Physiological Development and Vulnerability to *C. shasta* of Fall-run Chinook Salmon: a Candidate for Restoration in the Upper Klamath Basin

2006 Final Report

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October 30, 2007

Abstract

As an initial step in the evaluation of specific stocks suitable for the restoration of anadromous fish runs into the Upper Klamath River Basin, we monitored fall Chinook salmon (*Oncorhynchus tshawytscha*) in California's Iron Gate Hatchery to establish the progress of their development, and then held them in netpens at two sites—one in the Williamson River (WR) about 2.5 km above its mouth in Upper Klamath Lake (UKL) and one about 2 km downstream of the WR in UKL. Age 1+ fall Chinook salmon were transferred from hatchery to netpens in October 2005 and age 0+ fall Chinook salmon were transferred in May 2006. In the hatchery and after 3 and 14 days in the netpens, fish were removed and several physiological and morphological indices of smolt development were assessed. Based on gill Na^+, K^+-ATPase activity, plasma thyroxine (T4) concentration, and several measures of skin reflectance, age 1+ Chinook salmon were not developing smolt characteristics in the hatchery during October. Transferring these fish to WR or UKL had some expected physiological responses (i.e., increased plasma cortisol in response to stress, and increased T4 because of the change in water), but overall we do not think transfer altered the fish's development. The same variables in age 0+ Chinook salmon in 2006 indicated that the fish were smolting while in the hatchery. After transfer, fish in the WR netpens, however, lost weight and had the same gill ATPase activity as compared to fish in the hatchery on the day of transfer. Fish in UKL, on the other hand, after transfer gained weight and length, had reduced condition factor and had significantly higher gill ATPase when compared to WR fish. These results and measures of environmental variables suggest that conditions in UKL were conducive to smoltification and may have accelerated the development of Chinook salmon as compared to conditions in WR. The presence of *C. shasta* in the upper WR and lower Klamath River was confirmed using non-resistant rainbow trout exposed at those locations. None of the Chinook salmon in the hatchery or in the netpens in UKL or WR became infected with *C. shasta* during either trial, including Chinook salmon held for 90 d after a 10-d exposure in the netpens in May 2006. Our overall conclusion is that there is little evidence of physiological impairment or significant upriver vulnerability to *C. shasta* of Iron Gate Hatchery fall-run Chinook salmon stock that would preclude their consideration as a candidate for the restoration into the Upper Klamath basin.

INTRODUCTION

The Klamath River watershed once produced some of the largest runs of anadromous fish on the west coast of North America, including both fall and spring run Chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*O. kisutch*), chum salmon (*O. keta*), steelhead (*O. mykiss*), green sturgeon (*Acipenser medirostris*), eulachon (*Thaleichthys pacificus*), coastal cutthroat trout (*O. clarki clarki*), and Pacific lamprey (*Entosphenus tridentata*). These runs supported significant commercial, recreational, subsistence, and Tribal harvests. In particular, the Upper Klamath River Basin above Iron Gate Dam once supported the spawning and rearing of large populations of anadromous salmon and steelhead (Lane and Lane Associates 1981; Federal Energy Regulatory Commission (FERC) 1990). Prior to the completion of impassible barriers to anadromous fish on the main stem Klamath River (Copco 1 Dam in 1918, Copco 2 Dam in 1925, and Iron Gate Dam in 1962) anadromous fish runs accessed spawning, incubation, and rearing habitat in more than 350 miles of river and stream channel above the site of Iron Gate Dam (Hamilton et al. 2005). Iron Gate Dam, at River Mile 190, is the current limit of upstream passage. The Long Range Plan for the Klamath River Basin Conservation Area Fishery Restoration Program (LRP) (USDI Fish and Wildlife Service 1991) identified the lack of passage beyond Iron Gate Dam as a significant impact to the Klamath River anadromous fishery. At present, significant unused anadromous habitat exists upstream of Iron Gate Dam. Federal agencies have prescribed fishways for Klamath River dams as part of hydropower project relicensing.

One critical uncertainty to successful reintroduction of sustainable populations of anadromous fish into historical habitat above and within Upper Klamath Lake (UKL) is whether or not outmigrants will be able to pass through the lake. Because anadromous fish have been excluded from UKL, and habitat and water quality conditions have been altered over the past decades, it is possible that salmon put into the lake might be challenged physiologically, thus impairing their readiness to emigrate. Resistance to the pathogen *Ceratomyxa shasta*, present in the Upper Klamath River Basin, will be critical to the consideration of salmon stocks for reintroduction. To address these critical uncertainties, we assessed the physiological development of one salmonid stock proposed

for reintroduction, and determined the physiological impacts, including disease resistance, of transferring fish from a hatchery to UKL or the lower Williamson River (WR). We were unable to test more than one stock of Chinook salmon because of concerns about out-of-basin transfers. The objectives of this study were (1) determine if transferring fall Chinook salmon from California Department of Fish and Game's Iron Gate Hatchery (IGH) into netpens in the UKL or WR affects the fish's physiological development; (2) determine if the physiological effects of acclimation in the netpens differed in fish at UKL or WR; (3) determine if the fish become infected with the fish pathogen *C. shasta* during the acclimation period; and 4) determine if there is physiological impairment or vulnerability to *C. shasta* that might preclude the use of Iron Gate fall-run Chinook salmon from reintroduction into the Upper Klamath Basin. This study took place in the fall 2005 using age 1+ fall Chinook salmon (brood-year 2004) and was repeated in the spring 2006 using age 0+ Chinook salmon (brood-year 2005) from the same hatchery. Release of the two age groups represents two alternative approaches to producing juvenile salmon that are physiologically ready to outmigrate. Both groups of fish were sampled in the hatchery over several months to determine the physiological trajectory of their development. The fish were then transferred to the two locations and sampled again after three days. Previous studies have shown that within 24 to 72 hours after transport or other acute stress fish's physiological responses will have returned to baseline (Barton and Iwama 1991; Maule et al. 1988; Wendelaar Bonga 1997). We sampled the fish again after they had been in the netpens for 14 days to assess potential impacts of the transfer and the two locations on physiological development (Beckman et al. 2003; Hoffnagle and Fivizzani 1990). We monitored several physiological variables that have been shown to be important responses to stress and to the process of smoltification, which prepares salmon for emigration and pre-adapts them for entering the marine environment. We also examined fish for the pathogen *C. shasta*, which is responsible for mortalities of salmonids in the lower Klamath River system, and conducted a field-exposure experiment to determine the likelihood of these Chinook salmon being infected with *C. shasta* at various locations in the Klamath River Basin.

METHODS

Fish.

Rearing. Two groups of Chinook salmon from the Iron Gate Hatchery were used for this study. The first group of fish were progeny of 2004 brood-year adults that were spawned October 8, 2004. The eggs and larval fish were raised with the general population in the hatchery building until May 15, 2005, when about 1500 fish were transferred to a separate holding tank (4.7 m long by 1.2 m wide and 0.4 m deep) positioned outside near the standard hatchery raceways. These fish were held separately because the hatchery fish were going to be released before this study was completed. The fish in the tank received a continuous flow of single-pass Klamath River water from the reservoir behind Iron Gate Dam. Water temperature varied from 6.1° C (43° F) at the beginning of the study to 13.3° C (56° F) when fish were transferred. The fish were fed daily with the same commercial salmon diet (BioOregon Starter, Bio-Moist Grower, and then Nelson and Sons Silver Cup) as the general hatchery population. The second group of fish were progeny of adults of the 2005 brood-year, which were spawned on October 19, 2005. Because these experimental fish were going to be transferred before the hatchery fish were to be released, these fish were not separated from the general population, but were sampled from the general hatchery (production) population in raceways. Our initial assumption that this change did not compromise the experimental design was confirmed; there were no differences in results from the first year comparing responses of fish in the small tanks to those of fish in the raceways (see Results). The 2005 brood-year fish were fed the same diets as above, and were put in hatchery raceways 30.5 m x 3.1 m x 1.5 m (100' x 10' x 5') on February 17, 2006. At this time water temperature was 4.5^{o} C, which increased to 14° C by the time fish were transferred to the netpens.

Sampling. Fish were sampled in the hatchery at the beginning of each month from August through October 2005. Fish were also sampled on October 17, prior to transferring them to netpens (see below). Four groups of fish (total = 20 fish) were randomly sampled from the holding tank and put into about 10 L water containing 50 mg L^{1} tricaine methanesulfonate (MS-222) and taken to the sampling area in the hatchery building. One or two fish at a time were transferred to water containing 80 mg L^{-1} MS-

222 until they were well anesthetized and then were removed to determine weights and lengths. Individual fish were then transferred to the holding aquarium where color and infrared digital photographs were taken (see below: **Skin Reflectance during Smoltification**). The fish were then bled by severing their caudal peduncle and collecting blood into heparinized tubes. The blood was subsequently centrifuged to separate plasma from cells and the plasma was collected for determining concentrations of plasma cortisol and thyroxine (T4). About 10 mg of gill filament was clipped from the first full gill arch on the right side of the fish for determining Na^+,K^+ ATPase activity. These parameters have been shown to be indicative of smoltification (Hoar 1976; Zaugg et al. 1985). The tubes containing plasma and gill filaments were rapidly frozen in liquid nitrogen, and subsequently stored in a -80° C freezer until assayed (see below: **Biochemical Measures**). Fish that were sampled in the hatchery before transport and those sampled on two occasions from the netpens were assessed for the presence of *C. shasta*, a myxosporean parasite found extensively in salmonid fish in the Klamath River Basin. This was accomplished by removing a piece of the fish's lower intestine and preserving it in 95 percent ethanol. The tissue was then sent to the Department of Microbiology, Oregon State University, Corvallis, Oregon (OSU) to assess the presence of *C. shasta* using polymerase chain reaction (PCR) based on the method of Palenzuela and Bartholomew (2002).

In 2006, fish were sampled four times from the hatchery raceways between March 9 and May 16. After the last sample in the hatchery, fish were transported to the netpens. Sample collection and variables assayed were the same in 2006 as described for 2005, except that the small size of the fish at the first two sample times prohibited the collection of an adequate volume of blood with which to assay either plasma variable. Plasma samples collected beginning in May were of adequate volume to assay only one variable (T4).

We did not continue to sample hatchery fish in either year after experimental fish were transferred to the netpens. Based upon the results of other work (cite), we assumed that for fish in the hatchery, variables would continue on the same trajectory during the next two weeks as that observed at the time of transfer. For example, in actively smolting

Chinook salmon we expected that gill ATPase activity and plasma T4 would continue to increase, and condition factor would continue to decrease (Beckman et al. 2003; Hoar 1976; Hoffnagle and Fivizzani 1990)

Transfer and holding. After sampling in the hatchery on October 17, 2005, 30 age 1+ Chinook salmon were put into each of four transport tanks filled with about 150 L (40 gallons) of hatchery water. Each tank was continuously aerated with air from a small aquarium pump via air stones. Ice in a plastic bag was added to each tank to ensure that the water temperature was not elevated during transport. The tanks were in the back of a pickup truck which was driven to UKL—a trip which took about three hours. No fish died during transport to the netpen sites. In 2006, transportation, holding, and sampling of age 0+ Chinook salmon on May 16, 2006, was identical to that of 2005.

Two netpens had previously been put in place in each of two locations, the first about 2.5 km upstream of the mouth of the WR and the second about 2 km east of the mouth in UKL. The netpens were 0.45-m cubes made of 6.4-mm bar mesh netting on all sides of a PVC pipe frame. The netpens were held about 1 m off the bottom by a combination of anchors and floats. On arrival at the transfer site, two tanks of fish were taken to each netpen location by boat and 30 fish were transferred to each netpen, which was sealed and re-suspended. Water quality (temperature, pH and dissolved oxygen) at each site was monitored hourly using YSI 600 XLM data sondes deployed 1 m off the bottom. We calibrated multiprobes prior to each deployment and checked parameter precision and accuracy against reference multiprobes upon retrieval, following U.S. Geological Survey (USGS) established protocols to collect data and maintain multiprobes (USGS National Field Manual, USGS 1997 to present). After three days (October 20) we sampled one group from each site to assess the fish's response to stresses of transportation and change in holding, and after 14 days (October 31) the group from each site was sampled to assess the influence of the holding locations on physiological development. At sampling, all of the fish in one netpen at each location were quickly removed, put into 50 mg l^{-1} MS-222 and taken to a sampling station setup on the shore; there, the fish were sampled as described above.

Disease Testing in 2006.

We coordinated our disease experiments with another study conducted by OSU and others who had an established design, which we could not match precisely (i.e., different numbers of fish and lengths of exposures). Data from that study, however, are incorporated in the present study in order to determine (1) the susceptibility to *C. shasta* of IGH fall Chinook salmon in different areas of the Klamath Basin and (2) the impacts of *C. shasta* to a salmonid species with known susceptibility held in locations through which IGH Chinook salmon might migrate if released in the upper Klamath Basin.

We used a known susceptible strain of rainbow trout (*O. mykiss*) to assess the presence and prevalence of *C. shasta*. On May 16, 2006 two additional groups of 35 0+ Chinook salmon were transported from IGH and transferred to a cylindrical netpen (0.3 m x 1.0 m) at WR and UKL. These additional fish were used to test exposure of the fish to pathogens in each of the holding locations. Although there were more fish in each transport tank, weight per fish in 2006 was just 10 percent of 2005 fish. The OSU study mentioned above used 3-d exposures, which have historically resulted in close to 100 percent mortality in "hot-spots" in the lower Klamath River. Because none of the fish in our study became infected with *C. shasta* in 2005, we wanted to maximize exposure in 2006. Scheduling conflicts prohibited the OSU staff from collecting fish on day 14, so we were forced to end the exposures after 10 d. Thus, the Chinook salmon in each of these cylindrical netpens were retrieved on May 26 (identified as: May/10-d Lower WR FCS and May/10-d UKL FCS in Table 1) and transported to the Oregon State University, John L. Fryer Salmon Disease Laboratory (OSU-FSDL), Corvallis, Oregon where they were held for 90 d to assess the presence and severity of *C. shasta* and monitor pathogen-related mortalities. During April, May, and June 2006, 3-d exposures of 40 of the disease susceptible rainbow trout were conducted. The exposure took place in the Williamson River approximately 6.0 km (about rkm 8.0) upstream from the netpen site (referred to in Table 1 as: April/3-d, May/3-d, and June/3d Upper WR RBT). During the June exposure, 40 IGH Chinook salmon were also exposed along with the 40 rainbow trout at this location (June/3d Upper WR FCS in Table 1). In May and June, 40 rainbow trout and 40 IGH Chinook salmon were also exposed (3 d) in the lower Klamath River above the

Beaver Creek confluence (rkm 259.1; Table 1 rows 5 and 6 and 9 and 10). This site is located about 46.7 km downstream from Iron Gate Dam in Northern California and about 141.0 km downstream of the Williamson River confluence. Water temperature readings were recorded for all exposure groups at each site.

After exposure, the rainbow trout and Chinook salmon to be tested for disease were retrieved, and the groups placed in individual coolers with bubbled oxygen. The fish were then transported to the OSU-FSDL where each exposure group was held in separate 100-L tanks on 13˚C specific pathogen-free water until about 90 d post-exposure (dpe) when all fish were euthanized. Preventative treatments for bacterial infections were administered within 1 dpe, and included a two-week diet of TM100 (Bio-Oregon, Warrenton, OR) medicated feed and 1.0 mg/L Furanase (Aquarium Products, Glenburnie, MD) bath treatment 1 hr daily for 3 d. After two weeks, fish received a 1-hr formalin bath at 125 – 170 mg/L for three consecutive days to remove external parasites. Dead or moribund fish were collected daily and examined for signs of infection. All groups, including unexposed control groups, were terminated with a lethal dose of MS222 (tricaine methanesulfonate). A sample of 10 fish per exposure group was visually examined for spores by microscopy. If any fish was identified as positive, an additional 15 fish were examined by microscopy. Dead or moribund fish as well as fish sampled for infection were first examined by wet-mount. The wet-mount was prepared by inserting a sterilized inoculating loop of the appropriate diameter into the anogenital pore to a depth of approximately 1.0 – 1.5 cm. The sample collected was smeared onto a glass microscope slide and observed at 100 X or 250 X magnifications for 3 min. Fish were considered positive if the characteristic kidney bean-shaped myxospore was observed. Fish not demonstrating clear spore stages were not considered visually positive due to the difficulty of differentiating early presporogonic stages from host cells or other myxozoans. If spores were not observed, intestinal tissue was excised, digested, and assayed by a single round PCR using methods described by Palenzuela and Bartholomew (2002). The following modifications were made to the protocol: an additional 2 – 3 mm segment of the alimentary canal, just posterior to the pyloric ceca attachment, was excised and included with the 5.0 mm segment of the posterior intestine. This was done

to ensure inclusion of representative portions of the intestine as the disease manifests somewhat differently in different species.

Percent prevalence of infection was calculated as the number of exposed fish that tested positive for infection (by microscopy and/or PCR analysis), including euthanized fish and mortalities, divided by the total number of fish examined for infection (X 100). Percent mortality was calculated as the number of fish that died during the 90-d holding period that were visually positive for *C. shasta* by microscopy, divided by the total number of fish that survived the prophylactic treatment period (> 5 dpe) also expressed as: [(# mortalities) / (# mortalities + # terminated) X 100]. The mean days-to-death for each exposure group was calculated as the geometric mean of all days with *C. shasta* positive mortalities within the 90-d holding period.

In addition to the fish exposures, 1.0-L water samples were collected, filtered, and assayed by quantitative PCR to quantify spore concentrations using methods described by Hallet and Bartholomew (2006). Before the fish were set in the water for exposure, three 1.0-L bottles of water were manually collected 30 cm below the water surface at 5-min intervals. The same process was repeated just prior to retrieving the fish. Water samples were not collected from the lower WR or UKL netpens. We used ISCO 3700 portable water samplers (Teledyne-Isco Inc, Lincoln, NE) to collect water samples in the lower Klamath River. The portable samplers were set to collect two 500-ml samples with a single purge cycle every 2.0 hrs during the course of the 3-d exposure period. When one sampler had run its 24-hr collecting cycle the sample bottles were retrieved and filtered while the other sampler continued the process.

Skin Reflectance.

Photography. It has long been observed that as juvenile salmon prepare to emigrate, they change from the cryptic brown and green colors of a stream bottom to more silvery colors characteristic of pelagic marine fish. Coloration and changes in skin reflectance as the fish become silvery have been used as a measure of smoltification (Haner et al. 1995). The development of digital cameras during the past 10 years led us to us determine if digital camera technology would provide a better means to non-lethally detect smolt development than was used previously (Haner et al. 1995). In order to explore the full

range of digital camera capability, we took a color photo and a near-infrared (IR) photo of each fish using a Nikon D70 digital, single-lens reflex (SLR) camera with 6.1 mega pixels resolution and two Sigma 28-70 mm zoom lenses. One lens was for color photography and the second lens was fitted with a Hoya R72 IR filter to minimize the time and handling required to shift between the two photographs. The camera was mounted on a tripod with the camera in manual mode and the lens aperture set at f-stop 22 (f22). When taking color photographs, the aperture on the camera body was set at f8 and the shutter speed was 1/250 s. At the beginning of the photo session, the shutter speed for IR photography was determined empirically between 1/8 to 2 s based on the lighting. Light was supplied by an Interfit Tungsten 3200K 1300 WATT continuous light (Paterson Photographic, Douglasville, GA), which contained two 650-watt tungsten bulbs in soft-boxes with front light diffusers. The light was on a stand to the right of the camera, which was situated close enough to the fish tank so that the fish filled the view finder. Unfortunately one of the bulbs burned out at the beginning of sampling on the day fish were transferred to the netpens (17 Oct 2006). We intentionally took all photos with a single 650-watt bulb without changing aperture or speed on that day and subsequent days so that fish sampled from the netpens could be compared to similarly-lighted fish at the hatchery.

Data capture and analyses. All images were saved as JPEG files and were taken into Photoshop 7.0 software to obtain data on total, red, blue, and green luminosity from the color images; grey scale data from converted color images; and IR. The color image was brought into the software and a rectangular section of the image was captured. The vertical sides of the rectangle were delineated by the posterior edge of the opercula and the insertion of the dorsal fin; the horizontal lines ran along the back and just above the lateral line. In Photoshop, we initially ran the Auto-Levels command, which adjusts the black-point and white-point in the image. This clips a portion of the shadows and highlights in each channel and maps the lightest and darkest pixels in each color channel to pure white (level 255) and pure black (level 0). The intermediate pixel values are redistributed proportionately, thus, increasing the contrast in the image because the pixel values are expanded. Because Auto Levels adjusts each color channel individually, it may remove color or introduce color casts. The Photoshop Histogram function was used to

measure luminosity and grey-scale of the captured rectangle. The histogram generated by the software is the distribution of the dark-to-light values for all pixels (range: ~ 100,000 to 350,000 pixels) within the cropped image, and varies from 0 (dark) to 255 (light); thus, the higher the value, the lighter the image. The IR photo was cropped in the same way as the color photo. The selected image was desaturated to remove the artificial red color imposed by the camera, and the mean and median dark-to-light value was determined via the Histogram function, as was done with the color image. Data collected from Histograms were mean and median luminosity.

Biochemical Measures.

Gill ATPase activity (Johnson et al. 1977), plasma thyroxine (T4; Jaklitsch et al. 1976) and plasma cortisol (Ogihara et al. 1977) were assayed by Biotech Research and Consulting, Inc. (Corvallis, OR) using standard methods as cited.

Statistical analyses.

Statistical comparisons of the mean and median values for total color luminosity; red, blue, and green luminosity; gray scale and IR for each fish; as well as mean weight, length, condition factor {K-factor = $[(\text{mass})(1000)/ (\text{length})^3] [100]$}, plasma T4, and plasma cortisol were conducted in Prism GraphPad software. Analyses consisted of parametric and nonparametric tests, and it was determined that the use of either medians or means were appropriate for the various reflectance measures. However, as the data were derived from histograms that were often skewed, we used medians in our analyses. Mean data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests where differences occurred. In the event of an unbalanced design, the statistical software automatically used the General Linear Models (GLM) approach, which is an ANOVA for unequal sample sizes. Median data were subjected to a nonparametric Kruskal-Wallis test followed by Dunn's multiple pairwise comparisons. These analyses were conducted independently on two groups of data for each variable. The first group established the trajectory of the various indices examined, and included data collected from fish sampled in the hatchery. The second group examined the impacts of the netpens, and included data collected from fish in the

hatchery on the day fish were transported to the netpen sites and from fish sampled from the netpens after 3 or 14 days. We also conducted two-way ANOVA (or GLM) on data from fish in netpens using location (UKL and WR) and days in the netpens (3 and 14 d) as the dependent variables. Environmental variables (temperature, DO, and pH) were collected as continuous data and were summarized as daily means after we determined that there were no differences between mean daytime (0601 to 1800 hours) and mean nighttime (1801 to 0600 hours) values for any of the variables (t-tests, $P > 0.05$). The mean daily values for the days each group of fish were in the netpens (3 d and 14 d) were compared using Kruskal-Wallis tests and Dunn's multiple pairwise comparisons. For all statistical tests, differences were considered significant when $P \leq 0.05$.

RESULTS

Gill ATPase

In 2005, gill ATPase activity decreased continuously in fish in the hatchery. There were no differences in gill ATPase activity between the experimental fish and the hatchery fish at any time (Figure 1, top). This was true of all of the variables examined, except for plasma cortisol, and indicated that fish could be held and sampled from either the raceways or the smaller holding tanks and obtain the same results. Decreasing gill ATPase activity is expected of Chinook salmon during the late summer and early fall when photoperiod and temperature are decreasing. When fish were transferred to the netpens in UKL and WR (arrows on all figures indicate time and group transferred) there was no effect on ATPase activity from either environment. The values did differ between locations when comparing fish at WR after 3 d to fish at UKL after 14 d — a comparison that we do not believe is as meaningful as comparing between fish held at the two locations for an equal amount of time.

In 2006, gill ATPase activity increased in fish in the hatchery (Figure 2, top). These Chinook salmon exhibited evidence of smoltification in anticipation of emigration. When the fish were transferred to the netpens, gill ATPase activity remained elevated in both locations; that is, it did not differ from values in fish in the hatchery on the day of transfer. However, activity in fish held in the WR for 14 d was significantly lower than

that of fish in UKL after 14 d. In both years, gill ATPase values differed based on location of netpens (WR or UKL) but not days in the netpens (3 d or 14 d; 2-way ANOVA or GLM, $P < 0.05$).

Plasma T4

In 2005, plasma T4 did not differ between experimental and production (general hatchery) groups. Although there were some significant differences between some dates (e.g., lower values on September 8), we do not believe that there were any biologically significant differences in fish in the hatchery through time (Figure 1, bottom). On the last day of sampling before fish were transferred to the netpens, a mistake in plasma sample tube labels kept us from separating experimental fish from production fish. As these groups did not differ at any time for any of the variables, we are comfortable pooling the results. Sample size for this pool is 40. After transfer to netpens, plasma T4 increased significantly for fish in the WR after 3 d. This increase in T4 after transferring salmon to a new water source was expected and has been previously documented (Grau et al. 1982; Lin et al. 1988). Fish in UKL netpens demonstrated a similar (though not significant) increase in T4 after 14 d. After 14 d, the T4 levels of fish held in WR decreased significantly and differed from that of fish in held in UKL for the same length of time and from those in WR after 3 d.

In early 2006 there was insufficient blood volume in fish (mean weight < 1.0 g) during the sampling to assay for T4. However, by early May mean plasma T4 was > 2.0 ng ml^{-1} (Figure 2, bottom) – a level not attained by any groups in 2005. Thus, we assume that plasma T4 was increasing during the early spring, similar to gill ATPase (Figure 2, top) as the fish went through smoltification. Three days after transfer to the netpens, plasma T4 in fish at both locations did not differ from that of fish in the hatchery. Plasma T4 in fish after 14 d in the netpen in the WR was lower than in fish before leaving the hatchery on May 16, but there were no differences in T4 between any of the groups of fish held in netpens for 3 or 14 d (Figure 2, bottom). While in 2005 there were no difference in plasma T4 based on netpen location or days in the netpens (2-way GLM), in 2006 fish in the UKL netpens had higher T4 levels than fish in WR (2-way GLM, $P < 0.05$).

Plasma Cortisol

In 2005, plasma cortisol in the pooled production and treatment fish on October 17 was significantly higher than the other samples collected in the hatchery except the experimental fish on August 9 (Figure 3, top). The range of mean values (9.2 to 30.8 ng ml^{-1}), however, were all lower than what is considered stressful in hatchery fish (Barton and Iwama 1991; Schreck 1982). Plasma cortisol was extremely high in fish in both netpens 3 d after transfer—indicative of the stress the fish experienced by being transported from the hatchery. Cortisol declined after 14 d in the netpens, but was still significantly higher than it had been in the hatchery. Elevated but declining plasma cortisol after 14 d suggests that the fish were stressed by the transfer, but were adapting to the new environment. Differences in fish density and the lack of food, as well as differences in water quality, were probably all contributing to high plasma cortisol. Plasma cortisol in fish in the WR was significantly lower than that of fish in UKL based on location and number of days in the netpens (2-way GLM, $P < 0.05$; Figure 3, top). In 2006, insufficient volume of plasma in the small fish sampled prohibited the assessment of plasma cortisol.

Condition Factor, Weight and Length

In 2005, there were no differences in condition factor between treatment and production groups in the hatchery (Figure 3, bottom). Condition factor was reduced in fish in both netpens as compared to the hatchery, with the exception that fish held for 3 d in WR did not differ from fish in the hatchery. By 14 d, however, condition factor in fish at WR was reduced significantly from the 3 d measurements (Figure 3, bottom).

In 2006, condition factor of fish sampled in the hatchery increased significantly between March and May, and then declined significantly in all groups after the fish were transferred to the netpens (Figure 4). Furthermore, condition factor of fish in the WR was significantly lower than that of fish in UKL. Based on 2-way GLM, there was no difference between netpen fish based on location or days in the netpens in 2005. However, in 2006 this variable differed based on both location and days in the netpens (2-way GLM, $P < 0.05$).

Weights and lengths of fish in the hatchery increased throughout rearing in both years (Figures 5 and 6). These variables did not change in fish transferred to netpens in 2005, nor were there any differences among the two netpen locations. There were, however, significant changes in these variables when fish were transferred to netpens in 2006. Weight of fish in WR declined significantly after 3 d in netpens and remained lower after 14 d. Weights in fish in UKL did not decline after transfer, and were in fact greater than those in the hatchery after 14 d (Figure 6, top). Lengths of fish in the netpens for 3 d did not differ from that of fish in the hatchery; however, by 14 d the fish in UKL were significantly longer than fish in the hatchery and those in WR (Figure 6, bottom). While there was no difference in lengths or weights based on location or days in the netpens in 2005, in 2006 both variables differed based on location of netpens and the length of time fish were in the netpens (2-way GLM, $P < 0.05$).

Skin Reflectance

In 2005, Chinook salmon had significantly greater IR luminosity in the hatchery during September and October than in August (Figure 7, bottom), but there were no significant changes in grey scale or color luminosity—including the red, blue or green luminosity during the same time (Figures 7 top, 8 and 9). Moreover, the IR values declined initially after the fish were transferred to netpens and then returned to values equal to those of fish in the hatchery after 14 d (Figure 7 bottom). Grey scale, color luminosity, red, blue, and green luminosity all declined on the last sample date in the hatchery (October 17); however, this was almost certainly an artifact of the burned-out bulb that reduced by half the illumination. We continued to use a single bulb when sampling fish from the netpens, and it appears that the increases in luminosity in these variables were related to moving the fish to the netpens. There were no differences in any of these measures of reflectance between fish held in the WR versus UKL in 2005, with the exception of blue luminosity, which was lower in fish held in WR for 3 d as compared to UKL (Figure 9, top). By 14 d, all measures of reflectance (except IR) at both locations were significantly higher than in fish in the hatchery and in fish at WR at 3 d. However, there was no difference in reflectance measures between netpen locations after 14 d.

In 2006, the reflectance variables measured in fish were generally unchanged during the rearing time in the hatchery (Figures 10, 11 and 12), with the exception that all were reduced significantly in samples taken on May 5. Three days after fish were transferred to netpens, values for all of these reflectance variables were significantly lower in fish in the UKL netpens than fish in the hatchery or in the WR netpens. After 14 d, none of the measures of reflectance differed between fish in the netpens or the hatchery on the day the fish were transported (Figures 10, 11, and 12). However, these same measures of reflectance were all greater in fish in WR than those in UKL based on 2-way ANOVA ($P < 0.05$) (Figures 10, 11, and 12). This was not true in 2005, when none of the reflectance measures differed by location, but all except IR differed based on days in the netpens (2-way GLM, $P < 0.05$; Figures 7, 8, and 9).

Juvenile Chinook Salmon Transport and Short Term Survival.

All of the fish transferred from Iron Gate Hatchery (120 in 2005 and 180 in 2006) survived transport to the Upper Klamath Basin and were successfully put into the netpens. In 2005, there were no mortalities among the 60 fish sampled from the netpens (30 fish from each site) after 3 d, and one mortality in the WR netpen after 14 d. In 2006, there were two mortalities in the WR netpen 3 d after transport and one mortality at the same location 10 d after transport when fish were collected for *C. shasta* susceptibility test. After 14 d, there were three more mortalities in the WR. While there was no mortality among the fish held at the UKL site, there were only 14 fish in that netpen after 14 d. Examination revealed an approximately 3-cm diameter hole in the mesh. As there was no evidence of dead fish in this netpen and all fish in the other netpens were accounted for as alive or recovered mortalities, we believe the 16 missing fish escaped into UKL.

Susceptibility to *C. shasta*

In 2005, there was no evidence of *C. shasta* infection in any of the Chinook salmon sampled from the hatchery or from the netpens in the WR or UKL after 3 or 14 d (data not shown). However, some fish had exophthalmia ("popeye") indicating the potential for systemic pathogen infection. Exophthalmia was seen in one fish from the

UKL netpen and three from the WR netpen after 3 d and in three fish from the UKL and five from the WR after 14 d. In 2006, there was no evidence of *C. shasta* infection in any fish sampled from the netpens after 3 or 14 d; however, we did not necropsy the six dead fish from the WR netpens because opportunistic microbes could have invaded the carcasses after the fish died.

The two groups of 35 Chinook salmon exposed for 10 d at WR and UKL in May 2006 were retrieved, transported to OSU–CFDR without loss, and successfully acclimated to lab conditions without mortality. During the 90-d holding period, no mortality occurred and, at termination of the experiment, all fish that were examined microscopically were negative for *C. shasta* (May / 10-d exposures in Table 1). Gross pathology characteristic of ceratomyxosis was not evident in fish from either the UKL or WR 10-d exposure groups. However, in the past no Chinook salmon exposed in the WR or UKL have become infected, even during the period of the year when parasite densities are highest (R. Stocking, Oregon State University, unpublished data).

Results from rainbow trout exposures in April at the upper WR site (April / 3-d exposure in Table 1) indicate that *C. shasta* was present in the WR when water temperatures averaged about 12.2 ˚C. Only one rainbow trout died during the 90-d holding period (at 49 dpe), but the prevalence of infection was > 95 percent. In May, rainbow trout exposed in the upper WR (May / 3-d Upper WR in Table 1) demonstrated high prevalence of infection (97.5 percent) and high mortality (97.5 percent) when water temperatures averaged 19˚C; those exposed lower in the Klamath River, at about the same water temperature (18.2˚C) suffered similar mortality (92.3 percent) and prevalence of infection (100 percent; May / 3-d KR in Table 1). Water temperatures during the June exposure in the WR had decreased to an average of 17.4˚C while that in the Klamath River remained high (20˚C). Prevalence of infection and mortalities of rainbow trout exposed at both sites remained high (> 96 percent; June / 3-d in Table 1). The mean days-to-death for rainbow trout in June (32.2 dpe) was about the same as it was in May (31.8 dpe). We detected no infection or mortality in the Chinook salmon exposed in the upper WR in June; however those exposed in the Klamath River suffered 16.7 percent mortality and had a moderate prevalence of *C. shasta* (37.5 percent; Table 1). In all tests, rainbow trout and IGH Chinook that were not exposed (i.e., control fish) tested negative for *C.*

shasta. Detection of *C. shasta* spores in the water collected from the upper WR and lower KR indicates a seasonality of presence at both locations, as there were at least an order of magnitude more spores at both locations in June (10 spores / L in Table 1) than May (> 1 spores / L in Table 1).

Water Quality

As would be expected, water temperature varied between the fall 2005 (Figure 13) and spring 2006 (Figure 14) and between the WR and UKL sites. Due to its shallow nature, UKL is very responsive to changes in ambient air temperature. At the beginning of the 2005 holding period, temperature in UKL was about 11° and 14 d later had decreased to about 7.5° C. In 2006 temperature in UKL started at about 16.5° C, increased to 20.5° C after 3 d of unseasonably warm weather and then declined to about 13° C at the end of the 14-d holding period. Similar temperature patterns occurred in the WR, but with different values. In the fall 2005, WR temperatures started at about 9° C and declined to about 6.5° C. In the spring 2006 WR temperatures were 18° C, and increased to 20.5° C before declining to about 12° C. Mean daily temperatures in 2005 did not differ significantly when comparing one location at different times (e.g., UKL at 3 d versus UKL at 14 d) or between sites after the same number of days (e.g., UKL at 14 d versus WR at 14 d; Figure 15, top). In 2006, however, mean daily temperature at WR was significantly higher after 3 d as compared to 14 d (Figure 15, bottom).

The mean daily pH was similar at both locations for a given year (2005 daily means = 8.01 to 8.14; 2006 daily means = 7.34 to 7.87; Figure 15); however, in both years the mean daily pH was higher in UKL than WR (Figure 15). Because we used daily mean values of the continuous water quality variables for statistical analyses, the true variation in the data was lost. However, it is quite evident from visual inspection that there was considerably more variation in the measures taken at the UKL site than at the WR site (Figures 13 and 14). The continuous data (Figures 13 and 14) show that pH in the UKL was considerably more variable (daily variation of almost 1.0 units) than in WR (daily variation of < 0.1 units in 2005 and < 0.5 units in 2006). Similarly, mean daily dissolved oxygen (DO) in both locations and years ranged from 7 – 10 mg L^{-1}. DO values at WR were often significantly lower than values at UKL (Figure 15). However,

daily variation was much greater in UKL (~2.0 mg L^{-1}) than at WR (~0.5 mg L^{-1}; Figures 13 and 14).

DISCUSSION

During the fall 2005 and spring 2006, we monitored a number of physiological variables over several months in fall Chinook salmon in the hatchery to establish a developmental trajectory and a baseline against which to judge possible impacts of transferring the fish to two sites in the upper Klamath Basin. Based on the lack of biologically significant changes in plasma cortisol, condition factor (Figure 3) or plasma T4 (Figure 1), and the declining gill ATPase activity (Figure 1) we conclude that in 2005 age 1+ juvenile Chinook salmon in the hatchery were not going through smoltification at the time they were transferred to the netpens. We also conclude that transferring those fish to the netpens had no long-term effect on their physiology or development. As would be expected, plasma cortisol was elevated (Figure 3) in response to the stress of transportation (Maule et al. 1988; Wendelaar Bonga 1997). Also not surprisingly, fish in the WR after 3 d and UKL after 14 d had elevated plasma T4 (Figure 1), in response to being transferred to a new water source (Lin et al. 1985; Hoffnagle and Fivizzani 1990). While weights and lengths increased significantly while fish were held in the hatchery, neither variable changed after fish were put in netpens (Figure 5).

Most of the measures of skin reflectance did not change in fish in the hatchery in 2005 (Figures 7, 8, and 9); however, IR reflectance did increase. After transfer to the netpens, IR reflectance decreased significantly after 3 d and then returned to pre-transport levels at 14 d. This change in IR reflectance may be related to the stress of transport, which can have significant effects on fish skin (Igar et al. 1992; Mazon et al. 2006); however, the biological significance of this reflectance metric, independent of other developmental changes, is not known (Maule and Batt 2006). The pattern of changes in grey scale and all measures of luminosity were identical in fish sampled from the netpens; compared to values in the hatchery, these measures of skin reflectance were unchanged after 3 d in WR and UKL but were significantly higher in netpen fish after 14 d (Figures 7, 8, and 9).

While increased grey scale reflectance has been associated with smolt development and migratory behavior (Haner et al. 1995), the absence of changes in other physiological indices of smolt development and the fact that red, blue, and green luminosity increased suggests that skin coloration changed to match new environmental colors (Donnely and Whoriskey 1991; Donnelly and Dill 1984). In 2006 all measures of skin reflectance were significantly decreased in fish sampled from UKL after 3 d, but did not differ from the hatchery values at any of the other sample locations or days.

The significance of these differences in skin reflectance is not clear, as they could be the result of many internal and external factors. Fish skin coloration changes in response to surroundings or in response to stress. Anadromous salmonids also change coloration in anticipation of going from freshwater to the ocean. In freshwater, most predators attack from above (e.g., birds) so fish assume the brown and green colors of the stream bottom. To "hide" from marine predators (e.g., other fish) attacking from below, pelagic fish such as salmon, assume a blue and silver coloration that mimics the sky above them. This shift to silvery coloration is caused by the deposition of guanine in the skin (Staley and Ewing 1992) and happens at the same time as the other biochemical and physiological changes (i.e., smoltification) that prepare salmonids for survival in the high salt, marine environment (Ewing and Birks 1982).

In an earlier study (Haner et al. 1995), this change in coloration could be detected in hatchery fish during smoltification and emigration by measuring the grey-scale reflectance of the skin. In a more recent study, however, grey scale did not differ between spring Chinook salmon that volitionally left a hatchery in the fall and those that remained in the raceway (Maule and Batt 2006). The migrants did, however, have greater IR luminosity than did the non-migrants. The differences in reflectance of fish at the two sites in 2006 may also have been caused by environmental factors (discussed further below).

When Chinook salmon were monitored in the hatchery and then transferred to the same locations in the upper Klamath Basin, results differed notably between fall 2005 and spring 2006. In the hatchery, gill ATPase activity increased significantly between March and May 2006 (Figure 2), suggesting that the 0+ fish were going through smolt development (Beckman et al. 2003; Hoar 1986). Condition factor, weight, and length

also increased significantly between March and May (Figures 4 and 6). Measures of skin reflectance did not differ between March and May; however, all measures were significantly lower in April than March or May (Figures 10, 11, and 12). Unfortunately the fish were too small to collect enough blood with which to measure both plasma cortisol and T4, so we do not have a measure of the fishes' response to the stress of transport. While in 2006 there were few differences in variables between hatchery fish and fish in netpens after 3 d (i.e., reduced condition factor at both sites, Figure 4; and reduced weight in WR fish, Figure 6), virtually all measures of fish physiology and morphology differed when comparing fish in UKL to fish in the WR after 3 d or 14 d in the netpens. It appears that UKL fish continued to go through smoltification, as evidenced by elevated gill ATPase activity (Figure 2, top) and decreasing condition factor (Figure 4) while gaining weight and length (Figure 6). On the contrary, fish in the WR netpens also had significantly lower condition factor than did fish in the hatchery or UKL netpens, but they concurrently had significantly lower body weight than both other groups of fish. The weight loss seen after 3 d persisted in fish after 14 d in the WR netpens (Figure 6), and we believe this, rather than continued smolt development, resulted in reduced condition factor. The loss in weight of fish at the WR site is even more notable considering that fish in the UKL netpens actually gained weight and length between the 3-d and 14-d sampling times (Figure 6). Furthermore, after 14 d UKL fish had higher gill ATPase activity than did WR fish (Figure 2) suggesting that conditions in the WR that led to reduced weight of juvenile Chinook salmon may have also affected their smolt development. While declining condition factor has been used as an index of smoltification in other studies, we do not believe that is the case for WR fish because of the lack of other changes suggesting smolting (i.e., gill ATPase or plasma T4).

Differences in environmental variables may account for the differences in fish responses between fall 2005 and spring 2006, and between the two netpen sites in 2006. Both pH and DO were much more variable at UKL than WR (Figures 13 and 14), but mean daily values were similar between seasons and locations (Figure 15). On the contrary, while water temperatures were also more variable at UKL than WR, average values at both sites were significantly higher in the spring 2006 (about 12 to 20° C; Figure 14) than in the fall 2005 (about 6 to 11° C; Figure 13). In 2006, fish transferred

from the hatchery (temperature = 14° C) to the WR were subjected to initial water temperatures (~ 18° C) greater than those experienced by the UKL fish (~16° C; Figure 14). Water velocities may also have varied between the netpen sites and seasonally at the WR site. Wood et al. (2006) reported that water velocity at several locations in UKL varied between 0 and 30 cm per second (cm s^{-1}), but averaged about 5 to 10 cm s^{-1}, during the summer 2003. A USGS gaging station in the WR near the mouth of the Sprague River (about 14 km up stream of our netpens) reported that the average daily discharge for May 16 – 30, 2006 was 2621 cubic feet per second (cfs) as compared to just 545 cfs during October 17 – 31, 2005 (data from USGS NWIS; http://waterdata.usgs.gov/or/nwis/inventory for gaging station number 11502500). Based on a discharge-to-velocity relation that we derived from 10 years of quarterly velocity measurements at this station, the water velocities in fall 2005 and spring 2006 were about 29 and 73 cm s^{-1}, respectively — 3- to 12-fold higher than in UKL. Although these measures of velocity were not taken directly at the netpen locations, they do suggest differences in the WR versus the UKL. Another environmental factor that may have contributed to these spatial and temporal differences in fish responses is the presence of natural food available as drift into the netpens. Wood et al. (2006) documented nitrogen (as nitrates or nitrites) and ortho-phosphate concentrations in UKL that were 100-fold higher than those reported in the WR (USGS NWIS data; http://waterdata.usgs.gov/or/nwis/inventory). Although these measurements were taken decades apart, they confirm the hypereutrophic condition of UKL as compared to the WR and the high likelihood that planktonic drift would be available to fish in the UKL netpens.

To summarize, in the spring 2006 fish at both netpen sites were exposed to high water temperatures (~20°C), which would have increased the fish's basal metabolism as compared to fish in the same location in the fall 2005, when water temperatures were lower (~10°C). The fish at the WR site would also be required to expend more energy in the spring than in the fall (and perhaps as compared to fish at UKL in the spring) due to higher water velocities. Natural fish food (i.e., planktonic drift) was more likely available to fish in the UKL netpens than the WR netpens by virtue of the 100-fold greater nutrient loads in the lake than the river. Thus, fish in the WR netpens experienced

a negative energy budget (increased bioenergetic demand and decreased food availability) and lost weight, while those in UKL had a positive energy budget (less of an increased demand and increased availability) and actually gained weight. Another significant factor when comparing fish in the fall 2005 and spring 2006 was the almost 10-fold greater weight of fish used in the fall. A 1- or 2-g change in weight would not have been significant (biologically or statistically) to a 50-g fish, but most certainly would be to a 5-g fish.

Results from the pathogen exposure portion of this study demonstrated that *C. shasta* was present in the WR and was sufficiently abundant — especially considering the 10-fold increase in spores in the water between May and June (Table 1) — to cause high mortality in a known susceptible strain of rainbow trout. Chinook salmon exposures conducted in the upper WR, lower WR, and UKL suggest that this species is sufficiently resistant to survive exposure at these sites during the spring. When Chinook salmon were exposed in the lower Klamath River in June, however, they did suffer considerable mortality and infection. For unknown reasons, experimental exposures of Chinook salmon in the upper Klamath Basin have never resulted in the detection of the pathogen in the fish (R. Stocking, Oregon State University, unpublished data).

The results of these exposure experiments suggest that *C. shasta* will not be a concern for reintroduced Chinook salmon outmigrants before June. Later migrants moving downstream into the Klamath River below Iron Gate Dam may be exposed to the same conditions, and experience the same mortality factors as extant lower Klamath River stocks. However, for all exposed fish held at OSU-FSDL, post-exposure temperatures (13° C) were reduced compared with natural river conditions (18 to 20° C) and this may have resulted in an underestimation of infection, as the fish would be better able to resist pathogens at the lower temperature.

These results provide a pilot comparison of two potential restoration approaches at two locations for one stock of fall Chinook salmon. Under these conditions, results suggest that yearling (age 1+) releases in the fall may not result in fish that are physiologically ready to outmigrate at that time of year. In contrast, the results suggest that age 0+ Chinook salmon released in the spring in UKL continued smolt development and physiological readiness to outmigrate. For this same age group, the release in the

lower WR may result in less physiological readiness to outmigrate. However, this does not mean that individual elements of the WR release location scenario could not be successfully matched with other variables. For example, had these fish been released directly into this area of sub-optimal (but not lethal) conditions, they could move in search of more appropriate conditions. Alternatively, in order to facilitate imprinting, which is necessary for spawning adults to find their natal stream, fish could be held in larger netpens in areas where temperature, flow, and prey are more optimal.

Furthermore, the effects we documented on age 0+ fall Chinook salmon may not adequately represent impacts on spring Chinook salmon. Sauter et al. (2001) documented that the temperature preferences of fall and spring Chinook salmon differ as they go through the process of smoltification, and Maule et al. (1988) found differences in how fall and spring Chinook salmon respond to stress during their migrations to the ocean.

In conclusion, these results provide initial documentation of differences in the response of one stock of fall Chinook salmon placed in netpens at different sites in the Upper Klamath River Basin in the spring. The results suggest that age 0+ fall Chinook salmon continued smoltification and that this transformation was more pronounced in UKL than in the WR. These results would not preclude the consideration of the reintroduction of fall-run Chinook salmon in the WR, but should be a point of departure for future investigations into the best approach for the restoration of fall run Chinook stocks into the Upper Klamath Basin.

Acknowledgements

We thank Kim Rushton and the staff at the California Department of Fish and Game Iron Gate Hatchery for their excellent cooperation and help throughout the study. We thank Rip Shively at the USGS Klamath Falls Field Station for help with study design and logistics. We also thank Christine Adelsberger, Summer Burdick, Heather Hendrixson, Mark Johnson, Aaron Walker, Katherine Webster, and Alex Wilkens at the USGS Klamath Falls Field Station for help collecting samples, and Jodi Charrier, Sally Sauter, and Tom Batt at the USGS Columbia River Research Laboratory for help with collection and analyses of the photographs used to measure skin reflectance. Scott Foott, Phil Detrich, and Roxanna Hinzman (U.S. Fish and Wildlife Service) contributed significantly

with their thoughtful reviews of the report. We thank Larry Dunsmoor (Fishery Biologist, Klamath Tribe) for his critical review. The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the United States Department of Interior or the United States Geological Survey of any product or service to the exclusion of others that may be suitable. This study was funded by the U.S. Fish and Wildlife Service under agreement number 113334H006.

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Table 1. Results of 3-d or 10-d field exposures of fall Chinook salmon (FCS) and a known susceptible strain of rainbow trout (RBT) in 2006 to *Ceratomyxa shasta* in the upper Williamson River (Upper WR; rkm 8), Lower WR (rkm 2), Upper Klamath Lake (UKL) and Klamath River (KR; 141 rkm below WR mouth). After exposure fish were transported to Oregon State University and held at the John L. Fryer Salmon Disease Laboratory for 90 days to monitor disease progression. Mortalities (Mort), days-to-death [geometric mean + (SD)] and prevalence of *C. shasta* (Prev) based either on PCR or microscopic examination were determined for groups of 35 or 40 fish. Water temperature [Water Temp; mean + (SD)] and mean number of *C. shasta* spores in the water (Spores/L) were measured on-site during each exposure.

Month/ Exposure Duration	Location	Species	Mort (%)	Days-to-Death	Prev (%)	Water Temp (˚C)	Spores / L
April / 3-d	Upper WR	RBT	2.5	49	96.3	12.2 (1.1)	BD
May / 3-d	Upper WR	RBT	97.5	31.8 (5.4)	97.5	19.3 (1.0)	> 1
May / 10-d	Lower WR	FCS	0.0	0	0.0	18.0	ND
May / 10-d	UKL	FCS	0.0	0	0.0	20.0	ND
May / 3-d	KR	RBT	92.3	52.0 (14.6)	100	18.2 (0.6)	> 1
May / 3-d	KR	FCS	0.0	0	0.0	18.2 (0.6)	> 1
June / 3-d	Upper WR	RBT	97.6	32.2 (4.5)	100	17.4 (0.3)	10
June / 3-d	Upper WR	FCS	0	0	0	17.4 (0.3)	10
June / 3-d	KR	RBT	96.2	37.9 (3.2)	98.1	20.0 (1.1)	10
June / 3-d	KR	FCS	16.7	46.1 (15.1)	37.5	20.0 (1.1)	10

BD = below detection; ND = not determined

Figure Captions

Figure 1. Gill ATPase enzyme activity (top) and plasma thyroxine (T4) concentrations (bottom) (mean + 1 SEM) of experimental and production fall Chinook salmon sampled at Iron Gate Hatchery and after transport to the Upper Klamath Basin, October 2005. Bars with letters in common denote values for fish sampled in the hatchery that do not differ. Bars with numbers in common denote values for fish in the hatchery before they were transported to netpens in Upper Klamath Lake and Williamson River (indicated by arrows), and fish in the netpens after 3 or 14 d (last four bars) that do not differ from each other. Sample sizes are 18 to 20. Data were analyzed by 1-way ANOVA or General Linear Models (GLM; $P < 0.05$). Based on a 2-way GLM ($P < 0.05$), gill ATPase differed between fish based on location of the netpens, but not based on days in the netpens.

Figure 2. Gill ATPase enzyme activity (top) and plasma thyroxine (T4) concentrations (bottom) (mean + 1 SEM) of fall Chinook salmon sampled at Iron Gate Hatchery and after transport to the Upper Klamath Basin in May 2006. Bars with letters in common denote values for fish sampled in the hatchery that do not differ. Bars with numbers in common denote values for fish in the hatchery before they were transported to netpens in Upper Klamath Lake and Williamson River (indicated by arrows), and fish in the netpens after 3 or 14 d (last four bars) that do not differ from each other. All sample sizes are 14 to 20, except on May 4, 2006 when sample size is 8. Gill ATPase data were analyzed by General Linear Models (GLM; $P < 0.05$). Both variables differed (2-way GLM, $P < 0.05$) between fish based on location of the netpens, but not based on days in the netpens.

Figure 3. Plasma cortisol (top) and condition factor (bottom) (mean + 1 SEM) of experimental and production fall Chinook salmon sampled at Iron Gate Hatchery and after transport to the Upper Klamath Basin in October 2005. Bars with letters in common denote values from fish sampled in the hatchery that do not differ. Bars with numbers in common denote values from fish in the hatchery before they were transported to netpens in Upper Klamath Lake and Williamson River (indicated by arrows), and fish in the netpens after 3 or 14 d (last four bars) that do not differ from each other. Sample sizes are 18 to 20, except for plasma cortisol on the first date of sampling where sample sizes were 7 for experimental fish and 10 for production. Data were analyzed by 1-way ANOVA or General Linear Models (GLM; $P < 0.05$). Based on a 2-way GLM ($P < 0.05$), plasma cortisol differed based on location of the netpens, but not based on days in the netpens.

Figure 4. Condition factor (mean + 1 SEM) of fall Chinook salmon sampled at Iron Gate Hatchery and after transport to the Upper Klamath Basin in May 2006. Bars with letters in common denote values in fish sampled in the hatchery that do not differ. Bars with numbers in common denote values from fish in the hatchery before they were transported to netpens in Upper Klamath Lake and Williamson River (indicated by arrows), and fish in the netpens after 3 or 14 d (last four bars) that do not differ from each other. All sample sizes are 14 to 20. Data were analyzed by 1-way ANOVA or General Linear Models (GLM; $P < 0.05$). Based on a 2-way GLM ($P < 0.05$), condition factor differed based on location of the netpens and number of days in the netpens.

Figure 5. Weight (top) and fork length (bottom) (mean + 1 SEM) of experimental and production fall Chinook salmon sampled at Iron Gate Hatchery and after transport to the Upper Klamath Basin in October 2005. Bars with letters in common denote values from fish sampled in the hatchery that do not differ. Bars with numbers in common denote values from fish in the hatchery before they were transported to netpens in Upper Klamath Lake and Williamson River (indicated by arrows), and fish in the netpens after 3 or 14 d (last four bars) that do not differ from each other. Sample sizes are 18 to 20. Data were analyzed by 1-way ANOVA or General Linear Models (GLM; $P < 0.05$). Based on a 2-way GLM ($P < 0.05$), neither variable differed based on location of the netpens nor days in the netpens.

Figure 6. Weight (top) and fork length (bottom) (mean + 1 SEM) of fall Chinook salmon sampled at Iron Gate Hatchery and after transport to the Upper Klamath Basin in May 2006. Bars with letters in common denote values in fish sampled in the hatchery that do not differ. Bars with numbers in common denote values from fish in the hatchery before they were transported to netpens in Upper Klamath Lake and Williamson River (indicated by arrows), and fish in the netpens after 3 or 14 d (last four bars) that do not differ from each other. All sample sizes are 14 to 20. Data were analyzed by 1-way ANOVA or General Linear Models (GLM; $P < 0.05$). Based on a 2-way GLM ($P < 0.05$), both variables differed based on location of the netpens and days in the netpens.

Figure 7. Skin reflectance as measured by grey scale (top) and infrared luminosity (bottom) of experimental and production fall Chinook salmon sampled at Iron Gate Hatchery and after transport to the Upper Klamath Basin in October 2005. The raw data were medians from histograms (see Methods); however, the data are summarized as means (of the medians) + 1 SEM. Bars with letters in common denote values from fish sampled in the hatchery that do not differ. Bars with numbers in common denote values from fish in the hatchery before they were transported to netpens in Upper Klamath Lake and Williamson River (indicated by arrows), and fish in the netpens after 3 or 14 d (last four bars) that do not differ from each other. Sample sizes are 18 to 20. Data were analyzed by Kruskal-Wallis test followed by Dunn's multiple pairwise comparisons ($P < 0.05$). Based on 2-way GLM ($P < 0.05$), there were no differences between fish based on location of the netpens, but measures of grey scale differed based on days in the netpens.

Figure 8. Skin reflectance as measured by total color (top) and red luminosity (bottom) of experimental and production fall Chinook salmon sampled at Iron Gate Hatchery and after transport to the Upper Klamath Basin in October 2005. The raw data were medians from histograms (see Methods); however, the data are summarized as means (of the medians) + 1 SEM. Bars with letters in common denote values from fish sampled in the hatchery that do not differ. Bars with numbers in common denote values from fish in the hatchery before they were transported to netpens in Upper Klamath Lake and Williamson River (indicated by arrows), and fish in the netpens after 3 or 14 d (last four bars) that do not differ from each other. Sample sizes are 18 to 20. Data were analyzed by Kruskal-Wallis test followed by Dunn's multiple pairwise comparisons ($P < 0.05$). Based on 2-way GLM ($P < 0.05$), there were no differences between fish based on location of the netpens, but both variables differed based on days in the netpens.

Figure 9. Skin reflectance as measured by blue (top) and green luminosity (bottom) of experimental and production fall Chinook salmon sampled at Iron Gate Hatchery and after transport to the Upper Klamath Basin in October 2005. The raw data were medians from histograms (see Methods); however, the data are summarized as means (of the medians) + 1 SEM. Bars with letters in common denote values from fish sampled in the hatchery that do not differ. Bars with numbers in common denote values from fish in the hatchery before they were transported to netpens in Upper Klamath Lake and Williamson River (indicated by arrows), and fish in the netpens after 3 or 14 d (last four bars) that do not differ from each other. Sample sizes are 18 to 20. Data were analyzed by Kruskal-Wallis test followed by Dunn's multiple pairwise comparisons ($P < 0.05$). Based on 2-way GLM ($P < 0.05$), there were no differences in blue luminosity based on location or days in the netpens, but green luminosity differed based on days in the netpens.

Figure 10. Skin reflectance as measured by grey scale (top) and infrared luminosity (bottom) of fall Chinook salmon sampled at Iron Gate Hatchery and after transport the Upper Klamath Basin in May 2006. The raw data were medians from histograms (see Methods); however, the data are summarized as means (of the medians) + 1 SEM. Bars with letters in common denote values from fish sampled in the hatchery that do not differ. Bars with numbers in common denote values from fish in the hatchery before they were transported to netpens in Upper Klamath Lake and Williamson River (indicated by arrows), and fish in the netpens after 3 or 14 d (last four bars) that do not differ from each other. Sample sizes are 18 to 20. Data were analyzed by Kruskal-Wallis test followed by Dunn's multiple pairwise comparisons ($P < 0.05$). Based on 2-way GLM ($P < 0.05$), there were no differences between fish based on location of the netpens, but measures of grey scale differed based on days in the netpens.

Figure 11. Skin reflectance as measured by total color (top) and red luminosity (bottom) of fall Chinook salmon sampled at Iron Gate Hatchery and after transport the Upper Klamath Basin in May 2006. The raw data were medians from histograms (see Methods); however, the data are summarized as means (of the medians) + 1 SEM. Bars with letters in common denote values from fish sampled in the hatchery that do not differ. Bars with numbers in common denote values from fish in the hatchery before they were transported to netpens in Upper Klamath Lake and Williamson River (indicated by arrows), and fish in the netpens after 3 or 14 d (last four bars) that do not differ from each other. Sample sizes are 18 to 20. Data were analyzed by Kruskal-Wallis test followed by Dunn's multiple pairwise comparisons ($P < 0.05$). Based on 2-way GLM ($P < 0.05$), color luminosity differed based on location of the netpens and days in the netpens, but red luminosity differed only based on location of the netpens.

Figure 12. Skin reflectance as measured by blue (top) and green luminosity (bottom) of fall Chinook salmon sampled at Iron Gate Hatchery and after transport the Upper Klamath Basin in May 2006. The raw data were medians from histograms (see Methods); however, the data are summarized as means (of the medians) + 1 SEM. Bars with letters in common denote values from fish sampled in the hatchery that do not differ. Bars with numbers in common denote values from fish in the hatchery before they were transported to netpens in Upper Klamath Lake and Williamson River (indicated by arrows), and fish

in the netpens after 3 or 14 d (last four bars) that do not differ from each other. Sample sizes are 18 to 20. Data were analyzed by Kruskal-Wallis test followed by Dunn's multiple pairwise comparisons ($P < 0.05$). Based on 2-way GLM ($P < 0.05$), blue luminosity of fish differed based on location but not days in the netpens; green luminosity differed based on location and days in the netpens.

Figure 13. Water quality [temperature, pH and dissolved oxygen (DO)] at the netpens in Upper Klamath Lake (top) and Williamson River (bottom) monitored hourly in 2005 using YSI 600 XLM data sondes deployed 1 m off the bottom at each location.

Figure 14. Water quality [temperature, pH and dissolved oxygen (DO)] at the netpens in Upper Klamath Lake (top) and Williamson River (bottom) monitored hourly in 2006 using YSI 600 XLM data sondes deployed 1 m off the bottom at each location.

Figure 15. Mean (+ SE) daily water quality [temperature (Temp), dissolved oxygen (DO), pH] at netpens in Upper Klamath Lake (UKL) and Williamson River (WR) monitored hourly in 2005 and 2006 using YSI 600 XLM data sondes deployed 1 m off the bottom at each location. Values are for the first three days (3 d) and full 14 days (14 d) that fish were held. Bars within each four-bar group with letters in common do not differ (Kruskal-Wallis test followed by Dunn's multiple pairwise comparisons, $P < 0.05$).

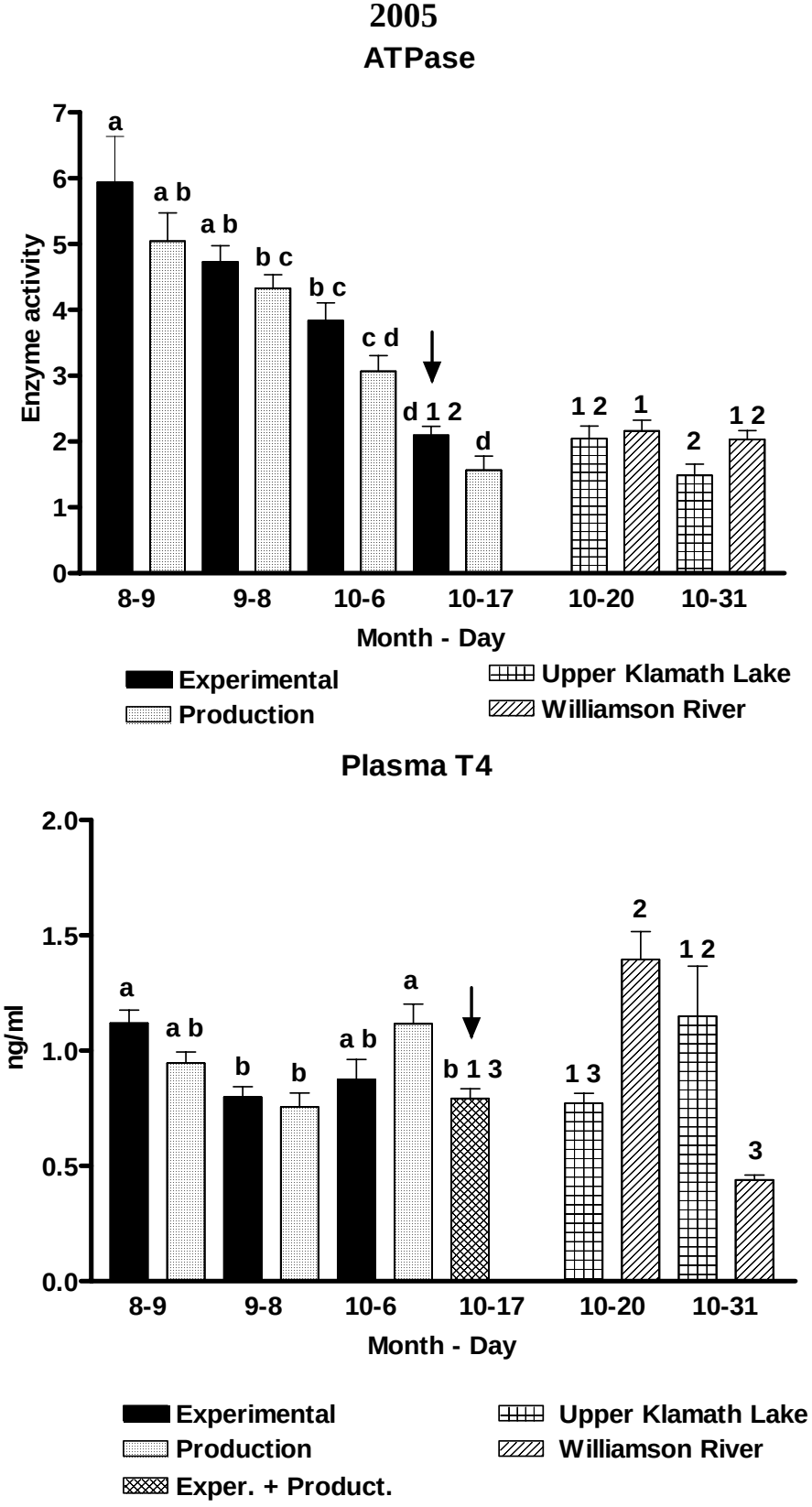

2005
ATPase
Enzyme activity
a
a b
a b
b c
b c
c d
d 1 2
d
1 2
1
2
1 2
8-9
9-8
10-6
10-17
10-20
10-31
Month - Day
Experimental
Production
Upper Klamath Lake
Williamson River
Plasma T4
ng/ml
a
a b
b
b
a b
a
b 1 3
1 3
2
1 2
3
Exper. + Product.

Figure 1.

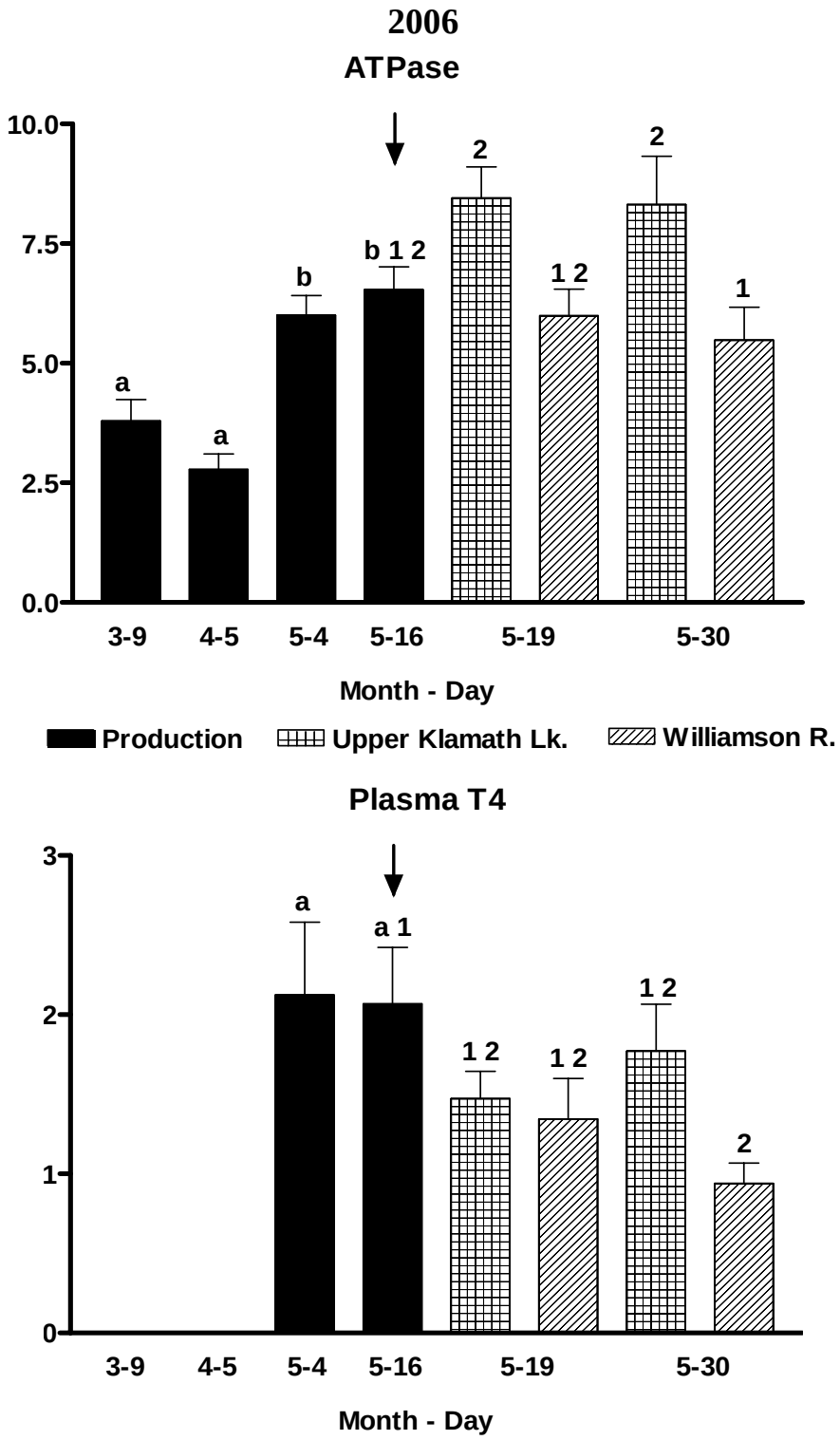
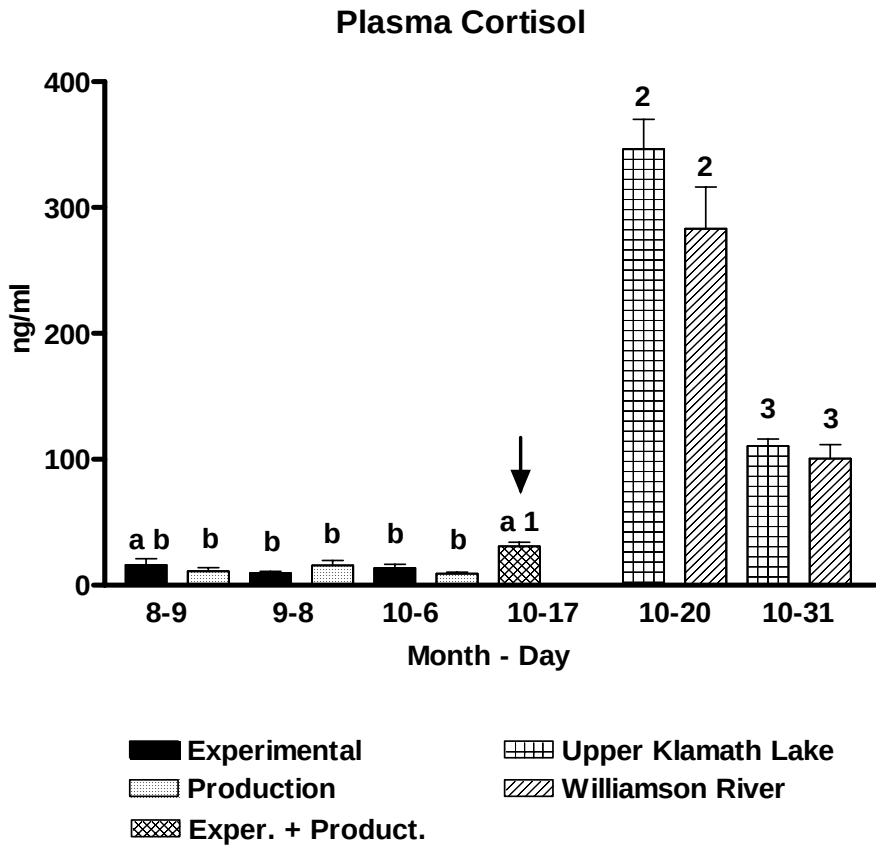

2006
ATPase
10.0
7.5
5.0
2.5
0.0
a
a
b
b 1 2
2
1 2
2
1
3-9
4-5
5-4
5-16
5-19
5-30
Month - Day
Production
Upper Klamath Lk.
Williamson R.
Plasma T4
3
2
1
0
a
a 1
1 2
1 2
1 2
2
3-9
4-5
5-4
5-16
5-19
5-30
Month - Day

Figure 2

2005



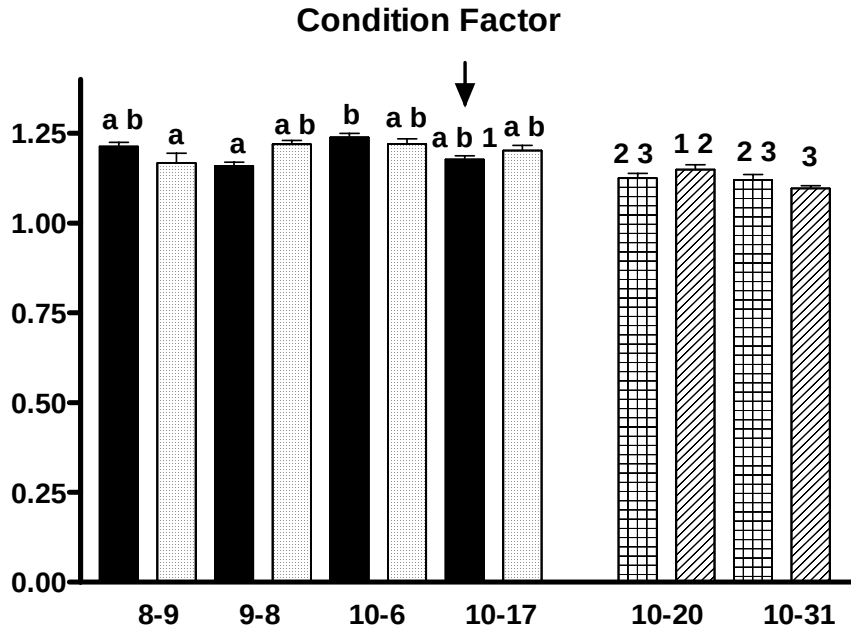


Figure 3.

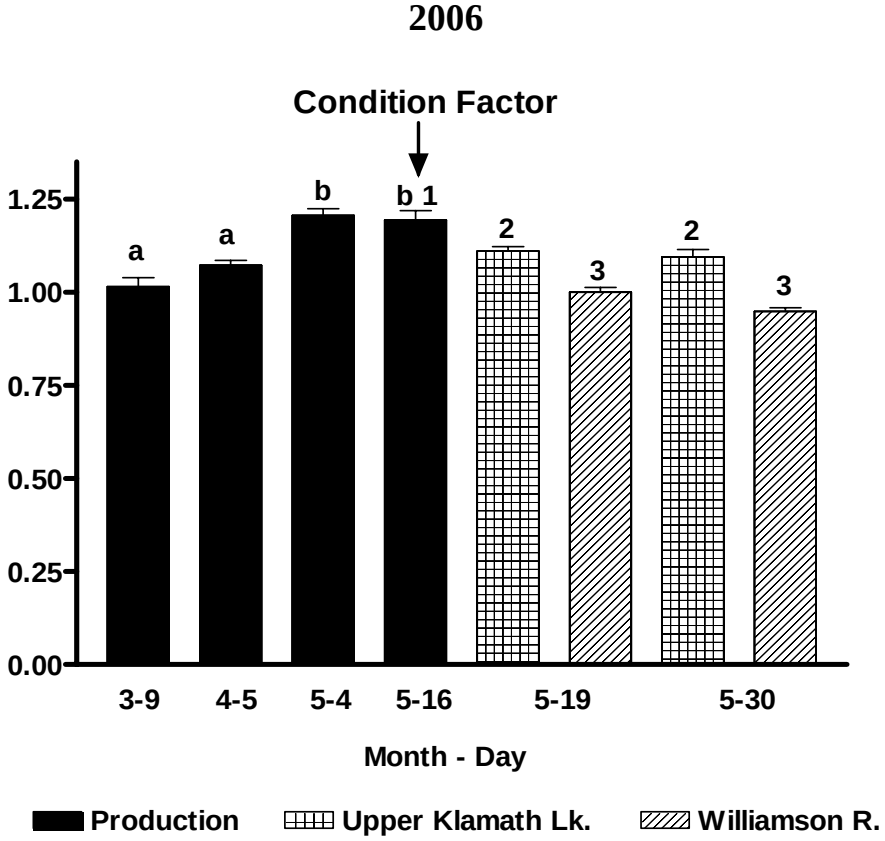

2006
Condition Factor
1.25
1.00
0.75
0.50
0.25
0.00
a
a
b
b 1
2
3
2
3
3-9
4-5
5-4
5-16
5-19
5-30
Month - Day
Production
Upper Klamath Lk.
Williamson R.

Figure 4

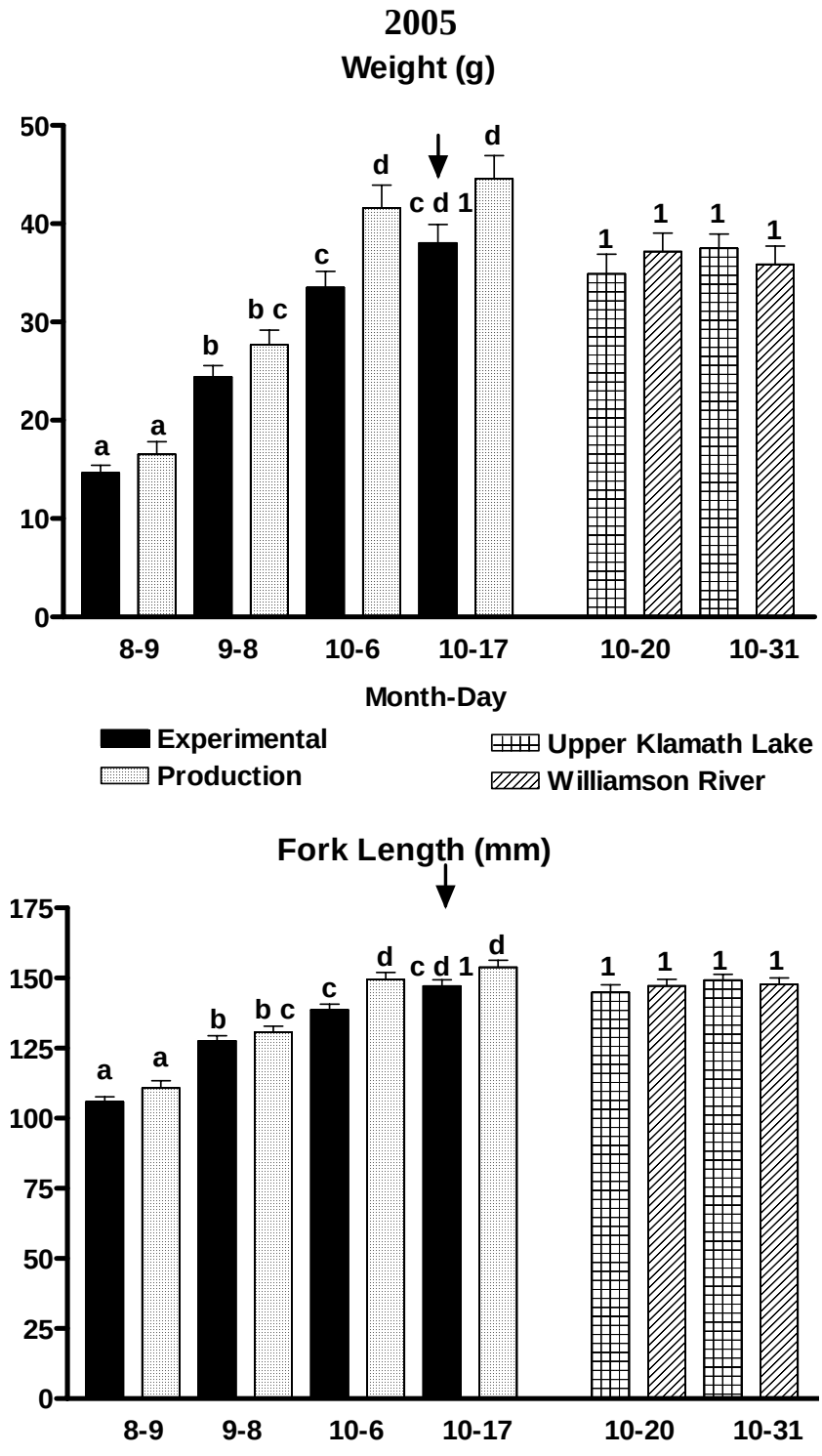

2005
Weight (g)
50
40
30
20
10
0
a
a
b
b c
c
d
c d 1
d
1
1
1
1
8-9
9-8
10-6
10-17
10-20
10-31
Month-Day
Experimental
Production
Upper Klamath Lake
Williamson River
Fork Length (mm)
175
150
125
100
75
50
25
0
a
a
b
b c
c
d
c d 1
d
1
1
1
1
8-9
9-8
10-6
10-17
10-20
10-31

Figure 5

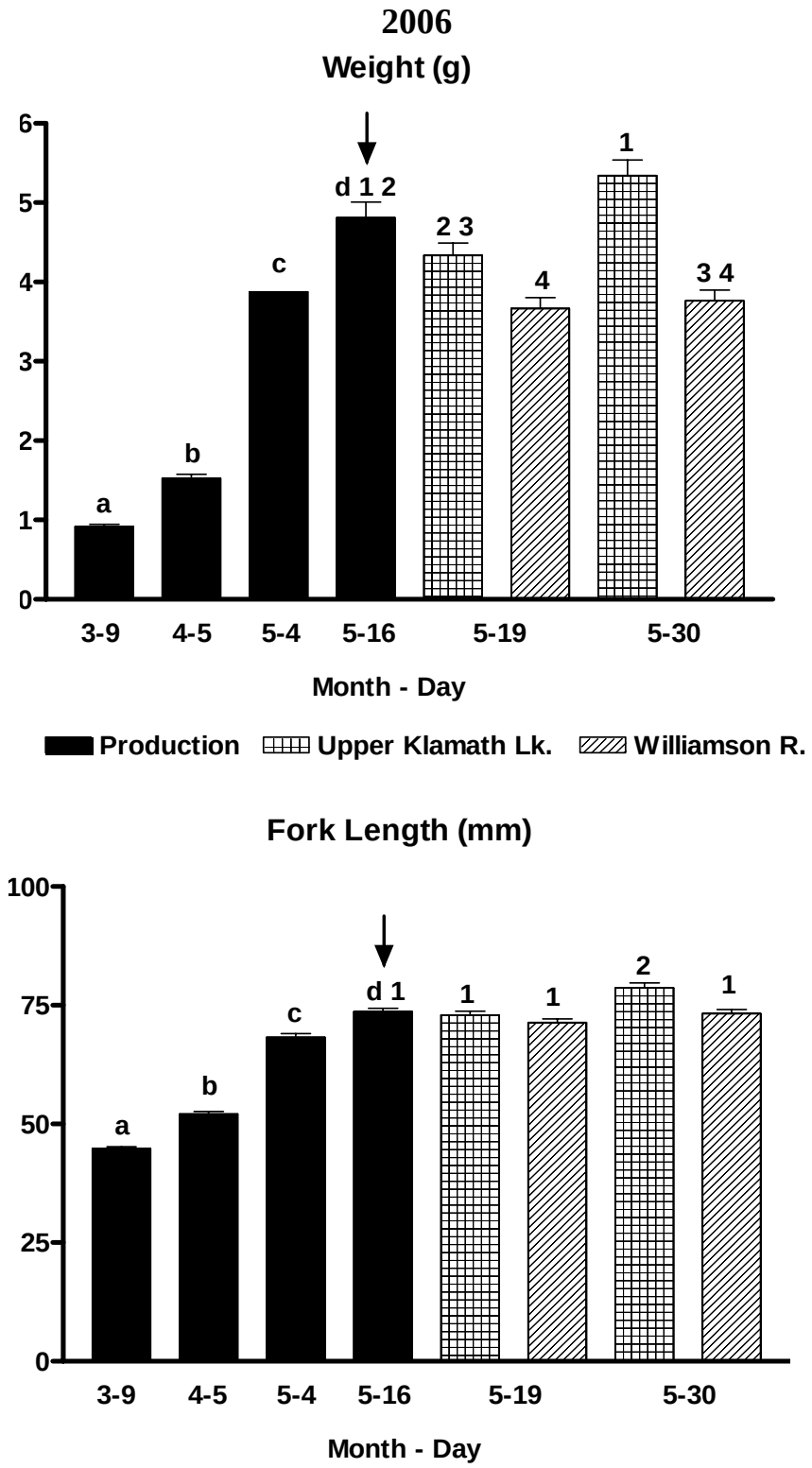

2006
Weight (g)
6
5
4
3
2
1
0
a
b
c
d 1 2
2 3
4
1
3 4
3-9
4-5
5-4
5-16
5-19
5-30
Month - Day
Production
Upper Klamath Lk.
Williamson R.
Fork Length (mm)
100
75
50
25
0
a
b
c
d 1
1
1
2
1
3-9
4-5
5-4
5-16
5-19
5-30
Month - Day

Figure 6

2005

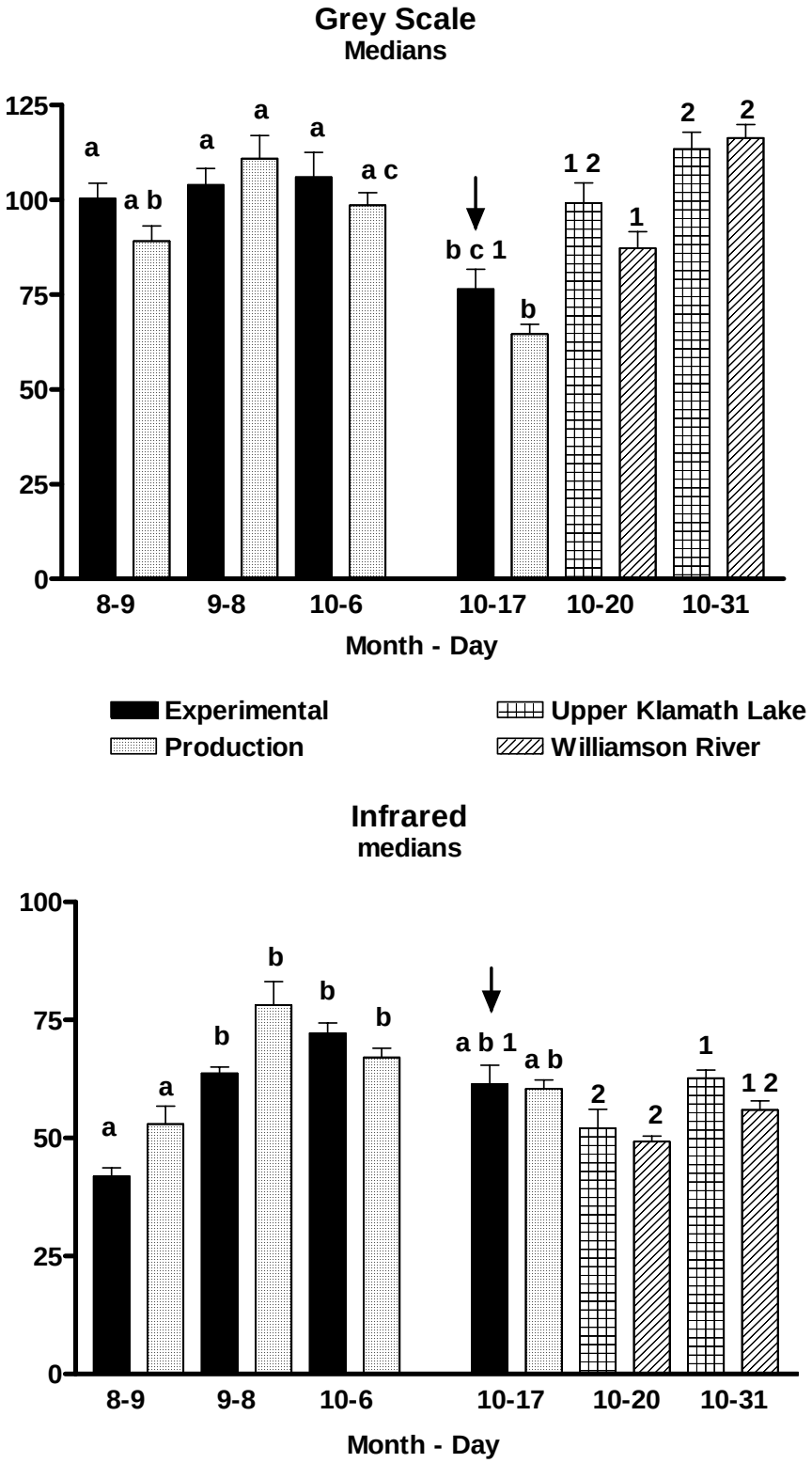


Figure 7.

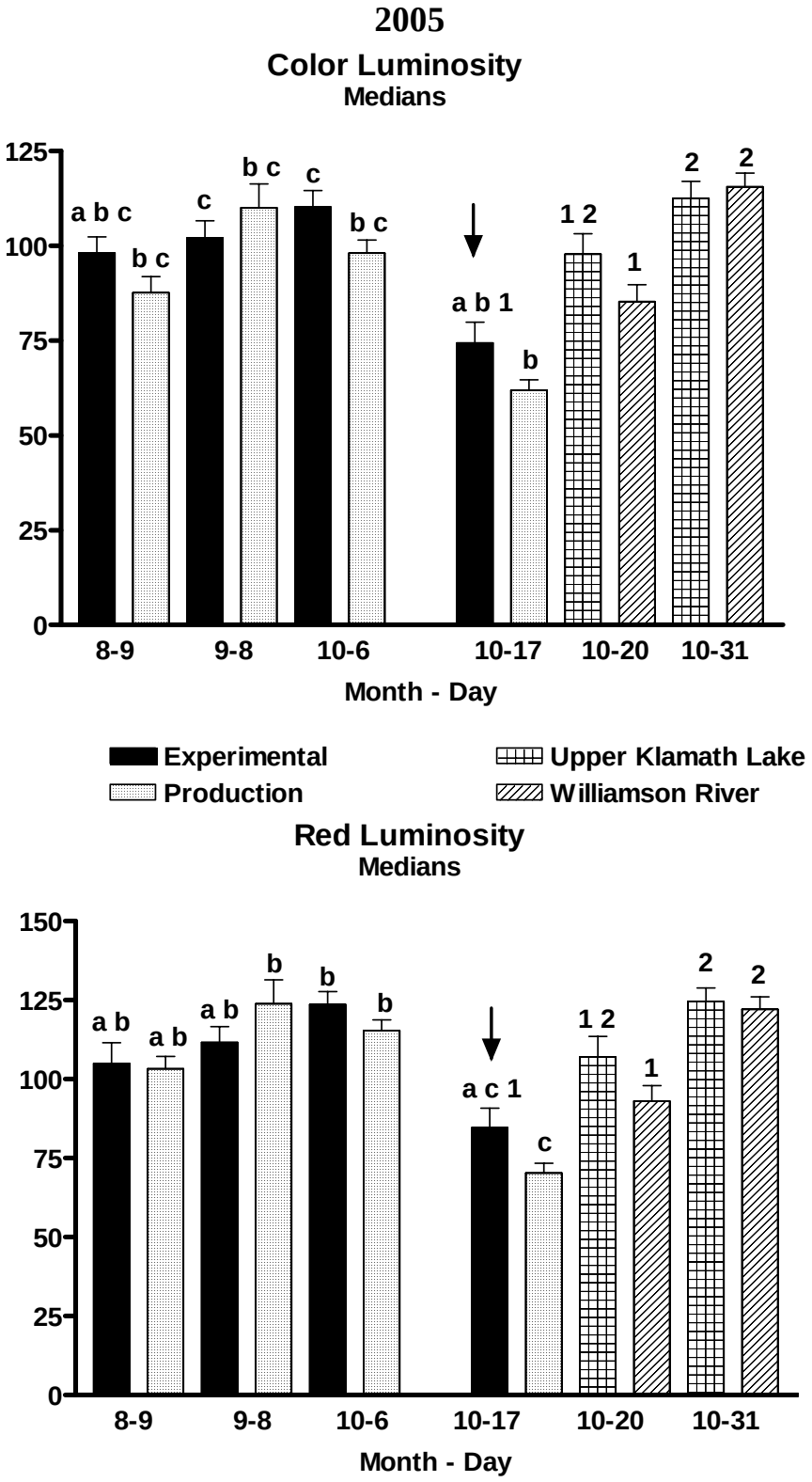
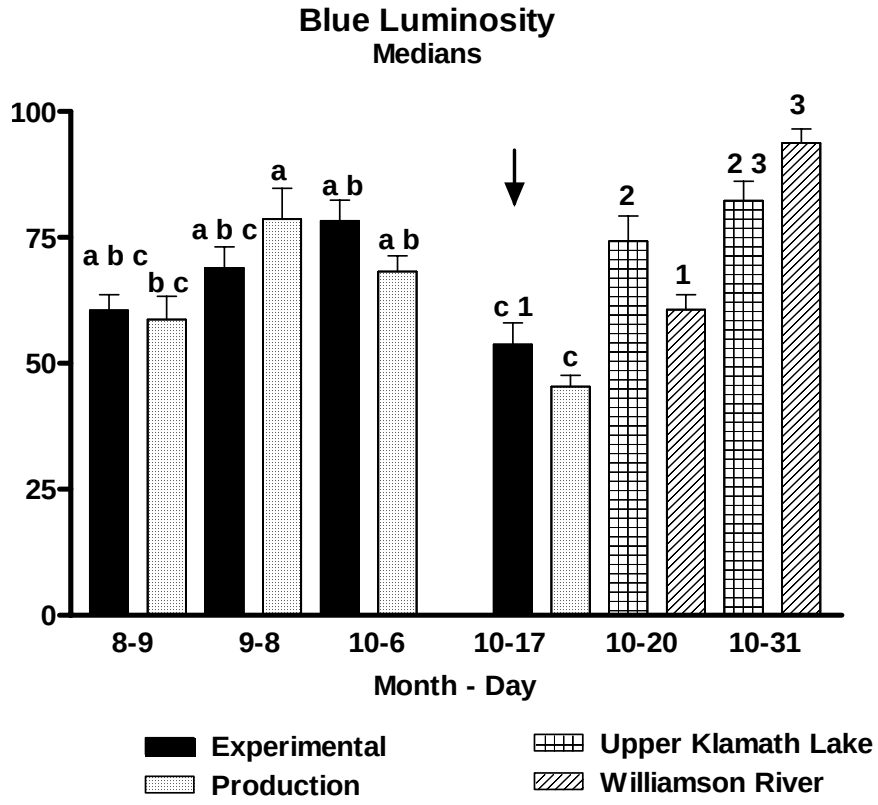

2005
Color Luminosity
Medians
125
100
75
50
25
0
a b c
b c
c
b c
c
b c
a b 1
b
1 2
1
2
2
8-9
9-8
10-6
10-17
10-20
10-31
Month - Day
Experimental
Production
Upper Klamath Lake
Williamson River
Red Luminosity
Medians
150
125
100
75
50
25
0
a b
a b
a b
b
b
b
a c 1
c
1 2
1
2
2
8-9
9-8
10-6
10-17
10-20
10-31
Month - Day

Figure 8.

2005



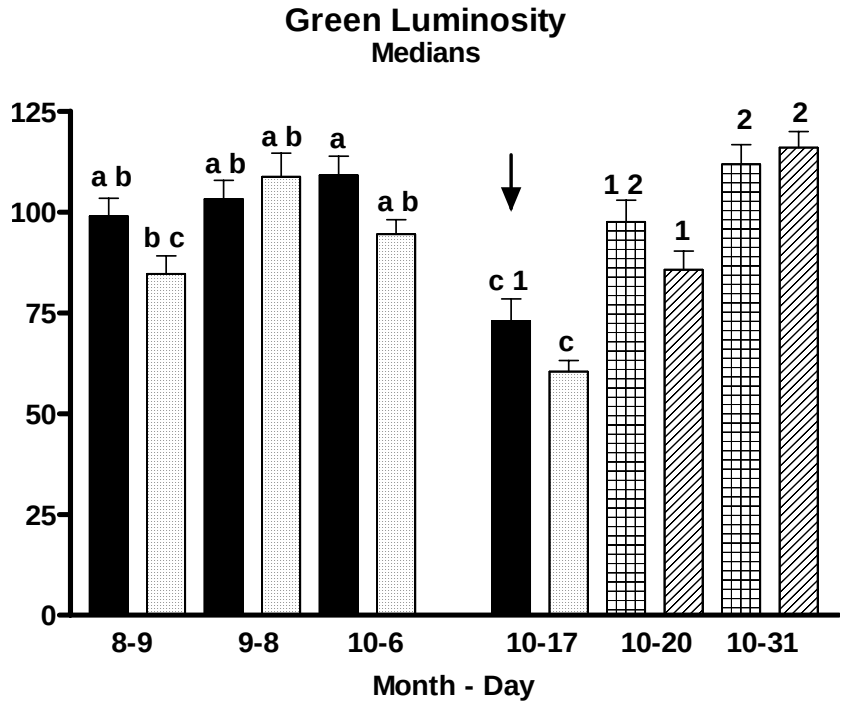


Figure 9.

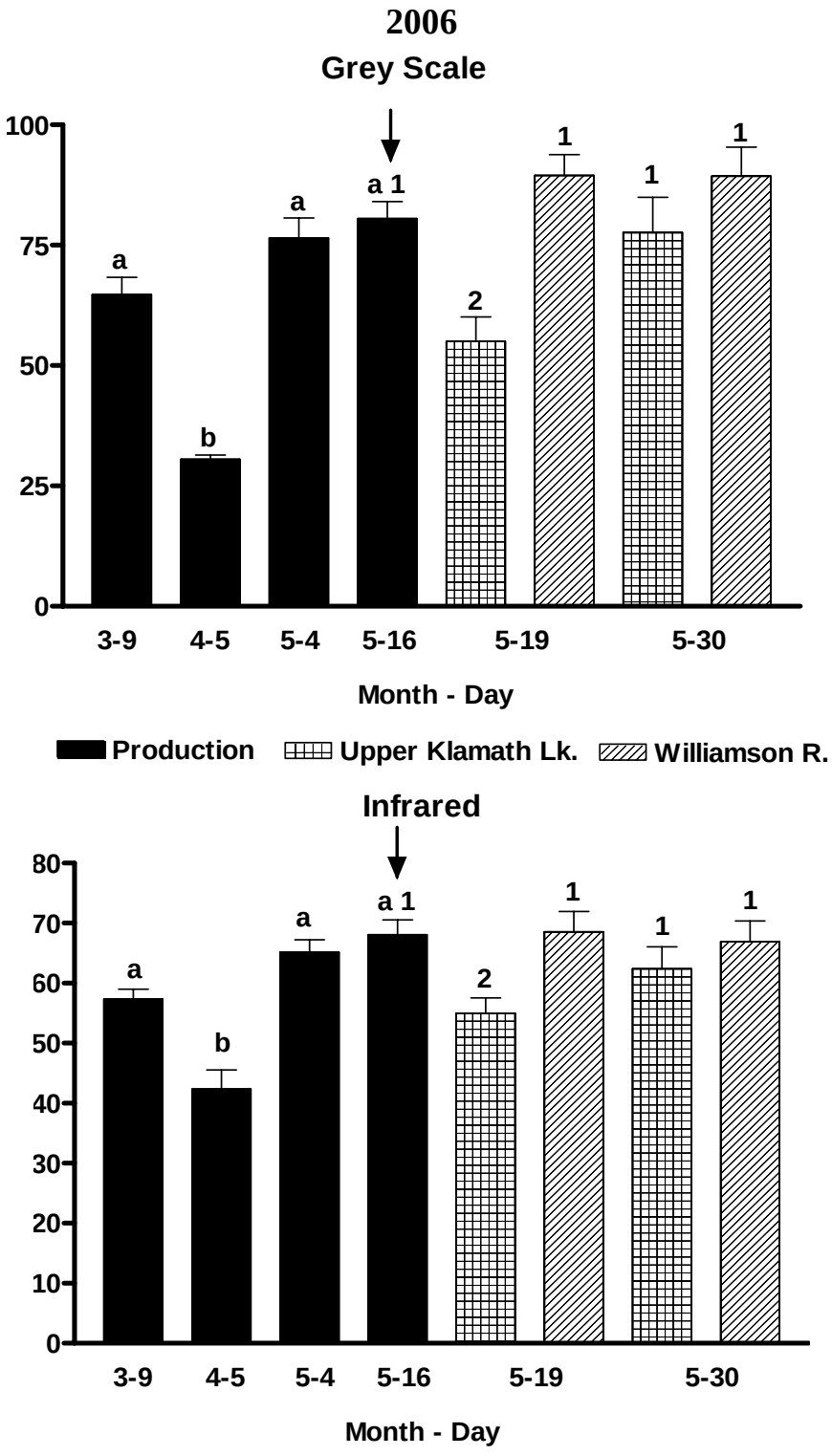
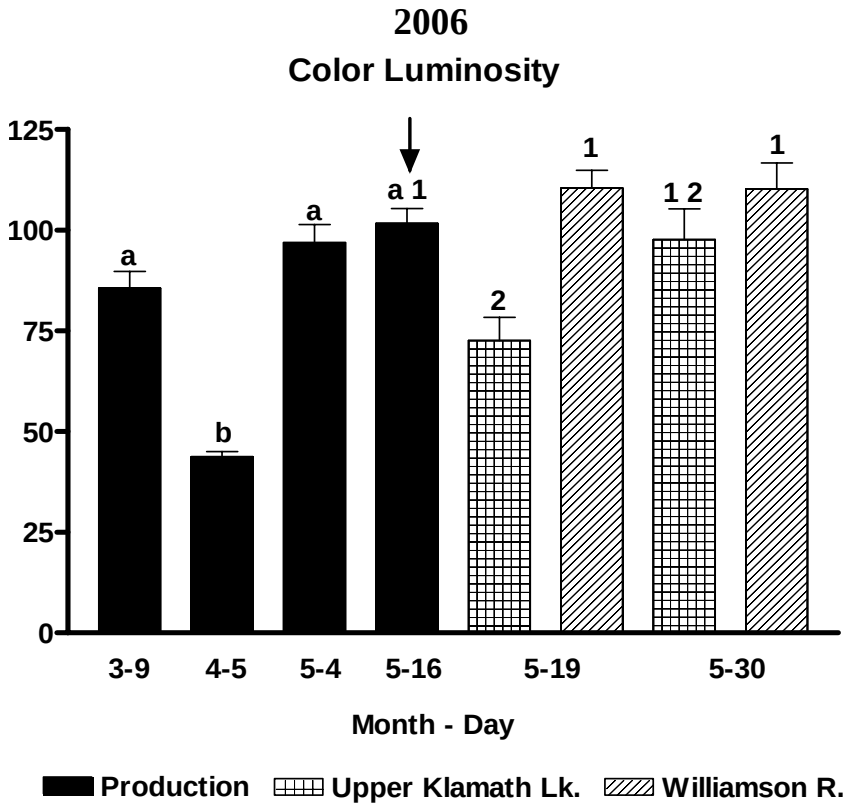

2006
Grey Scale
100
75
50
25
0
a
b
a
a 1
2
1
1
1
3-9
4-5
5-4
5-16
5-19
5-30
Month - Day
Production
Upper Klamath Lk.
Williamson R.
Infrared
80
70
60
50
40
30
20
10
0
a
b
a
a 1
2
1
1
1
3-9
4-5
5-4
5-16
5-19
5-30
Month - Day

Figure 10


2006
Color Luminosity
125
100
75
50
25
0
a
b
a
a 1
2
1
1 2
1
3-9
4-5
5-4
5-16
5-19
5-30
Month - Day
Production
Upper Klamath Lk.
Williamson R.

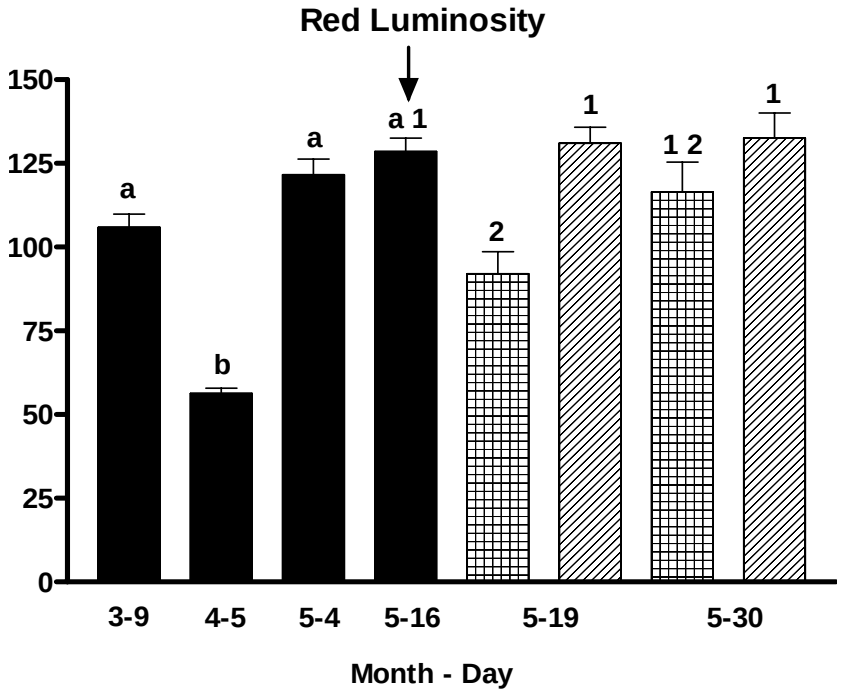
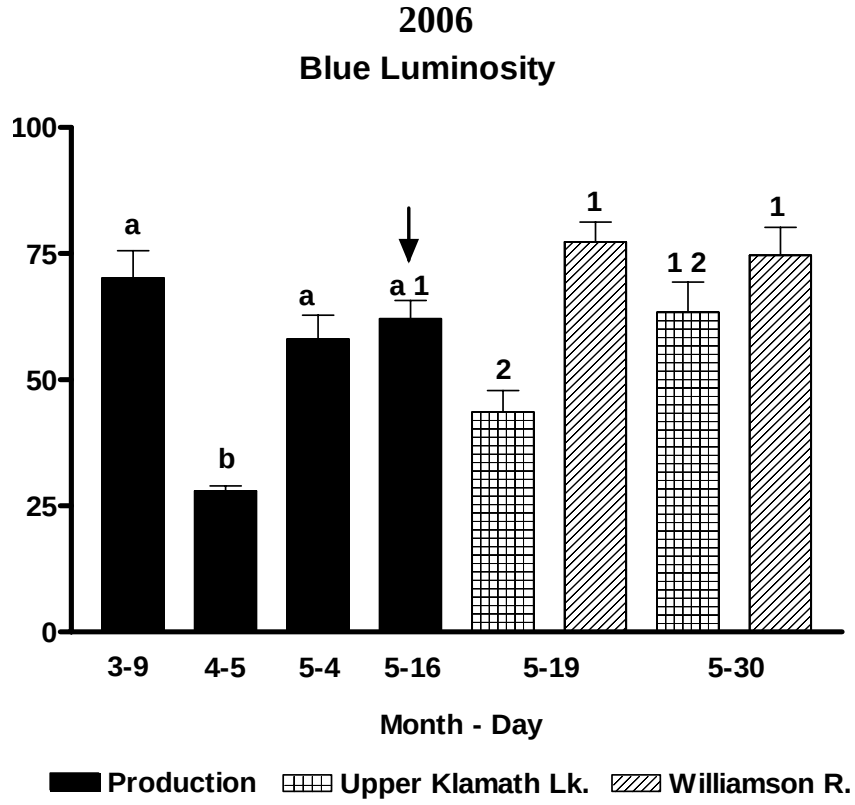

Red Luminosity
150
125
100
75
50
25
0
a
b
a
a 1
2
1
1 2
1
3-9
4-5
5-4
5-16
5-19
5-30
Month - Day

Figure 11


2006
Blue Luminosity
100
75
50
25
0
a
b
a
a 1
2
1
1 2
1
3-9
4-5
5-4
5-16
5-19
5-30
Month - Day
Production
Upper Klamath Lk.
Williamson R.

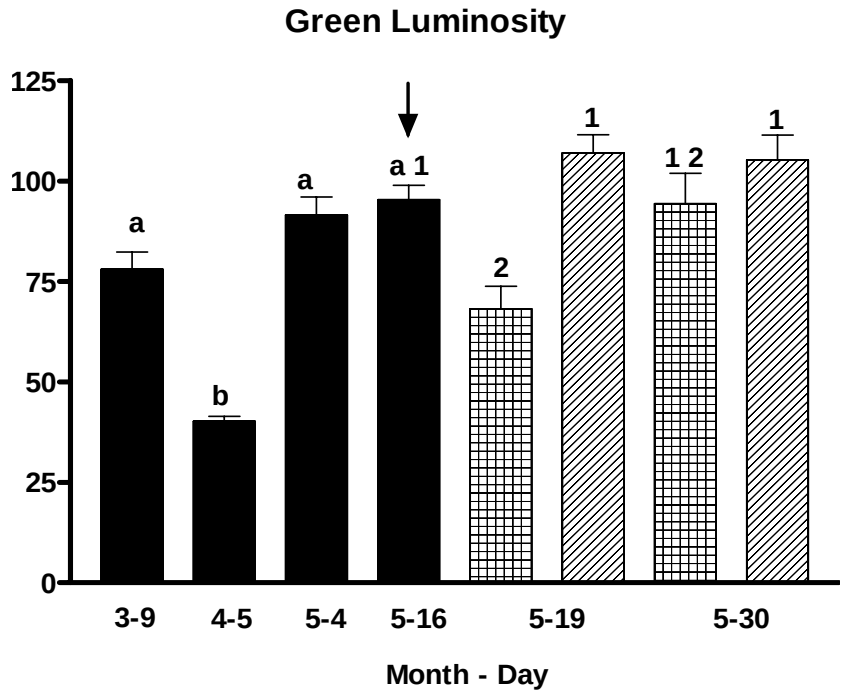
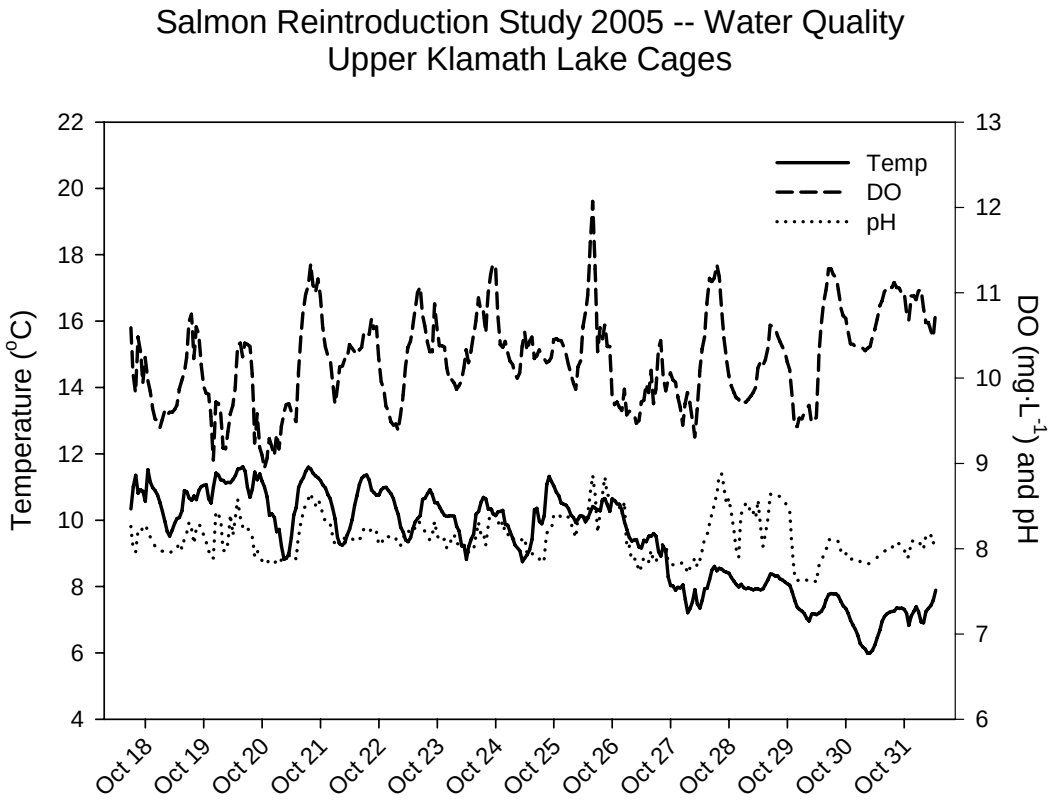

Green Luminosity
125
100
75
50
25
0
a
b
a
a 1
2
1
1 2
1
3-9
4-5
5-4
5-16
5-19
5-30
Month - Day

Figure 12


Salmon Reintroduction Study 2005 -- Water Quality
Upper Klamath Lake Cages
Temp
DO
pH
Temperature (°C)
DO (mg·L-1) and pH
22
20
18
16
14
12
10
8
6
4
13
12
11
10
9
8
7
6
Oct 18
Oct 19
Oct 20
Oct 21
Oct 22
Oct 23
Oct 24
Oct 25
Oct 26
Oct 27
Oct 28
Oct 29
Oct 30
Oct 31

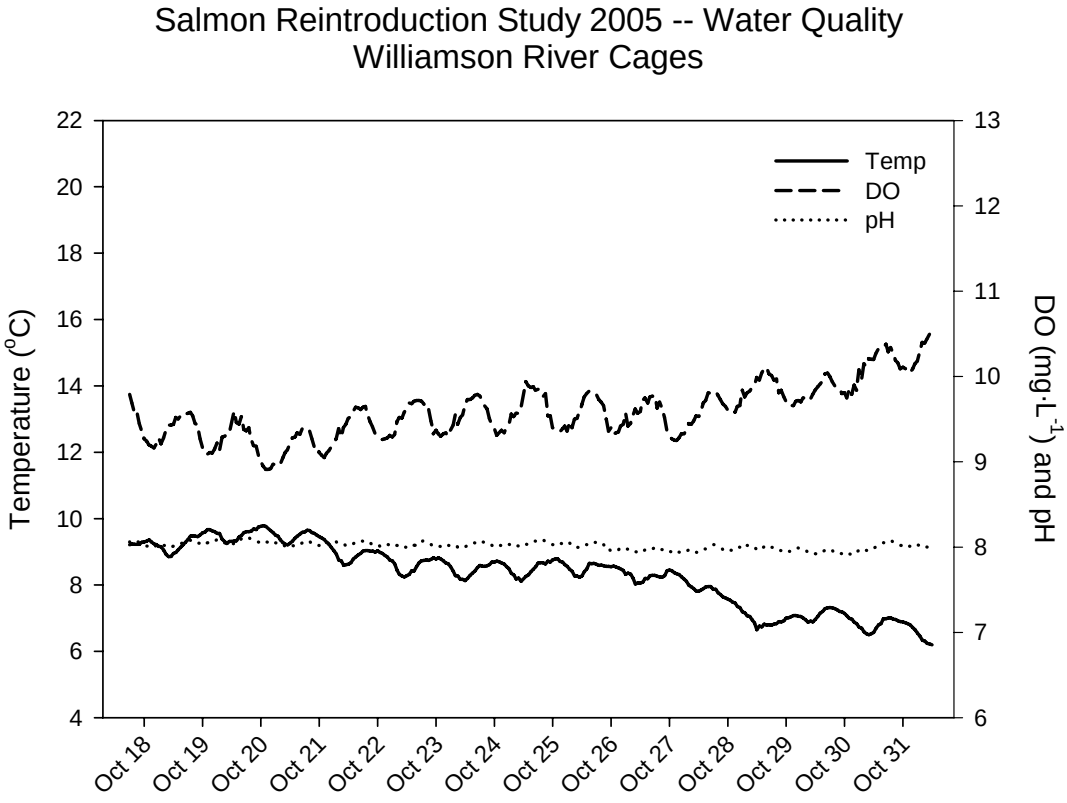

Salmon Reintroduction Study 2005 -- Water Quality
Williamson River Cages
Temp
DO
pH
Temperature (°C)
DO (mg·L-1) and pH
22
20
18
16
14
12
10
8
6
4
13
12
11
10
9
8
7
6
Oct 18
Oct 19
Oct 20
Oct 21
Oct 22
Oct 23
Oct 24
Oct 25
Oct 26
Oct 27
Oct 28
Oct 29
Oct 30
Oct 31

Figure 13.

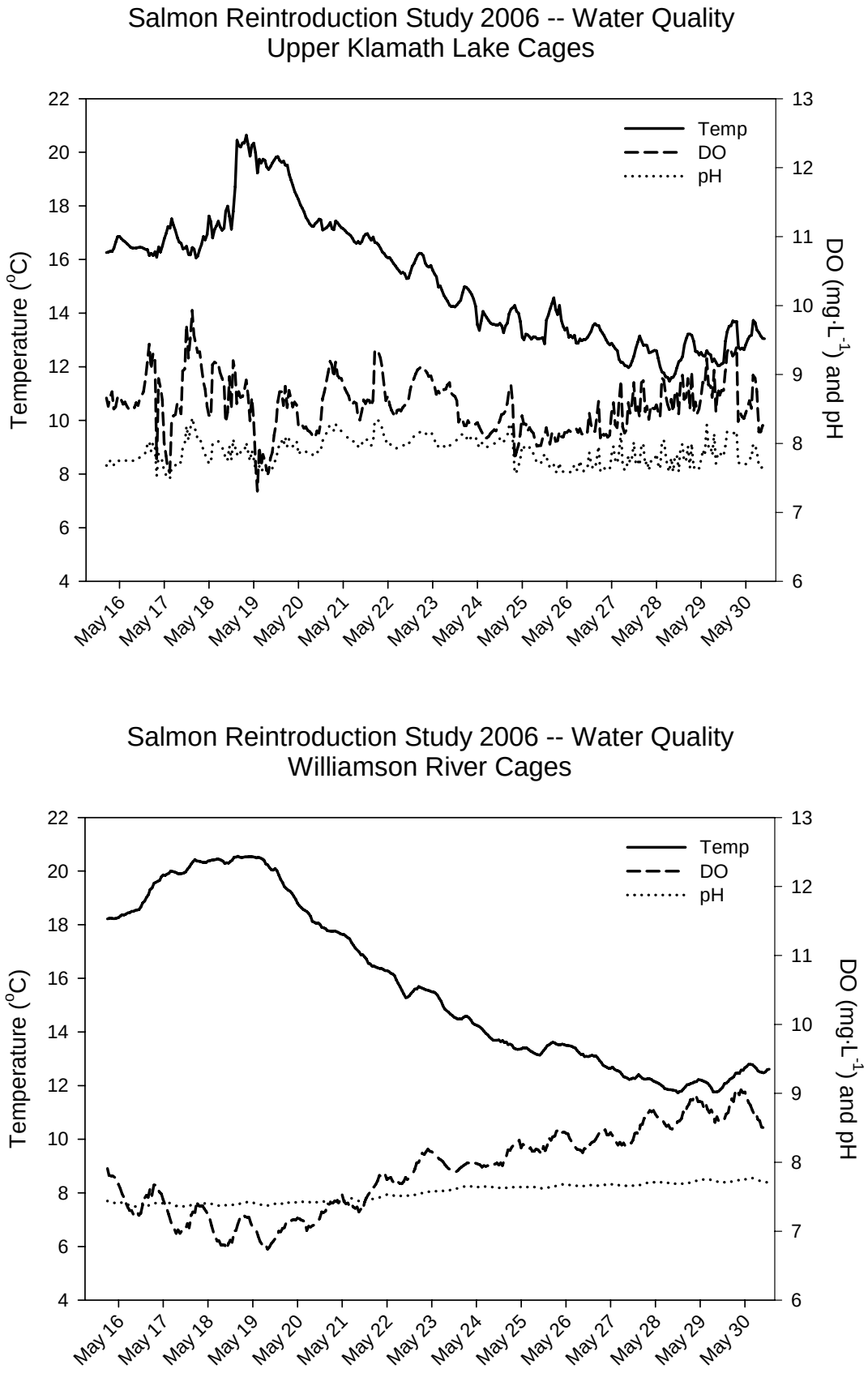

Salmon Reintroduction Study 2006 -- Water Quality
Upper Klamath Lake Cages
Temp
DO
pH
Temperature (°C)
DO (mg·L-1) and pH
22
20
18
16
14
12
10
8
6
4
13
12
11
10
9
8
7
6
May 16
May 17
May 18
May 19
May 20
May 21
May 22
May 23
May 24
May 25
May 26
May 27
May 28
May 29
May 30
Salmon Reintroduction Study 2006 -- Water Quality
Williamson River Cages
Temp
DO
pH
Temperature (°C)
DO (mg·L-1) and pH
22
20
18
16
14
12
10
8
6
4
13
12
11
10
9
8
7
6
May 16
May 17
May 18
May 19
May 20
May 21
May 22
May 23
May 24
May 25
May 26
May 27
May 28
May 29
May 30

Figure 14.

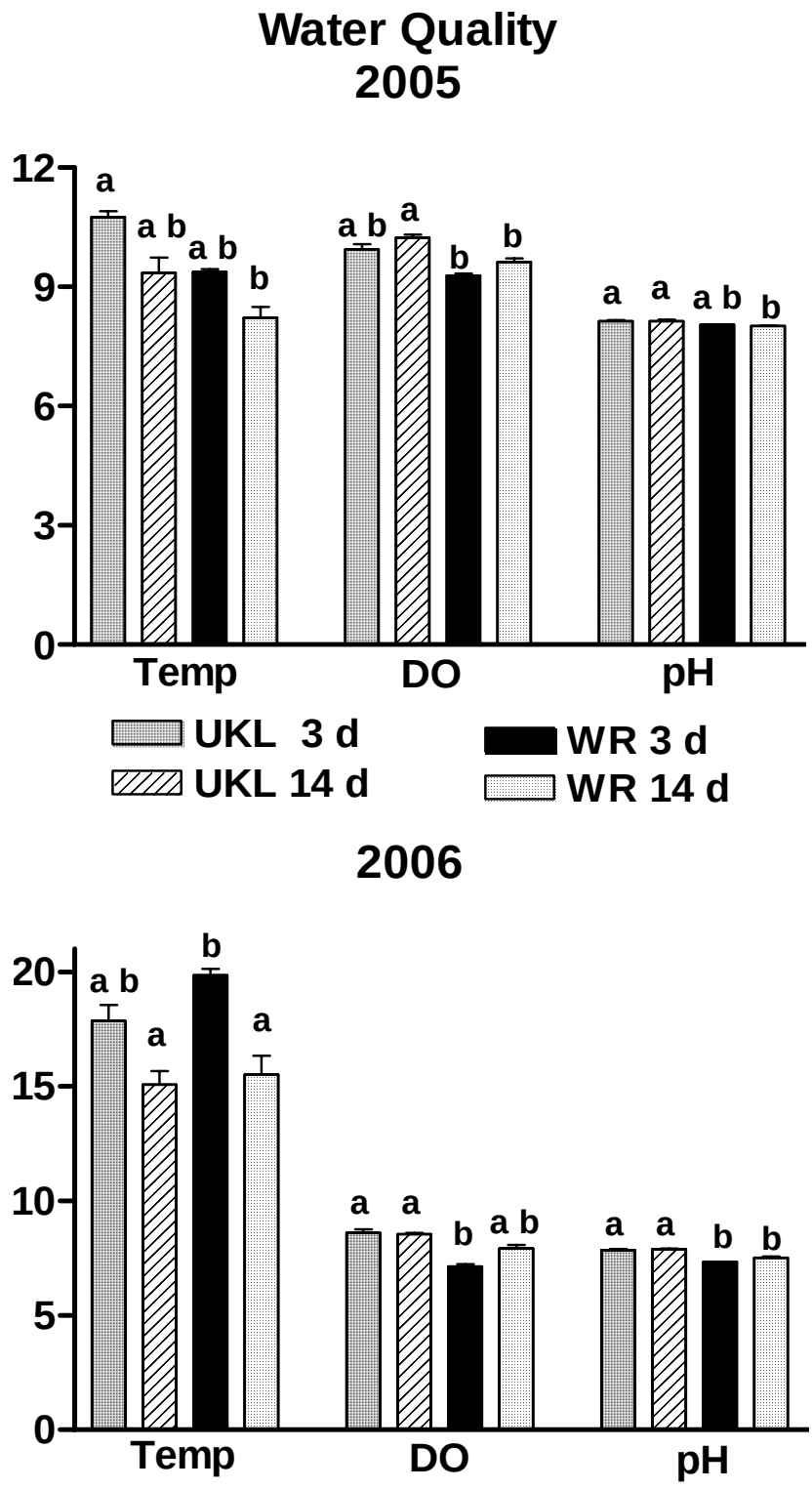

Water Quality
2005
12
9
6
3
0
a
a b
a b
b
a b
a
b
b
a
a
a b
b
Temp
DO
pH
UKL 3 d
UKL 14 d
WR 3 d
WR 14 d
2006
20
15
10
5
0
a b
a
b
a
a
a
b
a b
a
a
b
b
Temp
DO
pH

Figure 15