# Chinook salmon *Ichthyophonus* Investigations

Final Report to the Yukon River Panel

For project titled: "*Ichthyophonus* in Chinook salmon – Continuation of a baseline in Emmonak and Eagle, Alaska and potential links to fecundity and blood chemistry." URE 13-10

by

Larissa Horstmann-Dehn, Krista Nichols, Christopher Whipps

June 28, 2012

*Larissa Horstmann-Dehn, Krista Nichols, Christopher Whipps,*
*School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, 905 N. Koyukuk Drive, Fairbanks, Alaska 99775. email: lara.horstmann@alaska.edu*

## Abstract

*Ichthyophonus hoferi* is a protozoan parasite of various fish species, including salmonids, and infection has led to mass mortalities in species of economic significance. Prior evidence suggests that infection with *Ichthyophonus* leads to reduced endurance, increased pre-spawning mortality, and potentially low fecundity. Poor returns of Chinook salmon (*Oncorhynchus tshawytscha*) from adequate spawning escapements in 2007 to 2010 raise questions about involvement of disease in these declines. This study continued to establish baseline prevalence (1999-2009) of *Ichthyophonus* in Emmonak (river mouth) and Eagle (U.S.-Canadian border). In addition, potential effects of the parasite on female fecundity and egg quality were investigated. Further, blood plasma was analyzed for a variety of blood chemistry parameters including cortisol (as an indicator of stress) to aid in the development of potentially non-lethal biomarkers for *Ichthyophonus* infection. Prevalence of *Ichthyophonus* in Yukon Chinook salmon at the river mouth shows a cyclic variation over time. *Ichthyophonus* prevalence in 2010 was 8.7% in Emmonak and 7.0% in Eagle. Total egg counts did not differ between "healthy" and infected females ($P$=0.45). Egg quality (as determined by proximate composition analyses) showed some differences, mainly lipid contents was higher ($P$=0.003) in eggs from *Ichthyophonus*-positive females, while crude protein contents was significantly lower in eggs from infected compared to "healthy" females ($P$=0.02). However, only 6 of 63 females sampled were infected with *Ichthyophonus* and stock-specific differences in lipid contents in particular may confound any potential differences in egg quality. Analyzes of blood chemistry parameters revealed some differences between *Ichthyophonus*-infected and "healthy" fish. In Emmonak, blood chemistry parameters identified as indicators of inflammation and heart or liver disease were different between "healthy" and infected fish (i.e., CK, UA, ALP, ALT, and BUN). Cortisol was also significantly elevated in *Ichthyophonus*-positive fish from Emmonak ($P$=0.03). In Eagle, a different set of blood chemistry parameters showed significant differences between "healthy" and infected fish (i.e., AST, TBIL, and Na). Cortisol did not differ by health status of fish sampled in Eagle ($P$=0.24). However, cortisol levels were significantly lower in salmon sampled in Eagle compared to fish sampled in Emmonak ($P$=<0.0001), either related to capture method (set gillnet versus fish wheel) or adrenal fatigue in fish from Eagle. Nonetheless, blood chemistry profiles were distinguishable between "healthy" and infected Chinook salmon, making this a promising tool in the development of non-lethal methods to detect *Ichthyophonus* infection.

## Introduction

*Ichthyophonus hoferi* (*Ichthyophonus* here after) is a marine-derived protozoan parasite infecting a variety of marine and anadromous fish species including salmonids (Kocan et al. 2004; Tierney and Farrell 2004; Gavryuseava 2007). While the parasite is not harmful to humans, the effects on the fish host can be devastating and mass mortalities of herring have been attributed to infection with *Ichthyophonus* (Sindermann 1965; Mellergaard and Spanggaard 1997; Kocan et al. 1999). Continued poor returns of Chinook salmon from adequate spawning escapements raise questions about the potential contribution of *Ichthyophonus* to these declines either due to pathogen-induced mortality, reduced fecundity, or the inability of fish to successfully migrate and spawn in tributaries. Prior

research suggests that *Ichthyophonus* is a newly emerging parasite in the AYK region and may cause pre-spawning mortality of Yukon River Chinook salmon (Kocan et al. 2004, 2006, 2009). Moreover, Yukon River Chinook salmon appear to be more susceptible to *Ichthyophonus* than some British Columbia Chinook salmon populations (Jones and Dawe 2002) and exposure of fish with naive immune systems to *Ichthyophonus* results in high mortality (Kocan et al. 1999).

This study aims to:

1) Maintain the temporal baseline of *Ichthyophonus* prevalence at Emmonak and at border passage (i.e., Eagle) in Yukon Chinook salmon;

2) Determine fecundity in female Chinook salmon harvested in Eagle and analyze eggs for water, total lipid, and crude protein content to evaluate if infected Chinook salmon produce a similar number of eggs and allocate the same energy stores to ova as "healthy" fish;

3) Investigate biomarkers of disease in blood/plasma (e.g., tissue damage enzymes) to aid in the development of non-lethal *Ichthyophonus* testing

## Methods

A long-term data set (1999-2009) of *Ichthyophonus* prevalence has been established near the community of Emmonak, at the mouth of the Yukon River. In 2010, Chinook salmon *Ichthyophonus* sampling continued in Emmonak and Eagle, Alaska funded by the U.S./Canada Restoration and Enhancement Fund. Severe rain, flooding, debris, and inaccessibility (due to washout of the Taylor Highway) in the community of Eagle made the collection of samples challenging. In addition, conservation measures on the Yukon River asked fishermen to voluntarily reduce their catch, therefore reducing sampling opportunity. However, sampling in 2010 was successful and all sample targets were reached. Chinook salmon tissues were collected near the community of Emmonak (close to the mouth of the Yukon River) as part of the Big Eddy test fishery operated by ADF&G. The Big Eddy test fish project utilizes set gillnets with an 8.5" mesh size. Samples of cardiac muscle ($n$=150) were collected over the course of the Chinook salmon run from June 9 to July 12. Collection of samples over the entire run is critical as Kocan et al. (2004) noted that salmon returning early in the season seem to be relatively free of the typical clinically observed *Ichthyophonus* lesions, while fish tend to be more severely infected with these lesions later in the season.

In addition, samples of Chinook salmon ($n$=199) were collected in collaboration with subsistence fishermen in the community of Eagle near the U.S.-Canadian border. Samples were obtained over the course of the Chinook salmon run from July 9 to July 28. Subsistence fishing gear used at Eagle included a fish wheel (near Calico Bluff) and set gillnets with a mesh size of 6". Fishing sites varied and were dependent on the use of traditional sites. All Chinook salmon samples in both Emmonak and Eagle have been collected opportunistically and no fish were sacrificed for the research objectives of this

project. Institutional Animal Care and Use (IACUC) protocols required by UAF as part of vertebrate research were therefore not necessary for this study.

At both locations, samples and morphometric have been obtained on shore immediately after return of the test fish crew/subsistence fishermen. Age was estimated from all fish by scale pattern analysis (ADF&G aging laboratory) with scales collected from the preferred area on the left side of the fish above the lateral line (Bales 2007). Length was measured to the nearest 5 mm from mid-eye to fork of the tail. Sex was determined by internal examination of gonads. Girth was measured to the nearest millimeter anterior to the dorsal fin using a QM2000$^{TM}$ circumference measuring tape and fish weights were determined on shore using a tripod and a Chatillon scale (±0.05lbs). Axillary fin clips were taken for genetics in Eagle and made available to the Department of Fisheries and Oceans in Canada.

Presence of *Ichthyophonus* 18S rDNA has been evaluated using polymerase chain reaction (PCR) with DNA extracted from cardiac muscle and following the procedure described by Whipps et al. (2006). PCR tests have been conducted at Purdue University, West Lafayette, IN for both Emmonak and Eagle samples, and at the State University of New York, College of Environmental Science and Forestry, Syracuse, NY for Emmonak samples only. Samples from Emmonak have been analyzed by both institutions to assure inter-laboratory comparability and maintain a consistent temporal baseline at this site. The PCR method is highly sensitive and specific for *Ichthyophonus* (detection limit = $10^{-5}$ parasite spores/reaction, Whipps et al. 2006) and samples can be stored indefinitely, thus allowing for analysis of archived samples and storage of samples in the field. PCR analysis is equally sensitive to tissue culture in identifying the presence or absence of the parasite (Whipps et al. 2006). In addition, 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 infection.

To address fecundity of infected and healthy Canadian-origin female Chinook salmon, both ovaries have been weighed to the nearest 0.1g following the method of Kinnison et al. (1998). Two sub-samples of fresh ova from each skein (approximately 10% of the total skein weight) have been weighed to the nearest 0.1g in the field and preserved in 10% buffered formalin for later egg counts. Mean egg weight (the ratio of sub-sample fresh weight to number of eggs in the sub-sample) has then been determined. Fecundity was defined as the ratio of total fresh skein weight and mean egg weight. Eagle was selected as the sampling site to determine fecundity, due to the high degree of variability in maturity of the mixed Chinook salmon stocks obtained in Emmonak at the Yukon River mouth.

To evaluate resource allocations of eggs, a sub-sample of roe was collected into pre-weighed vials, frozen, and shipped to the Ecophysiology Laboratory at the University of Alaska Fairbanks (UAF) where they were homogenized and freeze-dried (VirTis Sentry) to a constant weight. Water content of tissues was determined as loss of mass during the freeze-drying procedure. Tissues were then lipid-extracted using chloroform:methanol 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. Ash content was determined via combustion at 550ºC 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. Caloric density of tissues was determined using bomb calorimetry (Parr Model 1281) on lyophilized tissues (~0.5-1g).

Approximately 7 ml of blood were taken by caudal vein puncture and transferred to sodium-heparin vacutainers. Whole blood was centrifuged for 10min at 3000rpm, and plasma separated and frozen at -20°C until analysis. 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), bile acids (BA), blood urea nitrogen (BUN), calcium (Ca), creatine kinase (CK), creatinine (CRE), globulin (GLOB), glucose (GLU), potassium (K), sodium (Na), phosphorous (P), total bilirubin (TBIL), total protein (TP), and uric acid (UA).

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.

## Results and Discussion

Sex composition of the acquired samples was 46% and 32% female for Emmonak and Eagle, respectively, as determined by internal examination of gonads. Fish sampled at Emmonak had a mean length of 804 ± 83 mm (mideye to tail fork), mean weight of 19.1 ± 5.0 lbs, and the mean girth was 492 ± 48 mm. At Eagle, the mean length was 742 ± 91 mm and mean weight was 12.5 ± 4.7 lbs; mean girth was 391 ± 56 mm. Age was estimated by scale pattern analysis with scales collected from the preferred area on the left side of the fish above the lateral line (Bales 2007). At Emmonak, age-5 (52%) fish were strongly represented, followed by age-6 (38%). Age-3 and age-7 fish were present at 0.7% and 4%, respectively. Due to scale resorption, 3.3% of fish in Emmonak could not be aged. Age composition of the fish sampled in Eagle was 8% age-4, 58.5% age-5, and 23.5% age-6 fish. Proportions of age-7 fish were small in Eagle with 2.5%. Due to advanced resorption of scales at later migratory stages, 7.5% of Chinook salmon collected in Eagle could not be aged. Heart samples were fixed in 95% ethanol at time of collection and were analyzed for the presence of *Ichthyophonus* 18s rDNA using polymerase chain reaction (PCR) following the procedure described by Whipps et al. (2006). Clinical signs typical for *Ichthyophonus* infection were noted at the time of

collection in 6.7% (10 of 150) of fish sampled in Emmonak. Clinical signs of *Ichthyophonus* infection were recorded in 4.0% (8 of 199) of Chinook salmon collected in Eagle. However, white, granulomatous lesions are a general inflammatory response of fish to foreign bodies and do not necessarily establish actual infection with the parasite (Corbel 1975). Both PCR analysis and culture of tissues obtained in Emmonak indicated a low infection prevalence of 8.7% (13 of 150) and 7.0% in Eagle (14 of 199). Both sexes were equally infected with *Ichthyophonus* in both Emmonak and Eagle ($P$=0.26 and $P$=0.38, respectively). In addition, there was no difference in morphometric measurements between infected and uninfected salmon ($P$=0.5 for length, girth, and weight) in Emmonak and ($P$=0.75, $P$=0.95, and $P$=1.0 for length, girth, and weight, respectively) in Eagle. *Ichthyophonus* prevalence over time for both Emmonak and Eagle is provided in Figure 1. Cyclic *Ichthyophonus* epizootics have been described in herring (Sindermann 1965) and a similar cyclic pattern is noticeable in the Chinook salmon time series data from Emmonak. Almost identical fluctuations in *Ichthyophonus* prevalence have been observed on the Yukon River (at 1,170 river km), approximately halfway between our two study sites (Zuray et al. 2012). While reasons for this temporal variability are poorly understood, a potential correlation with ocean conditions appears likely. For four consecutive years, the average summer and winter water temperatures in the Eastern Bering Sea have been cold (NOAA mooring M2, NOAA's Pacific Marine Environmental Laboratory) and coincide with a noticeable drop in *Ichthyophonus* prevalence over this time period (Figure 1). Environmental change can have direct impacts on the physiological response of fish to parasite or pathogen exposure as the inflammatory and stress responses in general are temperature dependent in poikilotherms (Finn and Nielsen 1971; Strange et al. 1977). In addition, temperature appears to influence the activity of *Ichthyophonus* in the host (e.g., higher overall parasite load with increased temperature, Kocan et al. 2009), and therefore has noticeable effects on host stamina, in particular once they enter the warmer river systems. In the light of rapidly changing Arctic and sub-Arctic ecosystems, temporal trends (such as disease monitoring near Emmonak) become crucial in documenting and understanding response and adaptation potential of salmonid populations as well as identifying mechanisms preceding these changes.

The effect of *Ichthyophonus* on salmon health, egg quality, and juvenile survival remains poorly understood. Fish egg and embryo vitality is correlated to body condition of spawning females (Harel et al. 1994). Condition is in turn dependent on physiological status and energy demands and generally fish exposed to stress or disease show an increase in energetic costs (King et al. 2003; Rand et al. 2006). Lipids may therefore be re-routed from gonads of *Ichthyophonus*-positive fish to complete the spawning migration and then they may either produce less or lower quality eggs. Collections in Eagle were therefore paired with egg counts and egg quality (as determined by proximate analyses; %water, %lipid, %crude protein, ash, and caloric density) data to assess fecundity, gonad energy storage, and potential links to *Ichthyophonus* infection (Table 1). In 2010, 63 females (32% of sampled fish) were available for study showing a significant positive correlation between fecundity and girth, fecundity and weight, and fecundity and length ($P$=<0.0001, Figure 2). Total egg count between *Ichthyophonus* infected salmon and uninfected fish was not significantly different ($P$=0.45, Table 1). In addition, egg quality (as determined by proximate analyzes) was investigated between "healthy" and infected females. Statistically significant differences were found in %lipid and %crude

protein contents, with eggs of infected salmon having higher lipid stores compared to eggs of uninfected females, while crude protein contents in eggs from *Ichthyophonus*-positive females was lower (Table 1). It should however be noted that a careful balance of fatty acids and phospholipids is required for viable offspring (Rainuzzo et al. 1997). There is some evidence that Vitamin A (in the form of retinol and carotenes) is bound to egg yolk protein and adequate concentrations are required for egg viability (Palace and Werner 2006). Vitamin A in turn is sequestered by the female from muscle and liver tissue. While higher lipid and lower crude protein levels in Chinook salmon eggs from infected females and potential links to vitamin concentrations should be further investigated, any effect on hatching success in this study as a consequence of *Ichthyophonus* infection is speculative.

In Emmonak, some blood parameters are different between *Ichthyophonus*-infected and "healthy" salmon. Enzymes, such as CK and ALP are significantly higher in infected fish, and so is UA, while ALT and BUN are significantly lower (Table 3). High levels of ALP in the blood can indicate hepatic damage or disease. CK is released in the blood stream when muscle tissue (cardiac, smooth, or skeletal) is damaged (Wells et al. 1986; Wagner and Congleton 2004). In addition, hyperuricaemia (high levels of UA) have been associated with chronic inflammation and heart failure (Leyva et al. 1998). In sea bass (*Dicentrarchus labrax*) a decrease in BUN has been associated with effects on protein metabolism (Roche and Boge 2000) providing a curious link to the observed lower protein contents in eggs from *Ichthyophonus* infected females. Interestingly, ALT levels have been associated with cell injury in general (Wagner and Congleton 2004) and concentrations were lower in *Ichthyophonus* infected fish compared to "healthy" salmon.

Blood chemistry profiles in Eagle were different between healthy and infected salmon compared to patterns observed in Emmonak. It is likely that differences in migration distance (i.e., river mouth to Eagle is approximately 1,200 kilometers) account for many of the observed differences. For example, CK is an indicator for muscle damage, including cardiac muscle, but CK is also an enzyme that catalyzes the conversion of creatine to phosphocreatine while consuming ATP. This is a reversible process, such that ATP can be generated from PCr and thus acts as a tissue energy pool. Salmon are not feeding during their spawning migration and energy stores towards the end of the migration will be exhausted and the enzyme (i.e., CK) is likely downregulated. Thus, any potential differences between "healthy" and *Ichthyophonus* infected salmon in Eagle are confounded by the geographical differences and physiological processes as part of the migration. Differences between "healthy" and infected Chinook salmon were found in three blood parameters. AST was higher in *Ichthyophonus*-infected salmon compared to "healthy" fish. This was somewhat expected as AST in blood is directly correlated to the degree of tissue damage, such as damage to heart or liver (Wagner and Congleton 2004). While AST levels in Emmonak were not different between infected and uninfected salmon, it was one of three variables driving the separation between groups in a principal components analysis (PCA, Figure 3) together with ALT and BUN. In Eagle, the separation between *Ichthyophonus* infected and "healthy" salmon in a PCA was similarly driven by a positive loading of AST, BUN, and ALT, but ALP was also an important factor (Figure 4).

Plasma cortisol was different between Emmonak and Eagle ($P$<0.0001) with cortisol in fish from Eagle being significantly lower. While method of capture is not directly comparable between the locations (gillnet in Emmonak and fish wheel in Eagle), migratory distance could also explain this difference. Fish migrating for extensive distances (i.e., Eagle) may have reached adrenal exhaustion (Heim et al. 1999). This could then also explain the lack of difference in cortisol concentration in "healthy" and infected salmon from Eagle, while Ichthyophonus infected salmon from Emmonak showed a significantly higher stress response compared to uninfected individuals. As such, blood chemistry parameters and stress hormone levels are promising tools in assessing damage and/or stress caused by *Ichthyophonus* and to further develop non-lethal disease monitoring in salmonids.

***Table 1:*** *Fecundity and proximate composition of Chinook salmon eggs from Ichthyophonus-infected and "healthy" females taken for subsistence use near Eagle, Alaska in 2010.*

| | Fecundity [total # of eggs] | Water [g%] | Lipid dry weight [g%] | Lipid wet weight [g%] | Ash/Inorganic [g%] | Crude Protein [g%] | Caloric Density [kcal/g dry weight] |
|---|---|---|---|---|---|---|---|
| ***Ichthyophonus* -negative** | 7,582 ± 1,726 | 56.9 ± 3.1 | 23.9 ± 6.3 | 15.3 ± 4.2 | 3.9 ± 0.7 | 20.5 ± 2.5 | 6.3 ± 0.4 |
| **Range** | 3,900 to 12,500 | 52.9 to 70.3 | 12.6 to 51.1 | 8.0 to 33.2 | 2.8 to 6.0 | 12.3 to 26.2 | 5.0 to 7.3 |
| ***n=57*** | | | | | | | |
| ***Ichthyophonus* -positive** | 7,022 ± 1,721 | 55.9 ± 2.9 | 33.0 ± 11.6 | 21.1 ± 7.5 | 3.8 ± 0.5 | 17.8 ± 3.1 | 6.3 ± 0.3 |
| **Range** | 4668 ± 9082 | 50.8 to 59.0 | 19.1 to 46.9 | 12.0 to 29.9 | 3.3 to 4.5 | 13.7 to 21.2 | 5.7 to 6.5 |
| ***n=6*** | | | | | | | |
| ***P* -value** | 0.45 | 0.45 | **0.003** | **0.004** | 0.74 | **0.02** | 1.00 |

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

***Table 2:*** *Blood chemistry parameters (mean ± SD) of Ichthyophonus-infected and uninfected Chinook salmon taken in Emmonak, Alaska in 2010.*

| | ***Ichthyophonus*** **-positive** | ***Ichthyophonus*** **-negative** | ***P*** **-value** |
|---|---|---|---|
| AST [u/l] | 366.6 ± 273.0 | 596.6 ± 413.5 | |
| Range | 0 to 993 | 0 to 1696 | *0.09* |
| *n* | 11 | 33 | |
| BA [umol/l] | 61.8 ± 23.9 | 65.0 ± 52.8 | |
| Range | 30 to 99 | 0 to 179 | *0.85* |
| *n* | 11 | 33 | |
| **CK [u/l]** | **3789.8 ± 3854.2** | **1558.4 ± 2743.4** | |
| **Range** | **0 to 9748** | **0 to 9311** | ***0.04*** |
| ***n*** | **11** | **33** | |
| **UA [mg/dl]** | **1.6 ± 0.8** | **0.8 ± 0.5** | |
| **Range** | **0.9 to 3.7** | **0.1 to 2.2** | ***0.004*** |
| ***n*** | **11** | **33** | |
| GLU [mg/dl] | 47.2 ± 28.7 | 82.4 ± 67.7 | |
| Range | 15 to 114 | 13.5 to 238 | *0.79* |
| *n* | 13 | 50 | |
| PHOS [mg/dl] | 18.7 ± 3.8 | 20.8 ± 4.7 | |
| Range | 15.6 to 26.4 | 12.3 to 29.1 | *0.15* |
| *n* | 13 | 49 | |
| TP [g/dl] | 5.8 ± 0.9 | 5.9 ± 1.0 | |
| Range | 4.4 to 7.5 | 2.7 to 8.4 | *0.83* |
| *n* | 13 | 50 | |
| ALB [g/dl] | 4.6 ± 0.6 | 4.6 ± 0.7 | |
| Range | 3.5 to 5.8 | 2.6 to 6.1 | *0.84* |
| *n* | 13 | 43 | |
| GLOB [g/dl] | 1.2 ± 0.6 | 1.2 ± 0.6 | |
| Range | 0.3 to 2.4 | 0.1 to 2.6 | *0.96* |
| *n* | 13 | 43 | |
| **ALP [u/l]** | **91.0 ± 63.4** | **46.2 ± 47.2** | |
| **Range** | **20.0 to 222** | **0 to 242** | ***0.02*** |
| ***n*** | **12** | **31** | |
| **ALT [u/l]** | **8.8 ± 13.8** | **56.2 ± 58.0** | |
| **Range** | **0 to 37.0** | **0 to 213** | ***0.008*** |
| ***n*** | **12** | **31** | |
| **BUN [mg/dl]** | **4.9 ± 1.4** | **6.0 ± 1.9** | |
| **Range** | **3.0 to 7.0** | **3.0 to 11.0** | ***0.02*** |
| ***n*** | **12** | **31** | |
| CRE [mg/dl] | 0.07 ± 0.1 | 0.5 ± 0.8 | |
| Range | 0 to 0.3 | 0 to 3.3 | *0.09* |
| *n* | 12 | 31 | |
| AMY [u/l] | 25.3 ± 9.4 | 17.8 ± 11.6 | |
| Range | 11.0 to 39.0 | 2.0 to 65.0 | *0.06* |
| *n* | 12 | 31 | |
| TBIL [mg/dl] | 0.2 ± 0.1 | 0.2 ± 0.1 | |
| Range | 0.1 to 0.4 | 0 to 0.5 | *0.62* |
| *n* | 12 | 31 | |
| K [mmol/l] | 4.7 ± 3.5 | 2.8 ± 2.9 | |
| Range | 0 to 9.1 | 0 to 10.8 | *0.06* |
| *n* | 13 | 48 | |
| Na [mmol/l] | 155.1 ± 11.4 | 147.4 ± 14.7 | |
| Range | 128 to 169 | 122 to 172 | *0.09* |
| *n* | 13 | 48 | |
| **Cortisol [ug/dl]** | **43.0 ± 29.8** | **26.0 ± 22.7** | |
| **Range** | **2.4 to 83.7** | **0.1 to 78.7** | ***0.03*** |
| ***n*** | **12** | **49** | |

***P*** **-value:** Testing $H_0$ no difference between "healthy" and infected Chinook salmon

***Table 3:*** *Blood chemistry parameters (mean ± SD) of Ichthyophonus-infected and uninfected Chinook salmon taken in Eagle, Alaska in 2010.*

| | ***Ichthyophonus*** **-positive** | ***Ichthyophonus*** **-negative** | ***P*** **-value** |
|---|---|---|---|
| **AST [U/l]** | **834.9 ± 698.6** | **293.6 ± 319.5** | |
| **Range** | **0 to 2086** | **0 to 987** | ***0.02*** |
| ***n*** | **11** | **14** | |
| BA [umol/l] | 6.0 ± 13.0 | 7.8 ± 14.8 | |
| Range | 0 to 35.0 | 0 to 35.0 | *0.74* |
| *n* | 13 | 14 | |
| CK [U/l] | 117.3 ± 406.5 | 982.6 ± 2036.8 | |
| Range | 0 to 1408 | 0 to 6281 | *0.16* |
| *n* | 12 | 14 | |
| UA [mg/dl] | 0.5 ± 0.6 | 0.9 ± 0.8 | |
| Range | 0 to 1.6 | 0 to 2.4 | *0.12* |
| *n* | 13 | 14 | |
| GLU [mg/dl] | 16.9 ± 19.5 | 20.2 ± 16.5 | |
| Range | 0 to 76.5 | 0 to 71.5 | *0.64* |
| *n* | 13 | 14 | |
| Ca [mg/dl] | 14.1 ± 4.9 | 15.4 ± 5.3 | |
| Range | 0 to 20.0 | 0 to 20.0 | *0.51* |
| *n* | 13 | 14 | |
| PHOS [mg/dl] | 16.0 ± 5.1 | 14.4 ± 6.4 | |
| Range | 0 to 20.3 | 0 to 20.0 | *0.48* |
| *n* | 13 | 14 | |
| TP [g/dl] | 6.2 ± 0.9 | 6.6 ± 0.9 | |
| Range | 4.0 to 7.4 | 4.9 to 8.4 | *0.18* |
| *n* | 13 | 14 | |
| ALB [g/dl] | 3.4 ± 2.0 | 3.9 ± 1.7 | |
| Range | 0 to 4.9 | 0 to 5.5 | *0.44* |
| *n* | 13 | 14 | |
| GLOB [g/dl] | 1.2 ± 1.0 | 1.7 ± 1.0 | |
| Range | 0 to 3.2 | 0 to 3.1 | *0.24* |
| *n* | 13 | 14 | |
| ALP [U/l] | 122.5 ± 68.7 | 98.7 ± 10.3 | |
| Range | 22.0 to 219 | 36.0 to 276 | *0.38* |
| *n* | 13 | 14 | |
| ALT [U/l] | 28.0 ± 56.3 | 2.6 ± 7.4 | |
| Range | 0 to 188 | 0 to 27.0 | *0.11* |
| *n* | 13 | 14 | |
| BUN [mg/dl] | 4.6 ± 3.4 | 5.1 ± 3.1 | |
| Range | 0 to 9.0 | 0 to 11.0 | *0.68* |
| *n* | 13 | 14 | |
| CRE [mg/dl] | 0.3 ± 0.7 | 0.3 ± 0.5 | |
| Range | 0 to 2.4 | 0 to 1.7 | *1.00* |
| *n* | 13 | 14 | |
| AMY [U/l] | 27.5 ± 10.5 | 26.9 ± 29.0 | |
| Range | 10.0 to 40.0 | 9.0 to 125 | *0.95* |
| *n* | 13 | 14 | |
| **TBIL [mg/dl]** | **0.7 ± 0.4** | **1.1 ± 0.3** | |
| **Range** | **0.1 to 1.5** | **0.3 to 1.4** | ***0.004*** |
| ***n*** | **12** | **14** | |
| K [mmol/l] | 0.1 ± 0.4 | 1.2 ± 2.6 | |
| Range | 0 to 1.5 | 0 to 7.5 | *0.15* |
| *n* | 13 | 14 | |
| **Na [mmol/l]** | **130.7 ± 13.2** | **141.5 ± 10.3** | |
| **Range** | **100 to 150** | **111 to 155** | ***0.03*** |
| ***n*** | **13** | **14** | |
| Cortisol [ug/dl] | 9.8 ± 12.7 | 5.4 ± 3.3 | |
| Range | 0.8 to 44.7 | 1.4 to 10.7 | *0.24* |
| *n* | 14 | 13 | |

***P*** **-value:** Testing $H_0$ no difference between healthy and infected Chinook salmon

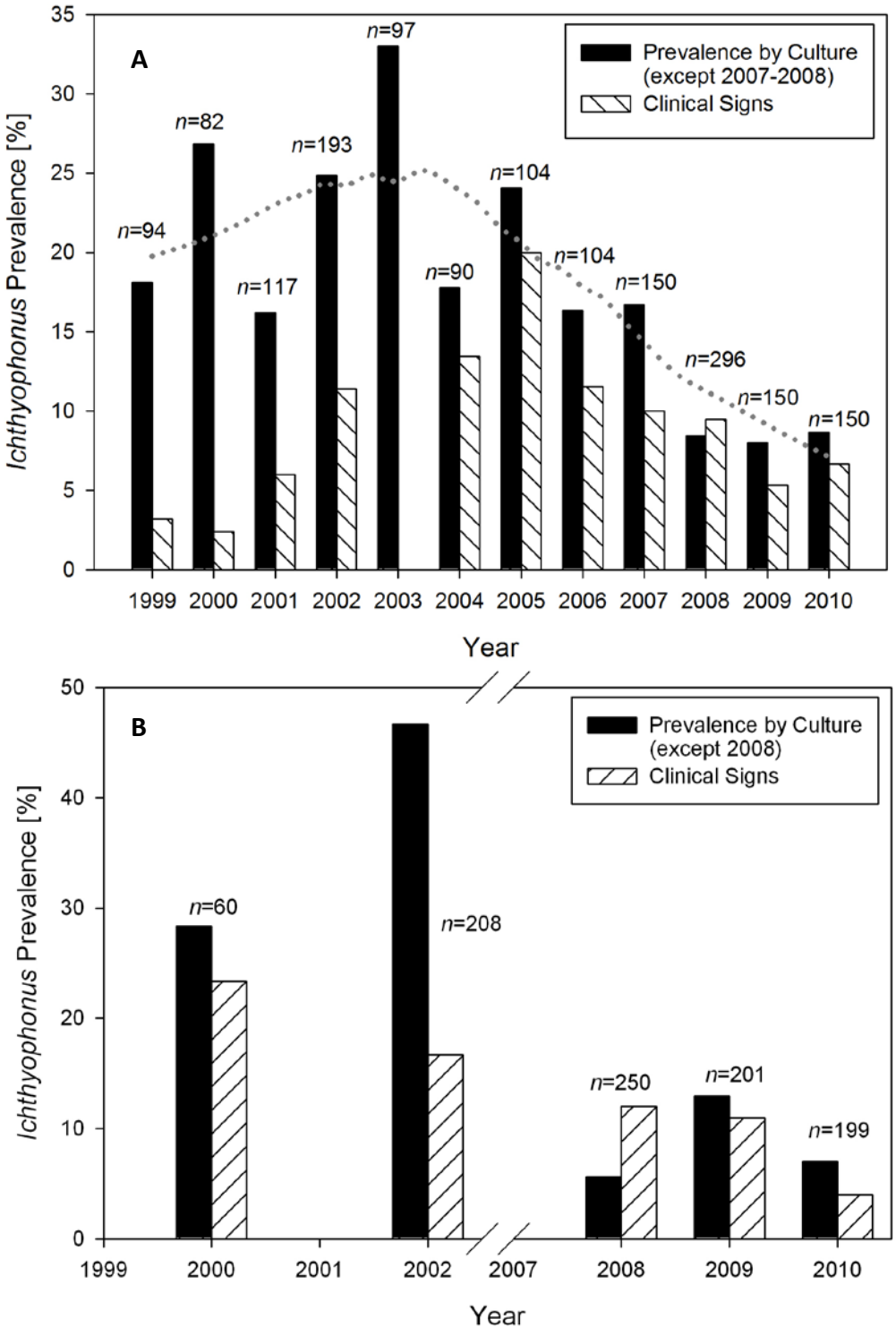


***Figure 1:*** *Time-series of Ichthyophonus prevalence at Emmonak (A) and Eagle (B) based on heart culture and PCR in Chinook salmon (n = sample size). LOESS non-parametric smoothing (dashed gray line) was applied to visualize temporal trends of parasite prevalence. Data from 1999 to 2003 is based on studies by Kocan et al. (2004), Kocan and Hershberger (2006) in Eagle and Emmonak, data from 2004-2006 in Emmonak after Kahler et al. (2007), and 2007-2010, this study.*

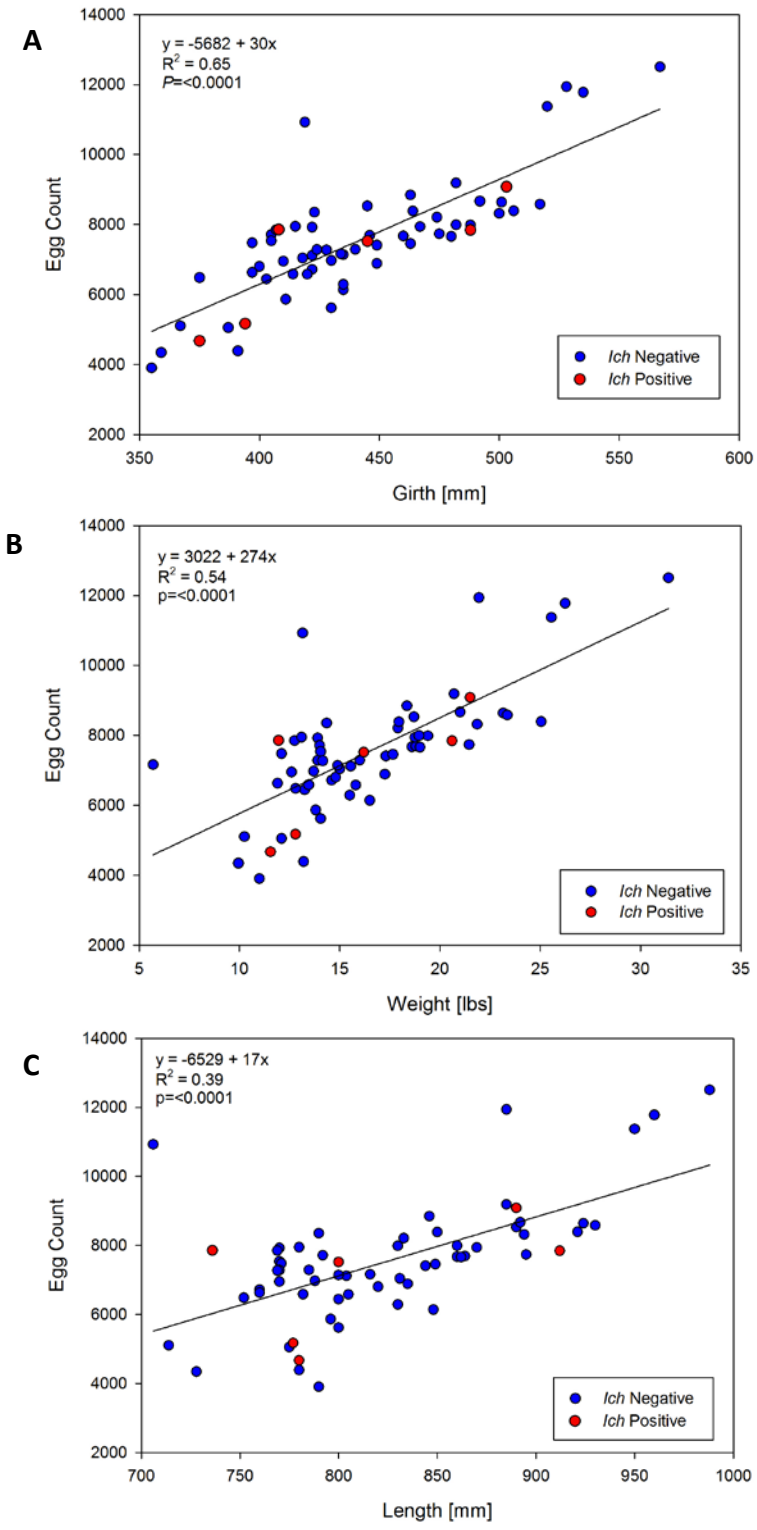


***Figure 2:*** *Total egg count versus girth (A), weight (B), and length (C) in Yukon River Chinook salmon caught for subsistence use in Eagle, Alaska in 2010. Red symbols indicate Ichthyophonus-positive females as determined by culture. Linear regression parameters are provided in the graph.*

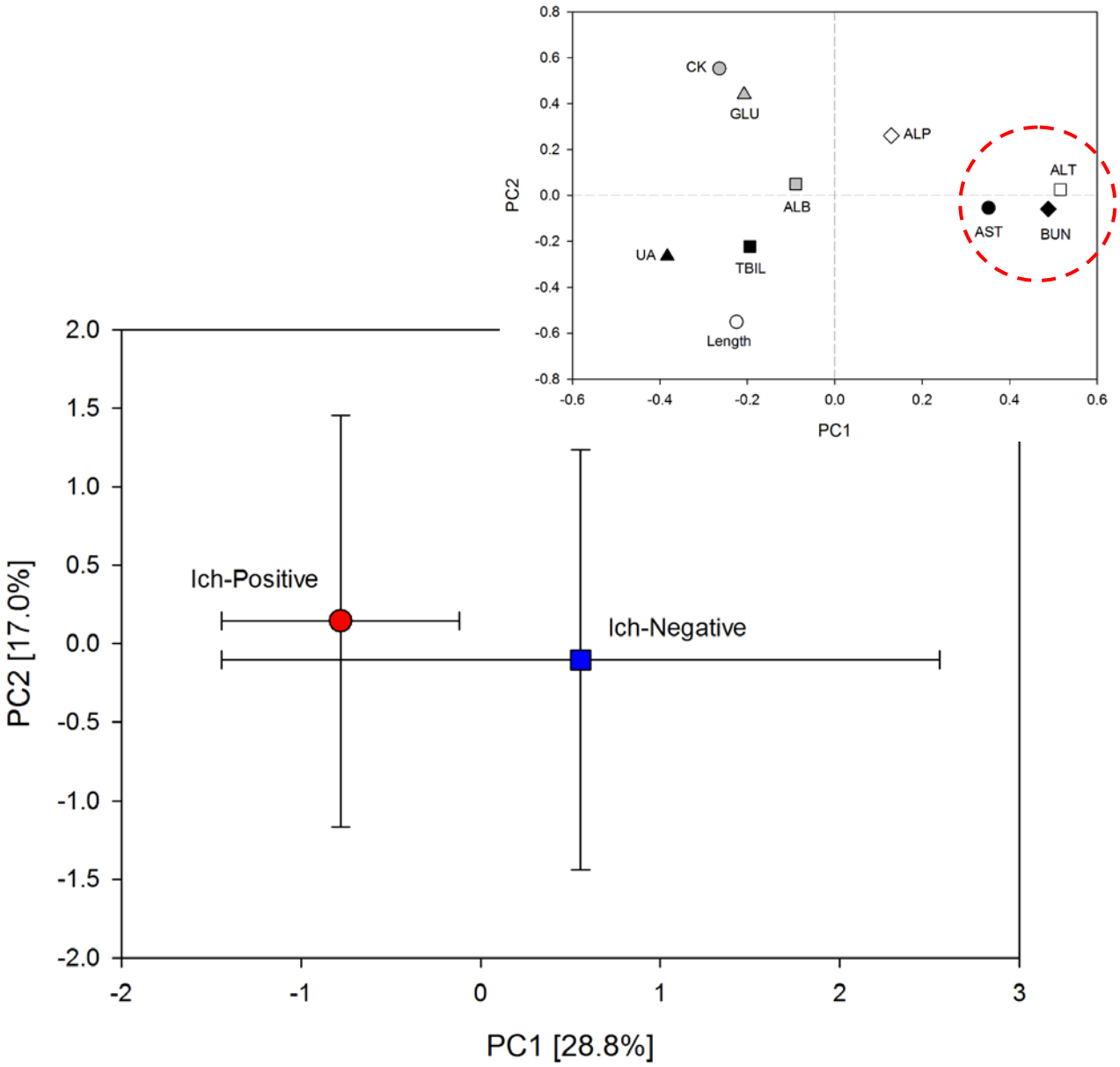


***Figure 3:*** *Principal component (PC) 2 versus 1 for Chinook salmon harvested in Emmonak, Alaska in 2010 by infection status. Symbols present the mean values and error bars show the standard deviations. The graph in the upper right illustrates the contribution of variables associated with the principle components in a loading plot and the red dashed circle highlights the variables driving the separation in PC1.*

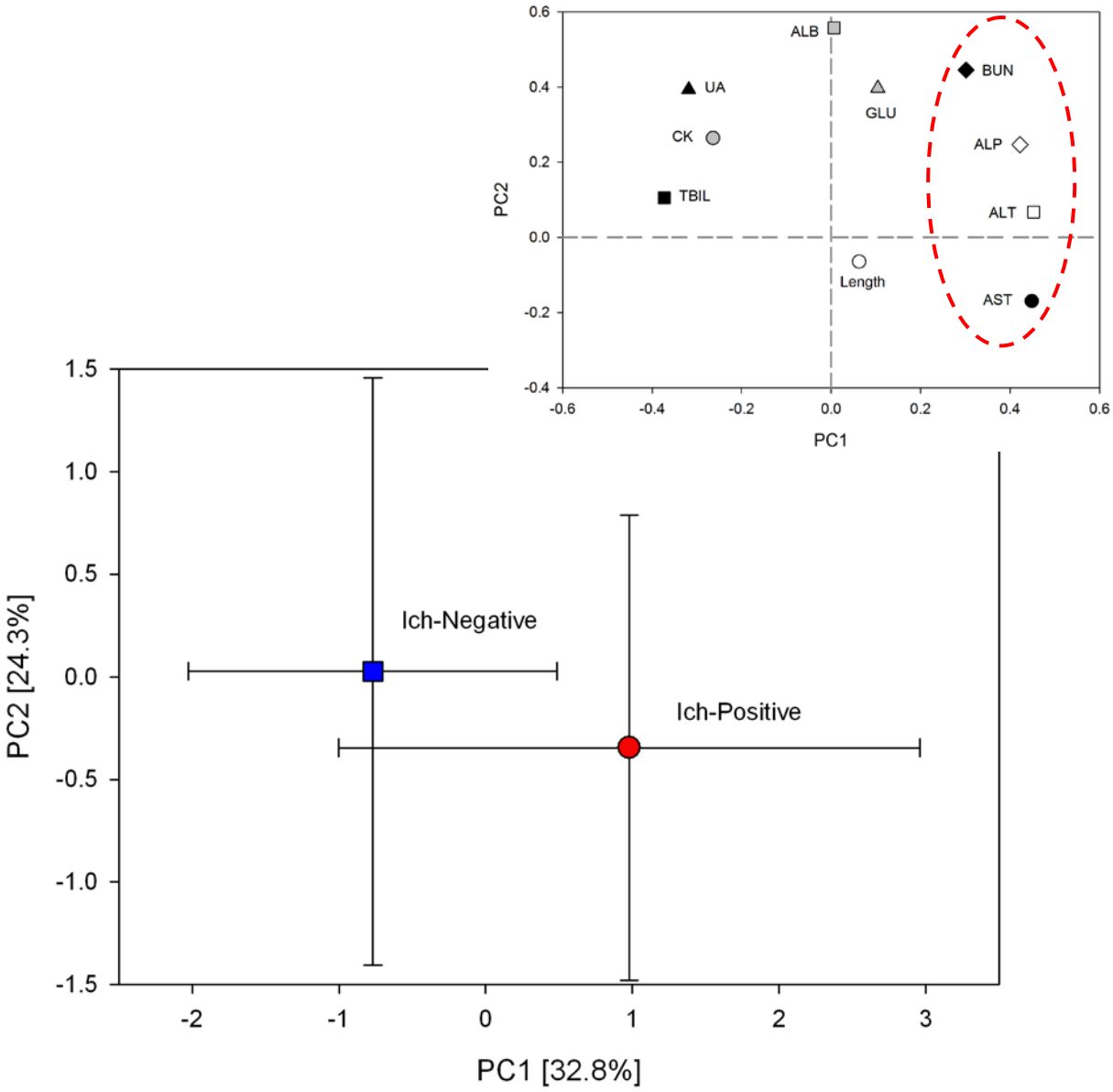


***Figure 4:*** *Principal component (PC) 2 versus 1 for Chinook salmon harvested in Eagle, Alaska in 2010 by infection status. Symbols present the mean values and error bars show the standard deviations. The graph in the upper right illustrates the contribution of variables associated with the principle components in a loading plot and the red dashed circle highlights the variables driving the separation in PC1.*

References

Bales, J. 2007. Salmon age and sex composition and mean lengths for the Yukon River area, 2005. Alaska Department of Fish and Game. Fishery Data Series 07-04: 111 pp.

Corbel, M.J. 1975. The immune response in fish: a review. Journal of Fish Biology 7: 539-563.

Finn, J.P. and N.O. Nielsen. 1971. The effect of temperature variation on the inflammatory response of rainbow trout. Journal of Pathology 105:257-268.

Gavryuseva, T.V. 2007. First report of *Ichthyophonus hoferi* infection in young coho salmon *Oncorhynchus kisutch* (Walbaum) at a fish hatchery in Kamchatka. Russian Journal of Marine Biology 33: 43-48.

Harel, M., A. Tandler, G.W. Kissil, S.W. Applebaum. 1994. The kinetics of nutrient incorporation into body tissues of gilthead seabream (*Sparus aurata*) females and the subsequent effects on egg composition and egg quality. British Journal of Nutrition 72: 45-58.

Heim, C., U. Ehlert, D.H. Hellhammer 1999. The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. Psychoneuroendocrinology 25: 1-35.

Jones, S.R.M. and S.C. Dawe. 2002. *Ichthyophonus hoferi* Plehn & Mulsow in British Columbia stocks of Pacific herring, *Clupea pallasi* Valenciennes, and its infectivity to Chinook salmon, *Oncorhynchus tshawytscha*. Journal of Fish Diseases 25: 415-421.

Kahler, E., T. Burton, T. Hamazaki, B.M. Borba, J. Jasper, and L.-A. Dehn. 2007. Assessment of *Ichthyophonus* in Chinook salmon within the Yukon River drainage, 2004. Alaska Department of Fish and Game, Fishery Data Series 07-64: 47 pp.

King, H.R., N.W. Pankhurst, M. Watts, and P.M. Pankhurst. 2003. Effect of elevated summer temperatures on gonadal steroid production, vitellogenesis and egg quality in female Atlantic salmon. Journal of Fish Biology 63: 153-167.

Kinnison, M.T., M.J. Unwin, W.K. Hershberger, and T.P. Quinn. 1998. Egg size, fecundity, and development rate of two introduced New Zealand Chinook salmon (*Oncorhynchus tshawytscha*) populations. Canadian Journal of Fisheries and Aquatic Sciences 55: 1946-1953.

Kocan, R., P. Hershberger, and J. Winton. 2004. Ichthyophoniasis: An emerging disease of Chinook salmon in the Yukon River. Journal of Aquatic Animal Health 16:58-72.

Kocan, R.M., P. Hershberger, T. Mehl, N. Elder, M. Bradley, D. Wildermuth, and K. Stick. 1999. Pathogenicity of *Ichthyophonus hoferi* for laboratory-reared Pacific herring

*Clupea pallasi* and its early appearance in wild Puget Sound herring. Diseases of Aquatic Organisms 35: 23-29.

Kocan, R. and P. Hershberger. 2006. Differences in *Ichthyophonus* prevalence and infection severity between upper Yukon River and Tanana River Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), stocks. Journal of Fish Diseases 29: 497-503.

Kocan, R., S. LaPatra, J. Gregg, J. Winton, and P. Hershberger. 2006. *Ichthyophonus*-induced cardiac damage: a mechanism for reduced swimming stamina in salmonids. Journal of Fish Diseases 29: 521-527.

Kocan, R., P. Hershberger, G. Sanders, J. Winton. 2009. Effects of temperature on disease progression and swimming stamina in *Ichthyophonus*-infected rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of Fish Diseases 29: 835-843.

Leyva, F., S.D. Anker, I.F. Godsland, M.Teixeira, P.G. Hellewell, W.J. Kox, P.A. Poole-Wilson, A.J.S. Coats. 1989. Uric acid in chronic heart failure: A marker of chronic inflammation. European Heart Journal 19: 1814-1822.

Maule, A. G., Tripp, R. A., Kaattari, S. L., C.B. Schreck. 1989. Stress alters immune function and disease resistance in Chinook salmon (*Oncorhynchus tshawytscha*). Journal of Endocrinology 120: 135-142.

Mellergaard, S. and B. Spanggaard. 1997. An *Ichthyophonus hoferi* epizootic in herring in the North Sea, the Skagerrak, the Kattegat and the Baltic Sea. Diseases of Aquatic Organisms 28: 191-199.

Palace, V.P. and J.A. Werner. 2006. Vitamins A and E in the maternal diet influence egg quality and early life stage development in fish: a review. Recent advances in the study of fish eggs and larvae 70: 41-56.

Pickering, A.D. and T.G. Pottinger, T. G.1989. Stress responses and disease resistance in salmonid fish: Effects of chronic elevation of plasma cortisol. Fish Physiology and Biolchemistry 7: 253-258.

Rainuzzo, J.R., K.I. Reitana, Y. Olse. 1997. The significance of lipids at early stages of marine fish: a review. Aquaculture 155: 103–115.

Rand, P.S., S.G. Hinch, J. Morrison, M.G.G. Foreman, M.J. MacNutt, J.S. MacDonald, M.C. Healey, A.P. Farrell, D.A. Higgs. 2006. Effects of river discharge, temperature, and future climates on energetics and mortality of adult migrating Fraser River Sockeye salmon. Transactions of the American Fisheries Society 135: 655–667.

Roche, H. and G. Boge. 2000. In vivo effects of phenolic compounds on blood parameters of a marine fish (*Dicentrarchus labrax*). Comparative Biochemistry and Physiology 125C: 345-353.

Schlechtriem, C., U. Focken, and K. Becker. 2003. Effect of different lipid extraction methods on $\delta^{13}C$ of lipid and lipid-free fractions of fish and different fish feeds. Isotopes in Environmental and Health Studies 39: 135-140.

Sindermann, C.J. 1965. Effects of environment on several diseases of herring from the western North Atlantic. International Commission for the Northwest Atlantic Fisheries Special Publication 6: 603-610.

Strange, R.J., C.B. Schreck, and J.T. Golden. 1977. Corticoid stress responses to handling and temperature in salmonids. Transactions of the American Fisheries Society 106: 213-217.

Tierney, K.B. and A.P. Farrell. 2004. The relationships between fish health, metabolic rate, swimming performance and recovery in return-run sockeye salmon, *Oncorhynchus nerka* (Walbaum). Journal of Fish Diseases 27: 663-971.

Wagner, T. and J.L. Congleton. 2004. Blood chemistry correlates of nutritional condition, tissue damage, and stress in migrating juvenile Chinook salmon (*Oncorhynchus tshawytscha*). Canadian Journal of Fisheries and Aquatic Science 61: 1066-1074.

Wells, R.M.G., R.H. McIntyre, A.K. Morgan, P.S. Davie. 1986. Physiological stress responses in big gamefish after capture: observations on plasma chemistry and blood factors. Comparative Biochemistry and Physiology 84A: 565-571.

Whipps, C.M., T. Burton, V.G. Watral, S. St-Hilaire, and M.L. Kent. 2006. Assessing the accuracy of a polymerase chain reaction test for *Ichthyophonus hoferi* in Yukon River Chinook salmon *Oncorhynchus tshawytscha*. Diseases of Aquatic Organisms 68:141-147.

Zuray, S., R. Kocan, P. Hershberger. 2012. Synchronous cycling of ichthyophoniasis with Chinook salmon density revealed during the annual Yukon River spawning migration. Transactions of the American Fisheries Society 141: 615-623.