Life after precocious male maturation in a semelparous salmonid: A physiological perspective in Chinook salmon, *Oncorhynchus tshawytscha*

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Abstract

Spring Chinook salmon, *Oncorhynchus tshawytscha*, are typically semelparous, dying after a single lifetime spawning event. However, observations of survival following maturation in males that mature at age-1 (common names: precocious parr or microjacks) have been documented, but little is known regarding their post-maturation physiology. In this investigation, mature microjacks were fed either a high or low winter ration to examine potential effects of feeding regime on mortality, smoltification and rematuration the following year. We measured a series of physiological factors including survival, size, percent whole body solid (an index of whole body lipid), and gill Na+/K+ -ATPase activity (an index of smoltification). We also analyzed a series of maturation indices including plasma 11-ketotestosterone (11-KT), gonadosomatic index (GSI), pituitary follicle stimulating hormone (*fshb)* and luteinizing hormone (*lhb*) beta-subunit mRNA, and testicular histology in male fish following maturation as microjacks. In both treatments, the proportion of males that survived (High Ration: 0.91, Low Ration: 0.87) and rematured (High Ration: 1.0, Low Ration: 1.0) was very high, more indicative of iteroparity than semelparity. The low winter ration may have caused a slight delay in rematuration as evidenced by delayed *fshb* expression and 11-KT secretion in the spring. However, there was no evidence microjacks smolted in the spring after maturation at age-1. From a fisheries conservation perspective, the potential for iteroparity in microjacks (survival to remature at age-2) means it is possible they contribute genetically more than once when breeding in the natural environment.

Keywords: Chinook salmon; iteroparity; smoltification; pituitary *fshb* and *lhb*; gill Na+/K+ -ATPase activity; 11-ketotestosterone (11-KT)

1. Introduction

Age of maturation in many salmonid species is phenotypically plastic and influenced by the interaction between genotype (Silverstein and Hershberger, 1992; Hankin et al., 1993; Heath et al., 1994; Larsen et al., 2019, 2022) and environmental factors, including water temperature and nutrient acquisition, and their associated influence on growth rate, body size and adiposity (Rowe et al., 1991; Silverstein and Shimma, 1994; Silverstein et al., 1997, 1998; Shearer and Swanson, 2000; Larsen et al., 2006; Shearer et al., 2006). Like many salmonid species, male Chinook salmon (*Oncorhynchus tshawytscha*) can mature precociously at 1 year of age (common names: precocious parr or microjack) or 2 years of age (minijack) (Rutter, 1904; Gebhards, 1960; Taylor, 1989; Larsen et al., 2004, 2006; Pearsons et al., 2009; Johnson et al., 2012). Anadromous males and females undergo smoltification (Hoar, 1988) and migrate to the ocean to grow for a year or more before returning to their natal spawning grounds. Conversely, precocious males generally remain in freshwater or migrate shorter distances downstream prior to returning (Beckman and Larsen, 2005; Larsen et al., 2010; Pearsons et al., 2023). Precociously mature males are orders of magnitude smaller than anadromous adults and employ a ‘sneak’ or ‘swarm’ strategy to spawn with full size anadromous females (Fleming, 1996). In wild salmon populations, precocious male maturation is a rare, but important, component of a diverse life-history portfolio (Hutchings and Myers, 1994; Hill et al., 2003; Moore et al., 2010, 2014; Schindler et al., 2010) allowing for genetic overlap among generations (Saunders and Schom, 1985; Saura et al., 2008). Still, overexpression of this life-history can occur in salmon aquaculture and hatchery supplementation programs where growing conditions

are optimized for fast growth on lipid rich diets which may have negative economic (Myers, 1984; Lundqvist et al., 1988; 1994; Kallio-Nyberg et al., 2011), genetic, ecological and management implications (Garant et al., 2003a, 2003b; Thorpe, 2004; Larsen et al., 2004, 2019; Ford et al., 2012, 2015; Harstad et al., 2014; Pearsons et al., 2023).

Much of the prior research regarding the ontogeny of precocious male maturation in salmonids has been conducted in Atlantic salmon (*Salmo salar*). Atlantic salmon are typically characterized as fully (or partially) iteroparous, dependent on adult migration distance (Shaffer and Elson, 1975), and possess the ability to repeat maturation (generally below 10% prevalence – Fleming and Reynolds, 2004; Quinn and Myers, 2004) over multiple years rather than die after their first spawning opportunity (Mills, 1989). Male Atlantic salmon are also capable of smoltification and rematuration in subsequent years following precocious maturation (Mitans, 1973; Thorpe and Morgan, 1980; Saunders and Schom, 1985; Myers and Hutchings, 1986; Berglund et al., 1992a, 1992b). By contrast, Chinook salmon are generally considered semelparous, dying after their first and only spawning opportunity (Quinn, 2018). However, observations of survival following age-1 microjack maturation have been documented in both spring ('stream-type') Chinook salmon (Rich, 1920; Robertson, 1957; Bernier et al., 1993) and fall ('ocean-type') Chinook salmon (Flain, 1971; Unwin et al., 1999). With the exception of these primarily descriptive studies and the physiological work of Bernier et al. (1993), little is known regarding potential for post maturation smoltification and rematuration of Chinook salmon as microjacks. Furthermore, to date, there is no information available on the effects that variation in environmental conditions may have on post maturation

survival, smoltification and rematuration of this species.

Salmonid smoltification is the behavioral, physiological, and morphological process whereby a freshwater dwelling parr transforms into a seawater adapted smolt (reviewed in Hoar, 1988). Behavioral changes include schooling and downstream migration from freshwater to seawater. Morphological changes include decreased condition factor (*K*) as fish become more fusiform, loss of cryptic parr marks, increased body silvering, and darkening of the fin margins; all adaptations for life in the open ocean. The physiological ontogeny of smoltification has been well characterized and includes, but is not limited to: olfactory imprinting; increased activity of the thyroid, interrenal, and growth associated endocrine axes; elevations in metabolism; lipolysis; and increased gill and gut Na+/K+ -ATPase activity for maintenance of ionic homeostasis in seawater (Hoar, 1988; Bjornsson et al., 2011). Among this suite of developmental markers, elevation in gill Na+/K+ -ATPase activity, entrained by seasonal photoperiod and modified by temperature (Duston and Saunders, 1990; Fuhrman et al., 2025) is generally considered the most universally accepted indicator ofsmoltification progression in both migrating and captively held fish (Zaugg, 1981, 1989; McCormick, 1993, 1995; Beckman et al., 2000; Larsen et al., 2010; McCormick et al., 2013).

Chinook salmon typically spawn in the autumn and male maturation is initiated in response to growth rate and energetic status. The maturation process is physiologically initiated approximately 8-12 months prior to spermiation for each age class ranging from 1 to 5 years post parental spawning (Silverstein et al., 1998; Shearer and Swanson, 2000; Campbell et al., 2003; Shearer et al., 2006). Thus, maturation as an age-1 microjack is essentially initiated in response to environmental cues shortly after hatching

and emergence (Beckman et al., 2007; Winsor et al., 2021; Fuhrman et al., 2025). Mechanistically, puberty is the process whereby the brain-pituitary-gonadal axis develops functional competency and a vertebrate animal first becomes capable of reproduction (reviewed in Taranger et al., 2010). Puberty in males is regulated by reproductive hormones, including pituitary gonadotropins (i.e. follicle-stimulating hormone, FSH, and luteinizing hormone, LH) and the primary male androgen in teleost fish, 11-ketotestosterone (11-KT) (Schulz et al., 2010; Taranger et al., 2010). In salmonid males that have initiated puberty, pituitary *fsh* beta-subunit (*b*) mRNA, plasma FSH, and plasma 11-KT levels are elevated first, followed by pituitary *lhb* mRNA and plasma LH levels with a concomitant increase in the gonadosomatic index (GSI) (Chinook salmon - Shearer and Swanson, 2000; Campbell et al., 2003; Atlantic salmon - Maugars and Schmitz, 2008a, 2008b; Melo et al., 2015; Rainbow trout, *Oncorhynchus mykiss* - Gomez et al., 1999; Kusakabe et al., 2006).

The goal of this investigation was to improve upon our basic understanding of the physiological ontogeny and life-history of precociously mature male Chinook salmon. Expanded information on microjacks may have utility in aquaculture and hatchery supplementation efforts, as well as resource management of wild populations. We reared a population of spring Chinook salmon microjacks for a period of one year, from the time of spermiation in October at age-1 until the following October at age-2. The fish were divided into two rearing regimes: a high ration regime, to approximate optimal post-maturation overwinter rearing conditions typical of captive culture, and a low ration regime, to approximate a resource limited environment more typical of a natural river. In addition, a subset of fish from each treatment were lethally sampled on a monthly basis

for a variety of physiological indicators to assess size, whole body lipid, smolt development, and maturation status. Based on the previously described observations of smoltification and rematuration in precocious male Atlantic salmon (Myers and Hutchings, 1986; Berglund et al., 1992a, 1992b), in the current investigation we endeavored to examine three working hypotheses in Chinook salmon microjacks:

H_1: Chinook salmon microjacks that survive under a low winter ration, will not remature, but undergo smoltification the following spring for maturation in the future (iteroparity).

H_2: Chinook salmon microjacks reared under high winter ration will survive and undergo repeat maturation as minijacks the following autumn (iteroparity).

H_3: Most Chinook salmon microjacks reared under a low winter ration will die within a few months of spermiation (semelparity).

2. Methods

2.1. Fish rearing

The current investigation was supplemental to a larger investigation focused on age-2 minijack maturation in spring Chinook salmon (Larsen et al., 2011). For that study, approximately 5,000 spring Chinook salmon embryos at the eyed stage of development were obtained from the Levi George Hatchery (Yakima River Supplementation and Research Facility), Cle Elum, WA, USA (lat: 47.194681, long: -120,936366) in November 2009 and transported to the Northwest Fisheries Science Center, Seattle, WA (lat: 47.644950, long: -122.308733) where they were incubated in Heath trays (MariSource, Burlington, WA) at 11°C under 24 hr darkness. Beckman et al. (2007) demonstrated that a combination of early emergence timing (via elevated embryo incubation temperature and photoperiod manipulation) and high rations can increase

the proportion of winter-run Chinook salmon males that mature as age-1 microjacks and age-2 minijacks. Similarly, we manipulated emergence time via high incubation temperature (11°C) to advance the ponding date to 1 January 2010. We did not manipulate photoperiod in this study. Fish were reared on a natural photoperiod regime for Seattle, WA using artificial lights on automatic timers that were adjusted weekly. Following yolk sac absorption, fry were ponded in ten 1.2 m tanks (500/tank) and fed commercial feed (Bio-Oregon, Longview, WA) by hand, daily. Feed ration was approximately 0.5% body weight/day in excess of manufacturer's recommended rate to ensure very high growth and potentially increase the proportion of age-2 mature males (minijacks).

While it was not the focus of the primary study, the first year of accelerated growth produced a total of 370 age-1 microjacks (~15% of all males). These males were sorted out of the 10 rearing tanks based on olive pigmentation, white fin margins and, in most cases, active milt expression (determined by a small release of milt when applying minimal pressure near the gonopore). Fish were then randomly assigned to one of two, 1.2 m diameter tanks (185 fish per tank) providing the opportunity to examine the effect of high and low "winter ration" (15 October 2010 to 15 February 2011) on post maturation survival, physiology and life-history. The High Ration (HR) treatment was fed by hand once daily (5 days/week) at the feed manufacturer's recommended ration as a percentage of body weight per day and the number of fish in each tank. The Low Ration (LR) treatment was fed once daily (5 days/week) at 1/3 that of the HR treatment. Rations were adjusted monthly based on manufacturer's recommendations, average body weight of sampled fish, and number of fish per tank. Following the designated

“winter ration period” both groups were fed the HR regime (5 days/week) from 16 February to the end of the study on 15 October 2011. Fish were monitored five days per week (Monday - Friday) for bodily condition, morbidity and mortality to determine cumulative mortality over the course of the entire investigation. All fish were reared and sampled according to the University of Washington Institutional Animal Care and Use Committee (Protocol #2313-90).

Artificial spawning of adult male salmonids typically entails manual expression of milt by applying even (anterior-to-posterior) pressure along the belly wall. Initially, in this study, microjacks were not manually stripped of milt under the assumption it would be resorbed over time. However, within two months of the initiation of the study (December 2010) a large number of fish in both treatments became visually bloated with excess fluid (ascites) likely resulting from the degradation of unexpressed sperm (Figure 1A). Therefore, the decision was made that, during daily health checks, any bloated fish encountered would be netted, lightly anesthetized in a buffered solution of 0.05% solution of tricaine methanesulfonate (MS-222: Syndel, Ferndale, WA), manually stripped, and returned to the tank. Following this procedure their abdomen appeared flaccid and they were visually distinguishable swimming in the tanks (Figure 1B). However, within 24-48 hr fish appeared fully recovered and were no longer visually distinguishable among the rest of the fish. By February 2011, bloated fish were no longer observed in either treatment.

2.2. Sampling

Throughout the investigation, fish were lethally sampled for a variety of physiological indicators on a monthly basis. At each sampling event, eight fish were

randomly selected from each treatment, euthanatized in a buffered 0.1% solution of MS-222, blotted dry with a paper towel and measured for fork length (to the nearest 1 mm) and body weight (to the nearest 0.1 g). Fulton's condition factor (*K*) was calculated as: $K = \frac{W}{L^3}$ where *W* = body weight in g and *L* is fork length in cm (Ricker, 1975). After severing the caudal vasculature with a single-edge razor blade, blood was collected in a heparinized Natelson tube (Fisher Scientific, Waltham, MA) and transferred to a 0.4 ml microcentrifuge tube. Blood was centrifuged at 3,000 g for 5 min and the plasma was transferred to a 0.5 ml tube and stored frozen at -80°C for future measurement of plasma 11-KT. Filaments from the first gill arch on the left side of each fish were placed in a solution of sucrose, EDTA, and imidazole according to methods described by Zaugg (1982) and then frozen on dry ice before being stored at -80°C for future analysis of gill Na+/K+ -ATPase activity. A separate set of gill arches were collected monthly from each of 16 immature Chinook salmon from the original 5,000 fish research population used to create microjacks for this study. These gill samples from known smolts served as a positive control for comparative purposes with those of the microjacks. Paired testes from microjacks were dissected and weighed to the nearest 0.001 g to calculate gonadosomatic index (GSI = [testes weight/body weight] × 100). One testis was fixed in Bouin's fixative for 48 hr prior to storage in 70% ethanol for subsequent histological analysis. Pituitary glands were dissected from the cranium, transferred to a 2.0 ml polypropylene tube, frozen in liquid nitrogen, and stored at -80°C for future analysis of gonadotropin beta-subunit mRNAs. The remaining fish bodies were then transferred to individual freezer bags and stored at -80°C for future analysis of percent whole body solid.

2.3. Laboratory analysis

2.3.1. Whole body solid

In salmonids, whole body moisture is inversely related to whole body lipid (Shearer et al., 1994; Spangenberg et al., 2023). Thus, whole body solid ("percent solid" hereafter) provides a relative index of whole body lipid. To determine percent solid, frozen fish were cut into 0.5 cm^3 pieces, placed in aluminum weighing pans, and dried in an oven at 40°C to a consistent weight for ~ 48 hr. Wet and dry weights were then used to calculate the percent solid for each fish.

2.3.2. Gill Na+/K+ -ATPase activity

Gill Na+/K+ -ATPase activity was measured according to the method of McCormick (1993), and all values are reported as μmol ADP · mg $protein^{-1}$ · hr^{-1}.

2.3.3. Plasma 11-Ketotestosterone (11-KT)

Each plasma sample was diluted 1:3 with sterile water (25 μl plasma + 75 μl water) and heated in an 80°C water bath for one hr, similar to the method described by Schulz et al. (1994). Samples were then centrifuged at 18,000 g for 6 min and supernatants were stored at -20°C until time of assay. 11-KT (ng/ml) was measured by enzyme-linked immunosorbent assay (ELISA) similar to the method previously described by Cuisset et al. (1994). Acetylcholinesterase tracer and pre-coated (mouse anti-rabbit IgG) 96-well plates were obtained from Cayman Chemical (Ann Arbor, MI). Primary antibody was provided by Dr. David Kime (University of Sheffield, retired).

2.3.4. Pituitary transcription

Relative expression for pituitary *fshb* and *lhb* mRNA, including RNA extraction methods, reverse transcription (RT) reaction conditions, and real-time quantitative

polymerase chain reaction (qPCR) conditions, followed the methods of Middleton et al. (2019, 2025) for Rainbow trout. Primer and probe sequences for real-time RT-qPCR assays for *fshb*, *lhb* and a reference gene, elongation factor one alpha (*ef1a*), were previously reported by Middleton et al. (2019) with the exception of the *fshb* reverse primer which differs for Chinook salmon. Sequence identity of primers and probes designed for Rainbow trout were verified for 100% homology to Chinook salmon (GenBank Accession numbers: *lhb*, XM_024388176; *ef1a*, FJ890356). Attempts to verify sequence identity of primers and the probe for *fshb* resulted in a single bp mismatch in the reverse primer. A Chinook salmon-specific reverse primer was designed for 100% homology using Chinook salmon sequence data (GenBank Accession number: AY493564; Chinook specific reverse primer sequence: 5'-AGTTCAGGTCCGTCGTTTCG -3') and Primer Express 1.5 software (Applied Biosystems - ABI, Foster City, CA). All primers and probes were purchased from Integrated DNA Technologies (San Jose, CA).

All assays were run on an ABI 7900 HT Fast Real-Time PCR System using 384-well plates and TaqMan Universal PCR Master Mix (ABI). All samples were analyzed on a single plate, thereby eliminating plate-to-plate variation. For each transcript measured, five randomly selected total RNA samples were analyzed without RT reaction to test for amplification due to genomic DNA contamination (no amplification control, NAC). NAC samples showed no, or negligible, DNA contamination with all samples amplifying > 7 cycles (< 1% DNA contribution) beyond those of their reverse transcribed counterparts. No template controls (NTC), consisting of an RT reaction containing no RNA template, were also performed and showed no template

contamination in the reagents used. PCR efficiency for each transcript was measured using a serial dilution of pituitary samples pooled across timepoints and treatments as a reference. Standard curve dilutions were run in triplicate; samples were run in singles. Stable expression of *ef1a* was observed across time points and treatments (data not shown). Therefore, transcript levels were efficiency corrected using the pooled, serially diluted total RNA standard curve and normalized with the reference gene (*ef1a*) using the method described by Pierce et al. (2004). Relative expression values were calculated by setting the mean of efficiency corrected values on the first sampling date to a value of 1.

2.3.5. Testis histology

Fixed testes were processed through a series of ethanol and xylene substitute, followed by embedding in paraffin. Sections were cut at a thickness of 5 μm on a Leica RM2165 rotary microtome (Leica, Teaneck, NJ). Sections were stained with hematoxylin (incubation period: 4 minutes) and eosin (incubation period: 10 seconds). Microscope images were captured at 40x magnification on a Nikon Eclipse Ci-L compound microscope using a Nikon digital camera and NIS Elements image software version 2.3 (Nikon, Melville, NY).

2.4. *Data analysis*

To control for heteroscedasticity, data for plasma 11-KT, GSI, and pituitary *fshb* and *lhb* were $\log_{10}$ transformed prior to statistical analyses. All data were graphed and statistically analyzed using Prism 9 software (GraphPad Software, La Jolla, CA). We focused our statistical analyses on differences between treatments within each date. Two sample t-tests of HR vs. LR treatments were performed on each sampling date for

all measures except gill ATPase activity. Because gill Na+/K+ -ATPase activity was analyzed for both treatments, as well as immature smolts for comparison, one-way ANOVAs were performed on each sampling date followed by Tukey's post hoc tests. The level of significance was set at $\alpha = 0.05$, with adjustments for multiple testing when applicable.

3. Results

3.1. General fish condition and mortality

In total, 82 (44.3%) and 103 (55.7%) of the HR and LR fish, respectively, from the original 185 fish per treatment required manual milt expression (Table 1). Most mortalities occurred within the first few months of the study, before the vast majority of the fish were lethally sampled (Table 1). A total of 96 out of 185 microjacks (51.9%) were lethally sampled from each treatment throughout the course of the study. All fish that were not lethally sampled (72 and 65 fish for the HR and LR treatments, respectively) were determined to have rematured as minijacks based on milt expression at the termination of the experiment. Assuming that all sampled fish would have survived based on their apparent health at the time of sampling, cumulative survival was very high and comparable between treatments ranging from 87.0% in the LR treatment to 90.8% in the HR treatment (Table 1).

Table 1. Numbers and percent of total for fish in each treatment, bloated with ascites, sampled for physiology, dissected at the termination of the experiment to determine percent rematuration, total mortalities, and estimated survival to rematuration.

	High Winter Ration		Low Winter Ration	
	N	**%**	**N**	**%**
Total microjacks	185		185	
Bloated (Dec 2010 - Feb 2011)	82	44.3	103	55.7
Sampled for physiology	96	51.9	96	51.9
Rematuration as minijack	72	100	65	100
Total mortalities (Oct 2010 - Oct 2011)	17	9.2	24	13.0
***Estimated survival**	168	90.8	161	87.0

*presuming that all physiologically sampled fish would have survived to remature based on their apparent health at the time of sampling

3.2. Growth and energetics

Fish in both treatment groups appeared to feed well throughout the investigation with minimal evidence of uneaten food at the bottom of the tanks. During the winter ration period (mid-October 2010 to mid-February 2011), both treatment groups showed little growth in either fork length (Figure 2A) or body weight (Figure 2B) and the mean size of fish at each sampling point was not significantly different between treatments, except in December 2010 when the HR treatment demonstrated slightly larger body weight ($t = 2.379$, $df = 10.580$, $p = 0.0374$). *K* decreased or remained steady over time in the LR group during the winter treatment period (Figure 2C). In the HR treatment, *K* was similar to the LR treatment during the winter period, with the exception of December 2010 and February 2011 when it was significantly higher ($t = 5.102$, $df = 14.000$, $p = 0.0002$ and $t = 2.246$, $df = 13.\ 009$, $p = 0.0427$, respectively). Finally, during the winter treatment period, percent solid decreased from October to December in the LR treatment and from October to November in the HR treatment before increasing in both treatments thereafter, although there were no significant differences within sampling dates (Fig. 2D). After the winter treatment period had ended, fork length, body weight, and percent solid were significantly different only in March and April 2011 (Figure 2A-B, D; fork length: March – $t = 4.573$, $df = 13.557$, $p = 0.0005$, April – $t = 3.159$, $df = 10.931$, $p = 0.0092$; body weight: March – $t = 5.326$, $df = 11.602$, $p = 0.0002$, April – $t = 2.794$, $df = 12.557$, $p = 0.0156$; percent solid: March – $t = 2.450$, $df = 8.659$, $p = 0.0377$, April – $t = 5.671$, $df = 12.954$, $p < 0.0001$). For *K*, after the completion of the winter treatment period the HR and LR treatments were significantly different only in

March and June 2011 (Figure 2C; t = 3.903, df = 12.216, p = 0.0020 and t = 2.236, df = 13.964, p = 0.0422, respectively).

3.3. Smoltification

Gill Na+/K+ -ATPase activity of the immature, smolting fish sampled as part of the companion investigation (Larsen et al., 2011) was characteristically low from October 2010 to February 2011, increased starting in March, and peaked in April before declining through the summer-fall (Figure 3). By contrast, gill Na+/K+ -ATPase activity of both the HR and LR treatments remained relatively low and was not significantly different between treatments throughout the entire investigation (Figure 3). Smolts had significantly higher gill Na+/K+ -ATPase activity than both the HR and LR treatments at every time point between March and October 2011, except in late August 2011 (Figure 3; March – $F(2,29) = 27.47$, $p < 0.0001$; April – $F(2,30) = 24.40$, $p < 0.0001$; May – $F(2,29) = 6.006$, $p = 0.0066$; June – $F(2,29) = 11.14$, $p = 0.0003$; July – $F(2,29) = 4.117$, $p = 0.0267$; October – $F(2,30) = 3.519$, $p = 0.0424$).

3.4. Maturation

3.4.1. Plasma 11-KT and GSI

Plasma 11-KT levels steadily declined following age-1 microjack maturation from October 2010 to the end of March 2011 before increasing steadily until rematuration in October 2011 (Figure 4A). Plasma 11-KT levels were significantly different between treatments in October and November 2010 and March 2011 (October 2010 – t = -3.078, df = 12.658, p = 0.0091; November 2010 – t = -2.313, df = 10.932, p = 0.0412; March 2011 – t = 2.556, df = 10.442, p = 0.0277), otherwise 11-KT levels were similar between the treatments. GSI was fairly stable between October 2010 and December 2010 before

steadily declining through April 2011, at which time it increased through rematuration in October 2011 (Figure 4B). GSI was only significantly different between treatments in November 2010 ($t = 2.263$, $df = 13.799$, $p = 0.0403$).

3.4.2. Pituitary transcripts

Following age-1 (microjack) maturation, relative expression of *fshb* mRNA was low and unchanged through December 2010, generally increased with some variation to a peak in late July, before declining by October 2011 in both the HR and LR treatments (Figure 4C). Furthermore, with the exception of the January 2011 sample date ($t = 3.088$, $df = 8.896$, $p = 0.0132$), there were no significant differences in relative expression for *fshb* mRNA between treatments. Relative expression for *lhb* mRNA declined steadily from October 2010 through early spring (March to April) 2011 before increasing steadily until rematuration in October 2011 (Figure 4D). Levels of *lhb* mRNA did not differ significantly between the two treatments on any sampling date.

3.4.3. Phenotypic and histological progression of rematuration

In order to assess qualitative changes over time, testicular development and associated histology were tracked and photographed in a subset of representative fish during from February to August 2011 (Figure 5A-J). In February, post-maturation testicular resorption was evidenced by the obvious decrease in testis size (Figure 5A) and decrease in density of spermatozoa (Figure 5B). By March testis size had decreased further; there was further reduction in spermatozoa, and a notable increase in type-A spermatogonia was observed (Figure 5C-D). By May the testes began to enlarge and type-B spermatogonia and spermatocytes predominated (Figure 5E-F). In July the testes filled the body cavity (Figure 5G) with a substantial presence of

spermatozoa (Figure 5H). Finally, in August the testes were very large (Figure 5I) and dominated by spermatids and spermatozoa (Figure 5J). Overall, the documented phenotypic and histological process of resorption and rematuration corresponded temporally with the decrease and subsequent increase in plasma 11-KT level, GSI, and relative expression of *Ihb* mRNA observed throughout the investigation in both treatment groups (Figure 4A-B, D).

4. Discussion

Findings from this investigation resulted in rejection of our first working hypothesis (H_1) that Chinook salmon microjacks that survive under a low winter ration will not remature, but undergo smoltification the following spring for repeat maturation in the future (iteroparity). Based on gill Na+/K+ -ATPase activity, we found no evidence of subsequent smoltification following precocious male maturation at age-1, unlike findings in precociously mature Atlantic salmon (Mitans, 1973; Thorpe and Morgan, 1980; Saunders and Schom, 1985; Myers and Hutchings, 1986; Berglund et al., 1992a, 1992b). This observation agrees, indirectly, with that of Unwin et al. (1999) who found at 12 months of age, mature parr performed poorly in a “hyper-salinity challenge” compared with normal parr suggesting that gill Na+/K+ -ATPase activity was not sufficiently up-regulated in those fish. Similarly, precociously maturing parr of several anadromous salmonid species, including Atlantic salmon (Lundqvist et al., 1985, 1989), Sea trout (*Salmo trutta* - Dellefors and Faremo, 1988) and Chinook salmon (Foote et al., 1991), have been shown to have lower seawater adaptability than non-maturing individuals. However, several other studies in Atlantic salmon have found that precocious male parr and immature Atlantic salmon have similar tolerance to high

salinity, and develop smolt characteristics to the same degree over the spring following maturation (Saunders et al., 1982; Jarvi et al., 1991; Berglund et al., 1992b). Bernier et al. (1993) found that 54.3% of mature Chinook salmon parr in their study survived for several months after seawater transfer at 20 months of age. Furthermore, they observed 100% rematuration at age-2 in all surviving fish indicative of osmoregulatory competency. We did not transfer our fish to seawater, making it difficult to directly compare these studies, but the consistently low levels of gill Na+/K+ -ATPase activity we observed suggest that the microjacks from our investigation would not have survived transfer to seawater. Since it is generally observed that age-1 precocious males (microjacks) forgo migration to seawater and remain in freshwater, from a life-history perspective, these conflicting results may have more to do with differences in experimental design and the advanced age and size at which the fish were transferred to seawater by Bernier et al. (1993) than true physiological differences.

We were able to clearly confirm our second working hypothesis (H_2) that Chinook salmon microjacks reared under high winter ration would survive and undergo repeat maturation as minijacks the following autumn (iteroparity) with ~91% of the HR fish surviving and 100% of those that survived rematuring as minijacks. However, our third working hypothesis (H_3) that Chinook salmon microjacks reared under a low winter ration would die within a few months of spermiation (semelparity) was not accepted. Estimated survival of the LR treatment was similarly high (87%) and repeat maturation, even under putative “resource limited” conditions, was 100% among survivors as well. The rapid senescence that occurs postspawning in both hatchery and naturally reared, full size, anadromous adult Pacific salmon is typically characterized by osmoregulatory

failure, increased adrenocorticosteroid activity and associated immunosuppression and organ deterioration (Dickhoff, 1989; Finch, 1990; Shrimpton et al., 2005; Hruska et al., 2010; Jefferies et al., 2011). Most of the microjacks in the current investigation did not succumb to these physiological processes even under the low winter ration conditions. The observation that microjacks in this, and previously cited studies, are capable of iteroparity under both natural and captive culture conditions indicates there are real physiological differences between the age classes that potentially operate at multiple organ levels in this species.

Our findings of repeat maturation confirm previous observations of Robertson (1957) who described gonadal resorption as well as repeat maturation in some male spring Chinook salmon as minijacks five months after spermiation, albeit at a much higher frequency in the current investigation than previously indicated. Additionally, as noted above, Bernier et al. (1993) found that some spring Chinook salmon microjacks could remature as minijacks while being reared in seawater. Finally, Unwin et al. (1999) demonstrated that captive held fall Chinook salmon microjacks could repeat maturation as age-2 minijacks and again at age-3 (common name: jacks). The proportion of precociously mature males that survive as well as remature at age-2 varied among all of these studies, likely due to differences in genotype and life-history (spring vs. fall run), as well as experimental conditions. But, overall, results from this and previous studies demonstrate a robust capacity for iteroparity in precociously mature Chinook salmon.

We note that due to a lack of available tank rearing capacity at the time of this investigation, we were unable to replicate the treatments at the tank level. However, we were able to sustain a sufficient number of biological replicates throughout the

experiment. The lack of significant differences in physiology, life-history and survival between the HR and LR treatments was somewhat surprising, but suggests our LR treatment was not “resource limited” enough or of an insufficient duration to result in significant mortality or prevent rematuration. Compared to the variable environmental conditions of a natural river, our experiment was conducted under constant 10°C water temperature, potentially limiting the need for thermal adaptation while balancing the needs of standard and active metabolism with somatic and gonadal growth. Fish were fed a high energy commercial fish feed as opposed to a natural insect diet typical of wild juvenile salmonids. Furthermore, fish were reared in a relatively stress-free (low activity, low density, pathogen- and predator-free) freshwater environment limiting the need for significant metabolic allocation to osmoregulation, immunological challenges and predator avoidance. Unwin et al. (1999) demonstrated that repeat maturing age-2 precocious males were significantly smaller than first time age-2 maturing males despite an abundant food supply, indicating that repeat maturing fish were incapable of allocating as much energy to somatic growth. Nonetheless, approximately 80% of the age-1 mature males survived to mature again at age-2 and all fish held for another year matured at age-3 (Unwin et al., 1999). These investigators suggest that, in the absence of other factors, low energy reserves do not inevitably result in death. In the current study, the differences in growth, *K* and percent solid (i.e. lipid) between the HR and LR treatments were only modestly different suggesting minimal effect on growth and energetic status. Based on our findings, and those of Unwin et al. (1999), approximating a resource limiting environment typical of a natural river, that may affect survival and capacity for repeat maturation, would likely require more extreme environmental

conditions than those employed in this investigation. Thus, while we failed to accept our third working hypothesis, finding the same results under more extreme experimental conditions may be required to fully reject it.

The observation that nearly half of the microjacks in this study became bloated with ascites shortly after spermiation was noteworthy. In the natural environment, precocious males employ a ‘sneaker’ or ‘swarm strategy’ to secure fertilization opportunities with anadromous females. Presumably, in the event that females are present on the spawning grounds, most precocious males find an opportunity to fertilize eggs and avoid germ cell degradation and ascites. Under laboratory culture conditions spontaneous milt expression seems unlikely. In conventional hatchery operations, precocious males are rarely (if ever) used for broodstock and, thus, not manually stripped of milt to minimize any potential additive genetic effects (heritability) of using precocious males as sires (but, see discussion in Unwin et al (1999) indicating evidence to the contrary). Even if they were used for broodstock they are not retained following milt expression. So, in the absence of manual milt stripping, a significant number of precocious males would likely die in culture. However, once we observed some early mortality in bloated fish, we manually stripped any fish we encountered that displayed this condition. Unexpectedly, they all seemed to recover very quickly and continue the testicular resorption and subsequent rematuration process. So, in those microjacks that became bloated from germ cell ascites, as long as they were manually expressed, mortality was prevented and rematuration was possible. Equally surprising was the observation that approximately half the microjacks in this investigation were capable of

testicular resorption in the absence of manual stripping and it is unclear what factor(s) contribute to this capacity.

To our knowledge this is the first study to systematically characterize a suite of reproductive indices throughout the rematuration process in Chinook salmon. In Atlantic salmon, Maugars and Schmitz (2008b) provide a comprehensive examination of the physiological and endocrinological ontogeny of initiation and completion of age-2 precocious male maturation. Similar to our investigation in Chinook salmon, they tracked seasonal changes in growth, *K*, plasma 11-KT, GSI, relative expression of pituitary *fshb* and *lhb* mRNA and, additionally, relative expression of testicular *fsh* and *lh* receptor (*r*) mRNA. In general, Maugars and Schmitz (2008b) observed significant increases in plasma 11-KT, GSI, and *fshb*, *fshr* and *lhr* mRNA all occuring in June/July approximately 3 months prior to spermiation and upregulation of *lhb* mRNA in August/September approximately 1-2 months prior to spermiation. By contrast, we observed significant increases in *fshb* mRNA as early as December 2010 through February 2011 and significant increases in plasma 11-KT, GSI and *lhb* mRNA starting in March 2011. Thus, in general the process from regression of age-1 maturation to rematuration at age-2 in Chinook salmon appears to commence earlier than simple initiation of age-2 maturation in Atlantic salmon. These differences in timing may be due to species differences between Chinook and Atlantic salmon and/or differences in thermal rearing regimes between studies as the current study was conducted at a constant 10°C whereas the Atlantic salmon in Maugars and Schmitz (2008a) were reared on seasonably variable temperatures. Nonetheless, this finding corroborates those of Unwin et al. (1999) who found evidence of some gonadal retention (based on

GSI) between microjack and minijack maturation in rematuring fall Chinook salmon suggesting that the physiological changes associated with microjack maturation were never fully reversed.

The very high survival and rematuration capacity we observed (i.e. iteroparity) in Chinook salmon microjacks has applicability for understanding and perhaps management of both wild and cultured salmon populations. The presence of microjacks among wild populations of spring Chinook salmon has been recognized since the work of Rutter (1904) in the Sacramento River, California (USA), Gebhards (1960) in the Salmon River Drainage, Idaho and quantified by Pearsons et al. (2009) in the Yakima River, Washington. But, the extent to which these fish may smolt and/or remature in nature is unclear, in part, because these fish are difficult to monitor in the field due to small size and cryptic coloration. Furthermore, the genetic contribution these wild males may make through multi-year breeding events, while likely low (Schroder et al., 2012), is relatively unknown. Alternatively, in some hatchery populations, both relatively high (Flain, 1970; Mullan, 1992) and very low (Larsen et al., 2004) levels of microjack maturation (high for minijack maturation) have been documented. In salmon hatchery smolt supplementation programs where microjacks are present it is important to note that these fish are not released until the following spring as putative smolts. Given that this investigation suggests microjacks do not smolt while also retaining sufficient energy reserves to remature, it is conceivable they residualize until the next spawning season when they may contribute genetically at rates disproportionate to natural occurrences (see Middleton et al., 2025). Understanding the extent to which these microjacks die,

smolt or remature as minijacks under variable rearing conditions may provide useful insight for hatchery management programs.

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FIGURE LEGENDS

Figure 1. Representative photos of the same age-1 microjack Chinook salmon “bloated” from ascites prior to (A) and after (B) manual expression of dilute milt. Following expression, all fish were returned to their respective rearing vessel for recovery.

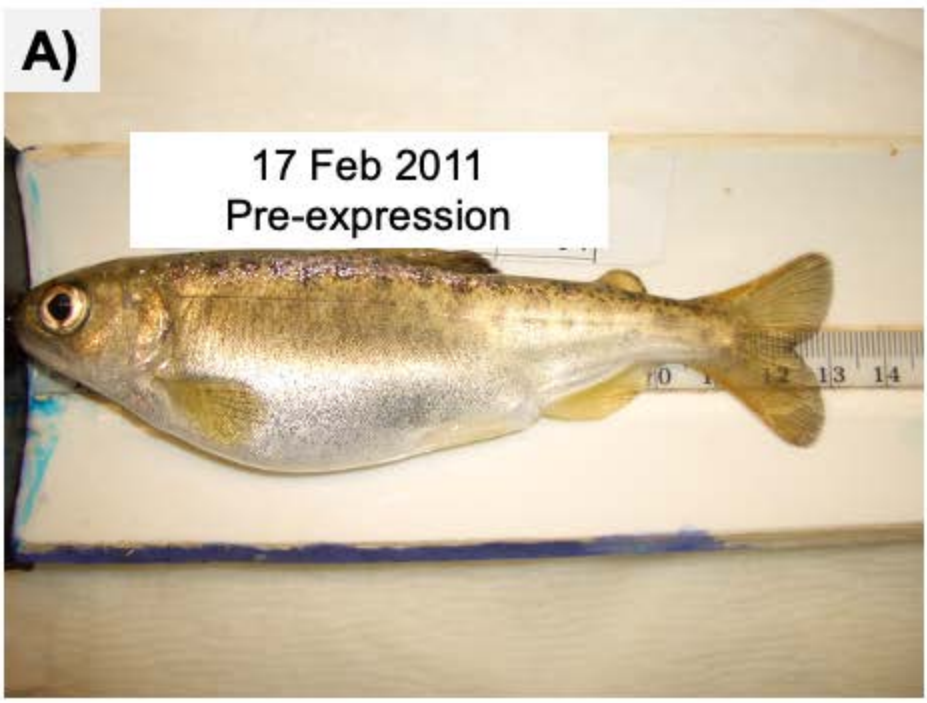
Figure 2. Fork length (A), body weight (B), condition factor (C), and percent (%) solid (D) in spring Chinook salmon age-1 microjacks reared under a high and low winter ration treatment. Arrows indicate the period of winter ration treatment (High or Low). An asterisk indicates a significant difference ($p < 0.05$) between treatments on a given date as determined by two sample t-test. Mean ± SEM; N = 8 per point.

Figure 3. Gill Na+/K+ -ATPase activity (measured as umol ADP $\cdot$ mg protein^{-1} $\cdot$ hr^{-1}) in spring Chinook salmon age-1 smolts (filled black triangles) and microjacks (labeled “mature”, filled black or white circles) reared under a high and low winter ration treatment. Arrow indicates the period of winter ration treatment (High or Low) applied to microjacks. An asterisk indicates a significant difference ($p < 0.05$) between smolts and microjacks (both High and Low treatment) on a given date as determined by one-way ANOVA with Tukey’s post hoc test. Mean ± SEM; N = 16 per point for smolts, N = 8 per point for microjacks.

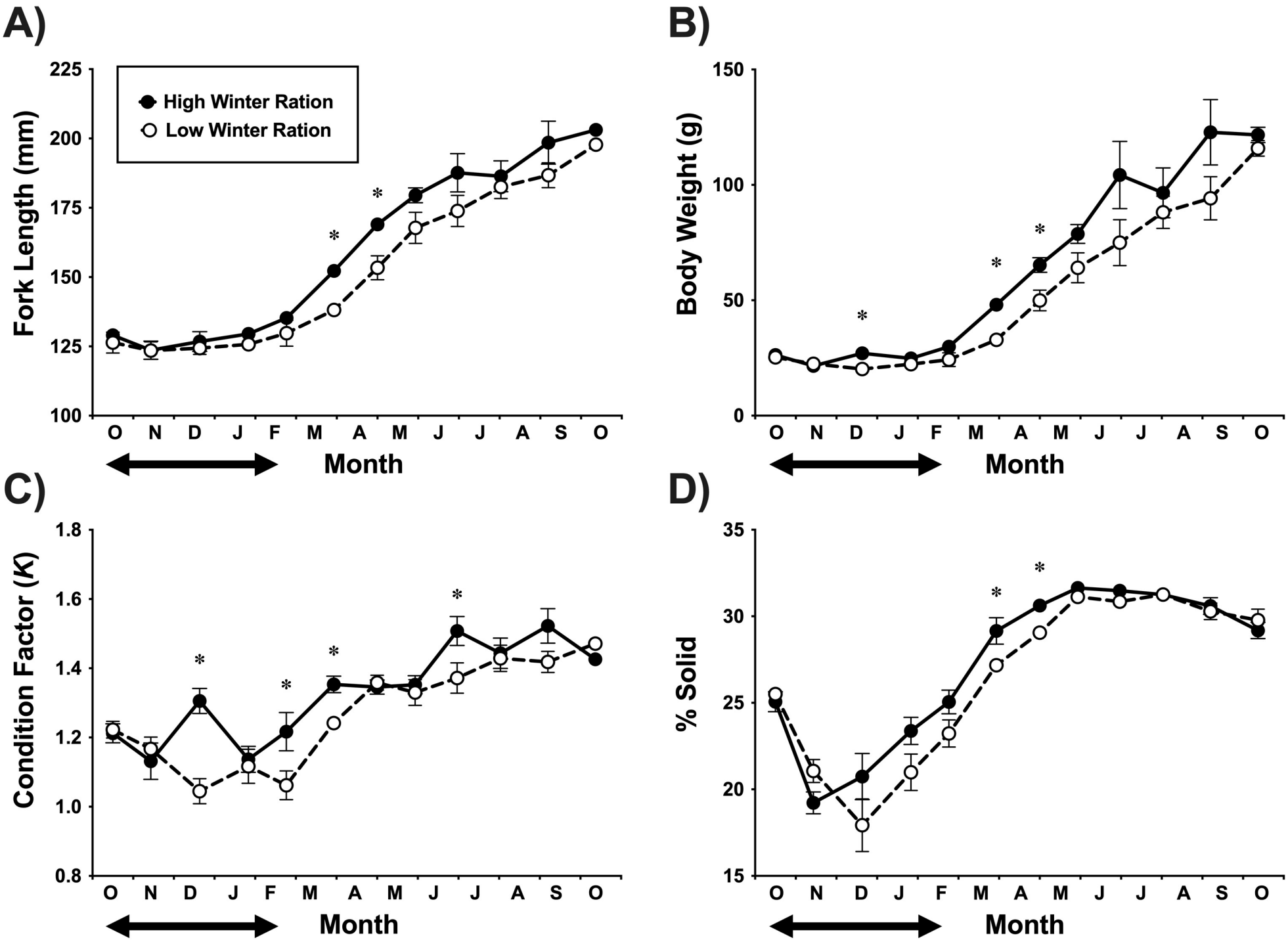
Figure 4. Plasma 11-ketotestosterone (11-KT) (A), gonadosomatic index (GSI) (B), and relative mRNA expression levels of pituitary *fshb* (C) and *lhb* (D) in spring Chinook

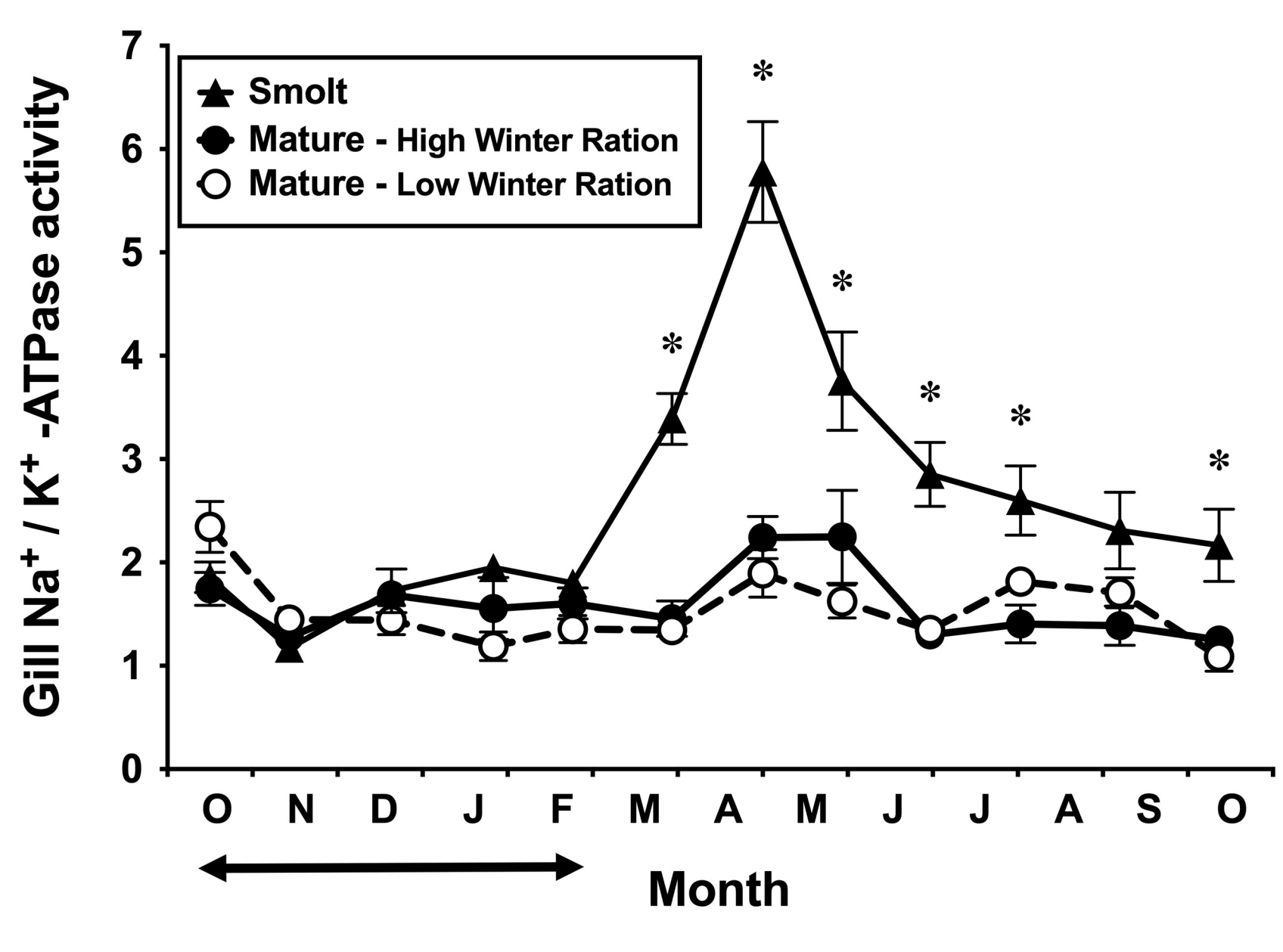
salmon age-1 microjacks reared under a high and low winter ration treatment. Arrows indicate the period of winter ration treatment (High or Low). An asterisk indicates a significant difference ($p < 0.05$) between treatments on a given date as determined by two sample t-test. Mean ± SEM; N = 8 per point.

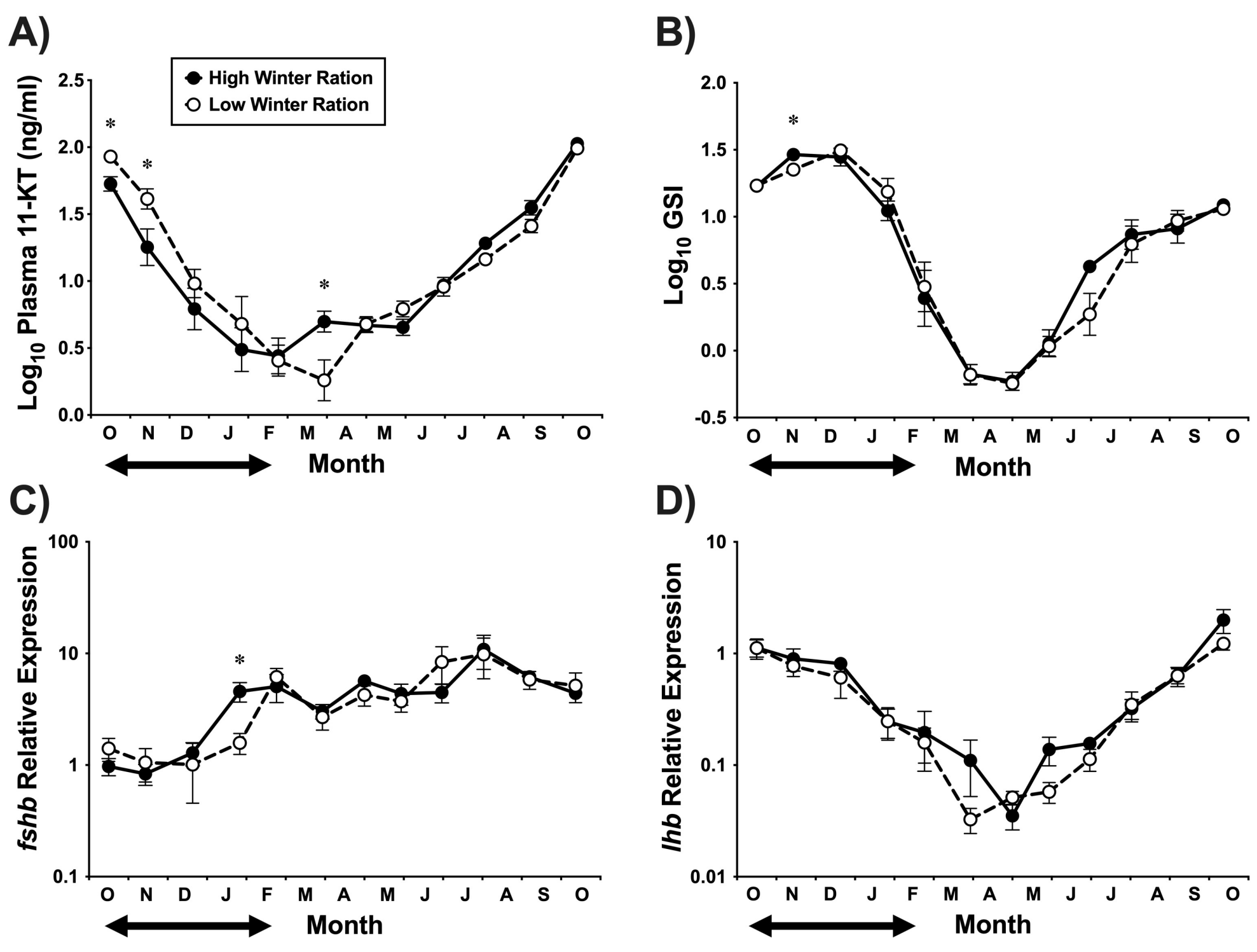
Figure 5. Representative photos of age-1 microjack Chinook salmon (left column) and light micrographs of their associated testes (right column) ranging from early to late testis resorption in Feb-Mar 2011 (A-D), early to mid-testis rematuration in May-July 2011 (E-H), to late rematuration in Aug 2011 (I-J). Abbreviations are as follows: RES, sperm resorption; SGA, type A spermatogonia; SGB, type B spermatogonia; SPC, spermatocyte; SPT, spermatid; SPZ, spermatozoa. All histological panels were imaged at 40x magnification.

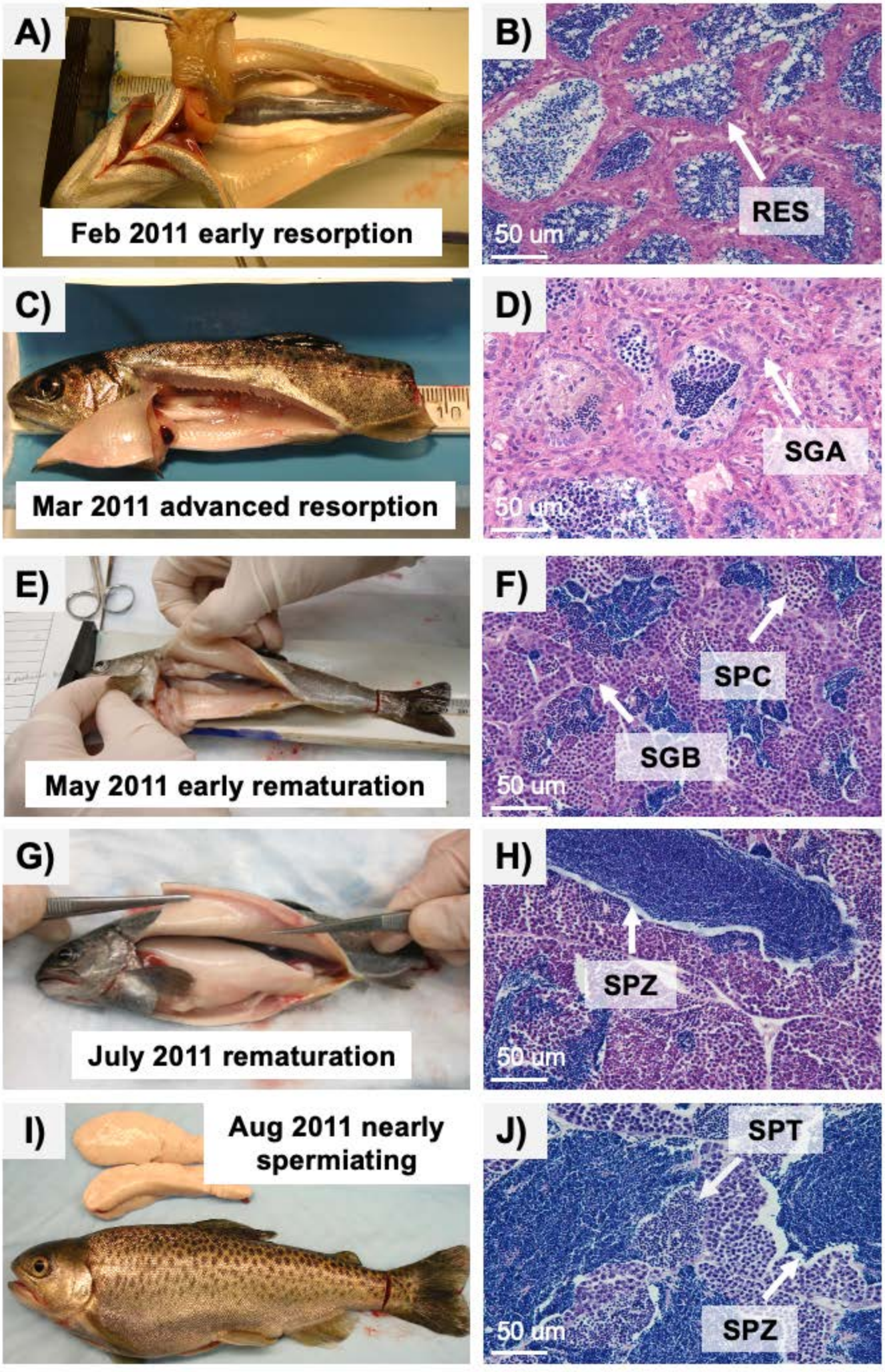

A)
17 Feb 2011
Pre-expression


B)
17 Feb 2011
Post-expression


A)
High Winter Ration
Low Winter Ration
Fork Length (mm)
225
200
175
150
125
100
O N D J F M A M J J A S O
Month
B)
Body Weight (g)
150
100
50
0
O N D J F M A M J J A S O
Month
C)
Condition Factor (K)
1.8
1.6
1.4
1.2
1.0
0.8
O N D J F M A M J J A S O
Month
D)
% Solid
35
30
25
20
15
O N D J F M A M J J A S O
Month


Smolt
Mature - High Winter Ration
Mature - Low Winter Ration
Gill Na+ / K+ -ATPase activity
0
1
2
3
4
5
6
7
O
N
D
J
F
M
A
M
J
J
A
S
O
Month
*


A)
High Winter Ration
Low Winter Ration
Log10 Plasma 11-KT (ng/ml)
2.5
2.0
1.5
1.0
0.5
0.0
*
*
*
O N D J F M A M J J A S O
Month
B)
Log10 GSI
2.0
1.5
1.0
0.5
0.0
-0.5
*
O N D J F M A M J J A S O
Month
C)
fshb Relative Expression
100
10
1
0.1
*
O N D J F M A M J J A S O
Month
D)
lhb Relative Expression
10
1
0.1
0.01
O N D J F M A M J J A S O
Month


A)
Feb 2011 early resorption
B)
RES
50 um
C)
Mar 2011 advanced resorption
D)
SGA
50 um
E)
May 2011 early rematuration
F)
SPC
SGB
50 um
G)
July 2011 rematuration
H)
SPZ
50 um
I)
Aug 2011 nearly spermiating
J)
SPT
SPZ
50 um