon for determination of *Ichthyophonus* prevalence, egg and milt quality, proximate composition, and blood parameters. ADF&G has further offered to share the %survival data of their eggs from known and sampled parents and will further share swim-up fry samples for blood chemistry and proximate composition analysis.

In accordance with ADFG's in-season salmon counts and management procedures, 24 males and 27 females (including 3 females perished in net pens that were not used for fertilization studies) were sampled in 2010. In 2011, ADF&G used and fertilized gametes of 7 males and 7 females as part of their brood stock take. The fresh carcasses were made available to us in the field and samples assessing physiological condition and gamete quality could be taken. An additional 8 females and 10 males were available for sampling as they were either spawned out or had died in net pens. Thus, 15 adult females and 17 adult males were sampled in 2011.

Boat electrofishing gear was utilized in collaboration with ADF&G Sportfish Division in Fairbanks as part of their brood stock takes in 2010 and 2011. Electroshockers send an electrical current through the water and temporarily stun the fish. Stunned fish were collected in gill nets with 50 m in length, 1-2 m in depth, and have a mesh size of 7-inch. After capture, fish were placed in holding pens until ripe. Males are ripe as soon as they reach the spawning grounds, however, females are at varying stages of maturity depending on arrival and/or transit time. Holding time in the pens was generally 1 to 2 days. Ripeness of females was characterized by loss of skein structure and was determined by gentle palpation of the belly and deposition of eggs through the ovipositor. Holding pens were placed in the river current for continuous water flow and were 4ft x 4ft x 8ft in size with a 1.5-inch mesh. Fish were held at a maximum density of 12 fish per pen with males and females separated. Ripe adult fish were euthanized by cranial concussion, and stripped of milt (males) by applying gentle pressure to both sides of the abdomen. Female gametes were harvested by opening the abdomen to remove eggs.

Both males and females were examined internally for typical clinical signs of *Ichthyophonus* infection after gametes were harvested. Clinical signs of *Ichthyophonus*-infection include white nodular lesions on heart or other highly vascularized tissues and muscle (*Figure 1*). To avoid cross-contamination with *Ichthyophonus* between fish and samples, tissues were collected with extreme care using sterile, disposable sampling supplies. The ventral surface of each fish was cleaned of mucus and blood and cut with a sterile blade to expose the heart. A piece of cardiac muscle was removed with a second sterile blade and cultured in MEM-5 supplemented with 5% fetal bovine serum, penicillin, streptomycin, and gentamycin after Kocan and Hershberger (2006). The tissue was then incubated at 14°C and examined daily for *Ichthyophonus* spores to confirm clinical infection at the State University of New York Syracuse in the laboratory of Dr. Whipps.

Harvested eggs were held separately in 1 gallon Nalgene containers until infection status (based on clinical signs of infection) was determined and fertilization could commence according to the treatment plan outlined in *Table 1*. A sub-sample of unfertilized eggs was collected for the determination of egg quality by analyzing eggs for total water, lipid, and protein as well as energy density via bomb calorimetry. A sub-sample of milt was collected from males in sterile weigh boats and placed in HEPES-buffer at a dilution of 1000:1. Samples were stored at 4°C

until analysis (as soon as possible after return from the field) at UAF. The HEPES buffered saline solution contains 10mM HEPES, 150 mM NaCl, and 10% BSA with the pH adjusted to 7.4. HEPES-buffer was used to maintain physiological pH balance, even when carbon dioxide concentrations (and consequently pH) change due to sperm-cell metabolism.

Approximately 10 g of epaxial muscle was removed from Chinook salmon for determination of body composition and resource allocation as determined by proximate composition analysis and bomb calorimetry. Further, approximately 10 mL of blood were taken by caudal vein puncture from each adult fish and transferred to sodium-heparin and EDTA vacutainers for laboratory analysis of hematology and plasma chemistry parameters, and cortisol. Heparinized blood was immediately centrifuged in the field at approximately 1000g for 10 minutes. Plasma was pipetted into cryovials and directly frozen in liquid nitrogen. Hematology parameters (i.e., hematocrit, hemoglobin, MCHC, and leukocytes) were determined directly from whole blood (EDTA treated) and analyzed with an Idexx QBC VetAutoread Hematology System in the field.

Figure 1. *Clinical signs of Ichthyophonus infection in heart (A) and muscle (B) of Chinook salmon. White focal lesions are visible in both tissues. (C) The cultured organism is illustrated at 50x magnification.*

Table 1. Fertilization plan for the hatchery experiment.

	***Ichthyophonus*-positive Male**	***Ichthyophonus*-negative Male**
***Ichthyophonus*-positive Female**	Scenario 1	Scenario 3
***Ichthyophonus*-negative Female**	Scenario 2	Scenario 4

Laboratory Analyses

Blood Chemistry

Blood chemistry parameters of plasma were analyzed using an Abaxis VetScan Classic. The following parameters were assessed: albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), amylase (AMY), aspartate aminotransferase (AST), blood urea nitrogen (BUN), calcium (Ca), creatine kinase (CK), creatinine (CRE), globulin (GLOB), glucose (GLU), potassium (K), sodium (Na), phosphorous (P), total bilirubin (TB), and total protein (TP). High levels of ALP and ALT in the blood can indicate hepatic damage or disease. Aspartate

aminotransferase in the blood is directly correlated to the degree of tissue damage, such as damage to heart or liver. Creatine kinase is an enzyme that catalyses the conversion of creatine (Cr) to phosphocreatine (PCr) while consuming ATP. This is a reversible process, such that ATP can be generated from PCr and thus acts as a tissue energy pool. CK is released in the blood stream when muscle tissue (cardiac, smooth, or skeletal) is damaged. As such, these parameters could be promising tools in assessing damage caused by *Ichthyophonus* in a potentially non-lethal fashion.

For yolk-sac fry, blood chemistry parameters were determined by using total body homogenates. In this process, approximately 1 g of tissue was placed in a glass homogenization tube, and then 9 mL of cold phosphate buffer (0.1 M, pH 7.4) was added. Using a glass rod the sample was homogenized for 2-3 minutes, or until all large particulates were dissolved. Next the samples are centrifuged for 20 minutes at 1000 g, and supernatants are removed. Samples are refrigerated at 4°C immediately after preparation and are analyzed within 4 hours of homogenization.

Cortisol is the leading bioindicator of stress and increases in cortisol are commonly associated with disease in salmonids (Maule et al. 1989; Pickering and Pottinger 1989). Cortisol levels have been determined using a radioimmunoassay (^{125}I) with a half-life of 60.2 days. Prior to analysis of individual plasma samples, the assay was validated for Chinook salmon plasma. Pooled plasma samples for both sexes were serially diluted 1:2 in appropriate buffer and run in duplicate in the assay to determine if displacement by the pool is parallel to that of the standard curve. Accuracy of the assay was determined by spiking the plasma pool at the appropriate dilution with known amounts of cortisol and assaying. Following validation, all individual plasma samples were run in duplicate at Dt. Atkinson's laboratory in Juneau. Any samples with a 10% or greater difference in counts were reanalyzed and any samples having counts outside the range of the gamma counter were diluted and reanalyzed.

Cortisol in yolk-sac fry was determined from total body homogenates. Steroids are lipophilic and thus an extraction protocol for steroid hormones from fat (i.e., blubber) was used (Kellar et al. 2006). Briefly, a sample of yolk-sac-fry (approximately 200 mg), or one individual (whichever was smaller), was homogenized. Samples undergo a multistep organic solvent extraction; the final residue will contain steroid hormones and can be frozen at -20°C until analysis. Currently, all 2010 swim-up fry have been extracted and await cortisol analysis in Juneau.

Proximate Composition

A sub-sample of adult muscle tissue, unfertilized eggs, live eyed eggs, dead eggs, yolk-sac fry, and swim-up fry was collected for determination of proximate composition (%water, %lipid, %crude protein) and energy contents. Water content of tissues was determined as loss of mass during the freeze-drying procedure (VirTis Sentry). Tissues were lipid-extracted using chloroform:methanol (2:1) in a modified Soxhlet procedure after Schlechtriem et al. (2003). Tissue nitrogen content was measured using a CNS 2000, Leco Combustion analyzer at the soil laboratory of the Alaska Agricultural and Forestry Experiment Station in Palmer, Alaska. Ash content was determined via combustion at 850ºC for 8 hours in a muffle furnace. The subtractions of ash content from dry matter allowed for calculation of organic matter in the sample and further subtraction of lipid provided lean dry mass. Crude protein was then calculated from nitrogen contents of lean dry mass assuming all nitrogen is bound to protein

(Mason et al., 2006). Caloric density of tissues was determined using bomb calorimetry (Parr Model 1281) on lyophilized tissues (~0.5-1g).

Milt Quality

The total number of sperm cells per mL was determined using InCyto disposable hemocytometers (10x10 grid measuring 0.1x0.1 mm each). For this procedure 10 µl of diluted sperm was pipetted into the viewing chamber of the hemocytometer. The grid was observed and sperm cells counted under 40x magnification on a Leica DM 1000 microscope. A live/dead sperm viability kit (L-7011, Molecular Probes) was used to analyze the viability and fertilizing capability of Chinook salmon milt. This fluorescence-based assay has a membrane-permanent nucleic acid, SYBR 14 dye, staining live cells a bright green color. A standard dead cell stain, propidium iodide was used to identify dead cells appearing bright red under the microscope. A chroma filter with emission maxima at 516nm for SYBR 14 and 617nm for propidium iodine allowed for differentiation of live and dead cells with visible-light excitation. Cells were enumerated under 40x magnification on a Leica DM 1000 microscope.

Hatchery Setup

In 2010, fertilized eggs were transported to the UAF Aquatic Research Laboratory within 12 hours of initial fertilization. After this 12 hour window eggs are sensitive to movement until they have reached the eyed stage of development. Fertilized eggs were treated with a 10% iodine solution at the hatching facility to reduce fungal or bacterial growth and were then placed into heath trays. The UAF hatchery system consists of a MariSource vertical incubator with 16 hatching trays (each tray can hold up to 10,000 eggs) on top of a 130 gallon raceway. The flow rate for the system is 6 gallons per minute. The system was closed with two 52 gallon sumps with one Frigid Units chiller in each. Water quality was monitored using a Hach test kit, model FF-1A, and an oxygen, conductivity, salinity, and temperature meter (model 451-85). The average water temperature was 10.5ºC. The pH of the system was consistently 7.5. The percent dissolved oxygen saturation stayed consistently at 100%. The salinity in the system varied between 2.5 ‰ to 4.5‰, as frequent water changes and adjustments using Instant Ocean were required to control for high ammonia levels. Ammonia levels varied between 1.4 mg/L and 3.5 mg/L, the bacterial population in the biological filter was not adequate to deal with the amount of ammonia produced in the system, as it had only been running for a few days prior to eggs being added to the system. A minimum of 6 weeks is generally required to generate enough bacteria to convert ammonia to a non-toxic form. The nitrite levels ranged between 0 and 0.004 mg/L. Total alkalinity was consistently 68.4 mg/L, and total hardness was 342 mg/L. Dead eggs were removed and enumerated weekly. After about 4 weeks of development, eggs reached the eyed stage and were shocked to recognize infertile eggs by bursting the yolk sac of lifeless eggs, and turning them white. Eggs identified as unviable were counted and removed. Following an additional 2-3 weeks of development, eggs hatch into yolk-sac fry. A sub-sample of 10-15 g of yolk-sac fry per tray was taken for morphometric measurements. Measurements included wet weight on individual fry, and measurements of total length, and height (including yolk sac) using a digital caliper. An additional sub-sample was taken for and proximate composition analyses and blood chemistry parameters.

As mentioned above, no eggs were taken for fertilization experiments in 2011 due to poor run strength of returning Chinook salmon.

Results

The overall prevalence of *Ichthyophonus* in the Salcha River was 7.8% (3 females, 1 male) in 2010, and 6.3% (2 males) in 2011. Analysis for 2011 samples is still in progress. The average percent survival of eggs to hatching for *Ichthyophonus*-negative offspring was 41.02%, while the average percent survival of eggs to hatching for *Ichthyophonus*-positive offspring was 24.44%. However, variability in survival was high among trays and results were not significantly different ($p>0.05$) between infected and "healthy" fish.

Average milt counts for *Ichthyophonus*-negative Chinook salmon in 2010 were $6.9 \pm 3.8 \times 10^9$ cells/ml, and $14 \pm 8.1 \times 10^9$ cells/ml in 2011. The proportion of live to dead cells was 70 ± 18%. The average milt counts for *Ichthyophonus*-positive Chinook salmon in 2011 were $14 \pm 3.6 \times 10^9$ cells/ml (the difference between "healthy" and infected fish was not significant, $p>0.05$), and the proportion of live to dead cells was 75 ± 15%. The milt sample from one *Ichthyophonus*-positive sampled in 2010 was lost in a refrigerator malfunction. Milt count and salmon size was not linearly correlated ($p>0.05$).

In 2011, 5 not-spawned-out females died in the net pens and could not be used for brood stock by ADF&G. It was therefore possible to determine fecundity of these fish. The average total number of eggs was 11,328 ± 3,741. This indicates that fecundity in Salcha River Chinook salmon females is higher than in the Yukon mainstem at Eagle, Alaska (Horstmann-Dehn, unpublished). However, female fecundity is positively related to Chinook salmon body length and girth and it is thus possible that morphometric factors play a role in this difference.

Blood parameters for all Chinook were analyzed using Vetscan rotors (*Table 2*). AST was higher in "healthy" fish compared to *Ichthyophonus*-positive salmon, but was not statistically significant ($p>0.05$). Levels of CK were significantly higher in *Ichthyophonus*-positive fish compared to "healthy" controls. Blood plasma cortisol levels where measured at three locations (Emmonak, Salcha, and Eagle, Alaska). Plasma cortisol of *Ichthyophonus*-negative and *Ichthyophonus*-positive fish were not significantly different within each location ($p>0.05$) (*Table 3*). However, plasma cortisol was different among the three locations with the Salcha River Chinook salmon being highest and salmon caught in Eagle being lowest ($p=0.001$) (*Figure 2*). While proximate composition of adult salmon muscle was not different between infected and "healthy" adult salmon (*Table 4*), the composition of eggs from "healthy" and infected Chinook salmon females showed some differences, most notably, %crude protein was significantly lower in fertilized eggs from *Ichthyophonus*-infected females ($p=0.02$; *Table 5* and *6*). Once eggs hatch into swim-up fry all analyzed parameters were similar between offspring from "healthy" and infected parents ($p>0.05$ for all parameters; *Table 7* and *8*).

Table 2. Blood chemistry parameters for adult Chinook salmon given as mean ± SD.

	GLU [mg/dl]	Hb [g/dl]	HCT [%]	AST [U/l]	CK [U/l]	ALT [U/l]	CRE [mg/dl]
***Ichthyophonus*-Negative**	191±106	13.3±2.7	45±7.5	342±683	0±0	311±149	<0.2±0
***Ichthyophonus*-Positive**	188±90.1	14.1±.7	46±7.9	941±842	1187±2904	247±306	28.4±84.6
p-value*	0.47	0.61	0.81	0.13	0.02	0.27	0.32

* testing H_0: no difference between "healthy" and infected Chinook salmon

Table 3. Plasma cortisol [mg/dl] of adult Chinook salmon at three locations given as mean ±SD

	Emmonak	Salcha River	Eagle
***Ichthyophonus*-Negative**	26.4±1.3	61.6±46.0	5.4±1.2
***Ichthyophonus*-Positive**	42.9±3.0	104±0.0	9.8±3.1
p-value*	0.09	0.37	0.23

* testing H_0: no difference between "healthy" and infected Chinook salmon

Table 4. Proximate composition of adult Chinook salmon epaxial muscle given as mean ± SD.

	%crude protein	%lipid	%water	kcal
***Ichthyophonus*-Negative**	17.6±1.6	14.5±16.4	79.3±2.9	5.2±0.2
***Ichthyophonus*-Positive**	16.6±3.8	14.2±17.0	81.2±2.8	5.2±0.3
p-value*	0.33	0.96	0.26	0.77

* testing H_0: no difference between "healthy" and infected Chinook salmon

Table 5. Proximate composition of unfertilized eggs from Chinook salmon given as mean ± SD.

	%crude protein	%lipid	%water	kcal
***Ichthyophonus*-Negative**	23.6±2.9	23.8±3.6	62.2±12	6.1±0.1
***Ichthyophonus*-Positive**	20.7±2.2	30.2±2.3	66.0±3.5	6.1±0.01
p-value*	0.18	0.008	0.15	0.22

* testing H_0: no difference between eggs from "healthy" and infected Chinook salmon

Table 6. Proximate composition of fertilized eggs from Chinook salmon given as mean ± SD.

	%crude protein	%lipid	%water	kcal
***Ichthyophonus*-Negative**	20.9±0.7	23.5±6.7	66.6±12.3	6.2±2.3
***Ichthyophonus*-Positive**	16.7±2.5	21.6±10.6	73±6.9	6.6±0.7

p-value*	0.02	0.64	0.03	0.04

* testing H_0: no difference between eggs from "healthy" and infected Chinook salmon

Table 7. Swim-up fry morphometrics and proximate composition given as mean ± SD.

	Weight [g]	**Length [mm]**	**Height w yolk sac [mm]**	**%crude protein**	**%lipid**	**%water**	**kcal**
***Ichthyophonus*-Negative Parents**	0.3±0.04	24.4±1.3	7.1±0.7	15.1±1.2	28.6±1.8	74±1.2	6.1±0.3
***Ichthyophonus*-Positive Parents**	0.3±0.03	23.9±1.7	7.1±0.5	14.6±1.8	31.0±2.5	76±3.6	6.1±0.1
p-value*	0.58	0.59	0.76	0.64	0.51	0.25	0.33

* testing H_0: no difference between offspring from "healthy" and infected Chinook salmon

Table 8. Blood chemistry parameters for swim-up fry given as mean ± SD.

	GLU [mg/dl]	**AST [U/l]**	**CK [U/l]**	**ALT [U/l]**
***Ichthyophonus*-Negative Parents**	39.1±12	59.1±27	5109±2938	31.1±14
***Ichthyophonus*-Positive Parents**	47.6±19	67.1±44	6146±4812	31.3±17
p-value*	0.40	0.73	0.68	0.98

* testing H_0: no difference between offspring from "healthy" and infected Chinook salmon

Preliminary Conclusions

Hatching success of eggs from *Ichthyophonus*-infected parents appears to be somewhat lower, although results were not significant (41% survival for offspring from *Ichthyophonus*-negative parents, and 24% survival for offspring from *Ichthyophonus*-positive parents). This is likely due to sample size limitations and more research is necessary to draw any final conclusions. Once hatched, yolk-sac fry have the same composition and chance of survival as offspring from "healthy" parents. Some blood parameters are different between *Ichthyophonus*-infected and "healthy" adult salmon, and organ damage indicators, e.g., CK and AST are unexpectedly higher in "healthy" fish. Low plasma AST has been correlated with Vitamin B6 deficiency (Chiang et al., 2005) and is in turn associated with inflammation, i.e., *Ichthyophonus*, thus explaining a different and potentially counter-intuitive mechanism in the expression of these indicators. Plasma cortisol between three locations along the Yukon River and its tributaries was different (p-value<0.05) by location. However, method of capture is not directly comparable among the locations (gillnet in Emmonak, fishwheel in Eagle, and net pens in the Salcha River). It is however possible that fish migrating for extensive distances (i.e., Eagle) may have reached adrenal fatigue, thus explaining the lowest stress hormone levels in fish from this location. The use of blood chemistry parameters to identify fish with diseases, such as *Ichthyophonus* is promising, but a larger sample size will be required. This could then provide a tool for

minimally-invasive monitoring and rapid turn-around of results for proactive in-season monitoring of *Ichthyophonus*-infection in returning Chinook salmon stocks.

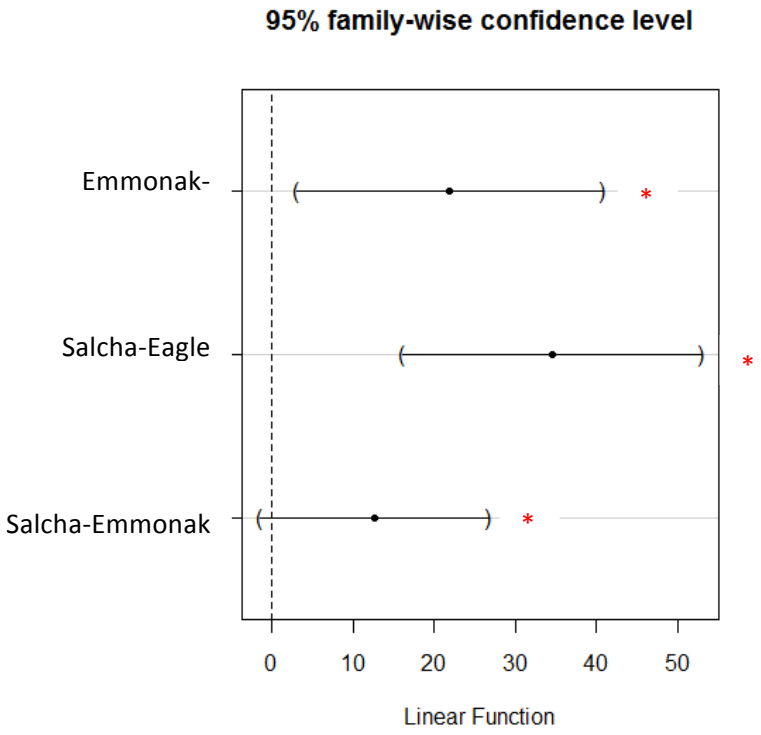

Figure 2. Differences in Chinook salmon plasma cortisol levels by location, as a graphic representation of the Tukey Test (p=0.001). Boxes represent maximum and minimum values, and whiskers are 95% confidence intervals. * Indicates a statistically significant result.

Caveats and Draw-Backs

In 2010, we collected and fertilized ova of *Ichthyophonus* positive and "healthy" fish. Offspring were reared in Marisource vertical incubators in the UAF hatching facility that had only recently been constructed and finished. On September 19, 2010 half of the water (about 150 gallons) drained from the system over night, and our fail safe phone, which should contact a list of people on the project upon any system malfunction, failed. As a result we lost all of our yolk-sac fry.

In 2011, the Chinook run in the Salcha River (and the remainder of the Yukon River) was poor and the biological escapement goal was not reached. We therefore did not receive permission from ADF&G to sacrifice and sample fish for this project. We were fortunate enough to be invited by ADF&G to participate in the egg takes for the State hatchery. While we were not able to obtain and fertilize gametes for our study, we were able to sample the adult salmon for determination of *Ichthyophonus*, egg and milt quality, proximate composition, and blood parameters. ADF&G has further offered to share the %survival data of their eggs from known and sampled parents and will further share swim-up fry samples for blood chemistry and proximate composition analysis.

Future plans

Due to poor run strength in 2011 we did not get permission to collect the anticipated samples. This effectively puts our progress back a year, although we were able to collect some data in 2011. If possible, we are planning to sample the Salcha River in 2012 if we can acquire additional funding for Ms. Floyd to support her graduate studies. We do not require additional funds for sampling, analyzes, or PI salaries.

Presentations

Floyd, T., Horstmann-Dehn, L., Sutton, T. 2011. Effects of *Ichthyophonus* on Chinook salmon reproductive success in the Yukon River drainage. Abstract and poster at the American Fisheries Society 141st Annual Meeting, Seattle, Washington.

Floyd, T., Horstmann-Dehn, L., Sutton, T. 2011. Effects of *Ichthyophonus* on Chinook salmon reproductive success in the Yukon River drainage. Abstract and poster at the Alaska Chapter of the American Fisheries Society Meeting, Girdwood, Alaska.

We also plan to present at these meetings in 2012.

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Component Title: Is freshwater growth is correlated with recruitment of Chinook salmon in the Yukon and Kuskokwim Rivers?

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MS Student: Justin Leon

Introduction

Recruitment of Chinook salmon *Oncorhynchus tshawytscha* in the Yukon-Kuskokwim (Y-K) region of western Alaska has been highly variable over the last century. Recruitment failures, coupled with poor markets for wild salmon, caused severe economic hardship for residents of this region from 1997-2002 (AYK SSI 2006). Continued concern over Chinook salmon returns in the Yukon River, particularly related to meeting escapement goals to Canadian tributaries up-river, indicate that fishery restrictions will be common in the future (e.g., Bue and Hayes 2009). Therefore, biologists, managers, and stakeholders all seek to better understand the factors affecting run size in Chinook salmon in the Y-K region.

Salmon exhibit complex life histories, occupying both freshwater and marine environments over diverse spatial and temporal scales. This complexity, while lending resilience to salmon populations at the regional scale (e.g., Hilborn et al. 2003), also makes predicting how salmon populations will respond to environmental variation difficult (McPhee et al. 2009). A logical way to approach this challenge is to parse salmon life history into three phases (freshwater, smolt migration, and marine) and examine the processes that regulate abundance at each phase (e.g., Thompson 1959, Mobrand et al. 1997). Most such studies to date have been relatively small in spatial scale and have focused on the freshwater phase (e.g., Holtby 1988, Bradford et al. 2001); however, studies focusing on the marine phase of salmon recruitment have revealed the importance of marine climatic variables on salmon abundance at intermediate (Mueter et al. 2002, 2005) and large (Mantua et al. 1997) spatial scales.

It is often thought that salmon abundance is mediated by size-dependent mortality and that the time shortly after entry into marine conditions is critical phase governing recruitment (Beamish & Mahken 2001, Farley et al. 2007). Indeed, smolts that are larger during early ocean phase often show higher survival (Holtby et al. 1990, Cross et al. 2008). In some populations, processes in freshwater contribute to marine survival via freshwater growth and subsequent effects of smolt size on survival through critical phases in marine life (e.g., sockeye salmon *O. nerka*, Henderson & Cass 1991; Koenings et al. 1993; steelhead *O. mykiss*, Ward et al. 1989). In others cases, benefits of greater size are not detected (Bradford et al. 2001), are only apparent during years of low marine survival (Holtby et al. 1990), or disappear with changing environmental conditions (Ward 2000). Additionally, compensatory growth (where low growth is followed by high growth, Ali et al. 2003) can effectively de-couple freshwater growth rates from early marine growth rates (Friedland et al. 2009). Given that oceanic conditions are a major factor controlling salmon recruitment (Briscoe et al. 2005), the idea that freshwater processes directly determine

overall recruitment from egg to returning adult is not a general principle, but rather an assumption that must be tested on a case-by-case basis.

Disentangling freshwater and marine contributions to salmon recruitment has proven difficult, particularly for remote regions such as western Alaska, where field research is especially challenging. However, long-term escapement projects conducted by Alaska Department of Fish and Game (ADF&G) have resulted in age, sex, and length (ASL) data and archived scales samples for Chinook salmon in a number of tributaries of the Yukon and Kuskokwim rivers. As scales record the growth-at-age for salmon (Bilton 1985), long-term scale collections promise to be a useful tool for understanding the link between freshwater and marine growth, survival, and recruitment of salmon. Indeed, this technique has been used to study salmon survival and recruitment in a number of systems (e.g., steelhead *O. mykiss*, Ward et al. 1989; coho salmon *O. kisutch*, Holtby et al. 1990, sockeye salmon *O. nerka*, Farley et al. 2007, Ruggerone et al. 2007a; Atlantic salmon *Salmo salar*, Friedland et al. 2009).

Recently, Ruggerone and colleagues (Ruggerone et al. 2007b, 2009) used Chinook salmon scale archives from commercial and test fisheries on the Yukon and Kuskokwim rivers in order to examine links between freshwater and marine growth, climate, and abundance. They found that growth in a given year was positively correlated with growth in the previous year, and this relationship was strongest when growth in the previous year was relatively low (Ruggerone et al. 2007a, b, 2009). They also found greater growth in females than in males and in individuals who returned after three years at sea versus those who returned after four years. Interestingly, the stage at which growth divergence between males and females and between 1.3 and 1.4 individuals differed between river systems, with growth diverging in freshwater stages in the Yukon River and in marine stages in the Kuskokwim River (Ruggerone et al. 2007b).

In the present study, we are using similar methods in order to examine the importance of freshwater processes on Chinook salmon recruitment in the Yukon and Kuskokwim Rivers. Rather than pooling samples at the regional level, we are conducting analyses at the individual tributary level, increasing the likelihood of detecting mechanisms connecting growth to population regulation. Importantly, this will allow us to track the effects of freshwater growth on recruitment within each population by following cohorts from time of spawning to return as mature adults, and will allow us to detect intergenerational effects of freshwater growth (e.g., whether large females produce offspring that grow faster). Second, we are analyzing marine growth and the degree to which it is correlated with freshwater growth in each of these populations, as found at the regional scale by Ruggerone et al (2009).

Objectives

The overall goal of this component is to determine if freshwater growth is correlated with recruitment of Chinook salmon in the Yukon and Kuskokwim rivers using archived scale samples.

Within this goal, we will address five specific questions:

Question 1) Is there evidence for a minimum size at which smolts are able to survive to maturity, and does this size change annually?

Question 2) Is there a correlation between freshwater growth and the number of females returning to spawn?

Question 3) Is there a correlation between freshwater growth and the size/age at which females mature?

Question 4) Is there a correlation between the size and number of females and the freshwater growth of their offspring?

Question 5) Is marine growth correlated with freshwater growth in Chinook females?

For this component, we are conducting retrospective scale analyses from two ADF&G escapement projects: the Andreafsky River weir (Yukon) and the Kogrukluk Weir (Kuskokwim). Originally we had planned to include two escapement projects per drainage but later determined that the scale reading could not be completed in the time allotted for the project. Therefore we focused on the Andreafsky and Kogrukluk river weirs, as both of these populations have been monitored for over 25 years by weir counts and have considerable long-term data on escapement, age composition, and sex ratio, in addition to long-term scale sample archives (L. DuBois, ADF&G, pers. comm.; D. Molyneaux, ADF&G, pers. comm.). The Andreafsky River population is particularly suited to our objectives; being low on the system, escapement is less affected by subsistence, commercial, and sports fisheries (D. Evenson, ADF&G, pers. comm.). Thus, we can use escapement counts as a reasonable proxy for recruitment. The Kwethluk, river site is more heavily influenced by fisheries and therefore we are using escapement data, harvest data, and estimates of harvest apportionment to arrive at approximations of recruitment each year.

Our analysis is limited to data from females. From a population dynamics perspective, females (and the number of eggs they deposit) determine the initial number of offspring produced in each brood year. Furthermore, the relationship between body size and reproductive success of females is expected to be linear, with larger females laying more and larger eggs, at greater burial depths (reviewed in Quinn 2005). The relationship between body size and reproductive success in males is less straightforward, complicating the interpretation of relationships between growth, age at maturity, and recruitment. Finally, female Chinook salmon show fewer age classes at maturity than do males in the Y-K region, further simplifying analysis.

In order to address the five questions outlined above, we are using recruitment estimates, sex-ratio, age-frequency, and size-at-age data from weir projects and previously published size-fecundity relationships to estimate the number of females spawning and the number of eggs spawned in each brood year. This is our starting point for estimating survival from egg to maturity. We used scales and body length (mid-eye to fork of the caudal fin, MEF) of adult

females to determine each female's age and to estimate smolt size (measured as the freshwater scale annulus width, FAW) and to estimate marine size-at-age (measured as the width of scale annulus for each year of marine growth; MAW). This was accomplished by obtaining acetate impressions of scales from the ADF&G sample archive, digitizing these impressions, and implementing a semi-automated image analysis routine, following the methods outlined in Hagen et al. (2001). Scale samples passed quality standards used by ADF&G prior to digitization (e.g., from the preferred region, no evidence for regeneration or significant resorption). We sampled up to 50 females per year per site depending on availability of samples.

Objective 1: Is there evidence for a minimum size at which smolts are able to survive to maturity, and is there annual variation in this pattern?
When studying growth patterns from scales of adult salmon, analysis is complicated by the fact that we only can acquire data from those individuals that survived. Assuming that juvenile size is distributed normally prior to size-dependent mortality, a truncated frequency distribution of FCW would indicate a minimum size below which smolts failed to survive to maturity. We will use quantile-quantile (Q-Q) plots to detect deviations from normality due to loss of small FAW classes (e.g., Post and Evans 1989, Finstad et al. 2004). We will also examine annual variation in the distribution, as well as minimum FAW, to determine whether there are years where size-dependent mortality appeared to act, years in which it appeared to be absent, and how much annual variation exists in minimum FAW.

Objective 2: Is there a correlation between freshwater growth and the number of females returning to spawn?
We estimated the number of females recruited from each brood year (FR), summing across adult female age classes. Escapement estimates were based on weir counts. For the Andreafsky River, where harvest rates are negligible, escapement data was assumed to be proportional to recruitment and directly compared to FAW. For the Kogrukluk, harvest estimates were based on commercial and subsistence harvest records with the assistance of knowledgeable biologists and managers working on the system. Harvest and escapement estimates were then be summed to estimate total recruitment.

We will then compare the FAW of females produced from each brood year to the corresponding estimate or index of recruitment to determine whether female recruitment is positively correlated, negatively correlated, or insignificantly correlated with freshwater growth.

Objective 3: Is there a correlation between freshwater growth and the size/age at which females mature?
The correlation between freshwater growth and recruitment can be complicated by the effects of growth rate on age/size at maturity. A growing body of evidence suggests that life history patterns in fish are determined via inhibitory effects on sexual maturation. Depending on size and/or physiological state at a decisive period, a fish may initiate sexual maturity or delay it for another year, and often it is larger and/or faster growing fish that initiate maturation (Thorpe 2007, Mangel and Satterwaithe 2008). Given the correlated growth found by Ruggerone et al. (2007b), we might expect that females who experienced faster freshwater growth matured at younger ages. In support of this hypothesis, Ruggerone et al. (2007b) found that age-1.3 Chinook salmon of both sexes grew faster than did age-1.4 individuals. We will test whether FAW differs

among size-at-maturity classes in an ANOVA framework. Secondly, as the effect of freshwater growth on sexual maturity might diminish with the number of years spent in saltwater, we will use logistic regression to determine if FAW predicts the probability of maturing early (as 1.2 adult females).

Objective 4: Is there a correlation between the size and number of females and the freshwater growth of their offspring?
We will examine the potential for density-dependent freshwater growth in Chinook salmon by testing for a correlation between the size of the spawning population and freshwater growth of their offspring. To accomplish this, we estimated the number of eggs deposited in each brood year by summing the number of females in each age class weighted by the mean fecundity of each age class (estimated by mean size of each age class and published relationships between size and fecundity for Chinook salmon). The number of eggs laid in each brood year will be correlated with the mean FAW of females resulting from that brood year across years to determine whether there is a negative correlation, suggesting density-dependent growth. If we detect a positive correlation, we will dissect the relationship further to ascertain whether brood years producing females experiencing high freshwater growth were dominated by older, larger female spawners (suggesting that larger females produce larger smolts).

Objective 5: Is marine growth correlated with freshwater growth?
Ruggerone et al. (2009) found that at the regional scale in the Yukon and Kuskokwim drainages, Chinook salmon growth (measured as scale annulus width) was positively correlated with growth in the previous year. These correlations held between years at sea and also between freshwater growth and growth during the first at sea, suggesting that freshwater growth contributes to marine growth (and thus possibly survival). We will test for these correlations between FAW and MAW in the first year, and between MAW for sequential years at sea. We expect that the findings of Ruggerone et al. (2009) will be upheld at the tributary scale. We will also extend our analysis of growth effects on recruitment to the marine phase, by repeating analyses in Objective 2b using marine growth (MAW) rather than FAW as a predictor variable.

Progress to Date:
2,925 scales were digitized through this project; 1,501 from the Andreafsky River and 1,424 from the Kuskokwim River. These scale digitizations represent data from 1967, 1968, and 1980-2010 with the exception of 2006 for the Andreafsky River and 1978, 1981-2010 for the Kogrukluk River (samples missing). Databases were compiled and are being maintained at the Alaska Department of Fish and Game Mark, Tag, and Age Laboratory in Juneau, Alaska for further use. Acetate impressions are also being held there with the possibility that more scales can be analyzed in the future.

Estimates of recruitment and total fecundity per brood year have been completed. We have begun using these estimates along with the increment widths (representing freshwater and marine growth) to test the hypotheses outline in our objectives. Because these analyses are very preliminary at this time, we chose to postpone reporting these results until we provide a final project update in January 2012.

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Component Title: An analysis and comparison of growth-increment formation using otoliths and scales of juvenile Chinook salmon

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Introduction:

Freshwater growth of juvenile Chinook salmon *Oncorhynchus tshawytscha* has been found to strongly influence survival and recruitment to the adult population. Retrospective analysis using daily increments on otoliths and circuli on scales has emerged as a tool to measure salmon growth at previous ages. Fish size and growth is assumed to be accurately reflected by otolith increments and scale circuli, but this assumption is rarely validated.

Therefore, in order to accomplish the following objectives, we have conducted an experiment to validate the relationship between body size, growth, and width between otolith growth increments and scale circuli in juvenile Chinook salmon.

Objective 1: Validate the relationship between body size and growth and width between daily otolith growth increments and scale circuli in juvenile Chinook salmon.

Testable hypotheses for Objective 1:

H_1: The width between hard structure increments in both otoliths and scales will reflect the growth rate of juvenile Chinook salmon.

H_2: Otolith and scale circuli increments will be directly dependent on fish growth rate, with growth increments for each structure growing in a constant, measurable proportion to each other.

H_3: Larger otolith and scale growth increments will be positively correlated with lower fish density and higher food ration.

Objective 2: Determine how growth is recorded on otoliths and scales of wild Chinook salmon relative to the same structures in laboratory-reared fish of the same species.

Testable hypotheses for Objective 2:

H_1: The width between hard structure increments in both otoliths and scales will reflect the growth rate of wild juvenile Chinook salmon.

H_2: Proportional growth between otolith increments and scale circuli in wild Chinook salmon will be equal to proportional growth between increments of the same structures in laboratory-reared fish of the same species.

Methods:

Six hundred eyed eggs were received from Alaska Department of Fish and Game's (ADF&G) Fort Richardson Hatchery on October 1, 2010. Hatching occurred from October 8-15, and the fish were reared to experimental size. On March 17, 2011, 360 juvenile chinook salmon were stocked into twenty-four 110-L aquaria at densities of 10 or 20 fish (12 aquaria for each density) and tanks were assigned a feeding ration of 1%, 2%, or 4% of total fish body weight to simulate low growth, maintenance, and high growth conditions, respectively. The 122-d experiment began on March 31, and all fish were sacrificed on July 30, 2011.

Sagittal otoliths and a scale sample were taken from each experimental fish (N = 360) during the week following the conclusion of the experiment. Otolith samples required a week to dry, with sample preparation beginning immediately after drying was complete. Preparation (mounting, taking measurements, and grinding) and digitization of a single otolith requires approximately 2 hours, and is scheduled to be completed at the end of November 2011. Following preparation, growth increments on the otoliths and scales will be counted and measured to examine the periodicity of otolith increment and scale circuli formation and to determine the effects of density and food ration on increment and circuli deposition. Increment counts and measurements will be completed by mid-December 2011.

Samples of otoliths and scales from wild juvenile chinook salmon in the Chena and Salcha rivers have also been collected and will be prepared according to identical methods for comparison to the laboratory results. Wild chinook salmon were collected once per month from June through September 2011 in each river. Field work was greatly hindered due to the prevalence of high water conditions during summer 2011, reducing capture efficiency of traps and nets and requiring extra days to conduct fieldwork (especially during September). A total of 79 juveniles were captured from the Chena River, with 30 fish being sacrificed and retained. One hundred and sixty fish were sampled in the Salcha River, with 40 individuals being retained. The 70 wild otolith and scale samples will be processed in November 2011, and increment counts and measurements are scheduled for completion by the end of December 2011.

Preliminary Results:

Preliminary laboratory results, shown below in Figures 1 and 2, have shown a strong correlation between both fish length and otolith diameter ($r^2 = 0.6413$) and fish weight and otolith diameter ($r^2 = 0.5961$). As this study is focused on growth and its impacts on increment formation, most of the analyses will take place following sample preparation.

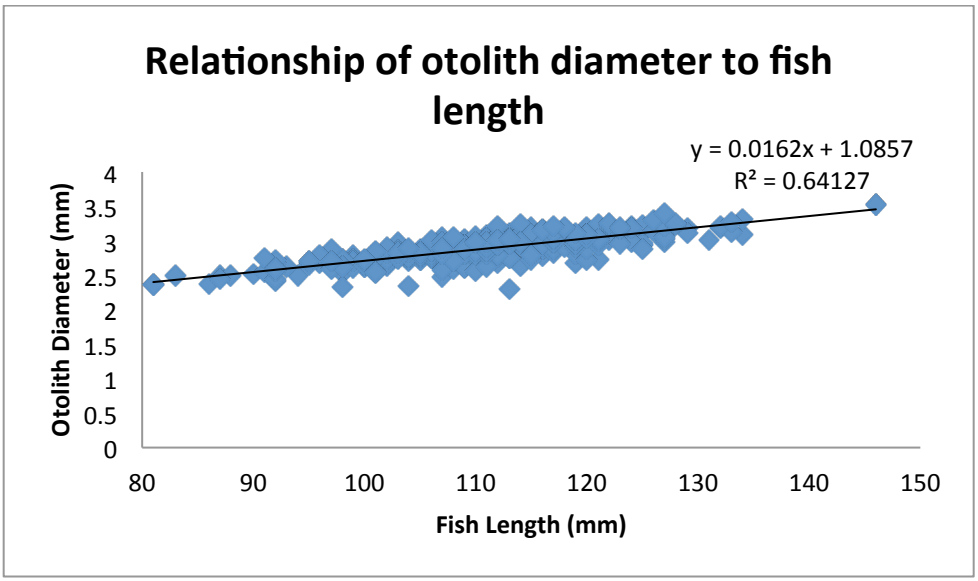

Figure 1: The relationship of otolith diameter to fish length for Chinook salmon reared under experimental conditions.

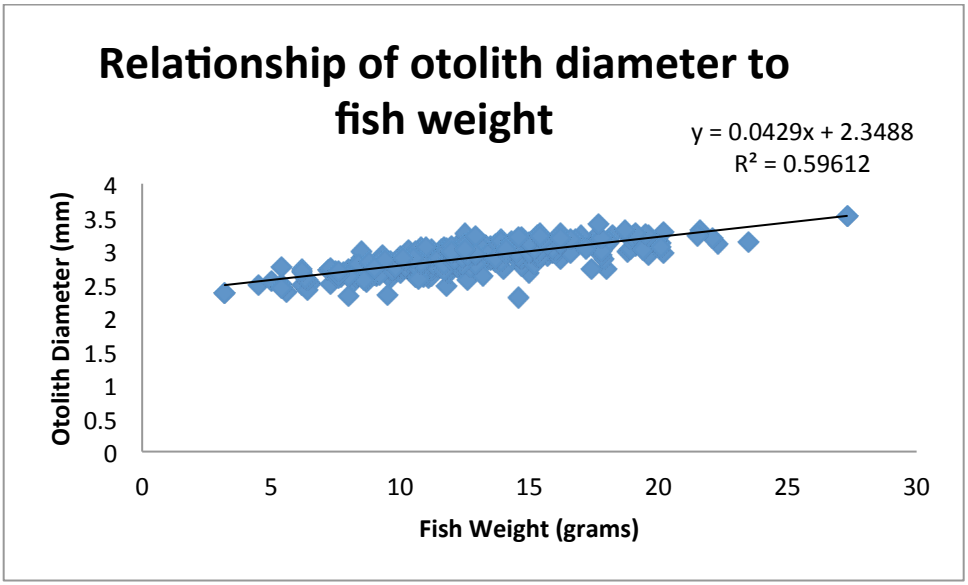

Figure 2: The relationship of otolith diameter to fish weight for Chinook salmon reared under experimental conditions.

Discussion:

This study will assist fisheries managers by testing the accuracy of the assumption that fish body size and growth is reflected by otolith and scale size and growth-increment formation. If the relationship between body size, hard structure size at age, and growth chronology can be validated, the findings of my study can be used to ascertain body size at past ages and interpret the meaning of retrospective growth.

Products:

This project has resulted in one oral presentation at ADF&G's Otolith Workshop in April 2011. A poster detailing progress to date is scheduled to be presented at the Alaska Chapter of the American Fisheries Society meetings in November 2011. Sampling for the wild cohort comparison resulted in the discovery of juvenile coho salmon in the Chena River (confirmed by ADF&G, 10/10/11), a species unknown to that water body previously.